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The Relative Toxicity of Waterborne Inorganic Contaminants to the Rio Grande Silvery Minnow (*Hybognathus amarus*) and Fathead Minnow (*Pimephales promelas*) in a Water Quality Simulating that in the Rio Grande, New Mexico

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Rio Grande silvery minnow
Fathead minnow
Acute toxicity
Aluminum
Ammonia
Arsenic
Chlorine
Copper
Nitrate
Site-specific mixture

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EXECUTIVE SUMMARY

Point and non-point source discharges into the Rio Grande in New Mexico degrade water quality, which may adversely affect the only existing populations of the endangered Rio Grande silvery minnow (*Hybognathus amarus*). Laboratory studies were conducted to determine the relative toxicity of aluminum, ammonia, arsenic, chlorine, copper, and nitrate, individually and in an environmentally realistic mixture (excluding chlorine), to larval Rio Grande silvery minnow and larval fathead minnow (*Pimephales promelas*), a standard warm-water test fish. Larvae of both species were exposed to a given chemical in side-by-side tests to minimize temporal variability in test conditions. The test solutions were prepared in a non-standard reconstituted water that simulated the major water quality characteristics in the Rio Grande, New Mexico. The measures of acute toxicity were the median effective concentration (EC50) based on the combined effects of impaired mobility and death and median lethal concentration (LC50) based on death.

The overall rank order of toxicity to both species from most to least toxic was: chlorine > copper > un-ionized ammonia > total ammonia = arsenate > nitrate (aluminum). Rio Grande silvery minnow were more sensitive to copper than fathead minnow during the first 72 h of exposure, but not at 96 h. Differences in sensitivity to copper between the two minnow species after 96 h were 1.6 fold based on lethality, but only 1.1 fold based on impaired mobility. Fathead minnow were more sensitive to arsenic than Rio Grande silvery minnow at all time periods. Both species exhibited similar sensitivities to the other individual chemicals and the mixture. The environmentally realistic contaminant mixture was more than additive in toxicity based on

the combined effects of impaired mobility and death (96-h EC50s) and additive in toxicity based

on death alone (96-h LC50s) to both species. The primary toxic components in the mixture were copper and un-ionized ammonia.

Comparisons of the 96-h EC50 and LC50 values to measured concentrations in the Rio Grande indicate that these chemicals individually or combined at environmentally relevant concentrations do not pose an acute hazard to populations of Rio Grande silvery minnow and fathead minnow. However, the margins of difference between the acutely toxic concentrations of copper and ammonia in the mixture and those measured in the Rio Grande were less than two orders of magnitude (35-66), which indicates that this mixture may pose a chronic hazard to both species. The acutely toxic concentrations of total ammonia in the mixture to both minnow species (4.5-7.7 mg/L as N) are close to or lower than current national and New Mexico acute criterion concentrations (4.5-10.1 mg/L as N) for ambient pH values (7.9-8.1), which indicates that site-specific criteria for ammonia may be more appropriate for mixtures of these chemicals in the Rio Grande.

INTRODUCTION

The Rio Grande silvery minnow (*Hybognathus amarus*) was formerly one of the most widespread and abundant fish species in the Rio Grande basin of New Mexico, Texas, and Mexico. The species has been extirpated from most of its historic range and is now found only in the middle Rio Grande from Cochiti Dam to the headwaters of Elephant Butte Reservoir in New Mexico (USFWS 1994, 1999). The decline of the Rio Grande silvery minnow has been attributed to a number of factors including modification of stream flows and channel desiccation by impoundments, water diversions, stream channelization, competition with and predation by nonnative fishes, and water quality degradation. The species is listed as endangered in the United States, New Mexico, Texas, and the Republic of Mexico (USFWS 1994, 1999).

The only existing populations of Rio Grande silvery minnow continue to be threatened by land use practices in the basin. Point and non-point source discharges into the Rio Grande degrade water quality, which may adversely affect populations of *H. amarus*. The contaminants of concern in the Rio Grande include, but are not limited to, trace elements (aluminum, arsenic, cadmium, copper, lead, mercury, nickel, selenium, and zinc), chlorine, ammonia, and nutrients (NMWQCC 1998, 2000a). Although the toxicity of these contaminants to fish has been well studied, little is known about the sensitivity of Rio Grande silvery minnow to these (and other) waterborne contaminants. It is not known if the sensitivities of standard test organisms, such as fathead minnow (*Pimephales promelas*), are representative of those of the Rio Grande silvery minnow.

In a previous study that compared the relative sensitivity of fathead minnow, two endangered minnows (family, Cyprinidae), and one endangered sucker (family, Catostomidae) to five toxicants (with different modes of action) using side-by-side static tests, at least one

endangered species was more sensitive to three of the toxicants than fathead minnow (Dwyer et al. 1995). Interestingly, copper was one the toxicants evaluated in that study and it was found to be more toxic to two of the endangered fishes than to fathead minnow. Consequently, the use of fathead minnow in standard regulatory testing programs designed to protect aquatic life from toxic discharges, such as in whole effluent toxicity testing (Weber et al. 1989, Weber 1993), may not provide adequate protection of endangered fish species inhabiting waters receiving point and non-point source discharges. Moreover, it is not known if current single chemical water quality criteria derived from toxicity studies with standard test species, such as fathead minnow, provide adequate protection for *H. amarus* exposed to mixtures of these chemicals in the Rio Grande.

Objective

The purpose of this study was to determine the relative sensitivity of larval Rio Grande silvery minnow and larval fathead minnow to aluminum, ammonia, arsenic, chlorine, copper, and nitrate in a water quality simulating that of the Rio Grande. The larvae were tested because they are often the most or among the most sensitive life stage to chemicals (McKim 1985, Weber 1993). The chemicals were selected based on consultations with the U.S. Fish and Wildlife Service and were believed to be representative of inorganic contaminants in the middle Rio Grande. In addition, the acute toxicity of a mixture of the same inorganics (except chlorine), combined at a ratio of their measured concentrations in the Rio Grande was determined with both species. To minimize temporal variability in test conditions and maximize comparability of test results, larvae of both species were exposed to a given chemical in side-by-side tests. Acutely toxic concentrations of the chemicals tested singly and in a mixture were compared to measured concentrations in the Rio Grande to assess their potential hazard to the endangered Rio Grande silvery minnow and native fathead minnow.

METHODS AND MATERIALS

Test Fish

Both species were cultured in 67-L glass aquaria filled with about 48 L of reconstituted test water. Each aquaria received 1 L of fresh reconstituted test water every 15 min, which produced an intermittent flow rate through the aquaria of about 2-volume additions per day. All aquaria were placed in the same temperature-controlled water bath under a photoperiod representative of that for Albuquerque, NM in May (13.5 h light and 10.5 h dark). All fish were held in reconstituted test water at 25 ± 1°C for at least 2 d before they were tested and they were not fed during the tests.

Fertilized eggs of Rio Grande silvery minnow were obtained from feral adults collected in the Rio Grande near Albuquerque, NM (Sandoval and Socorro Counties) and spawned at the Yankton Field Research Station (FRS) by personnel of the University of New Mexico and the City of Albuquerque Aquarium, Albuquerque, NM. The adults (18) were collected with seines and packed in plastic bags filled with about 4 L of water and inflated with oxygen. The bags were placed in coolers and transported to the Yankton FRS within 24 h of capture. Upon arrival, the fish were partially acclimated to the reconstituted test water over a 2-h period. The adults were examined for general health and reproductive condition and then subjected to the spawning procedures of Platania and Altenbach (1998). The semi-buoyant eggs were incubated in aquaria at 22.5°C and kept in suspension by vigorously aerating the water with a large airstone (15.2 x 3.8 cm) placed on the bottom and across the width of each aquarium. The resulting larvae were cultured in the same aquaria and the temperature was gradually increased to 25°C over a 30-h period. The larvae were fed a commercial fish diet (Fry Feed Kyowa A, Kyowa Hakko Kogyo Inc., Tokyo, Japan) after they inflated their swim bladders at 2 d posthatch (dph).

Fathead minnow larvae (1-dph) were obtained from Aquatic Biosystems, Inc. (Fort Collins, CO). The larvae were shipped by air in a plastic bag packed in a cooler. Upon arrival, the larvae were partially acclimated to the reconstituted test water over a 2-h period and then placed in an aquarium filled with reconstituted test water at 22.5°C. The temperature was gradually increased to 25°C over a 30-h period. The larvae were fed nauplii of brine shrimp (*Artemia* spp.) starting at 2 dph.

Dilution Water

Tests were conducted in a nonstandard reconstituted water designed to simulate the major water quality characteristics (without trace inorganic contaminants) of the Rio Grande at Isleta, NM in May (USGS Station ID 08331000; Table 1; Ortiz and Lange 1997). This site was selected because it is below the city of Albuquerque, NM and is in a reach of the Rio Grande currently inhabited by Rio Grande silvery minnow. The water quality data for May were used because Rio Grande silvery minnow spawn in May-June (USFWS 1999) and this water quality would be representative of that in which the larvae would be exposed to contaminants. The dilution water was prepared by adding appropriate amounts of calcium chloride, calcium sulfate dihydrate, magnesium carbonate, sodium bicarbonate, and potassium bicarbonate to deionized water (DI) in a 5,678-L polyethylene tank equipped with a recirculating pump to mix and aerate the water.

Each tank of reconstituted water was analyzed for selected water quality characteristics prior to use in fish culture or testing. Calcium, total alkalinity, and total hardness were measured using titration methods of APHA (1995). Magnesium was determined indirectly by the difference between total hardness and calcium. Chloride was measured using the mercuric nitrate titration method of Hach (1997). Sulfate was determined by the turbidimetric method

described in APHA (1995) using Hach SulfaVer 4 reagent (Hach 1997) and a Turner Designs (Mountain View, CA) model 40 nephelometer. Conductivity (corrected to 25°C) was measured with a YSI (Yellow Springs Instruments, Yellow Springs, OH) model 31 conductivity bridge with a cell constant of K = 1.0 ± 1%.

Test Chemicals

The chemicals (percent inorganic toxicant) and their sources used to prepare the test solutions were as follows: aluminum chloride 6-hydrate (11.0% Al), ammonium chloride (26.2% NH_3 as N), sodium hypochlorite solution (5.4% available Cl), sodium arsenate dibasic 7-hydrate (23.5% As), and sodium nitrate (16.5% NO_3 as N) were obtained from Baker Chemical (Phillipsburg, NJ) and copper sulfate pentahydrate (25.2% Cu) was obtained from Aldrich Chemical (Milwaukee, WI). The percentage of inorganic toxicant in each chemical was determined from the certificate of analysis provided by the supplier. Arsenate was used in this study because this form is favored under conditions of high dissolved oxygen (DO), high redox potential (Eh), and basic pH (NRCC 1978), which occur in the Rio Grande at the Isleta Site (DO, 7.4 mg/L and pH, 7.9; Ortiz and Lange 1997).

Test solutions of all chemicals, except for nitrate, were prepared by pipetting appropriate aliquants of stock solution (prepared in DI water) into 2-L beakers containing 900 or 1,800 ml of tempered dilution water and gently mixing with plastic stirring rods. Nitrate solutions were prepared by adding appropriate amounts of sodium nitrate directly to the 2-L beakers and mixing each solution until the chemical was dissolved. After all solutions were prepared, the beakers were covered with plastic wrap and placed in a water bath at 25 ± 1°C until used. This test solution (in the 2-L beakers) was used in both pairs of side-by-side tests and for chemical analysis.

The initial pH of aluminum test solutions decreased in proportion to the amount of aluminum chloride added, and ranged from 4.0 to 7.8. To remove the effect of low pH on aluminum toxicity, the pH of the test solutions was adjusted to within 0.1 unit of the control treatment with 1N NaOH prior to testing. In addition, a pH-adjusted control treatment was prepared by adding the largest amount of 1N NaOH used to adjust the pH of the highest aluminum concentration and then adding 1N and 10N HCl to lower the pH to its original value.

The mixture tested with both species was composed of ammonia, aluminum, arsenate, copper, and nitrate combined at a concentration-ratio representative of that in Rio Grande. The ratio was based on measured concentrations in the Rio Grande at Isleta, NM [USGS Station 08331000], on May 20-21, 1996 (Ortiz and Lange 1997). Chlorine was not included in the mixture because the City of Albuquerque Wastewater Treatment Plant dechlorinates its effluent during routine operations. Nine mixture treatments were tested and in each treatment, the chemicals were combined at a fixed ratio (environmental concentration) of: 1 copper (0.002 mg/L):1.5 arsenic (0.003 mg/L):1.5 aluminum (0.003 mg/L):65 total ammonia-nitrogen (0.130 mg/L):160 nitrate-nitrogen (0.320 mg/L). The ammonia concentrations in the mixture were based on total ammonia instead of un-ionized ammonia because the concentration of un-ionized ammonia varies with pH (which was not controlled) and temperature (discussed below). Concentrations of copper in the mixture treatments were the same as those used in the individual copper tests. The mixture solutions were prepared as described above using the same or diluted stock solutions of ammonia, aluminum, arsenate, and copper prepared for the individual tests. Nitrate was added to the mixture solutions either directly as sodium nitrate for the two highest concentrations or as a stock solution for the remaining concentrations. Hereinafter, the mixture will be referred to as the Isleta Site mixture.

Experimental Design

Acute toxicity tests were conducted under static-renewal conditions following the procedures recommended by the American Society for Testing and Materials (ASTM 1996). Each test consisted of exposing groups of 10 fish to a geometric series of 7-9 test concentrations (with a 60% dilution factor between concentrations) and a control treatment for 96 h. Both species were exposed to the same test solutions (as prepared above) in side-by-side tests conducted in 1-L glass beakers containing 600 mL of solution. At 24, 48, and 72 h of exposure, test solutions with live fish were renewed using a 50% volume replacement with freshly prepared test solution. Water renewals were accomplished by siphoning down the water level to the

300-mL calibration line using a glass pipet with the submerged end covered with a polypropylene filter cloth (285 µm openings) attached with a latex band. The filter cloth was used to prevent larvae from being siphoned out of the beaker. After siphoning, 300 ml of tempered newly prepared test solution was added by slowly pouring the solution down the side of the beaker.

Exposure beakers were randomly assigned within water baths maintained at 25 ± 1°C and both pairs of a given side-by-side test were placed in the same water bath. Tests were conducted under normal laboratory lighting from fluorescent bulbs and light intensity measured 10 cm above the water surface at the middle and both ends of each water bath averaged 15 ft-candles.

Tests were initiated with Rio Grande silvery minnow at 3 to 5 dph and fathead minnow at 4 to 6 dph. For each test, about 200-300 larvae were siphoned from the culture tank into a plastic pan (23 cm x 18 cm x 9.5 cm). Groups of five fish were counted into medium-size plastic

weigh boats partially filled with tempered control water using wide-bore glass pipets. After each weigh boat received five fish, most of the water was carefully decanted, leaving just enough water for total immersion of the larvae. The larvae were recounted and inspected for general condition (replaced if necessary) and then poured into the test beakers. After each beaker contained five fish, the procedure was repeated so that 10 fish were stocked into in each beaker. The fish were impartially allocated to the exposure beakers and the order of the species stocked was randomly assigned for each test. An attempt was made to stock the larvae within 30 minutes after preparing the test solutions. The remaining larvae in the plastic pan were returned to their culture tank.

Observations on mortality and overt changes in mobility of the fish were made at 24-h intervals, just before the water renewals. Each beaker was removed from the water bath and placed on a light table for the observation. The criterion for death was the absence of a heart beat in fish for a 10-second observation period examined under 30X magnification. The dead fish were not returned to the beaker. Behavioral responses used to characterize impaired mobility were immobilization, loss of equilibrium, and pronounced lethargy. These responses were considered to be ecologically important because they decrease the individual's ability to survive in the wild. Total length and body weight were measured on the control fish at the end of the test (Table 2). Total length was measured to the nearest 0.1 mm using an ocular micrometer (Reichert model 426C filar micrometer eyepiece, Reichert Scientific Instruments, Buffalo, NY) fitted on a stereoscope. Body weight was determined to the nearest 0.1 mg by weighing a pooled sample of all live fish from a given beaker on a Mettler (Highstown, NJ) model PE 360 balance.

Water Quality

Dissolved oxygen, pH, and temperature were measured directly in the exposure vessels using portable meters (YSI model 58 dissolved oxygen meter, Yellow Springs Instruments, Yellow Springs, OH and Orion model 250A pH meter and Orion model 9107 pH electrode, Orion Research, Boston, MA). In all tests, DO was measured in the control, low, medium, and high test concentrations with live fish at 0, 48, and 96 h of exposure. The pH and temperature were measured in all test concentrations of aluminum, ammonia, and Isleta Site mixture and in the control, low, medium, and high test concentrations of arsenic, chlorine, copper, and nitrate with live fish at 0, 48, and 96 h of exposure. Hardness, alkalinity, and conductivity were measured once during each test in newly prepared solutions for the control, low, medium, and high treatments. Reliable results could not be obtained for hardness measured in the medium and high copper and Isleta Site mixture treatments due to matrix interferences with the colorimetric method used. Test solutions of aluminum, arsenic, copper, and Isleta Site mixture were also analyzed for boron, calcium, iron, potassium, magnesium, manganese, molybdenum, and zinc. The collection of samples and analytical methods used are described in the Toxicant Analysis section below.

