Chronic Toxicity of Ammonia to Early Life Stage Rainbow Trout

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Abstract.—A 90-d ammonia toxicity test for early life stage rainbow trout *Oncorhynchus mykiss* was conducted using newly fertilized eggs from a wild strain of fish. The toxicity test was conducted at a pH of 7.75 and temperature of 11.4°C. Hatch success and survival of sac fry were not affected by ammonia exposure. Survival, growth, and biomass of swim-up fry were significantly reduced at an ammonia-nitrogen (NH_3-N) concentration of 16.8 mg NH_3-N/L of water but were unaffected by exposures to 7.44 mg NH_3-N/L or lower concentrations. The chronic toxicity value was 11.2 mg NH_3-N/L, and the EC20 (concentration estimated to cause a 20% reduction in organism performance compared with the control) based on biomass at test termination was 7.72 mg NH_3-N/L. Development of sac fry to the swim-up stage was retarded by ammonia, but fry exposed to 7.44 mg NH_3-N/L or lower concentrations appeared to recover by the end of the test. Histological analysis of the gills of exposed fry did not detect any gill pathology. U.S. Environmental Protection Agency chronic ammonia criteria are protective of early life stages of rainbow trout under test conditions.

Ammonia enters the aquatic environment from point sources, such as sewage treatment plants, and non-point sources, such as agricultural runoff. Ammonia discharged from domestic and industrial treatment facilities is regulated throughout the United States based on periodically updated criteria developed by the U.S. Environmental Protection Agency (USEPA; USEPA 1985, 1998, 1999). The current criteria document was most recently updated in 1999. Ammonia differs from most other regulated toxicants in that ammonia is produced endogenously by fish, which often rely on a diffusion gradient for excretion. Elevated concentrations of ammonia in the surrounding water may reduce or prevent ammonia excretion, leading to a buildup of ammonia in the plasma of fish and ultimately leading to death. Several physicochemical factors have been found to affect the toxicity of ammonia to rainbow trout *Oncorhynchus mykiss*, including pH, temperature, ionic strength, and ionic composition. Of these factors, pH is by far the most important factor, and water quality criteria for ammonia are adjusted for pH (USEPA 1999). Effects of ionic strength and composition are small compared with the effect of pH, and temperature is unimportant if ammonia is expressed as total ammonia (USEPA 1999; Randall and Tsui 2002; Eddy 2005). Biotic factors affecting ammonia

toxicity to rainbow trout include life stage (Calamari et al. 1981), size (Thurston and Russo 1983), forced exercise (Wicks et al. 2002), and feeding (Wicks and Randall 2002).

Acute toxicity of ammonia to rainbow trout has been extensively studied, and results are largely consistent among studies (see Appendix 4 in USEPA 1999). In contrast, chronic toxicity data for rainbow trout are limited and have a wide range of toxicity thresholds. The most ambitious of the chronic tests exposed multiple generations of rainbow trout for more than 5 years (Thurston et al. 1984). No effects on survival or growth were detected at the highest ammonia-nitrogen (NH_3-N) concentration tested (5.4 mg NH_3-N/L of water; normalized to pH 8.0). Other chronic toxicity values for rainbow trout considered in the USEPA criteria document include less than 1.34 mg NH_3-N/L, 1.44 mg NH_3-N/L, and less than 18.7 mg NH_3-N/L (all normalized to pH 8.0). A species-specific mean chronic value was not calculated for rainbow trout because several toxicity values were expressed as "less than" or "greater than" values and because results varied widely. Chronic toxicity values from some tests suggest that USEPA criteria for ammonia may be toxic to rainbow trout, while other values indicate the criteria are protective. The first objective of the present study was to determine the chronic toxicity of ammonia to early life stage rainbow trout. The second objective was to compare the toxicity threshold to current ammonia criteria to determine whether rainbow trout are protected.