Toxicant Analysis

The initial concentration of each toxicant was measured in unfiltered water samples collected from the test solution preparation beakers. Water samples for total residual chlorine (TRC) analysis were collected in 250-ml beakers at the time of analysis, which usually began within 2 h of test initiation or water renewal, and covered with a watch glass. Due to the instability of chlorine in aqueous solutions (APHA 1995), TRC was also measured in freshly prepared test solutions with live fish at 24, 48, and 72 h of exposure. Water samples for ammonia and nitrate analysis were collected in tightly capped low density polyethylene (LDPE)

bottles (125 or 250 mL) and analyzed within 4 hours of collection. Analysis of ammonia, nitrate, and TRC were performed at the Yankton FRS.

Unfiltered water samples from tests with aluminum, arsenic, copper, and Isleta Site mixture were collected in 250-mL LDPE bottles and preserved in 5% ultrapure HNO_3 (10 ml acid:190 mL sample). A second set of samples was collected from each aluminum treatment and filtered through a 0.4 μm polycarbonate filter prior to acidification, because a white precipitate formed in most of the test concentrations. The samples were stored under refrigeration until they were packed with blue ice in a cooler and shipped by overnight delivery to Research Triangle Institute, Research Triangle, NC. All samples were analyzed for the three test chemicals (aluminum, arsenic, and copper) and also for boron, calcium, iron, potassium, magnesium, manganese, molybdenum, and zinc.

Total ammonia-nitrogen (TA-N) and nitrate-nitrogen (NO_3-N) were measured using ion-selective electrodes connected to an Orion model 901 Ionalyzer (Orion Research, Boston, MA) following the procedures of Orion (1990, 1991). Un-ionized ammonia-nitrogen (NH_3-N) concentrations were calculated using the ammonia equilibrium equations of Emerson et al. (1975) and measured values of total ammonia, pH, and temperature at 0 h. Detection limits of the methods, based on the lowest ammonia and nitrate standard analyzed, were 0.1 mg/L as TA-N and 1.0 mg/L as NO_3-N. Quality assurance methods included analysis of duplicate samples and sample spikes. Relative percent difference for duplicate analyses of two samples were 0.8% for TA-N and 0.0 and 1.3 % for NO_3-N. Spike recoveries (n = 1) were 98.4% for TA-N and 99.0% for NO_3-N.

Total residual chlorine was measured in triplicate 200-mL samples using a Bailey, Fisher, and Porter (Wickliffe, OH) model 17T2000 amperometric titrator and proprietary reagents, with

a sensitivity of 0.005 mg/L. Total residual chlorine is a measure of both free and combined chlorine in water and is the form of chlorine upon which water quality criteria were derived (USEPA 1985a). Accuracy of the method was verified by analyzing a certified quality control standard (Environmental Resource Associates, Arvada, CO) and a 10-fold dilution of the standard at 0 h. The 10-fold dilution was used to achieve a TRC concentration that was within the range of the exposure concentrations. Also, one in-house chlorine standard was prepared and analyzed at 72 h. The measured concentration of TRC in the commercial standard (2.88 mg/L) was within the performance acceptance limit of 2.00-3.15 mg/L and was 7.5% greater than the certified value (2.68 mg/L). Measured concentrations of TRC in the diluted commercial standard at 0 h and in-house standard at 72 h were within 11.1 and 3.3%, respectively of the expected values (0.268 and 0.120 mg/L).

Concentrations of aluminum, boron, calcium, copper, iron, magnesium, manganese, molybdenum, and zinc were determined by inductively coupled plasma (ICP) emission using a Leeman Labs Spec I sequential or ES2000 simultaneous spectrometer. Arsenic and potassium were measured by graphite furnace atomic absorption (GFAA) using a Perkin-Elmer Zeeman 3030 or 4100ZL atomic absorption spectrometer. Prior to analysis, the samples were digested according to standard procedures of the contract laboratory. Quality control measures for ICP and GFAA analysis included the preparation and analysis of procedural blanks (to measure contamination and determine detection limits), duplicate samples (to measure precision), predigestion sample spikes (to measure matrix effects and loss of analyte), and blank spikes (used in place of standard reference material to measure accuracy). Quality control results for all 11 elements were within acceptable limits of the analytical laboratory and are given in Table 3. For the three test chemicals, concentrations in the procedure blanks were below the detection

limits, which were 0.0222 mg/L for aluminum and 0.0056 mg/L for arsenic and copper. The relative percent differences for duplicate analyses were 1.7 and 4.9% for aluminum and 0.0% for arsenic and copper. Spike recoveries in samples and procedure blanks ranged from 104 to 110% for aluminum, 104 to 112% for arsenic, and 105 to 115% for copper.

Data Analysis

The measure of acute toxicity was expressed as both median effective concentrations (EC50s; concentration of a chemical producing an effect in 50% of test fish) and median lethal concentrations (LC50s; concentration of chemical lethal to 50% of test fish). The EC50s were based on the total number of dead fish plus those exhibiting impaired mobility (defined above) at each concentration. The use of EC50s, based on a combination of severe adverse responses that are likely to result in death or reduced fitness of an organism in the wild, would better reflect the total toxic impact of the chemical and provide a more realistic assessment of its acute hazard potential compared to using lethality alone (Stephan 1982).

The EC50 and LC50 values along with their 95% confidence intervals (C.I.) were calculated for each time period by the moving average-angle method (Peltier and Weber 1985). In cases where no partial kills occurred, the 95% confidence intervals were estimated as follows: the lower limit was the highest concentration with 0% mortality and the upper limit was the lowest concentration with 100% mortality. The criterion of nonoverlapping 95% C.I. was used to determine significant differences ($P = 0.05$) between LC50 or EC50 values (APHA 1995). All LC50 and EC50 values were based on measured total concentrations at 0 h as recommended by ASTM (1996) for static-renewal tests. Although TRC concentrations were measured daily in fresh test solutions with live fish, initial TRC concentrations were used for calculating the EC50 and LC50 values because most mortality occurred during the first 24 h of exposure and the

relative percent difference between initial TRC concentrations and those measured at 24, 48, or 72 h ranged from 0 to 19%. The maximum acceptable variation in the concentration of a toxicant in any treatment during an acute toxicity test for regulatory applications is 20% (Weber 1993), which is greater than the temporal variation in measured TRC concentrations observed in this study.

Routine statistical analyses of water chemistry data, including correlation and regression tests, were performed using Statistical Analysis System (SAS 1990a, 1990b) software. Concentrations of elements measured by ICP and GFAA spectroscopy were compared using analysis of variance (ANOVA) and when significant differences were detected, individual treatment means were compared using Tukey's multiple comparison test. Before the final analysis, the assumptions of normality and equal variance were tested on the residuals by Shapiro-Wilk's test (SAS 1990a) and Levene's test, (Milliken and Johnson 1984) respectively. If the data did not meet both assumptions at a significance level of 0.01 (Weber et al. 1989), analogous comparisons were made using the nonparametic Kruskal-Wallis method (SAS 1990b) and Dunn's test (Hollander and Wolfe 1973). Statistically significance was declared at P values of ≤ 0.05.

The joint toxicity of the Isleta Site mixture was analyzed using the toxic unit concept of Sprague (1970) and additive index of Marking and Dawson (1975). The toxic unit (TU) concept was used to determine the contribution of the individual chemicals to the toxicity of the mixture. Toxic units are theoretical toxic contributions of the components to a mixture and are calculated by dividing the LC50 or EC50 of each chemical in the mixture by its individual LC50 or EC50 value. Toxic units are summed to estimate the toxicity of the mixture; if the summed TU value is ≥ 1.0, the mixture is predicted to be lethal. The additive index (AI) and range of Marking and

Dawson (1975) were used to quantitatively assess the type of joint toxicity exhibited by the Isleta Site mixture to both species. The AI was derived from the summed TU value according to following equations:

For $\sum TU \leq 1.0$, the AI = $(1 \div \sum TU) - 1.0$

For $\sum TU \geq 1.0$, the AI = $(\sum TU \times -1) + 1.0$

Marking and Dawson (1975) used the variable S (sum of the biological activity), instead of $\sum TU$ in their equations. The range of the AI was calculated using summed TU values derived by substituting the 95% confidence intervals for the EC50s or LC50s as follows: The lower value of the range was calculated by substituting the lower limits (of the 95% C.I.) of the individual tests and the upper limits of the mixture test for the EC50 or LC50 values. The upper value of the range was calculated by substituting the upper limits of the individual tests and the lower limits of the mixture test for the EC50 or LC50 values. The mixture was judged to be additive in toxicity when the range of the AI overlapped zero. A mixture with AI and range values > 0 was considered to be more than additive in toxicity (synergistic) and a mixture with AI and range values < 0 was considered to be less than additive in toxicity (antagonistic).

RESULTS

Test Conditions

Initial measured toxicant concentrations in unfiltered water from the individual tests were within 12% of nominal values, except for chlorine (Appendix A). Differences between measured and nominal concentrations (expressed as a percentage of nominal value) averaged (range) -0.7% (-3.9 to 3.7%) for aluminum, -3.0% (-5.5 to 0.0%) for TA-N, -8.5% (-12.5 to 1.1%) for arsenic, -0.4% (-7.2 to 6.4%) for copper, and -2.2% (-4.8 to 0.9%) for NO_3-N. Measured aluminum concentrations in filtered water (operationally defined as dissolved

aluminum) from aluminum test solutions were similar across treatments and ranged from only 1.06 to 1.30 mg/L (four of five values were 1.06-1.10 mg/L). These dissolved aluminum concentrations were only about 2 to 14% of the total aluminum concentrations in the same waters.

For chlorine, the difference between measured and nominal concentrations increased with decreasing exposure concentrations. In the three highest chlorine treatments, initial measured TRC concentrations were within 90-94% of nominal values; whereas in the three lowest treatments, measured TRC concentrations were only 32-49% of nominal values. Average TRC concentrations measured in solutions prepared at 24, 48, and 72 h of exposure were 5 to 13% higher than those measured at test initiation. The slightly higher TRC observed at 24 to 72 h compared to those at 0 h may be partly due to differences in holding times for the water samples and the instability of chlorine in aqueous solutions, particularly at low concentrations (APHA 1995). Test solutions prepared at 0 h were analyzed for TRC about 1.5-2.0 hours later than those prepared at 24 to 72 h, because of the time required to stock fish in exposure vessels.

For the Isleta Site mixture, measured concentrations of the components were generally within 10% of the nominal values except for aluminum (Appendix B). Differences between measured and nominal concentrations (expressed as a percentage of nominal value) averaged (range) -3.0% (-8.7 to 1.4%) for arsenic, -2.3% (-5.6 to 3.8%) for TA-N, 9.7% (2.2 to 29.8%) for copper, and 10.2% (-2.9 to 22.5%) for NO_3-N. Differences between measured and nominal concentrations exceeded the 20% variability criterion (Weber 1993) for copper and nitrate in the two lowest mixture treatments. Measured aluminum concentrations in the same mixture solutions ranged from 102 to 324% of nominal values. However, when the aluminum concentration in the control treatment was subtracted from those in the test treatments, measured

concentrations averaged 108% (96-143%) of nominal concentrations.

Initial concentrations of each test chemical in the control treatments were below the detection limit, except for copper in the aluminum control treatment and aluminum in all control treatments. Copper concentration in the aluminum control treatment (0.0060 mg/L) was only 7% above the detection limit (0.0056 mg/L). Aluminum concentrations in the control treatments ranged from 0.079 to 0.254 mg/L and were similar to those in test solutions of arsenic and copper (range, 0.075 to 0.197 mg/L). Arsenic concentrations in test solutions of aluminum and copper were below the detection limit (0.0056 mg/L). Copper was detected in 3 of 15 test solutions of aluminum and arsenic, but these concentrations were only 7 to 25% higher than the detection limit (0.0056 mg/L).

Dissolved oxygen concentrations in the exposure beakers, before renewals, were ≥ 75% air saturation at 48 and 96 h (Appendix C). The initial pH of control solutions ranged from 8.0 to 8.4 for both species (Appendix C). In test solutions of arsenic, chlorine, copper, and nitrate the initial pH was within 0.2 unit of the controls and ranged from in 8.1 to 8.5. Initial pH of ammonia and Isleta Site mixture test solutions ranged from 7.2 to 8.3 and was inversely related to ammonia concentration ($r^2 = 0.953$ and 0.996, $P < 0.0001$, for pH values converted to hydrogen ion concentration).

Alkalinity and conductivity, but not hardness, were affected by at least one of the test chemicals (Appendix D). Alkalinity increased with increasing arsenic concentration and the highest Isleta Site mixture treatment had the lowest alkalinity (114 mg/L as $CaCO_3$). Mean alkalinity for the remaining test solutions was the same as that for the control treatments (128 as $CaCO_3$). Conductivity of test solutions of aluminum, arsenic, ammonia, nitrate, and Isleta Site mixture increased with increasing toxicant concentration and except for nitrate, the relation

between conductivity and test concentration was highly significant ($r = 1.000$, $P \leq 0.01$, N = 3). Hardness of the test solutions was similar to that of the controls and averaged 141 (range, 140-143) mg/L as $CaCO_3$ across all test concentrations. Calculated hardness values for test solutions of aluminum, arsenic, copper, and Isleta Site mixture, based on measured concentrations of calcium and magnesium and conversion factors of Hem (1970), averaged 148 (range, 142 to 153) mg/L as $CaCO_3$. Differences between measured and calculated hardness values for the same control or toxicant treatment were less than 10%.

Concentrations of iron, molybdenum, and zinc in test solutions of aluminum, arsenic, copper, and Isleta Site mixture were generally below detection. Iron was detected in only 3 of 34 treatments and the highest concentration was 0.056 mg/L. Zinc was only detected in the pH-adjusted aluminum control treatment. The source of zinc contamination in this sample is not known, but it probably was not due to the sodium hydroxide used to adjust the pH because the same sodium hydroxide solution was added to each aluminum treatment. Also, zinc was not detected in filtered water from this control, which indicated that the hydrochloric acid used to re-adjust the pH was not the source of zinc contamination.

There were no differences in mean concentrations of calcium or potassium in test solutions of aluminum, arsenic, copper and the Isleta Site mixture (Appendix D). Isleta Site mixture test solutions had the highest magnesium and lowest manganese concentrations compared to those of aluminum, arsenic and copper, but these differences were not considered biologically significant because the largest difference between means was less than 1 mg/L for magnesium and lower than the detection limit for manganese (0.0022 mg/L). Mean concentrations of boron in unfiltered aluminum solutions were higher than those in the other solutions, but the largest difference between means (0.020 mg/L) was lower than the detection

limit (0.022 mg/L).

Individual Toxicity Tests

There were no mortalities in the control treatments, except in the Rio Grande silvery minnow-chlorine test where one fish died between 72 and 96 h. In tests with aluminum, a precipitate was observed in all but the lowest test concentration and the highest test concentration only killed 20-30% of the fish after 96 h. Consequently, the EC50 and LC50 values of aluminum for both species are reported as being greater then the highest concentration tested (59.1 mg/L as total aluminum and 1.3 mg/L as dissolved aluminum).

Relative toxicity

The acute lethality of the inorganic chemicals to larval Rio Grande silvery minnow and fathead minnow varied greatly; chlorine (as TRC) was the most toxic chemical (96-h LC50s, 0.114-115 mg/L) and nitrate was the least toxic chemical (96-h LC50s, 1,278-1,519 mg/L) to both species (Table 4). After 96 h of exposure, un-ionized ammonia was 14-15 times more lethal than total ammonia to both species at a pH range of 7.6 to 8.3.

The use of EC50s as the measure of acute toxicity did not change the rank order of toxicity of these chemicals to either species, but the number of fish exhibiting impaired mobility varied among chemicals and with exposure time (Table 4). In the nitrate tests, most surviving fish in concentrations bracketing the LC50s at 48 to 96 h of exposure exhibited impaired mobility and the 48-, 72-, and 96-h EC50 values were significantly lower (38-59%) than the corresponding LC50 values for both species. Conversely, impaired mobility of fish was rarely observed in test solutions of ammonia and arsenic as evidenced by the similarity in corresponding EC50 and LC50 values. In the chlorine tests, impaired mobility of fish was only observed after 72 h of exposure, but differences between corresponding EC50 and LC50s were

not significant. In the copper tests, two concentrations that killed 100% of the Rio Grande silvery minnow after 24 h only killed 30-40% of the fathead minnow. However, most of the surviving fathead minnow in these copper treatments exhibited impaired mobility and the 24-h EC50 was significantly lower than the corresponding 24-h LC50.

To assess the time course of effects, toxicity curves were constructed for each test by plotting LC50 values against exposure time on logarithmic scales and fitting a line to the points by eye (not shown; Rand and Petrocelli 1985, APHA 1995). The curves (not shown) for arsenic, ammonia, and nitrate tested with both species and copper tested with fathead minnow did not appear to become asymptotic to the time axis. Because lethal thresholds were not apparent within 96 h, it is not known if the 96-h LC50s adequately estimate incipient or threshold LC50s for these chemicals. Consequently, additional mortality of larvae might have occurred after 96 h of exposure to these chemicals (Sprague 1969, Rand and Petrocelli 1985). However, interpretation of the toxicity curves is subjective as there are no criteria for determining whether an incipient LC50 has been achieved (Sprague 1990).

With the exception of chlorine, the LC50 values decreased from 24 to 96 h, but the magnitude of reduction varied among chemicals and species (Table 4). The toxicity of copper and ammonia (total and un-ionized) to both fishes increased 23-57% between 24 and 96 h, and these differences were significant in tests with copper and fathead minnow and ammonia and Rio Grande silvery minnow. The toxicity of arsenic and nitrate to both fishes increased by only 10 to 17% during the same time period.

The time course of toxicity for the individual chemicals based on EC50s was similar to those based on LC50s, except for nitrate (Table 4). The 24-h EC50s of nitrate for both species were greater than those at 48, 72 and 96 h and nitrate toxicity increased by about 56-65% from

24 to 96 h.

Comparative sensitivity

Based on lethality, Rio Grande silvery minnow were more sensitive (2.1- to 2.5-fold) to copper than fathead minnow during the first 72 h of exposure (Table 4). After 96 h, the difference between LC50 values for copper was not significant, but Rio Grande silvery minnow were 1.6 times more sensitive than fathead minnow. Fathead minnow were twice as sensitive to arsenic as Rio Grande silvery minnow at all time periods. The LC50s of chlorine, total ammonia, un-ionized ammonia, and nitrate for fathead minnow were numerically lower than those for Rio Grande silvery minnow at each time interval, but the differences were not significant.

Sensitivity comparisons based on EC50s were similar to those based on lethality, except for copper (Table 4). Differences in copper EC50s between species were only significant at 48 and 72 h and the difference between 96-h EC50s was only 1.1-fold.

Mixture Toxicity

The type of joint toxicity produced by the Isleta Site mixture varied with exposure duration and measure of toxicity (Table 5). Based on lethality at 24 h (i.e., 24-h LC50s), the mixture was judged to be less than additive in toxicity (additive index and range < 0) to Rio Grande silvery minnow, but more than additive in toxicity (range of additive index > 0) to fathead minnow. After 96 h, the mixture was considered to be additive in joint lethality to both species (range overlaps 0). When mixture toxicity was based on the combined effects of impaired mobility and lethality (i.e., EC50s), it was judged to be more than additive in toxicity to both minnow species at 96 h.

Based on EC50 and LC50 values of copper and un-ionized ammonia in the Isleta Site

mixture, fathead minnow were more sensitive to the mixture than Rio Grande silvery minnow at 24 h, whereas both species were equally sensitive to the mixture at 96 h (Table 5). The toxicity of the Isleta Site mixture to both species increased significantly with exposure duration. Differences between 24-h and 96-h EC50s or LC50s ranged from 1.9- to 4.1-fold for copper and 1.5- to 2.2-fold for un-ionized ammonia. Toxicity curves (not given) for the mixture based on copper or un-ionized ammonia did not appear to become asymptotic to the time axis, indicating that additional mortality may occur in both species after 96 h of exposure.

To determine the major toxic components in the Isleta Site mixture, the TU of each inorganic was expressed as a percentage of the summed TU value. Based on this analysis, copper and un-ionized ammonia were the primary toxic components in the mixture because together they contributed about 93-98% of the mixture toxicity to both species at each observation time. Based on 96-h EC50 and LC50 values, copper contributed 49-62% and un-ionized ammonia 36-50% of the mixture's toxicity to both species. The relative toxic contribution of the other three chemicals to the mixture was believed to be minor because each chemical contributed $\leq$ 5% of the observed toxicity.

DISCUSSION

The results of this study indicate that fathead minnow may be an acceptable surrogate species for estimating the acute hazard potential of chlorine, nitrogenous chemicals, and certain metals or metalloids, individually and combined, to the endangered Rio Grande silvery minnow. With the exception of copper tested individually, fathead minnow had numerically lower EC50 and LC50 values for each chemical and the Isleta Site mixture at all observation times compared to Rio Grande silvery minnow. For copper tested individually, Rio Grande silvery minnow were statistically more sensitive than fathead minnow during the first 72 h of exposure. However,

after 96 h of exposure, interspecific differences in sensitivity to copper tested individually (based on EC50 or LC50 values) were not significant.

The observed interspecific difference in copper sensitivity during the early part of the exposure may be due to differences in the rate of intoxication processes (i.e., time required for the fish to expire) rather than to inherent differences in tolerance. Lethal effects of copper occurred rapidly in Rio Grande silvery minnow as most mortality occurred within 24 h of exposure. In contrast, lethality of fathead minnow in the high copper treatments occurred gradually over the test period. The time course of copper toxicity (plotted as toxicity curves) indicated that an apparent threshold for lethality was reached within 72 h for Rio Grande silvery minnow, whereas no apparent lethal threshold was attained at 96 h for fathead minnow. Sprague (1970, 1973) and Fogels and Sprague (1977) advocated the use of threshold LC50 values as the measure of acute toxicity rather than those at predetermined times (i.e., 96-h LC50), because threshold LC50s represent time-independent measures of species tolerance to test materials, whereas LC50s measured at predetermined exposure times may only reflect the rate of response at that time period as was observed in this study with copper. Moreover, this difference in the rate of copper intoxication may partly explain the seemly conflicting results obtained for the mixture exposures where fathead minnow were more sensitive to the copper in the mixture, but less sensitive to copper individually, than Rio Grande silvery minnow during the first 48 h of exposure.

The use of EC50 values as the measure of acute toxicity may provide a more meaningful comparison of species sensitivity to copper in this study, particularly at the early exposure periods because this measure incorporates behavioral effects that are likely to precede death. After 24 and 48 of exposure, sensitivity differences to copper between the two minnow species

were 2.4-fold based on lethality alone. However, when acute sensitivities to copper were based a combination of severe adverse effects (i.e., EC50s), sensitivity differences at 24 and 48 h were ≤ 1.7-fold. Similarly, sensitivity differences to copper at 96 h were only 1.1-fold based on EC50s, compared to 1.6-fold based on LC50s. It is unlikely that fish exhibiting these impairments would be able to survive in the Rio Grande due to their inability to locomote in current, escape predators, capture prey, and avoid exposure conditions that ultimately lead to death.

Interlaboratory Comparisons

Comparisons of the relative sensitivity of the fish in this study with other cyprinids for which toxicity data have been published should viewed in light of the expected variation in response for a given species-toxicant combination tested repeatedly in the same lab and at different laboratories. Formal round-robin studies are designed to evaluate intra- and interlaboratory variability in test results and should provide an estimate of inherent variability in the sensitivity of the test organism to a given toxicant if the participating laboratories follow prescribed protocols and test conditions (e.g., water quality, age of the test organism, and lighting) conform to specific criteria (Parkhurst et al. 1992). DeGraeve et al. (1991) segregated intralaboratory variation into two components, spatial variability and temporal variability. Spatial variability represents the variation in results from replicate tests conducted concurrently, whereas temporal variability arises from differences in results of repeated tests conducted at different times.

The magnitude of difference in sensitivity between Rio Grande silvery minnow and fathead minnow to the chemicals tested in this study (≤ 1.9-fold) falls within the intralaboratory variation in fathead minnow LC50s derived from round-robin studies. Lemke (1981) conducted

a round-robin study with fathead minnow and rainbow trout (*Oncorhynchus mykiss*) exposed to silver and endosulfan under static and flow-through conditions. Using the fathead minnow data evaluated by Lemke (1981), calculated differences in 96-h LC50 values between duplicate tests (i.e., spatial variation) were ≤ 2.0-fold and differences in 96-h LC50s among laboratories that used soft water for each toxicant-test type combination were ≤ 4.5-fold. Similarly, Rue et al. (1988) reviewed the variability in acute effluent toxicity tests and reported that the spatial variation (ratio of high-to-low 48-h LC50s) in four replicate static tests with fathead minnow was ≤ 1.5-fold. DeGraeve et al. (1989, 1991) conducted a multilaboratory study of the intra- and interlaboratory variability of the 7-d fathead minnow larval survival and growth test and reported that the coefficient of variation (C.V.) for 7-d LC50s from duplicate tests (spatial variability) ranged from 0 to 21% for potassium dichromate and 0 to 43% sodium pentachlorophenate. The 7-d LC50s for the duplicate tests reported in DeGraeve et al. (1989) differed by a factor of ≤ 1.4-fold for potassium permangenate and ≤ 1.9-fold sodium pentachlorophenate. Further analysis of their data showed that the temporal variability in 7-d LC50s (pooled values for duplicate tests) was ≤ 1.9-fold for both toxicants and the interlaboratory variation in 7-d LC50s across all laboratories was 2.1-fold for potassium dichromate and 5.5-fold for sodium pentachlorophenate. The above findings support the conclusion drawn by Schimmel (1981) that LC50 values obtained from repeated tests (for a given species-toxicant combination) at the same laboratory generally fall within a factor of two, whereas those obtained from different laboratories should fall within a factor of four.

Aluminum

The addition of sodium hydroxide to aluminum test solutions (to normalize the pH) resulted in the rapid formation of a precipitate, presumably aluminum hydroxide ($Al(OH)_3$; Hem

and Roberson 1967, Gensemer and Playle 1999). Measured dissolved aluminum concentrations (operationally defined as aluminum that passes through a 0.4 micron filter; 0.45 microns in APHA 1995) in solutions adjusted to pH 8.2 seemed to reach a plateau at about 1.0 mg/L irregardless of the total amount of aluminum sulfate added, which indicates that the solubility of aluminum in simulated Rio Grande water at pH 8.2 is at or below 1.0 mg/L. The actual dissolved aluminum concentrations in these solutions were probably lower than 1.0 mg/L, because the size range of aluminum particles formed exhibit a continuous distribution and the distinction between dissolved and particulate fractions is dependent on filter pore-size (Driscoll and Postek 1996). The theoretical solubility of aluminum at pH 8.2 was estimated to be 0.83 mg/L, using the mean solubility product ($K_{S4} = [Al(OH)_3] \times [H]$) of 1.93×10^{-23} for precipitated $Al(OH)_3$ given in Hem and Roberson (1967). This value is in good agreement with the measured concentrations of dissolved aluminum in this study, especially considering that the dissolved aluminum fraction measured in this study probably included aluminum hydroxide particles smaller than 0.4 microns.

Aluminum is a gill toxicant (Gensemer and Playle 1999) and its toxicity to fish is believed to be due primarily to the soluble forms (Freeman and Everhart 1971, Gundersen et al. 1994, Gensemer and Playle 1999). However, several investigators have hypothesized that the mode of acute aluminum toxicity to fish in soft acid water may involve the polymerization or precipitation of aluminum on the gills in response to changes in pH and solubility (Baker and Schofield 1982, Poleo 1995, Gensemer and Playle 1999). The results of this study demonstrated that aluminum in simulated Rio Grande water was not acutely toxic to either minnow species and the lack of aluminum toxicity was probably related to low solubility of aluminum at pH 8.0-8.2. Based on measured filterable and total aluminum concentrations, up to about 98% of the

aluminum added to the test water was precipitated from solution and presumably was not available for uptake by the gills.

Results of this study indicate that larval Rio Grande silvery minnow and fathead minnow are not acutely sensitive to dissolved aluminum concentrations up to about 1.3 mg/L. It is unlikely that the mortalities in the highest aluminum concentration (20-30%) were due to dissolved aluminum alone as the concentrations of dissolved aluminum were similar across all aluminum treatments and cumulative larval mortality in 13 of 18 aluminum treatments was ≤ 10%. It is more probable that the mortalities were partly due to the relatively large amount of precipitate present in this treatment.

Due to concerns about acid precipitation, most studies on aluminum toxicity to fish have been conducted in soft water at acidic pH. Considering that aluminum speciation and toxicity are pH dependent, comparison of results of this study with those of other investigators is limited to studies conducted in waters with similar pH values. Furthermore, these comparisons are confounded by the test methods used because of the effect of aging on aluminum polymerization and precipitation. Aging increases the size and orderliness of the aggregates formed (Hem and Roberson 1967), which may alter their effects on the gill.

The results of this study are in general agreement with those obtained by other investigators for fathead minnow exposed at neutral or alkaline pH levels. Palmer et al. (1988, 1989) exposed three life stages of fathead minnow to aluminum in soft water (20-25 mg/L as $CaCO_3$) at pH 7.5 for 96 hours and observed 88-100% survival of 1-dph larvae and 4-week old juveniles at nominal total aluminum concentrations of 0.05-0.40 mg/L. In the same treatments, 12-d old larvae exhibited an inverted concentration-survival response, ranging from about 82% at 0.40 mg/L to 68% at 0.05 mg/L; survival of the control fish was about 91%. As was observed

in present study, they reported the occurrence of aluminum precipitation in their test system and low ratios of measured dissolved aluminum to nominal concentrations (0.13-0.81 in juvenile test, Palmer et al. 1988). Call et al. (1984) exposed juvenile fathead minnow (32- to 33-d old) to aluminum in soft lake water (hardness, 47 mg/L as $CaCO_3$) adjusted to a pH of 7.6 and 8.0 and reported that total aluminum concentrations of 2.9 to 49.8 mg/L killed ≤ 10% of the fish after 96 h. They also noted the formation of a precipitate after adjusting the pH of the test solutions at total aluminum concentrations ≥ 3.12 mg/L. In their summary of aluminum toxicity values, USEPA (1988) reported a 96-h LC50 of 35 mg/L for juvenile fathead minnow tested in hard water (220 mg/L as $CaCO_3$) at pH 7.34.

Although the aluminum solutions were not acutely lethal to the minnows, prolonged exposures to precipitated aluminum may adversely affect the fish as a result of the precipitated material becoming embedded in the gill filaments and impairing gill function (gas and ion exchange). Moreover, some dissolution of precipitated aluminum may occur as result of transient pH changes at the gill-water interface resulting from the excretion of carbon dioxide and ammonia (reviewed by Gensemer and Playle 1999). Precipitated aluminum deposited on the surface of the gills might also be taken up by phagocytosis. Martens and Servizi (1993) exposed juvenile Pacific salmon (*Oncorhynchus* spp.) to high concentrations of suspended sediments and reported that sediment particles could be phagocytosed by gill epithelial cells. They speculated that this process may serve as a route of uptake for particulate-bound contaminants.

Ammonia

In aqueous solution, ammonia is present in the ionized (NH_4^+) and un-ionized form, and the relative percentage of each form is governed primarily by pH and temperature and to a lesser extent by dissolved solids (Emerson et al. 1975, USEPA 1985b). Ammonia toxicity to aquatic

biota is generally attributed to the un-ionized form and its toxicity (expressed as total ammonia) usually increases with increasing pH due to an increase in the relative concentration of the un-ionized form (USEPA 1985b, 1998a). Consequently, published ammonia toxicity values obtained at pH levels outside the range of those in this study (7.7-8.3) may not be directly comparable to the findings in this study.

The acutely toxic concentrations of ammonia (expressed as NH_3-N and TA-N) determined in this study for larval Rio Grande silvery minnow and fathead minnow are within a factor of two of those obtained by other investigators for larval fathead minnow. Reported 72-h LC50s for 2-d old larvae exposed under static-renewal conditions in well water at 22-23°C with a pH range of 8.2-8.5 were 0.78 mg/L as NH_3-N and 8.17 mg/L as TA-N (A. Allert, U.S. Geological Survey, written communication). The 72-h LC50s of ammonia for both minnow species in this study (1.13-1.19 mg/L as NH_3-N; 17.2-18.6 mg/L as TA-N,) are about twofold higher than those values. Nimmo et al. (1989) conducted flow-through ammonia tests with larvae (age not reported) in well water and river water at 20-21°C with a pH of 7.8-7.9 and reported 96-h LC50s of 1.12 and 0.94 mg/L as NH_3-N, respectively. These values encompass those obtained for both minnow species in this study (1.01-1.12 mg/L as NH_3-N). Nimmo et al. (1989) also tested larval fathead minnow in well water at 6°C (pH, 8.2) and found that they were about 6 times more sensitive to ammonia in cold water (96-h LC50, 0.19 mg/L as NH_3-N) than in warm water (96-h LC50, 1.12 mg/L as NH_3-N). Dwyer (1998) reported total ammonia 7-d LC50s ranging from 7.3 to > 17.0 mg/L as N in five static-renewal tests with larvae exposed in ASTM (1996) hard water at 25°C (pH not reported). Estimated un-ionized ammonia 7-d LC50s for three of his tests ranged from 0.277 to 0.652 mg/L as N, but no comparative values were given for the two tests in which there were insufficient mortalities to calculate LC50s.

The 96-h LC50s of ammonia (1.01-1.12 mg/L as NH_3-N and 14.4 -16.9 mg/L as TA-N) obtained for larval Rio Grande silvery minnow and fathead minnow are lower than, but within a factor of four, of those reported for older fathead minnow tested at comparable pH levels and temperatures. Thurston et al. (1983) conducted 35 flow-through tests with different sizes of fathead minnow (0.1-2.3 g) exposed to ammonia at temperatures ranging from 12 to 22°C. The 96-h LC50s for their tests conducted at 20-22°C and pH 7.8-8.2 (n = 8; fish size, 0.22-1.7 g) ranged from 1.85 to 3.44 mg/L as NH_3-N and 36.2 to 55.3 mg/L as TA-N. These authors also reported that ammonia toxicity was not correlated with fish size, but was inversely correlated with temperature. Mayes et al. (1986) reported a 96-h LC50 of 1.50 mg/L as NH_3-N for 0.22-g fathead minnow tested in river water with a pH range of 7.9 to 8.4 and mean temperature of 22.0°C. In other ammonia studies conducted with fathead minnow in river water with a pH of 8.0-8.1, Nimmo et al. (1989) obtained a 96-h LC50 of 1.40 mg/L as NH_3-N for 25-mm juveniles tested at 20°C and Arthur et al. (1987) reported a higher 96-h LC50 of 2.55 mg/L as NH_3-N for 1.7-g fish at 26°C.