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Methods

Test fish.—The strain selected for testing was Colorado River rainbow trout. A wild strain is expected to be more sensitive to ammonia than a domesticated strain and also to better represent environmental exposures. Adult rainbow trout were collected from the Colorado River, taken to the Glenwood Springs Hatchery (Colorado Division of Wildlife [CDOW]), and held until they were ready to spawn. Eggs from six females were stripped, fertilized, and then allowed to water harden for 1 h. These newly fertilized eggs were immediately transported in a cooler to the CDOW Aquatic Toxicology Laboratory in Fort Collins. Temperature in the cooler was maintained using a 12-V chiller (Coolworks, Inc., San Rafael, California). Aeration was provided with a small air pump. Water temperature was 3.0°C upon departure from the hatchery and 4.6°C upon arrival in Fort Collins 4 h later. Water quality and temperature were slowly adjusted over the next 90 min to test conditions before random allocation of eggs to incubation cups. Eggs started hatching 23 d after initiation of exposure. Swim-up of fish in the exposure control occurred approximately 15 d later. Upon swim-up, fry were fed a concentrated suspension containing nauplii of brine shrimp *Artemia* spp. supplemented with trout starter chow (Silver Cup; Nelson and Sons, Inc., Murray, Utah). Fry were fed four times daily on weekdays and two times daily on weekends and holidays at an estimated rate of 4% body weight per day. Exposure chambers were cleaned as needed.

Test methods.—Source water for the test consisted of a mixture of on-site well water and reverse-osmosis water. A conductivity controller maintained a constant mixture with water hardness near 45 mg/L. The pH of the source water was maintained at 7.7 using a pH controller (Oakton Instruments, Vernon Hills, Illinois). A continuous-flow serial diluter (Benoit et al. 1982) delivered exposure solutions. The diluter was constructed of Teflon, polyethylene, and polypropylene components. Food-grade vinyl tubing (Nalge Nunc International Corp., Rochester, New York) delivered test solutions to exposure chambers. Test solutions overflowed from the exposure chambers into a water bath maintained at 12°C using a recirculating chiller. An ammonia stock solution was prepared by dissolving a calculated amount of analytical reagent-grade ammonium chloride (Mallinckrodt, Inc., Hazelwood, Missouri) in deionized water. New stock solutions were prepared as needed during the exposure period. The ammonia stock solution was delivered to the diluter via a peristaltic pump at a rate of 2.0 mL/min. The diluter delivered five concentrations of ammonia with a 50%

dilution ratio and a control. Target total ammonia concentrations were 13.0, 6.6, 3.3, 1.6, 0.8, and 0.0 mg NH_3-N/L. The target concentrations represented approximately 4×, 2×, 1×, 0.5×, and 0.25× of the chronic criteria for ammonia at the test pH and temperature. A flow splitter allocated each concentration equally among four replicate exposure chambers at a rate of 40 mL/min for each chamber. Exposure chambers consisted of 2.8-L polypropylene containers. Fluorescent lights suspended 1.3 m above the exposure chambers provided a photoperiod of 12 h light : 12 h dark. Dark lids placed over part of the chambers served to screen some of the light. Diluters and toxicant flow rates were monitored daily to ensure proper operation.

Twenty eggs were randomly assigned and distributed to each incubation cup. Incubation cups were constructed from polyvinyl chloride (PVC) pipe (53-mm internal diameter; 75-mm length) and a 1,000-micron nylon screen, which was affixed to the PVC pipe with aquarium-grade silicone adhesive. Incubation cups were suspended in the exposure chamber and received exposure water from the diluter at a flow rate of 40 mL/min. Eggs were monitored daily to measure hatching success. The first 10 eggs to successfully hatch were removed from the incubation cup and placed in the exposure chamber. Remaining eggs were monitored for hatching and removed after hatching was completed. Thus, hatching success was based on 20 organisms in each incubation cup, while fry survival and growth were based on 10 organisms transferred to the exposure chamber. Duration of exposure was 90 d total (~73 d posthatch; ~52 d after swim-up). At the end of the test, surviving organisms were euthanized with an overdose of tricaine methanesulfonate (MS-222) and blotted dry with a paper towel; weights (nearest 0.001 g) and lengths (nearest 1 mm) were measured and recorded. Fish were then preserved in 10% buffered formalin for histology.

Water quality.—The pH and conductivity of the dilution water were monitored and recorded daily to ensure correct adjustment of pH and proper mixture of source waters. Additional water quality characteristics were measured weekly in all exposure containers within a replicate. Different replicates were selected each week for sampling. Hardness and alkalinity were determined titrimetrically according to standard methods (APHA et al. 1998). A Thermo Orion Model 635 meter was used to measure pH and conductivity. Dissolved oxygen was measured using an Orion Model 1230 dissolved oxygen meter. The conductivity, pH, and dissolved oxygen meters were calibrated before each use. Water samples for ammonia analysis were collected weekly in all exposure containers within a replicate. Different replicates were selected each week

TABLE 1.—Mean, standard deviation (SD), and range of water quality characteristics present during ammonia toxicity tests conducted with early life stage rainbow trout (number of measurements = 78; pH is given in standard units).