The sensitivity of the two minnow species tested in this study to ammonia is similar to that of other endangered nonsalmonid species. Saiki et al. (1999) determined the acute toxicity of ammonia to larvae (35-d old) and juveniles (0.53-2.00 g) of two endangered suckers, Lost River sucker (*Deltistes luxatus*) and shortnose sucker (*Chasmites brevirostris*), in a reconstituted site-specific water at 20°C with a pH of 8.0. The 96-h LC50s of un-ionized ammonia they reported for larvae and juveniles of both species (0.39 and 0.64 mg/L as N [reported as 0.48 and 0.78 mg/L NH_3] for Lost River sucker and 0.87 and 0.44 mg/L as N [reported as 1.06 and 0.53 mg/L NH_3] for shortnose sucker) are within a factor of three of those obtained in this study. Reported 72-h LC50s for ammonia and 60-d old Colorado pikeminnow (*Ptychocheilus lucius*)

exposed in well water at 22-23°C with a pH of 8.2-8.5 were 1.62 mg/L as NH_3-N and 12.3 mg/L as TA-N (A. Allert, U.S. Geological Survey, written communication). The range of 7-d LC50s obtained by Dwyer (1998) for total ammonia and larval Colorado pikeminnow (4.44-22.6 mg/L as N) and razorback sucker (*Xyrauchen texanus*; 7.34 - > 17 mg/L as N) tested in ASTM (1996) hard water at 25°C (pH not reported) encompass the 96-h LC50s of total ammonia obtained for both minnow species in this study. The range of corresponding 7-d LC50s for un-ionized ammonia in tests with sufficient mortality (0.229 to 1.416 mg/L as N) also include the 96-h LC50s of un-ionized ammonia obtained in this study.

Arsenic

Previously published data on arsenate toxicity to aquatic biota are not as extensive as those for arsenite as evidenced by the fact that current national recommended water quality criteria for arsenic were derived from toxicity data for arsenite (USEPA 1996), because of insufficient data for arsenate (USEPA 1985c). In general, arsenite has been shown to be more acutely toxic to fish than arsenate, however some incongruities exist. Based on a review of the early literature, the USEPA (1985c) reported that arsenate was slightly more toxic than arsenite to rainbow trout, but arsenite was about twice as toxic as arsenate to fathead minnow. However, studies that tested both inorganic forms under the same conditions found that arsenite was about 3-6 times more acutely toxic than arsenate (as arsenic pentoxide, As_2O_5) to rainbow trout and chinook salmon (*O. tshawytscha*; McGeachy and Dixon 1989, Hamilton and Buhl 1990). Based on these findings, comparisons of the results in this study are limited to published data on arsenate toxicity.

Fathead minnow (96-h LC50, 17.9 mg/L) were about twice as sensitive as Rio Grande silvery minnow (96-h LC50, 34.3 mg/L) to arsenate. The acute toxicity of arsenate to larval

fathead minnow in the present study is similar to that reported for juvenile fathead minnow (25.6 mg/L, USEPA 1985c) and larval razorback sucker (17.8 mg/L, Hamilton and Buhl 1997a) tested with sodium arsenate (Na_2HAsO_4). The relative sensitivity of Rio Grande silvery minnow to arsenate in this study is very close to that for larval flannelmouth sucker (*Catostomus latipinnis*; 33.1 mg/L) tested with the same arsenate compound under similar conditions (Hamilton and Buhl 1997b). Acutely toxic arsenate concentrations reported for larval Colorado pikeminnow tested with Na_2HAsO_4 (105 mg/L, Hamilton and Buhl 1997a) and postlarval northern pikeminnow (*P. oregonensis*) tested with arsenic pentoxide (As_2O_5; 73 mg/L; Beleau and Bartosz 1982) are higher those obtained for two minnow species in this study. Palawski et al. (1985) exposed 63-d-old striped bass (*Morone saxatilis*) to As_2O_5 in hard water (285 mg/L as $CaCO_3$) and soft water (40 mg/L as $CaCO_3$) and obtained 96-h LC50s of 30.5 and 40.5 mg/L, which encompass that obtained for Rio Grande silvery minnow tested at a hardness of 149 mg/L as $CaCO_3$ in this study.

Acutely lethal concentrations of arsenate (as Na_2HAsO_4) reported for rainbow trout differ by an order of magnitude and encompass those obtained for minnows in this study (17.9-34.3 mg/L). Hale (1977) exposed juveniles in creek water and obtained a 96-h LC50 of 10.8 mg/L, whereas McGeachy and Dixon (1989) exposed to 3.5-g fish in ground water and reported 144-h LC50s of 58 mg/L at 15°C and 114 mg/L at 5°C.

Chlorine

Chlorine was the most toxic chemical tested in this study (96-h LC50s, 0.114-0.115 mg/L) and its rate of lethality to both species was rapid, with all mortalities occurring within the first 24 hours of exposure, except for one larval Rio Grande silvery minnow that died at 72 hours of exposure. The lack of toxicity after 24 hours of exposure may be partly due to the short

half-life of chlorine residuals in water (APHA 1995). Although the decay of chlorine residuals were not verified in the test solutions, it seems likely that larvae were exposed to progressively decreasing concentrations until the solutions were renewed.

Chlorine toxicity expressed as TRC is a measure of the joint toxic action of the free forms (hypochlorous acid and hypochlorite ion) termed free residue chlorine (FRC) and combined forms (monochloramine and dichloramine) referred to as combined residual chlorine (CRC; Mattice et al. 1981, Brooks and Bartos 1984). The relative concentration of each form at a given TRC concentration depends mainly on pH, temperature, and ammonia concentrations (Zillich 1972, APHA 1995). Current water quality criteria for chlorine are expressed as TRC, which implies that the USEPA (1985a) considers the toxicity of these compounds to be similar and additive to aquatic biota. However, several investigators have observed differences in toxicity among these forms to fish and suggested that separate criteria should be developed for these components of TRC (Heath 1977, Mattice et al. 1981, Brooks and Bartos 1984). It is noteworthy that these investigators used intermittent exposure regimes that simulated chlorination practices at electric power generating plants.

Although FRC was not measured in this study, it seems likely that this was the predominant form of TRC initially present in the test solutions as the reconstituted dilution water had no known source of ammonia (or organic matter) contamination. However, the proportion of TRC present as CRC probably increased during the test due to the excretion of ammonia by the fish. For the FRC, the ratio of hypochlorous acid (HOCl) to hypochlorite ion (OCl^-) decreases as pH increases and their relative proportions at a given pH can be estimated from the dissociation constant (pK_a) for HOCl (Mattice et al. 1981). Using a pK_a of 7.5 for HOCl at 25°C (Snoeyink and Jenkins 1980), the ratio of HOCl to OCl^- in chlorine solutions at pH of 8.0-8.1

was about 0.3:1.

Comparisons of the toxicity values obtained in this study with those reported in the literature are confounded by differences in exposure conditions (e.g., static-renewal versus flow-through tests) and the relative proportions of FRC and CRC in the chlorine solutions, if they differ in toxicity to fish as discussed above. The exposure regime used in this study is similar to a series of daily pulse exposures. Wilde et al. (1983) exposed juvenile and adult fathead minnow and young-of-year bluegill (*Lepomis macrochirus*) to pond water spiked with NaOCl for about 2 hours/day and reported that juvenile fathead minnow were considerably more sensitive
(4- to 8-fold) than the other fish. The 96-h LC50 of 0.14 mg/L TRC (as mean maximum concentration) they obtained for juvenile fathead minnow is very close to those obtained for larvae of both minnow species in this study (0.114-0.115 mg/L).

The acutely toxic concentrations of TRC obtained for larval Rio Grande silvery minnow and fathead minnow (0.114-0.115 mg/L) in reconstituted Rio Grande water fall in the range of those obtained for fathead minnow tested under continuous-flow conditions. Ward and DeGraeve (1978, 1980) exposed juvenile fathead minnow (0.1-0.6 g) to serial dilutions of chlorinated effluent from two wastewater treatment plants and reported 96-h LC50s of 0.082-0.120 mg/L as TRC. Additional acute toxicity values reported by the USEPA (1985a) for fathead minnow range from 0.086 to 0.130 mg/L as TRC, which are within a factor of two of those obtained for both minnow species in this study.

Acute toxicity data are available for other minnow species, but the test conditions used in these investigations differed from those used in this study. Beleau and Bartosz (1982) determined the relative sensitivity of young Colorado pikeminnow and northern pikeminnow to

chlorine under static conditions and reported 96-h LC50s of 0.163 mg/L for post-larval Colorado pikeminnow, 0.304 mg/L for post-larval northern pikeminnow, and 0.366-1.322 mg/L for juveniles of both species, based on nominal chlorine concentrations. Their toxicity values for the larval stages are within a factor of three of those obtained for minnow larvae tested in this study. Fisher et al. (1999) determined the acute toxicity of chlorine to young golden shiner (*Notemigonus crysoleucas*; 3.2-3.3 g) under continuous and daily intermittent exposure regimes and found that continuous exposures (96-h LC50, 0.304 mg/L TRC) were more toxic than intermittent exposures (96-h LC50, 0.572 mg/L TRC). Continuous exposure TRC LC50 values summarized by the USEPA (1985a) for cyprinids, excluding goldfish (*Carassius auratus*) and fathead minnow, range from 0.040 to 0.190 mg/L. In lieu of differences in test conditions, the TRC 96-h LC50s obtained in the present study for larval Rio Grande silvery minnow and fathead minnow fall neatly in the center of this range in toxicity values.

Copper

The difference in sensitivity to copper between the surrogate and endangered species in this study (1.6 fold) is less than the intralaboratory variability reported for repeated tests with fathead minnow and copper. In a set of five flow-through and six static tests with < 24-h old larvae exposed in unmodified Lake Superior water (hardness, 46 mg/L as $CaCO_3$ calculated from calcium and magnesium concentrations using conversion factors of Hem [1970]), the high-to-low ratios of 96-h LC50s were 2.6 and 2.4, respectively (Erickson et al. 1996). Dwyer et al. (1995) obtained a similar high-to-low ratio of 2.8 for pooled 96-h LC50s derived from six static tests with juvenile fathead minnow conducted over a two-year period in standard hard water (173 mg/L as $CaCO_3$). Calculated high-to-low ratios of published copper 96-h LC50s for larval Colorado pikeminnow and razorback sucker from two year classes were 1.2 and 1.7 respectively

(Buhl and Hamilton 1996, Hamilton and Buhl 1997a).

The relative toxicity of copper to aquatic animals is affected by various characteristics of the exposure water, most notably water hardness, alkalinity, and pH (Sprague 1985, USEPA 1985d, 1998b, 1999). Due to the ameliorating effect of water hardness and alkalinity on copper toxicity, direct comparisons of lethal copper concentrations obtained in this study to those reported in the literature are limited to studies conducted in similar water qualities.

The relative sensitivity of Rio Grande silvery minnow to copper observed in this study (96-h LC50, 0.250 mg/L) is similar to that of endangered fishes in the Colorado River basin. Buhl and Hamilton (1996) and Hamilton and Buhl (1997a) exposed larvae of three endangered species to copper in reconstituted river water of similar hardness (144-199 mg/L as $CaCO_3$) and alkalinity (82-107 mg/L as $CaCO_3$) and obtained 96-h LC50s of 0.364 mg/L for bonytail (*Gila elegans*), 0.293-0.363 mg/L for Colorado pikeminnow, and 0.231-0.404 mg/L for razorback sucker. In tests with juveniles of the same species exposed to copper in standard reconstituted hard water (173 mg/L as $CaCO_3$; Dwyer et al. 1995) and reconstituted river water (199 mg/L as $CaCO_3$; Buhl and Hamilton 1996), reported 96-h LC50s ranged from 0.200 to 0.663 mg/L. Differences in sensitivity to copper between Rio Grande silvery minnow and the three endangered species above are less than twofold for the larvae and less than threefold for the juveniles.

No published studies with larval fathead minnow and copper were found that are directly comparable to the results of this study due to differences in dilution water quality (hardness, alkalinity, and pH) and biological conditions. However, toxicity data are available for larval fathead minnow exposed to copper in soft dilution water and these toxicity values can be normalized for hardness by using the copper LC50-hardness equation developed by the USEPA

(1985d) for deriving criterion maximum concentrations (ln LC50 at hardness 2 = 0.9422 [ln hardness 2 - ln hardness 1] + ln LC50 at hardness 1). Reported 96-h LC50s for larval fathead minnow tested in Lake Superior water (average hardness, 46-47 mg/L as $CaCO_3$) ranged from 0.042 to 0.149 mg/L (Carlson et al. 1986, Erickson et al. 1996). When normalized to a hardness of 140 mg/L as $CaCO_3$, the adjusted 96-h LC50 values of 0.120-0.426 mg/L encompass those obtained for larvae of both species in this study (0.250-0.393 mg/L). Diamond et al. (1997) tested 3- to 7-d old larvae in diluted mineral water with a hardness of 72-80 mg/L as $CaCO_3$ and reported 48-h LC50s of 0.146-0.297 mg/L as total recoverable copper; the hardness adjusted toxicity values of 0.273-0.503 mg/L fall between the 48-h LC50s determined for larval Rio Grande silvery minnow (0.262 mg/l) and fathead minnow (0.621 mg/L) in the present study.

The relative sensitivity of larval Rio Grande silvery minnow and fathead minnow to copper determined under static-renewal conditions in this study is quite similar to that reported for larval fathead minnow tested under different exposure regimes. Scudder et al. (1988) reported a 96-h LC50 of 0.250 mg/L for larvae that were pre-exposed, starting as embryos, to the copper in hard water (202 mg/L as $CaCO_3$, calculated from calcium and magnesium concentrations using the conversions factors of Hem [1970]). Pickering and Lazorchak (1995) evaluated the effects of age, source of embryos, and feeding regimes on the sensitivity of larval fathead minnow to copper in a series 7-d static renewal toxicity tests conducted in hard water (176-184 mg/L as $CaCO_3$). The range of the lowest observed effect concentrations for survival they obtained from nine tests (0.150-0.400 mg/L) encompasses the copper 96-h LC50s for both species in this study.

The relative sensitivity of larval fathead minnow in this study (96-h LC50, 0.393 mg/L) is similar (within a factor of two) to older life stages exposed in hard water. In a series of six

tests with juveniles (0.32-0.56 g) exposed to copper in standard hard water (173 mg/L as $CaCO_3$), Dwyer et al. (1995) reported a geometric mean 96-h LC50 of 0.47 mg/L with a range of 0.29 to 0.81 mg/L. Birge et al. (1983) and Bennett et al. (1995) tested fathead minnow in moderately hard water (101-103 mg/L as $CaCO_3$) and obtained copper 96-h LC50s of 0.21 and 0.36 mg/L. Reported 96-h LC50s for juveniles and adults tested with copper in diluted spring-fed pond water at a hardness of 182-216 mg/L as $CaCO_3$ ranged from 0.43 to 0.49 mg/L (Mount 1968, Geckler et al. 1976).

The acutely toxic concentrations of copper observed for larval Rio Grande silvery minnow and fathead minnow in this study (0.250-0.393 mg/L) are within a factor of four of those obtained for other cyprinids. Reported copper 96-h LC50s for feral and laboratory reared bluntnose minnow (*P. notatus*) tested in laboratory water (hardness, 172-230 mg/L as $CaCO_3$) ranged from 0.26 to 0.29 mg/L under static conditions (Geckler et al. 1976) and from 0.21 to 0.27 mg/L under flow-through conditions (Horning and Neiheisel 1979). Lewis (1978) tested juvenile longfin dace (*Agosia chrysogaster*), a native fish of desert streams in the Southwest, in creek water (hardness, 221 mg/L as $CaCO_3$) and obtained a 96-h LC50 of 0.86 mg/L. Tests with feral blacknose dace (*Rhinichthys atratulus*), creek chub (*Semotilus atromaculatus*), and central stoneroller (*Campostoma anomalum*) in standard water (hardness, 196-205 mg/L as $CaCO_3$) produced copper 96-h LC50s of 0.29 to 0.32 mg/L, whereas those with striped shiner (*Luxilus [Notropis] chrysocephalus*) yielded higher 96-h LC50s of 0.79-1.9 mg/L (Geckler et al. 1976).

Nitrate

Nitrate was relatively non-toxic to larval Rio Grande silvery minnow and fathead minnow with all LC50s values exceeding 1,000 mg/L as N. The low order of nitrate toxicity to aquatic organism may account for the relatively small number of published toxicity values

available for fish, compared to ammonia and nitrite. Nitrate toxicity to fish is dependent on the cationic component of the salt tested. In an earlier investigation, Trama (1954) tested three nitrate salts with bluegill and found that potassium nitrate (415 mg/L as N, reported as 1,840 mg/L NO_3) was about 4-5 times more toxic than calcium nitrate (1,708 mg/L as N, reported as 7,558 mg/L NO_3) and sodium nitrate (1,978 mg/L as N, reported as 8,753 mg/L NO_3). Based on these findings, interlaboratory comparisons were limited to studies that tested sodium nitrate.

The acutely lethal concentrations of nitrate obtained for the two minnow species tested in this study (1,278-1,519 mg/L as N) fall in the range of those reported by Scott and Crunkilton (2000) for larval (< 8 d old) fathead minnow (1,010-1,607 mg/L as N) tested under similar conditions. Thus, the interspecific difference in tolerance to nitrate between Rio Grande silvery minnow and fathead in this study (1.2-fold) is lower than the intraspecific variation in 96-h LC50s (1.6-fold) observed for three tests with fathead minnow (Scott and Crunkilton 2000).

The relative sensitivities of larvae of both minnow species to nitrate observed in this study (96-h LC50s, 1,278-1,519 mg/L as N) are within a factor of two of those determined for older life stages of other fishes. Reported 96-h LC50s of nitrate for juvenile rainbow trout and chinook salmon range from 1,310-1,658 mg/L as N (Westin 1974 [reported as 5,800 and 6,000 mg/L as NO_3], Buhl and Hamilton 2000). Colt and Tchobanoglous (1976) exposed juvenile channel catfish (*Ictalurus punctatus*) to nitrate solutions at three temperatures (22, 26, 30°C) and reported that the incipient LC50s were not influenced by temperature and ranged from 1,355-1,423 mg/L as N (reported as 6,000-6,300 mg/L as NO_3). Trama (1954) obtained a high nitrate 96-h LC50 of 1,978 mg/L as N (reported as 8,753 mg/L as NO_3) for bluegill 5-9 cm in total length.

The toxicity of sodium nitrate solutions to fish may be related to the osmotic effect of the

high salt content rather than to the nitrate ion concentrations per se (Colt and Armstrong 1981). In this study, total solute concentrations of $NaNO_3$ calculated at the 96-h LC50s of nitrate were about 7,750 and 9,210 mg/L, which are similar to sodium chloride 96-h LC50s of 6,020-7,070 mg/L obtained for larval fathead minnow in moderately hard water (80-100 mg/L as $CaCO_3$; Mount et al. 1997). Moreover, when these 96-h LC50s are expressed on the basis of sodium, the toxicities of the two salts overlap (2,100-2,490 mg Na/L for $NaNO_3$ and 2,370-2,780 mg Na/L for NaCl).

Isleta Site Mixture

Contamination of surface waters from anthropogenic activities usually involves more than a single pollutant or chemical and aquatic biota inhabiting these waters would be exposed to a mixture of substances. The hazard posed by mixtures of contaminants can be particularly insidious in that the contaminants may be present at concentrations below their individual threshold values, but may interact to produce adverse effects in aquatic biota. In this study, simultaneous exposures to the five chemicals combined at concentration ratios representative of those at the Isleta Site in the Rio Grande were more toxic to both minnow species than any of the five chemicals tested alone. Lethal concentrations of the two major toxic components, copper and un-ionized ammonia, in the mixture to both species were 47 to 71% lower than their individual 96-h LC50s.

Although national water quality criteria have been established for each component in the Isleta Site mixture (except for nitrate, USEPA 1998b, 1999), no criteria exist for mixtures of these chemicals. Based on a review of mixture studies for the European Inland Fisheries Advisory Commission, Alabaster et al. (1988) concluded that for the purpose of deriving water quality criteria, the concentration addition model or toxic unit approach is adequate for

describing the joint lethal effects of mixtures of chemicals commonly found in wastewaters (including ammonia, nitrate, and copper) to fish and aquatic invertebrates. They further commented that deviations from additive interaction of mixture components may be due to several factors including the type of response measured, such as lethality versus nonlethal end points. The findings of this study, which showed that the Isleta Site mixture exhibited additive toxicity to both species based on lethality and more than additive toxicity based on the combination of death and major impairment, are in agreement with the conclusions of Alabaster et al. (1988).

The use of lethality alone did not accurately characterize the toxicity of the Isleta Site mixture to both species. If mixture toxicity was based on mortality alone, the toxicity of could be predicted by summing the relative toxic contributions of each component due to the additive interaction of the components. However, when toxicity was expressed as a combination of lethality and impairments likely to result in death in the field, the mixture was more than additive in toxicity. Thus, a risk assessment based on the assumption of simple additivity of mixture components would probably underestimate the acute hazard posed by this mixture to both minnows. These findings demonstrate the usefulness of measuring acute toxicity as EC50s based on a combination of severe adverse effects or "functional death" as advocated by Stephan (1982).

The toxic unit approach implies that each component in a mixture contributes toxicity in proportion to their individual toxicity values for the same effect (Sprague 1970). Based on this approach, the toxicity of the Isleta Site mixture to both species was probably due to combined effects of copper and ammonia because they accounted for over 90% of the summed toxic units. The relative toxic contribution of arsenic, aluminum, and nitrate to the mixture was probably

insignificant because each chemical only contributed 0.03 TU or less, which accounted for ≤ 5% of the summed toxic units (based on either EC50s or LC50s). Sprague (1970) postulated that there may be a threshold for the joint toxic action of mixture components, below which the components do not contribute toxicity to the mixture. The no effect TU value for a given mixture component is likely to be dependent on the type of chemicals (modes of action) and their proportions in a mixture. For mixtures composed of chemicals combined at ratios representative of specific environmental conditions, it is difficult to assess the significance of components that contribute ≤ 5% of the summed TU (nitrate, arsenic, and aluminum in this study) compared to those contributing over 90% of the toxicity (copper and ammonia in this study) due to the variability in acute toxicity tests (Lemke 1981, Schimmel 1981). To determine the influence of chemicals present at low TU on mixture toxicity, additional tests are needed whereby mixtures formulated without the chemicals of interest are tested along with the full mixture. Using this approach, Hamilton and Buhl (1990) were able to demonstrate that concentrations of selenate and selenite at 1-3% of their 96-h LC50s (0.01-0.03 TU) did not affect the acute toxicity of two 13-component mixtures that simulated irrigation drainwater to young chinook salmon.

No studies were found that tested a mixture of the same chemicals for toxicity to aquatic biota, but data are available for binary mixtures of these chemicals. Of these, the study of Herbert and Vandyke (1964) is the most relevant because they tested a mixture of ammonia and copper, which were the major toxic components in the Isleta Site mixture. These authors reported that the joint toxicity of an equitoxic mixture of copper and total ammonia (1:98) to rainbow trout was additive based on threshold 48-h LC50s. In the present study, the mean ratio of copper to total ammonia in the Isleta Site mixture solutions (1:58) was intermediate to the equitoxic ratios (copper 96-h LC50:total ammonia 96-h LC50) derived from individual tests with

fathead minnow (1:37) and Rio Grande silvery minnow (1:68). Thus, the concentration ratio of copper to total ammonia in the Isleta Site mixture approximated equitoxic proportions for both species and its additive joint lethal action is in agreement with the results Herbert and Vandyke (1964).

A similar comparison of the copper to un-ionized ammonia ratio in the mixture is complicated by the inverse relation between pH and total ammonia concentration. For both minnow species, the ratio of copper to un-ionized ammonia in the Isleta Site mixture ranged from 1:0.5 at the high treatment to 1:4 at the low treatment. However, the copper to un-ionized ammonia ratio at the 96-h LC50 of the mixture (1:2) was the same or nearly the same as the equitoxic ratio of copper to un-ionized for individual tests with fathead minnow (1:2) and Rio Grande silvery minnow (1:3). A similar analysis of the data of Herbert and Vandyke (1964) revealed further concordance between the two studies as the calculated equitoxic ratio of copper (48-h LC50, 0.27 mg/L) to un-ionized ammonia (48-h LC50, 0.52 mg/L as N calculated using equations of Emerson et al. (1975) at pH 7.8 and 17.0°C) in their mixture was also 1:2.

Environmental Considerations

Assessing the hazard of chemical substances to aquatic biota is based on a simple concept of comparing the biological effect concentration (BEC) to the expected environmental concentration (EEC; Cairns et al. 1978, Kimerle et al. 1979). The complexity of this approach lies in accurately identifying the toxicological and exposure concentrations and predicting the hazard at an acceptable level of certainty. Most hazard evaluation programs are based on a tiered or sequential system of testing, which is designed to reduce this uncertainty by developing increasingly accurate estimates of the highest concentration of the chemical that does not cause adverse biological effects and the highest environmental exposure concentration expected from

the production and use of the chemical. At each tier or level, the two values are compared and the margin of difference between them provides decision points or criteria to estimate the potential hazard and to guide the need and direction of additional testing (Cairns et al. 1978, Maki 1979).

Maki (1979) compared 13 hazard evaluation programs and concluded that the most objective decision criterion for assessing hazard and guiding the need for additional data was the ratio of the LC50 as the BEC to the EEC. The ratio of the 96-h EC50 and LC50 value to its EEC is the margin of safety or in more recent literature, the margin of uncertainty (Slooff et al. 1986). In the absence of chronic toxicity data, the USEPA (1984) establishes environmental concentrations of concern as 0.01 times the lowest LC50 for a base set of acute toxicity data (fish, invertebrates, and algae) or 0.001 times a single LC50 value. The assessment factors (0.01 and 0.001 or margins of uncertainty of 100 and 1000, respectively) applied to acute toxicity data are based on the uncertainties associated with interspecific differences in sensitivity, estimating chronic effect concentrations from acute data, and extrapolating laboratory data to the field (USEPA 1984). Moreover, some federal laws administered by the USEPA require the development of chronic toxicity data for new chemicals with margins of uncertainty ≤ 100 prior to any further regulatory decisions (Akerman and Coppage 1979, Walker 1990, 1995).

The potential hazard of chemicals tested in this study to native Rio Grande silvery minnow and fathead minnow was estimated by comparing the acutely toxic concentrations (i.e., 96-h EC50 and LC50 values) of each chemical, except chlorine, to expected or measured environmental concentrations to derive the margins of uncertainty (Table 6). The margins of uncertainty for the chemicals tested individually were greater than 100 for both species when compared to their concentrations at the Isleta Site in the Rio Grande (Table 6). In contrast, the

margins of uncertainty for the mixture based copper or ammonia (total or un-ionized) ranged from 34 to 46 for impaired mobility and from 51 to 66 for lethality. Based on the criteria discussed above, prolonged exposures to a mixture of these chemicals at concentrations representative of those at the Isleta Site may adversely affect early life stages of both minnow species. Furthermore, additional information is needed on the chronic effects of this mixture along with a characterization of the duration, frequency, and magnitude of the exposures.

The Ecological Effects Branch of the USEPA uses this ratio-based approach to evaluate the acute risk of pesticide usage to non-target and endangered species and has developed regulatory risk criteria for these ratios (Urban and Cook 1986). In their standard evaluation procedure, the USEPA presumes that a pesticide does not pose an acute risk to non-target fish species when the ratio of the LC50 to ECC is greater than 10 (Urban and Cook 1986). The risk criteria for endangered fishes and pesticides are more stringent and minimal acute risk is presumed when this ratio or margin of difference is greater than 20 (when the slope of the concentration-response curve is not available). If the margin of difference is lower than the criterion value of 20, formal consultation with the listing agency is initiated. Based on these risk criteria, short-term exposures to mixtures of aluminum, ammonia, arsenic, copper, and nitrate at concentrations representative of those measured at the Isleta Site probably do not pose an acute hazard larval Rio Grande silvery and fathead minnow as the ratios of the presumed no acute risk concentrations (0.05 x L(E)C50 values) to the EEC are greater than 1 for both species and end points (Table 6). Based on this comparison, the presumed no acute risk concentrations for copper and ammonia in the mixture were 2-3 times greater then their respective environmental concentrations.

Considering that existing populations of Rio Grande silvery minnow are depressed and

not of sufficient size for recovery (USFWS 1999), any loss of individuals due to toxic exposures may jeopardize their persistence in the Rio Grande. Thus, margins of uncertainty based toxic end points may not provide adequate protection for this species. One conservative approach to estimate the potential acute hazard would be to derive margins of uncertainty based on the ratio of the highest non-toxic test concentrations for a given end point to the EEC. The no acute effect concentration (NAEC) and no acute lethal concentration (NALC) were established as the highest test concentration impairing the mobility of or killing ≤ 10% of the fish at 96 hours, respectively (Table 6). These concentrations were based on the acceptable level of control fish mortality in acute toxicity tests (Weber 1993, APHA 1995, ASTM 1996) and the sporadic occurrence of 10% mortality at low toxicant concentrations with no mortality at subsequent higher concentrations (Appendices A, B).

Based on the NAEC and NALC of copper and ammonia (total or un-ionized) in the mixture, the margins of uncertainty for larval Rio Grande silvery minnow were 15-23 for impaired mobility and 39-49 for lethality (Table 6). For larval fathead minnow, the ratios were 23-34 for impaired mobility and 39-48 for lethality. Moreover, the NAEC and NALC are about 8-17 times higher than the USEPA presumed no acute risk concentrations for impaired mobility and lethality (Table 6), which indicates that the USEPA acute risk criteria (0.05 x L[E]C50; Urban and Cook 1986) seems to provide adequate protection for the endangered Rio Grande silvery minnow from acutely toxic exposures to these chemicals in the Rio Grande. However, toxicity curves (not shown; plot of the LC50 values against exposure time on logarithmic scales) drawn for the mixture tests did not appear to become asymptotic to the time axis, indicating that toxic effects in Rio Grande silvery minnow and fathead minnow may continue to occur after 96 h (Sprague 1969, Rand and Petrocelli 1985). Consequently, additional tests using exposures

longer than 4 days are needed to assess the potential cumulative effects of prolonged exposures to mixtures of these chemicals on endangered Rio Grande silvery minnow.

Comparison with Criteria Concentrations

National water quality criteria have been recommended by the USEPA (1998b, 1999) for aluminum, (at pH values of 6.5-9.0), ammonia (as TA-N adjusted for pH), arsenic (as arsenite), chlorine (as TRC), and copper (adjusted for hardness). There are no recommended national criteria for nitrate. The criteria consist of two numbers (Table 7), the Criterion Continuous Concentration (CCC) or chronic safe concentration and Criterion Maximum Concentration (CMC) or acute lethal threshold concentration that are intended to protect 95% of the resident aquatic species. The CCC is 4-d (30-d for total ammonia) average concentration and CMC is the 1-hour average concentration that should not be exceeded more than once every three years. The criteria were derived form acute and chronic toxicity data for individual chemicals tested with specific numbers and types of aquatic organisms according to procedures described in Stephan et al. (1985).

The State of New Mexico has established water quality standards to protect fishery uses in surface waters based on national water quality criteria (NMWQCC 2000b). The acute and chronic water quality standards for aluminum, arsenic, chlorine, and copper are numerically identical to their respective national CMC and CCC. The New Mexico standards for total ammonia in waters at 25°C with a pH of 7.9-8.1 are about 28-41% lower (more stringent) than the national criterion concentrations (Table 7). Compliance with New Mexico water quality standards are based on measured dissolved concentrations in the surface water and the acute standard should not be exceeded and the chronic standard should not be exceeded more than once every three years (NMWQCC 2000b).

The ratios of the 96-h EC50 and LC50 values for both minnow species (exposed to the individual chemicals and the Isleta Site mixture) to the national CMC and New Mexico acute standards are greater than 1, except for total ammonia (Table 7). The acutely toxic concentrations of total ammonia in the Isleta Site mixture for both minnow species based on impaired mobility (96-h EC50, 4.48-4.54 mg/L as N) are lower then national CMC (6.95-8.41 mg/L as N) and similar to or lower than New Mexico acute standards for warmwater fishery use (4.5-5.6 mg/L as N) at the pH (8.0-8.1) and temperature (25°C) of test solutions that bracketed the 96-h EC50s. The acutely lethal concentrations of total ammonia (96-h LC50, 6.7-7.7 mg L as N) are lower than national CMC (8.41-10.1 mg/L as N) and higher than New Mexico acute standards (5.6-6.0 mg/L as N) at pH values (7.9-8.0) that bracketed the 96-h LC50 values. These results indicate that current national and New Mexico acute criteria for ammonia may not be protective of Rio Grande silvery minnow and fathead minnow exposed to mixtures of ammonia and copper in the Rio Grande (at a pH range of 7.9 to 8.1). Moreover, the development of site-specific criteria for ammonia in the Rio Grande seems to be justified to account for the combined toxicity of ammonia and copper to native fishes**.**

In order to properly assess if national and New Mexico criteria are protective of native minnow species in the Rio Grande, information on the effects of long-term exposures to mixtures are needed. Results of chronic toxicity tests are usually expressed as the maximum acceptable toxicant concentration (MATC) or the threshold concentration that falls somewhere between the highest test concentration at which no significant adverse effect was observed (NOEC) and the lowest test concentration at which a significant effect was observed (LOEC; Rand and Petrocelli 1985). When chronic toxicity data are not available for the species of interest, it can be estimated from acute toxicity data using ratios or statistical methods. If an acute-to-chronic

toxicity ratio has been empirically derived for one species (acute LC50 divided MATC), it can be applied to the acute LC50 for another species to estimate the MATC for that species and chemical substance.