Statistic	Hardness (mg/L)	Alkalinity (mg/L)	pH	Temperature (°C)	Conductivity (μS/cm)	Dissolved oxygen (mg/L)
Mean	44.6	42.9	7.75	11.4	192.7	8.50
SD	4.2	5.1	0.13	0.25	61.8	0.66
Range	34.8–54.6	32.6–51.2	7.46–7.96	10.8–11.9	109–377	6.73–9.17

for sampling. Water samples for ammonia determinations were preserved with 0.25% H_2SO_4 and refrigerated at 4°C until analysis. Ammonia concentrations were measured with a flow injection analyzer (Quik-Chem Model 8000; Lachat Instruments, Loveland, Colorado) using USEPA method 350.1. Sample splits, spikes, and blanks were collected weekly during each sampling event. The detection limit for ammonia analyses was 0.03 mg NH_3-N/L. Mean sample spike recovery was 106% ($N = 12$). Mean relative percent difference of sample splits was 3.0% ($N = 12$). Mean analysis of external quality assurance ammonia standard was 99% of the certified value ($N = 13$).

Histology.—Gills were preserved in 10% buffered formalin for analysis. The middle portions of the second or third left gill filament were embedded in paraffin. The arch was mounted and sectioned laterally. Sections (8 μm thick) were mounted on microscope slides and stained using hematoxylin and eosin (Presnell and Schreibman 1997). Gills were examined under a light microscope to assess morphometric criteria. The gills were examined for the incidence of interlamellar hyperplasia, lamellar hyperplasia, epithelial lifting, and epithelial sloughing using a 1–5 scale to score the degree of damage (1 = none, 2 = slight, 3 = moderate, 4 = high, 5 = extreme). The diffusion distance was measured from five consecutive secondary lamellae on each of two primary lamellae using National Institutes of Health image analysis software (rsb.info.nih.gov/nih-image/).

Statistical analysis.—Statistical analyses of toxicity test data were conducted using Toxstat version 3.5 (Western EcoSystems Technology 1996). Analysis of variance (ANOVA) was used to compare toxicity endpoints, which included hatching success, survival (through the sac fry stage and at test termination), biomass, lengths (mm), weights (g), and condition factor (K) of surviving fish at test termination. The K-value was calculated as (weight/length^3)/10^6. Hatching success and survival data were arcsine–square root transformed before ANOVA (Snedecor and Cochran 1980). Normality and homogeneity of variances were tested using chi-square analysis and Levene's test, respectively. Treatment means were compared with the control using Williams' one-tailed test (Williams 1971,

1972) at a significance level of 0.05. The nonparametric Steel's many-one rank test was used to compare sac fry survival treatment means because data failed assumptions of normality and homogeneity of variances. The highest ammonia concentration not associated with a treatment effect (e.g., decreased survival or decreased body weight) was designated as the no-observed-effect concentration (NOEC). The lowest concentration of ammonia associated with a treatment effect was designated as the lowest-observed-effect concentration (LOEC). Chronic values were calculated as the geometric mean of the LOEC and NOEC. The inhibition concentration estimated to cause a 20% reduction in organism performance compared with the control (EC20; USEPA 1993) was calculated using the combined weight of surviving fish from each treatment (biomass or standing crop).

Histologic data were analyzed in two ways. Raw scores for hyperplasia, epithelial lifting, and sloughing were analyzed qualitatively using the Kruskal–Wallis test. Diffusion distances across secondary lamellar membranes were analyzed quantitatively using one-way ANOVA for the main effect of exposure.

Results

Water Quality

Water quality characteristics were consistent for the duration of the experiment (Table 1). Mean pH was 7.75 and ranged between 7.46 and 7.96. Mean dissolved oxygen was near saturation levels (Fort Collins, Colorado; elevation = 1,519 m [4,984 ft] above sea level) and did not fall below 6.73 mg/L. Temperature of exposure chambers was maintained in a narrow range near the mean temperature of 11.4°C. Conductivity of exposure solutions increased in direct proportion to the amount of dissolved ammonium chloride at higher exposure levels. Measured ammonia concentrations of exposure solutions were slightly greater than target concentrations in the two higher exposure levels but otherwise were near target levels and constant over the 90-d duration of the test (Table 2).