Acute-to-chronic ratios for the primary toxic components in the Isleta Site mixture (copper and ammonia) have been determined in single chemical tests with fathead minnow. The USEPA (1996, 1998a) reported geometric mean acute-to-chronic ratios of 11.2 for copper and 10.9 for total ammonia. Using these ratios, the MATC for single chemical exposures to both minnow species (based on 96-h LC50s) would be 0.022-0.035 mg/L for copper and 1.32-1.55 mg/L as N for total ammonia. The extrapolated MATC for copper are higher than the national and New Mexico copper chronic criterion of 0.012 mg/L (Table 7). For total ammonia, extrapolated MATC are higher than the chronic criteria for New Mexico (0.73-1.0 mg/L as N), but overlap national criteria (1.09-1.46 mg/L as N) at pH 7.9-8.1 and 25°C. When the ratios were applied to the acutely lethal concentrations of copper and total ammonia in the Isleta Site mixture to both species (Table 5), the extrapolated MATC of 0.010-0.012 mg/L for copper and 0.615-0.706 mg/L as N for total ammonia are lower than or equal to national and New Mexico chronic criterion values (Table 7). These results raise concerns that national and New Mexico chronic criteria for total ammonia, and perhaps copper, may not provide adequate protection for these fish from long-term exposures to copper-ammonia mixtures in the Rio Grande.

As an alternative to using ratios to predict chronic toxicity from acute LC50s at one point in time, the accelerated life test (ACE) procedure and software developed by Sun et al. (1994, 1995) was used to predict chronic lethality of the Isleta Site mixture to both minnow species. The procedure utilizes the time course of lethality observed in acute toxicity tests to predict the concentration lethal to any given percentage of organisms (LCx) at any time interval selected by

the investigator. Sun et al. (1995) recommended using 1% mortality (LC1) as the measure of chronic lethality for the model, based on their findings that calculated LC1 values accurately predicted (within a factor of two) the NOEC for survival in 85% (22 of 26) of the data sets tested. They also noted that using smaller percentages of mortality (e.g., LC0.1 or LC0.01) in the model may overestimate chronic toxicity. Furthermore, LC1 values have been used to estimate lethal threshold concentrations of toxicants in short-term tests with embryo-larval stages of fish and amphibians (Birge et al. 1981, 1985). Accordingly, the LC1 was the degree of response used in the model to predict chronic lethality. Considering that the chronic test data used to validate the ACE procedure ranged from embryo-larval to full life cycle exposures (Mayer et al. 1994, Sun et al. 1995) and that Rio Grande silvery minnow and fathead minnow reproduce naturally within 1 year (APHA 1995, USFWS 1999), the survival time used in the model was 365 days. The extrapolated 365-d LC1 values of copper and total ammonia in the Isleta Site mixture were, respectively, 0.0004 mg/L and 0.023 mg/L as N for Rio Grande silvery minnow and 0.004 mg/L and 0.399 mg/L as N for fathead minnow. These predicted no-effect concentrations for an exposure over the entire life cycle of both minnow species are lower than national and New Mexico chronic criteria for copper (0.012 mg/L) and total ammonia (0.73-1.46 mg/L as N) in the Rio Grande. The results of these acute to chronic extrapolations, using either ratios or models, raise concerns that national criteria may not be protective of native fishes in Rio Grande from the combined cumulative effects of inorganic contaminants and that the development of site-specific criteria for these chemicals may be more appropriate.

CONCLUSIONS

The overall rank order of descending toxicity to both species, based on EC50s and LC50s was: chlorine > copper > un-ionized ammonia > total ammonia = arsenate > nitrate (aluminum).

Rio Grande silvery minnow were more sensitive to copper during the first 72 h of exposure and less sensitive to arsenic than fathead minnow at all time periods. Both species had similar sensitivities to the other chemicals. The Isleta Site mixture was more than additive in toxicity to both minnow species based on the combined effects of impaired mobility and lethality and was additive in toxicity based on lethality alone. The primary toxic components in the mixture were copper and ammonia.

The acutely toxic concentrations of the chemicals were compared to measured concentrations in the Rio Grande to derive margins of uncertainty. The margins of uncertainty ranged from 34 to 11,433, which indicates that the environmental concentrations of these chemicals do not pose an acute hazard to either the endangered Rio Grande silvery minnow or native fathead minnow. However, acutely toxic concentrations of total ammonia in the Isleta Site mixture are similar to or lower than their national and New Mexico acute criteria concentrations at ambient pH levels (7.9-8.1). These findings raise concerns that current acute criteria may be underprotective of endemic minnow species exposed to mixtures of these contaminants in the Rio Grande.

The results of this study suggest that fathead minnow may serve as a suitable surrogate for the relative sensitivity of Rio Grande silvery minnow to the chemicals examined thus far. Additional studies are needed to determine if fathead minnow are an acceptable surrogate for chronic effects of these contaminants on the endangered Rio Grande silvery minnow.

REFERENCES

Akerman, J.W. and D.L. Coppage. 1979. Hazard assessment philosophy: A regulatory viewpoint. Pages 68-73 *in* K.L. Dickson, A.W. Maki, and J. Cairns, Jr., editors. Analyzing the Hazard Evaluation Process. American Fisheries Society, Washington, DC.

Alabaster, J.S., D. Calamari, V. Dethlefsen, H. Konemann, R. Lloyd, and J.F. Solbe. 1988. Water quality criteria for European freshwater fish. Chemistry and Ecology 3:165-253.

APHA (American Public Health Association, American Water Works Association, and Water Environment Federation). 1995. Standard methods for the examination of water and wastewater, 19th edition. American Public Health Association, Washington, DC.

Arthur, J.W., C.W. West, K.N. Allen, and S.F. Hedtke. 1987. Seasonal toxicity of ammonia to five fish and nine invertebrate species. Bulletin of Environmental Contamination and Toxicology 38:324-331.

ASTM (American Society for Testing and Materials). 1996. Standard guide for conducting acute toxicity tests on test materials with fishes, macroinvertebrates, and amphibians. Pages 220-240 *in* Annual Book of ASTM Standards, Vol. 11.04. American Society for Testing and Materials, Philadelphia, PA.

Baker, J.P. and C.L. Schofield. 1982. Aluminum toxicity to fish in acidic waters. Water, Air, and Soil Pollution 18:289-309.

Beleau, M.H. and J.A. Bartosz. 1982. Acute toxicity of selected chemicals: Data base. Pages 242-254 *in* Colorado River Fisheries Project, Part 3, Final Report, Contracted Studies. U.S. Fish and Wildlife Service, Salt Lake City, UT.

Bennett, W.A., A. Sosa, and T.L. Beitinger. 1995. Oxygen tolerance of fathead minnows previously exposed to copper. Bulletin of Environmental Contamination and Toxicology 55:517-524.

Birge, W.J., J.A. Black, and B.A. Ramey. 1981. The reproductive toxicology of aquatic contaminants. Pages 59-115 *in* J. Saxena and F. Fisher, editors. Hazard Assessment of Chemicals - Current Developments, Volume 1. Academic Press, New York, NY.

Birge, W.J., W.H. Benson, and J.A. Black. 1983. The induction of tolerance to heavy metals in natural and laboratory populations of fish. Research Report No. 141. University of Kentucky, Water Resources Research Institute, Lexington, KY. 26 pages.

Birge, W.J., J.A. Black, and A.G. Westerman. 1985. Short-term fish and amphibian embryo-larval tests for determining the effects of toxicant stress on early life stages and estimating chronic values for single compounds and complex effluents. Environmental Toxicology and Chemistry 4:807-821.

Brooks, A.S. and J.M. Bartos. 1984. Effects of free and combined chlorine and exposure

duration on rainbow trout, channel catfish, and emerald shiners. Transactions of the American Fisheries Society 113:786-793.

Buhl, K.J. and S.J. Hamilton. 1996. Toxicity of inorganic contaminants, individually and in environmental mixtures, to three endangered fishes (Colorado squawfish, bonytail, and razorback sucker). Archives of Environmental Contamination and Toxicology 30:84-92.

Buhl, K.J. and S.J. Hamilton. 2000. Acute toxicity of fire-control chemicals, nitrogenous chemicals, and surfactants to rainbow trout. Transactions of the American Fisheries Society 129:408-418.

Cairns, Jr., J., K.L. Dickson, and A.W. Maki, editors. 1978. Estimating the hazard of chemical substances to aquatic life. ASTM Special Technical Publication 657. American Society for Testing and Materials, Philadelphia, PA. 278 pages.

Call, D.J., L.T. Brooke, C.A. Lindberg, T.P. Markee, D.J. McCauley, and S.H. Poirier. 1984. Toxicity of aluminum to freshwater organisms in water of pH 6.5-8.5. Technical Report 549-238-RT-WRD, prepared for Camp Dresser and McKee. University of Wisconsin-Superior, Center for Lake Superior Environmental Studies, Superior, WI. 46 pages.

Carlson, A.R., H. Nelson, and D. Hammermeister. 1986. Development and validation of site-specific water quality criteria for copper. Environmental Toxicology and Chemistry 5:997-1012.

Colt, J.E. and D.A. Armstrong. 1981. Nitrogen toxicity to crustaceans, fish, and mollusks. Pages 34-47 *in* L.J. Allen and E.C. Kinney, editors. Proceedings of the Bio-engieering Symposium for Fish Culture. Fish Culture Special Publication 1, American Fisheries Society, Bethesda, MD.

Colt, J. and G. Tchobanoglous. 1976. Evaluation of the short-term toxicity of nitrogenous compounds to channel catfish (*Ictalurus punctatus*). Aquaculture 8:209-224.

DeGraeve, G.M., J.D. Cooney, T.L. Pollock, N.G. Reichenbach, J.H. Dean, M.D. Marcus, and D.O. McIntyre. 1989. Precision of the EPA seven-day fathead minnow larval survival and growth test. Intra- and interlaboratory study. Final Report EA-6189, Research Project 2368-2, for the American Petroleum Institute. Battle Columbus Division, Columbus, OH.

DeGraeve, G.M., J.D. Cooney, D.O. McIntyre, T.L. Pollock, N.G. Reichenbach, J.H. Dean, and M.D. Marcus. 1991. Variability in the performance of the seven-day fathead minnow (*Pimephales promelas*) larval survival and growth test: An intra- and interlaboratory study. Environmental Toxicology and Chemistry 10:1189-1203.

Diamond, J.M., C. Gerardi, E. Leppo, and T. Miorelli. 1997. Using a water-effect ratio approach to establish effects of an effluent-influenced stream on copper toxicity to the fathead minnow. Environmental Toxicology and Chemistry 16:1480-1487.

Driscoll, C.T. and K.M. Postek. 1996. The chemistry of aluminum in surface waters. Pages 363-418 *in* G. Sposito, editor. The Environmental Chemistry of Aluminum, Second Edition. CRC Press, Boca Raton, FL.

Dwyer, F.J. 1998. USGS memorandum from Jim Dwyer to USFWS Utah Field Office Supervisor concerning ammonia toxicity to endangered fishes of the Colorado River. Basin, January 23, 1998. 4 pages.

Dwyer, F.J., L.C. Sappington, D.R. Buckler, and S.B. Jones. 1995. Use of surrogate species in assessing contaminant risk to endangered and threatened fishes. EPA 600/R-96/029, U.S. Environmental Protection Agency, Environmental Research Laboratory, Gulf Breeze, FL.
78 pages.

Emerson, K.E., R.C. Russo, R.E. Lund, and R.V. Thurston. 1975. Aqueous ammonia equilibrium calculations: Effects of pH and temperature. Journal of the Fisheries Research Board Canada 32:2379-2383.

Erickson, R.J., D.A. Benoit, V.R. Mattson, H.P. Nelson, Jr., and E.N. Leonard. 1996. The effects of water chemistry on the toxicity of copper to fathead minnows. Environmental Toxicology and Chemistry 15:181-193.

Fisher, D.J., D.T. Burton, L.T. Yonkos, S.D. Turley, and G.P. Ziegler. 1999. The relative acute toxicity of continuous and intermittent exposures of chlorine and bromine to aquatic organisms in the presence and absence of ammonia. Water Research 33:760-768.

Fogels, A. and J.B. Sprague. 1977. Comparative short-term tolerance of zebrafish, flagfish, and rainbow trout to five poisons including potential reference toxicants. Water Research 11:811-817.

Freeman, R.A. and W. H. Everhart. 1971. Toxicity of aluminum hydroxide complexes in neutral and basic media to rainbow trout. Transactions of the American Fisheries Society 100:644-658.

Geckler, J.R., W.B. Horning, T.M. Neiheisel, Q.H. Pickering, E.L. Robinson, and C.E. Stephan. 1976. Validity of laboratory tests for predicting copper toxicity in streams. EPA 600/3-76-116. U.S. Environmental Protection Agency, Environmental Research Laboratory, Duluth, MN. 192 pages.

Gensemer, R.W. and R.C. Playle. 1999. The bioavailability and toxicity of aluminum in aquatic environments. Critical Reviews in Environmental Science and Technology 29:315-450.

Gundersen, D.T., S. Bustaman, W.K. Seim, and L.R. Curtis. 1994. pH, hardness, and humic acid influence aluminum toxicity to rainbow trout (*Oncorhynchus mykiss*) in weakly

alkaline waters. Canadian Journal of Fisheries and Aquatic Sciences 51:1345-1355.

Hach Company. 1997. Water quality handbook, 3rd edition. Hach Company, Loveland, CO.

Hale, J.G. 1977. Toxicity of metal mining wastes. Bulletin of Environmental Contamination and Toxicology 17:66-73.

Hamilton, S.J. and K.J. Buhl. 1990. Safety assessment of selected inorganic elements to fry of chinook salmon (*Oncorhynchus tshawytscha*). Ecotoxicology and Environmental Safety 20:307-324.

Hamilton, S.J. and K.J. Buhl. 1997a. Hazard assessment of inorganics, individually and in mixtures, to two endangered fish in the San Juan River, New Mexico. Environmental Toxicology and Water Quality 12:195-209.

Hamilton, S.J. and K.J. Buhl. 1997b. Hazard evaluation of inorganics, singly and in mixtures, to flannelmouth sucker *Catostomus latipinnis* in the San Juan River, New Mexico. Ecotoxicology and Environmental Safety 38:296-308.

Heath, A.G. 1977. Toxicity of intermittent chlorination to freshwater fish: Influence of temperature and chlorine form. Hydrobiologia 56:39-47.

Hem, J.D. 1970. Study and interpretation of the chemical characteristics of natural water, 2nd edition. U.S. Geological Survey Water-Supply Paper 1473. 363 pages.

Hem, J.D. and C.E. Roberson. 1967. Form and stability of aluminum hydroxide complexes in dilute solution. Geological Survey Water-Supply Paper1827-A. 55 pages.

Herbert, D.W.M. and J.M. Vandyke. 1964. The toxicity to fish of mixtures of poisons. II. Copper-ammonia and zinc-phenol mixtures. Annals of Applied Biology 53:415-421.

Hollander, M. and D.A. Wolfe. 1973. Nonparametric statistical methods. John Wiley and Sons, New York, NY. 503 pages.

Horning, W.B. and T.W. Neiheisel. 1979. Chronic effect of copper on the bluntnose minnow, *Pimephales notatus* (Rafinesque). Archives of Environmental Contamination and Toxicology 8:545-552.

Kimerle, R. A., D. Branson, G. Baughman, K. Besch, G. Veith, K. Ahmed, C. Hutchinson, R. Purdy, P. Hodson, and W. Bishop. 1979. Discussion synopsis - hazard assessment philosophy and principles. Pages 119-121 *in* K.L. Dickson, A.W. Maki, and J. Cairns, Jr., editors. Analyzing the Hazard Evaluation Process. American Fisheries Society, Washington, DC.

Lemke, A.E. 1981. Interlaboratory comparison acute testing set. EPA 600/3-81-005. U.S. Environmental Protection Agency, Environmental Research Laboratory, Duluth, MN. 26 pages.

Lewis, M. 1978. Acute toxicity of copper, zinc and manganese in single and mixed salt solutions to juvenile longfin dace, *Agosia chrysogaster*. Journal of Fish Biology 13: 695-700.

Maki, A.W. 1979. An analysis of decision criteria in environmental hazard evaluation programs. Pages 83-100 *in* K.L. Dickson, A.W. Maki, and J. Cairns, Jr., editors. Analyzing the Hazard Evaluation Process. American Fisheries Society, Washington, DC. 160 pages.

Marking, L.L. and V.K. Dawson. 1975. Method for assessment of toxicity or efficacy of mixtures of chemicals. U.S. Fish and Wildlife Service Investigations in Fish Control 67:1-7.

Martens, D.W. and J.A. Servizi. 1993. Suspended sediment particles inside gills and spleens of juvenile Pacific salmon (*Oncorhynchus* spp.). Canadian Journal of Aquatic Sciences 50:586-590.

Mattice, J.S., S.C. Tsai, and M.B. Burch. 1981. Comparative toxicity of hypochlorous acid and hypochlorite ions to mosquitofish. Transactions of the American Fisheries Society 110:519-525.

Mayer, F.L., G.F. Krause, D.R. Buckler, M.R. Ellersieck, and G. Lee. 1994. Predicting chronic lethality of chemicals to fishes from acute toxicity test data: Concepts and linear regression analysis. Environmental Toxicology and Chemistry 13:671-678.

Mayes, M.A., H.C. Alexander, D.L. Hopkins, and P.B. Latvaitis. 1986. Acute and chronic toxicity of ammonia to freshwater fish: A site-specific study. Environmental Toxicology and Chemistry 5:437-442.

McGeachy, S.M. and D.G. Dixon. 1989. The impact of temperature on the acute toxicity of arsenate and arsenite to rainbow trout (*Salmo gairdneri*). Ecotoxicology and Environmental Safety 17:86-93.

McKim, J.M. 1985. Early life stage toxicity tests. Pages 58-95 *in* G.M. Rand and S.R. Petrocelli, editors. Fundamentals of Aquatic Toxicology. Hemisphere Publishing, Washington, DC.

Milliken, G.A. and D.E. Johnson. 1984. Analysis of messy data. Lifetime Learning Publications, Belmont, CA. 473 pages.

Mount, D.I. 1968. Chronic toxicity of copper to fathead minnows (*Pimephales promelas*, Rafinesque). Water Research 2:215-223.

Mount, D.R., D.D. Gulley, J.R. Hockett, T.D. Garrison, and J.M. Evans. 1997. Statistical models to predict the toxicity of major ions to *Ceriodaphnia dubia*, *Daphnia magna* and

Pimephales promelas (fathead minnows). Environmental Toxicology and Chemistry 16:2009-2019.

NMWQCC (New Mexico Water Quality Control Commission). 1998. Water quality and water pollution control in New Mexico 1998. NMED/SWQ-98/4. New Mexico Water Quality Control Commission, Santa Fe, NM. 149 pages + appendices.

NMWQCC (New Mexico Water Quality Control Commission). 2000a. Water quality and water pollution control in New Mexico 2000. NMED/SWQ-00/1. New Mexico Water Quality Control Commission, Santa Fe, NM. 112 pages + appendices.

NMWQCC (New Mexico Water Quality Control Commission). 2000b. State of New Mexico Standards for interstate and intrastate surface waters (as amended through December 16, 2001). 20.6.4 New Mexico Annotated Code. New Mexico Water Quality Control Commission, Santa Fe, NM. 35 pages.

Nimmo, D.W.R., D. Link, L.P. Parrish, G.J. Rodriguez, and W. Wuerthele. 1989. Comparison of on-site and laboratory toxicity tests: Derivation of site-specific criteria for un-ionized ammonia in a Colorado transitional stream. Environmental Toxicology and Chemistry 8:1177-1189.

NRCC (National Research Council of Canada). 1978. Effects of arsenic in the Canadian environment. Publication No. NRCC 15391. National Research Council of Canada, Ottawa, Canada. 349 pages.

Orion Research Incorporated. 1990. Model 95-12 ammonia electrode instruction manual. Boston, MA. 36 pages.

Orion Research Incorporated. 1991. Model 93-07 nitrate electrode instruction manual. Boston, MA. 31 pages.

Ortiz, D. and K.M. Lange. 1997. Water Resources Data New Mexico Water Year 1996. U.S. Geological Survey Water-Data Report NM-96-1. Pages 176-179.

Palawski, D., J.B. Hunn, and F.J. Dwyer. 1985. Sensitivity of young striped bass to organic and inorganic contaminants in fresh and saline waters. Transactions of the American Fisheries Society 114:748-753.

Palmer, R.E., R.J. Klauda, and T.E. Lewis. 1988. Comparative sensitivities of bluegill, channel catfish and fathead minnow to pH and aluminum. Environmental Toxicology and Chemistry 7:505-516.

Palmer, R.E., R.J. Klauda, M.A. Jepson, and E.S. Perry. 1989. Acute sensitivity of early life stages of fathead minnow (*Pimephales promelas*) to acid and aluminum. Water Research 23:1039-1047.

Parkhurst, B.R., W. Warren-Hicks, and L.E. Noel. 1992. Performance characteristics of effluent toxicity tests: Summarization and evaluation of data. Environmental Toxicology and Chemistry 11:771-791.

Peltier, W.H. and C.I. Weber. 1985. Methods for measuring the acute toxicity of effluents to freshwater and marine organisms, 3rd edition. EPA 600/4-85/013, U.S. Environmental Protection Agency, Cincinnati, OH. 216 pages.

Pickering, Q.H. and J.M. Lazorchak. 1995. Evaluation of the robustness of the fathead minnow, *Pimephales promelas*, larval survival and growth test, U.S. EPA Method 1000.0. Environmental Toxicology and Chemistry 14:653-659.

Platania, S.P. and C.S. Altenbach. 1998. Reproductive strategies and egg types of seven Rio Grande basin cyprinids. Copeia 1998:559-569.

Poleo, A.B.S. 1995. Aluminum polymerization-a mechanism of acute toxicity of aqueous aluminum to fish. Aquatic Toxicology 31:347-356.

Rand, G.M. and S.R. Petrocelli. 1985. Introduction. Pages 1-28 *in* G.M. Rand and S.R. Petrocelli, editors. Fundamentals of Aquatic Toxicology. Hemisphere Publishing, Washington, DC.

Rue, W.J., J.A. Fava, and D.R. Grothe. 1988. A review of inter- and intralaboratory effluent toxicity test method variability. Pages 190-203 *in* W.J. Adams, G.A. Chapman, and W.G. Landis, editors. Aquatic Toxicology and Hazard Assessment: 10th Volume. ASTM Special Technical Publication 971. American Society for Testing and Materials, Philadelphia, PA.

SAS Institute. 1990a. *SAS/STAT Procedures Guide*, Version 6, 3rd edition. SAS Institute, Cary, NC. 705 pages.

SAS Institute. 1990b. *SAS/STAT User's Guide*, Version 6, 4th edition, Volumes 1 and 2. SAS Institute, Cary, NC. 1,673 pages + appendix and index.

Saiki, M.K., D.P. Monda, and B.L. Bellerud. 1999. Lethal levels of selected water quality variables to larval and juvenile Lost River and shortnose suckers. Environmental Pollution 105:37-44.

Schimmel, S.C. 1981. Results: Interlaboratory comparison - acute toxicity tests using estuarine animals. EPA 600/4-81-003. U.S. Environmental Protection Agency, Environmental Research Laboratory, Gulf Breeze, FL. 14 pages.

Scott, G. and R.L. Crunkilton. 2000. Acute and chronic toxicity of nitrate to fathead minnow (*Pimephales promelas*), *Ceriodaphnia dubia*, and *Daphnia magna*. Environmental Toxicology Chemistry 19:2918-2922.

Scudder, B.C., J.L. Carter, and H.V. Leland. 1988. Effects of copper on development of the fathead minnow, *Pimephales promelas* Rafinesque. Aquatic Toxicology 12:107-124.

Slooff, W., J.A.M. van Oers, and D. de Zwart. 1986. Margins of uncertainty in ecotoxicological hazard assessment. Environmental Toxicology and Chemistry 5:841-852.

Snoeyink, V.L. and D. Jenkins. 1980. Water Chemistry. John Wiley and Sons, New York, NY. 463 pages.

Sprague, J.B. 1969. Measurement of pollutant toxicity to fish. I. Bioassay methods for acute toxicity. Water Research 3:793-821.

Sprague, J.B. 1970. Measurement of pollutant toxicity to fish. II. Utilizing and applying bioassay results. Water Research 4:3-32.

Sprague, J.B. 1973. The ABC's of pollutant bioassay using fish. Pages 6-30 *in* J. Cairns, Jr. and K.L. Dickson, editors. Biological Methods for the Assessment of Water Quality. ASTM Special Technical Publication 528. American Society for Testing and Materials, Philadelphia, PA.

Sprague, J.B. 1985. Factors that modify toxicity. Pages 124-163 *in* G.M. Rand and S.R. Petrocelli, editors. Fundamentals of Aquatic Toxicology. Hemisphere Publishing, Washington, DC.

Sprague, J.B. 1990. Aquatic toxicology. Pages 491-528 *in* C.B. Schreck and P.B. Moyle, editors. Methods in Fish Biology. American Fisheries Society, Bethesda, MD.

Stephan, C.E. 1982. Increasing the usefulness of acute toxicity tests. Pages 69-81 *in* J.G Pearson, R.B. Foster, and W.E. Bishop, editors. Aquatic Toxicology and Hazard Assessment: Fifth Conference. ASTM Special Technical Publication 766. American Society for Testing and Materials, Philadelphia, PA.

Stephan, C.E., D.I. Mount, D.J. Hansen, J.H. Gentile, G.A. Chapman, and W.A. Brungs. 1985. Guidelines for deriving numerical national water quality criteria for the protection of aquatic organisms and their uses. U.S. Environmental Protection Agency, Office of Research and Development, Duluth, MN. 98 pages.

Sun, K., G.F. Krause, F.L. Mayer, Jr., M.R. Ellersieck, and A.P. Basu. 1994. Predicting chronic toxicity based on the theory of accelerated life testing. EPA 600/R-94/058. U.S. Environmental Protection Agency, Environmental Research Laboratory, Gulf Breeze, FL. 33 pages.

Sun, K., G.F. Krause, F.L. Mayer, Jr., M.R. Ellersieck, and A.P. Basu. 1995. Predicting chronic lethality of chemicals to fishes from acute toxicity test data: Theory of accelerated life testing. Environmental Toxicology and Chemistry 14:1745-1752.

Thurston, R.V., R.C. Russo, and G.R. Phillips. 1983. Acute toxicity of ammonia to fathead minnows. Transactions of the American Fisheries Society 112:705-711.

Trama, F.B. 1954. The acute toxicity of some common salts of sodium, potassium and calcium to the common bluegill (*Lepomis macrochirus* Rafinesque). Proceedings of the Academy of Natural Sciences of Philadelphia 106:185-205.

USEPA (U.S. Environmental Protection Agency). 1984. Estimating "concern levels" for concentrations of chemical substances in the environment. Office of Pollution Prevention and Toxics, U.S. Environmental Protection Agency, Washington, DC. 47 pages.

USEPA (U.S. Environmental Protection Agency). 1985a. Ambient water quality criteria for chlorine-1984. EPA 440/5-84-030. Office of Water, U.S. Environmental Protection Agency, Washington, DC. 57 pages.

USEPA (U.S. Environmental Protection Agency). 1985b. Ambient water quality criteria for ammonia-1984. EPA 440/5-85-001. Office of Water, U.S. Environmental Protection Agency, Washington, DC. 217 pages.

USEPA (U.S. Environmental Protection Agency). 1985c. Ambient water quality criteria for arsenic-1984. EPA 440/5-84-033. Office of Water, U.S. Environmental Protection Agency, Washington, DC. 66 pages.

USEPA (U.S. Environmental Protection Agency). 1985d. Ambient water quality criteria for copper-1984. EPA 440/5-84-031. Office of Water, U.S. Environmental Protection Agency, Washington, DC. 142 pages.

USEPA (U.S. Environmental Protection Agency). 1988. Ambient water quality criteria for aluminum-1988. EPA 440/5-86-008. Office of Water, U.S. Environmental Protection Agency, Washington, DC. 47 pages.

USEPA (U.S. Environmental Protection Agency). 1996. 1995 updates: Water quality criteria documents for the protection of aquatic life in ambient water. EPA 820-B-96-001. Office of Water, U.S. Environmental Protection Agency, Washington, DC.

USEPA (U.S. Environmental Protection Agency). 1998a. Update of ambient water quality criteria for ammonia. Office of Water, U.S. Environmental Protection Agency, Washington, DC. 148 pages.

USEPA (U.S. Environmental Protection Agency). 1998b. National recommended water quality criteria; republication. Federal Register 63:68354-63864.

USEPA (U.S. Environmental Protection Agency). 1999. National recommended water quality criteria-correction. EPA 822-Z-99-001. Office of Water, U.S. Environmental Protection Agency, Washington, DC. 25 pages.

USFWS (U.S. Fish and Wildlife Service). 1994. Endangered and threatened wildlife and plants; Final rule to list the Rio Grande silvery minnow as an endangered species. Federal Register 59:36988-37001.

USFWS (U.S. Fish and Wildlife Service). 1999. Rio Grande silvery minnow recovery plan. U.S. Fish and Wildlife Service, Albuquerque, NM. 141 pages.

Urban, D.J. and N.J. Cook. 1986. Hazard evaluation division, standard evaluation procedure: Ecological risk assessment. EPA 540/9-85-001. Office of Pesticide Programs, U.S. Environmental Protection Agency, Washington, DC. 96 pages.

Walker, J.D. 1990. Chemical fate, bioconcentration, and environmental effects testing: Proposed testing and decision criteria. Toxicity Assessment 5:103-134.

Walker, J.D. 1995. Toxic Substances Control Act (TSCA) Interagency Testing Committee: Data development under Section 4 of TSCA. Pages 669-701 *in* G.M. Rand, editor. Fundamentals of Aquatic Toxicology: Effects, Environmental Fate, and Risk Assessment, 2nd edition. Taylor and Francis, Washington, DC.

Ward, R.W. and G.M. DeGraeve. 1978. Acute residual toxicity of several wastewater disinfectants to aquatic life. Water Resources Bulletin 14:696-709.

Ward, R.W. and G.M. DeGraeve. 1980. Acute residual toxicity of several disinfectants in domestic and industrial waste water. Water Resources Bulletin 16:41-48.

Weber, C.I., editor. 1993. Methods for measuring the acute toxicity of effluents and receiving waters to freshwater and marine organisms, 4th edition. EPA 600/4-90/027F. Office of Research and Development, U.S. Environmental Protection Agency, Cincinnati, OH. 293 pages.

Weber, C.I., W.H. Peltier, T.J. Norberg-King, W.B. Horning, F.A. Kessler, J.R. Menkedick, T.W. Neiheisel, P.A. Lewis, D.J. Klemm, Q.H. Pickering, E.L. Robinson, J.M. Lazorchak, L.J. Wymer, and R.W. Freyberg, editors. 1989. Short-term methods for estimating the chronic toxicity of effluents and receiving waters to freshwater organisms, 2nd edition. EPA 600/4-89/001. Office of Research and Development, U.S. Environmental Protection Agency, Cincinnati, OH. 249 pages.

Westin, D.T. 1974. Nitrate and nitrite toxicity to salmonoid fishes. Progressive Fish-Culturist 36:86-89.

Wilde, E.W., R.J. Soracco, L.A. Mayack, R.L. Shealy, T.L. Broadwell, and R.F. Steffen. 1983. Comparison of chlorine and chlorine dioxide toxicity to fathead minnows and bluegill. Water Research 17:1327-1331.

Zillich, J.A. 1972. Toxicity of combined chlorine residuals to freshwater fish. Journal of the Water Pollution Control Federation 44:212-220.

Table 1. Chemical characteristics of reconstituted Rio Grande water used in acute toxicity tests with larval Rio Grande silvery minnow and fathead minnow.

Parameter (unit)	Rio Grande site water[a]	Experimental design[b]	Control treatments (mean and range in parentheses)
pH	7.9	-	8.1 (7.9-8.4)[c]
Hardness (mg/L as $CaCO_3$)	140	138	140 (139-140)
Alkalinity (mg/L as $CaCO_3$)	127	127	128 (127-129)
Calcium (mg/L)	42	42	42[d]
Magnesium (mg/L)	8	8	8[d]
Sodium (mg/L)	33	40	40[e]
Potassium (mg/L)	5	6	6[e]
Chloride (mg/L)	15	18	19[d]
Sulfate (mg/L)	63	76	77[d]
Conductivity (µmhos/cm @25°C)	438	-	462 (461-464)

[a]Water quality in the Rio Grande at Isleta, NM on May 20, 1996: USGS Station ID 08331000 (Ortiz and Lange 1997).

[b]Desired quality of reconstituted test water based on the type and solubility of mineral salts used to prepare the water.

[c]Average and range across all tests and time periods.

[d]Measured in blending tank prior to use in tests.

[e]Nominal concentration based on the amount of salt (sodium bicarbonate and potassium bicarbonate) used to prepare the test water.

Table 2. Age and size of fish tested with selected inorganic chemicals in reconstituted Rio Grande water.

Species	Age (dph)[b]	Size (range of means)[a]	
		Weight[c] (mg)	Total length (mm)
Rio Grande silvery minnow	3-5	5.4-5.6	6.4-6.9
Fathead minnow	4-6	0.8-1.4	1.2-1.6

[a]Measured on control fish at end of test.

[b]dph = days posthatch at test intiation.

[c]Mean weight determined by dividing pooled weight by number of fish in sample.

Table 3. Quality control results for 11 elements analyzed in test solutions of aluminum, arsenic, copper, and Isleta Site mixture by inductively coupled emission (ICP) and graphite furnace atomic absorption (GFAA) spectroscopy.

Element	Method	Detection limit (mg/L)	Method precision (RPD)[a]	Digestion spikes (% recovery)[b]	
				Sample	Blank[c]
Aluminum	ICP	0.0222	1.7-4.9	104-107	108-110
Arsenic	GFAA	0.0056	0.0-0.0	107-112	104-107
Boron	ICP	0.0222	2.4-11.3	101-101	97.4-98.7
Calcium	ICP	0.111	2.0-5.5	74.9-115	97.8-98.7
Copper	ICP	0.0056	0.0-0.0	105-106	109-115
Iron	ICP	0.0222	0.0-0.0	104-109	111-118
Potassium	GFAA	1.11	0.0-4.2	81.0-90.4	91.5-91.6
Magnesium	ICP	0.0222	0.5-2.6	108-113	107-116
Manganese	ICP	0.0022	0.0-9.5	104-109	110-117
Molybdenum	ICP	0.0044	0.0-0.0	103-112	106-117
Zinc	ICP	0.0111	0.0-0.0	103-108	104-111

[a]RPD = relative percent difference between duplicate analyses for two samples calculated as:

$$\frac{\left|\text{Reading 2} - \text{Reading 1}\right|}{(\text{Reading 2} + \text{Reading 1})/2} \times 100$$

[b]Range of values for two samples and two blanks spiked at the same time prior to digestion.

[c]Concentration of each element in procedure blanks were below the detection limit.

Table 4. Acute toxicity (median effective concentration [EC50] and median lethal concentration [LC50], in mg/L)[a] of selected inorganic chemicals to larval Rio Grande silvery minnow (RGSM) and fathead minnow (FHM) in reconstituted Rio Grande water at 25°C.

Chemical[b]	Exposure time (h)	EC50 (95% confidence interval)			LC50 (95% confidence interval)		
		RGSM	FHM	Ratio RGSM/FHM[c]	RGSM	FHM	Ratio RGSM/FHM[c]
Ammonia: Total (as nitrogen)	24	25.3 (20.2-31.3)	20.4 (16.9-26.1)	1.24	30.7 (24.2-41.1)	22.0 (16.1-27.9)	1.40
	48	20.9 (17.3-26.9)	19.6 (16.3-24.8)	1.07	21.8 (16.7-26.7)	20.4 (16.9-26.1)	1.07
	72	17.8 (14.3-22.9)	17.2 (14.2-21.1)	1.03	18.6 (15.0-24.3)	17.2 (14.2-21.1)	1.08
	96	16.0 (12.6-20.1)	14.4 (10.4-18.5)	1.11	16.9 (13.5-21.5)	14.4 (10.4-18.5)	1.17
Ammonia: Un-ionized (as nitrogen[d])	24	1.44 (1.25-1.63)	1.25 (1.12-1.45)	1.15	1.61 (1.40-1.92)	1.31 (1.08-1.52)	1.23
	48	1.28 (1.14-1.50)	1.22 (1.09-1.41)	1.05	1.31 (1.12-1.48)	1.25 (1.12-1.45)	1.05
	72	1.16 (1.01-1.35)	1.13 (1.00-1.28)	1.03	1.19 (1.04-1.40)	1.13 (1.00-1.28)	1.05
	96	1.08 (0.93-1.25)	1.01 (0.83-1.18)	1.07	1.12 (0.97-1.30)	1.01 (0.83-1.18)	1.11

Table 4. Continued.

Chemical[b]	Exposure time (h)	EC50 (95% confidence interval) RGSM	EC50 (95% confidence interval) FHM	Ratio RGSM/FHM[c]	LC50 (95% confidence interval) RGSM	LC50 (95% confidence interval) FHM	Ratio RGSM/FHM[c]
Arsenic (as arsenate)	24	41.5 (31.5-54.6)	20.0 (14.7-24.8)	2.08*	41.5 (31.5-54.6)	20.0 (14.7-24.8)	2.08*
	48	38.3 (29.9-47.8)	19.1 (15.8-24.7)	2.01*	39.5 (29.6-51.4)	19.1 (15.8-24.7)	2.07*
	72	38.3 (29.9-47.8)	19.1 (15.3-25.8)	2.01*	38.3 (29.9-47.8)	19.1 (15.3-25.8)	2.01*
	96	34.3 (26.1-42.6)	16.2 (12.8-20.8)	2.12*	34.3 (26.1-42.6)	17.9 (14.3-23.7)	1.92*
Chlorine (as total residue chlorine)	24	0.126 (0.094-0.175)	0.114 (0.079-0.165)[e]	1.11	0.126 (0.094-0.175)	0.114 (0.079-0.165)[e]	1.11
	48	0.126 (0.094-0.175)	0.114 (0.079-0.165)[e]	1.11	0.126 (0.094-0.175)	0.114 (0.079-0.165)[e]	1.11
	72	0.115 (0.085-0.156)	0.088 (0.057-0.123)	1.31	0.115 (0.085-0.156)	0.114 (0.079-0.165)[e]	1.01
	96	0.087 (0.061-0.117)	0.076 (0.055-0.119)	1.14	0.115 (0.085-0.156)	0.114 (0.079-0.165)[e]	1.01

Table 4. Continued.

Chemical[b]	Exposure time (h)	EC50 (95% confidence interval) RGSM	EC50 (95% confidence interval) FHM	Ratio RGSM/FHM[c]	LC50 (95% confidence interval) RGSM	LC50 (95% confidence interval) FHM	Ratio RGSM/FHM[c]
Copper	24	0.330 (0.273-0.419)	0.495 (0.413-0.613)	0.67	0.379 (0.303-0.454)	0.914 (0.704-1.289)	0.41*
	48	0.250 (0.201-0.303)	0.427 (0.338-0.533)	0.59*	0.262 (0.212-0.318)	0.621 (0.445-0.808)	0.42*
	72	0.250 (0.201-0.303)	0.415 (0.306-0.540)	0.6*	0.250 (0.201-0.303)	0.487 (0.330-0.829)	0.51*
	96	0.250 (0.201-0.303)	0.276 (0.206-0.371)	0.91	0.250 (0.201-0.303)	0.393 (0.258-0.537)	0.64
Nitrate (as nitrogen)	24	1,721 (1,348-2,078)	1,483 (1,206-1,902)	1.16	1,792 (1,419-2,164)	1,547 (1,259-2,005)	1.16
	48	1,067 (827-1,311)	726 (583-891)	1.47	1,721 (1,348-2,078)	1,352 (1,094-1,700)	1.27
	72	760 (590-979)[e]	678 (515-856)	1.12	1,656 (1,347-2,190)	1,352 (1,094-1,700)	1.22
	96	760 (590-979)[e]	522 (428-660)	1.46	1,519 (1,237-1,960)	1,278 (1,027-1,593)	1.19

Table 4. Continued.

[a]Based on measured concentrations in unfiltered water collected at test initiation.

[b]Aluminum EC50 and LC50 values were greater than 59.1 mg/L as total aluminum and 1.30 mg/L as dissolved aluminum.

[c]Ratios with an asterisks (*) denote significantly different ($P = 0.05$) EC50 or LC50 values between species.

[d]Concentrations calculated from measured values of total ammonia, pH, and temperature at test initiation using equations of Emerson et al. (1975).

[e]No partial kills; 95% confidence intervals calculated as: lower limit = highest concentration with 0% mortality and upper limit = lowest concentration with 100% mortality.

Table 5. Acute toxicity of the Isleta Site mixture to larval Rio Grande silvery minnow and fathead minnow in reconstituted Rio Grande water at 25°C.

End point	Toxic concentration (mg/L)[a] in mixture			Toxic unit[b]					Additive index and range	Type of joint toxicity
	Copper	Ammonia (as nitrogen) Unionized	Ammonia (as nitrogen) Total[c]	Cu	NH_3-N	NO_3-N	As	Total[d]		
Rio Grande silvery minnow										
24-h EC50	0.305 (0.250-0.377)	0.562 (0.518-0.614)	18.5 (15.3-22.6)	0.92	0.39	0.03	0.01	1.35	-0.35 -0.94 to 0.05	Additive
24-h LC50	0.542 (0.425-0.725)	0.722 (0.648-0.822)	32.7 (25.6-44.0)	1.43	0.45	0.05	0.02	1.95	-0.95 -2.09 to -0.32	Less than additive
48-h EC50	0.235 (0.183-0.284)	0.504 (0.455-0.546)	14.4 (11.4-17.3)	0.94	0.39	0.04	0.01	1.38	-0.38 -0.96 to 0.06	Additive
48-h LC50	0.289 (0.236-0.355)	0.550 (0.505-0.599)	17.6 (14.5-21.4)	1.10	0.42	0.03	0.01	1.56	-0.56 -1.26 to -0.11	Less than additive
72-h EC50	0.137 (0.113-0.179)	0.356 (0.323-0.407)	7.99 (6.68-10.18)	0.55	0.31	0.03	<0.01	0.89	0.12 -0.35 to 0.59	Additive
72-h LC50	0.203 (0.167-0.258)	0.463 (0.406-0.541)	12.4 (10.0-16.1)	0.81	0.39	0.02	0.01	1.23	-0.23 -0.84 to 0.18	Additive

96-h EC50	0.078 (0.066-0.096)	0.256 (0.210-0.330)	4.54 (3.45-6.46)	0.31	0.24	0.02	<0.01	0.57	0.75 0.15 to 1.50	More than additive
96-h LC50	0.132 (0.108-0.170)	0.349 (0.316-0.396)	7.70 (6.45-9.71)	0.53	0.31	0.01	0.01	0.86	0.16 -0.29 to 0.64	Additive

Table 5. Continued.

End point	Toxic concentration (mg/L)[a] in mixture			Toxic unit[b]					Additive index and range	Type of joint toxicity
	Copper	Ammonia (as nitrogen) Unionized	Ammonia (as nitrogen) Total[c]	Cu	NH_3-N	NO_3-N	As	Total[d]		
Fathead minnow										
24-h EC50	0.159 (0.127-0.194)	0.393 (0.346-0.439)	9.44 (7.35-11.77)	0.32	0.31	0.02	0.01	0.66	0.52 0.10 to 1.13	More than additive
24-h LC50	0.220 (0.181-0.286)	0.471 (0.422-0.545)	13.5 (10.9-18.0)	0.24	0.36	0.02	0.02	0.64	0.56 0.02 to 1.22	More than additive
48-h EC50	0.137 (0.113-0.179)	0.361 (0.323-0.421)	7.99 (6.68-10.18)	0.32	0.30	0.03	0.01	0.66	0.52 0.01 to 1.13	More than additive
48-h LC50	0.156 (0.124-0.190)	0.388 (0.341-0.433)	9.21 (7.13-11.46)	0.25	0.31	0.02	0.01	0.59	0.69 0.15 to 1.44	More than additive

72-h EC50	0.121 (0.099-0.152)	0.335 (0.300-0.383)	7.11 (5.94-8.78)	0.29	0.30	0.03	0.01	0.63	0.59 0.06 to 1.27	More than additive
72-h LC50	0.132 (0.108-0.170)	0.353 (0.315-0.408)	7.70 (6.45-9.71)	0.27	0.31	0.02	0.01	0.61	0.64 0.02 to 1.50	More than additive
96-h EC50	0.077 (0.067-0.091)	0.249 (0.211-0.303)	4.48 (3.54-5.96)	0.28	0.25	0.03	0.01	0.57	0.75 0.16 to 1.63	More than additive
96-h LC50	0.113 (0.092-0.140)	0.323 (0.288-0.366)	6.70 (5.58-8.17)	0.29	0.32	0.02	0.01	0.64	0.56 -0.01 to 1.33	Additive

Table 5. Continued.

[a]95% confidence interval in parentheses.

[b]EC50 or LC50 in mixture ÷ individual EC50 or LC50; based on measured concentrations of copper (Cu), un-ionized ammonia nitrogen (NH_3-N), nitrate-nitrogen (NO_3-N), and arsenic (As) in unfiltered water collected at test initiation.

[c]Total ammonia-nitrogen values not used in calculating summed toxic units or addition indices.

[d]Toxic units for aluminum were < 0.01 in both tests.

Table 6. Hazard assessment ratios of five inorganic chemicals individually and combined in the Isleta Site mixture to larval Rio Grande silvery minnow (RGSM) and fathead minnow (FHM), based on end points of impaired mobility and death.

		Impaired mobility				Death			
			Ratio of				Ratio of		
Chemical [EEC][a]	Species	NAEC[b]	96-h EC50/EEC	NAEC/ EEC	PNARC[c]/ EEC	NALC[d]	96-h LC50/EEC	NALC/ EEC	PNARC/ EEC
Individual									
Ammonia: Total (as nitrogen)	RGSM	13.0	123	100	6	13.0	130	100	6
[0.130]	FHM	4.44	111	34	6	4.44	111	34	6
Ammonia: Un-ionized (as nitrogen)	RGSM	0.952	193	170	10	0.952	200	170	10
[0.0056][e]	FHM	0.428	180	76	9	0.428	180	76	9
Arsenic [0.003]	RGSM	19.6	11,433	6,533	572	19.6	11,433	6,533	572
	FHM	7.12	5,400	2,373	270	7.12	5,966	2,373	298
Copper [0.002]	RGSM	0.130	125	65	6	0.130	125	65	6
	FHM	0.130	138	65	7	0.130	196	65	10
Nitrate (as nitrogen)	RGSM	590	2,375	1,844	119	979	4,747	3,059	237
[0.320]	FHM	218	1,631	681	82	979	3,994	3,059	200

Table 6. Continued.

Chemical [EEC][a]	Species	Impaired mobility NAEC[b]	Impaired mobility Ratio of 96-h EC50/EEC	Impaired mobility Ratio of NAEC/ EEC	Impaired mobility Ratio of PNARC[c]/ EEC	Death NALC[d]	Death Ratio of 96-h LC50/EEC	Death Ratio of NALC/ EEC	Death Ratio of PNARC/ EEC
Isleta site mixture									
Copper [0.002]	RGSM	0.035	39	18	2	0.083	66	42	3
	FHM	0.061	38	30	2	0.083	56	42	3
Ammonia: Total (as nitrogen) [0.130]	RGSM	1.89	35	15	2	5.07	59	39	3
	FHM	3.03	34	23	2	5.07	51	39	3
Ammonia: Un-ionized (as nitrogen) [0.0056][e]	RGSM	0.131	46	23	2	0.277	62	49	3
	FHM	0.190	44	34	2	0.271	58	48	3

[a]EEC = expected environmental concentration (mg/L) based on dissolved concentrations measured in the Rio Grande at Isleta, NM, on May 21, 1996 (Ortiz and Lange 1997).

[b]NAEC = no acute effect concentration; highest test concentration (mg/L) affecting ≤ 10% of the fish at 96 hours.

[c]PNARC = presumed no acute risk concentration for endangered species (0.05 x 96-h EC50 for impaired mobility or 0.05 x 96-h LC50 value for death; Urban and Cook 1986).

Table 6. Continued.

[d]NALC = no acute lethal concentration; highest test concentration (mg/L) killing ≤ 10% of the fish at 96 hours.

[e]EEC for un-ionized ammonia nitrogen was calculated from measured values of total ammonia nitrogen (0.130 mg/L), pH (7.9), and temperature (25° C) in the Rio Grande at Isleta, NM, on May 21, 1996 (Ortiz and Lange 1997) using the ammonia equilibrium equations of Emerson et al. (1975).

Table 7. Comparison of national and New Mexico water quality criterion concentrations for selected chemicals in the Rio Grande, NM to acute toxicity values for larval Rio Grande silvery minnow (RGSM) and fathead minnow (FHM).

				Ratio of lowest acute value[a] to acute criterion			
	Criterion concentration (mg/L)			Individual		Isleta Site mixture	
Chemical	Scope	Chronic	Acute	RGSM	FHM	RGSM	FHM
Aluminum	National[b]	0.087	0.750	>79	>79	nc[d]	nc
	New Mexico[c]	0.087	0.750	>79	>79	nc	nc
Ammonia							
pH 7.9	National	1.46	10.1	1.6	1.4	0.45	0.44
	New Mexico	1.0	6.0	2.7	2.4	0.76	0.75
pH 8.0	National	1.27	8.41	1.9	1.7	0.54	0.53
	New Mexico	0.91	5.6	2.9	2.6	0.81	0.80
pH 8.1	National	1.09	6.95	2.3	2.1	0.65	0.64
	New Mexico	0.73	4.5	3.6	3.2	1.01	1.00
Arsenic	National	0.150	0.340	101	48	nc	nc
	New Mexico	0.150	0.340	101	48	nc	nc
Chlorine	National	0.011	0.019	4.6	4.0	nt[e]	nt
	New Mexico	0.011	0.019	4.6	4.0	nt	nt
Copper	National	0.012	0.018	14	15	4.3	4.3
	New Mexico	0.012	0.018	14	15	4.3	4.3

[a]Lowest 96-h EC50 or LC50 for each species based on measured concentrations in unfiltered water collected at test initiation.

[b]USEPA (1998b, 1999); criterion expressed as follows: aluminum as total recoverable aluminum at pH 6.5 to 9.0, ammonia as total ammonia-nitrogen at the pH value listed for waters where salmonids are absent, arsenic as total recoverable arsenic, chlorine as total residual chlorine, and copper as dissolved copper adjusted to a hardness of 140 mg/L as $CaCO_3$.

[c]NMWQCC (2000b); criterion for fishery use expressed as follows: aluminum and arsenic as dissolved concentrations; ammonia as total ammonia-nitrogen at 25°C and the pH value listed for warmwater fishery use; chlorine as total residual chlorine; and copper as dissolved copper adjusted to a hardness of 140 mg/L as $CaCO_3$.

[d]Not calculated; chemical was not a major toxic component in the Isleta Site mixture.

[e]Not tested in the Isleta Site mixture.