Toxicity Endpoints

Mean hatching success exceeded 78% at all exposure levels (Table 2). No effect of ammonia

TABLE 2.—Mean ammonia-nitrogen (NH_3-N) concentrations (SD in parentheses; $N = 12$) and associated means of rainbow trout hatching success, survival (through the sac fry stage or to the end of the 90-d trial), and growth metrics (individual weight, individual length, condition factor K, and combined biomass of surviving fish at the end of the trial; SE in parentheses; $N = 4$) observed during an ammonia toxicity test. Asterisks indicate values that were significantly lower than control values ($P < 0.05$).

Variable	Target concentration (mg NH_3-N/L)					
	0	0.8	1.6	3.3	6.6	13.0
Measured concentration (mg NH_3-N/L)	<0.02 (0.01)	0.81 (0.20)	1.74 (0.58)	3.34 (0.53)	7.44 (0.88)	16.8 (2.07)
Measured concentration normalized to pH 8 (mg NH_3-N/L)	<0.02 (0.019)	0.58 (0.14)	1.25 (0.41)	2.41 (0.38)	5.36 (0.62)	12.1 (1.50)
Hatching success (%)	83.8 (2.4)	82.5 (7.8)	86.3 (6.9)	78.8 (5.6)	88.8 (1.2)	83.8 (6.6)
Survival through sac fry stage (%)	100 (0)	100 (0)	100 (0)	95.0 (2.9)	100 (0)	97.5 (2.5)
Survival to end of test (%)	92.5 (4.8)	75.0 (10.3)	90.0 (7.0)	85.0 (8.6)	82.5 (2.5)	22.5* (4.8)
Final weight (g)	0.431 (0.020)	0.403 (0.011)	0.409 (0.015)	0.403 (0.025)	0.396 (0.012)	0.199* (0.014)
Final length (mm)	35.8 (0.6)	35.0 (0.4)	35.5 (0.4)	34.4 (1.0)	34.6 (0.4)	27.3* (1.0)
Final K	0.918 (0.012)	0.917 (0.017)	0.878 (0.015)	0.935 (0.003)	0.937 (0.013)	0.900 (0.022)
Final biomass (g)	3.97 (0.17)	2.95 (0.44)	3.65 (0.20)	3.48 (0.52)	3.27 (0.14)	0.44* (0.18)

exposure on hatching success was detected at any concentrations tested. Survival of sac fry released from the incubation cups into exposure chambers exceeded 95% at all exposure levels and was unaffected by ammonia concentrations used in this test (Table 2). Survival of fry to the end of the test ranged from a high of 92.5% in the control to a low of 22.5% at 16.8 mg NH_3-N/L (Table 2). Fry survival was not significantly affected by exposure to 7.44 mg NH_3-N/L or lower concentrations but was reduced at 16.8 mg NH_3-N/L. Length and weight at test termination were not significantly reduced at 7.44 mg NH_3-N/L or lower concentrations, but both variables were significantly reduced at 16.8 mg NH_3-N/L (Table 2). The K-value was not affected even though the highest ammonia concentration significantly reduced lengths and weights of surviving fry. Biomass at test termination (combined weight of surviving fish) was also unaffected at 7.44 mg NH_3-N/L or lower concentrations but was significantly reduced at 16.8 mg NH_3-N/L. The LOEC for each of the endpoints affected by ammonia (fry survival, length, weight, and biomass at test termination) was 16.8 mg NH_3-N/L. The NOEC for each of the endpoints was 7.44 mg NH_3-N/L. The chronic value was 11.2 mg NH_3-N/L. The EC20 based on biomass was 7.72 mg NH_3-N/L.

Casual observations near the end of the sac fry stage suggested a concentration-dependent effect of ammonia on development of fry. The number of free-swimming fry was compared with the number resting on the bottom in each exposure chamber to assess the transition from sac fry to the swim-up stage. The transition to the swim-up stage was delayed by a few days in fish exposed to between 0.81 and 7.44 mg NH_3-N/L (Figure 1). Higher concentrations caused greater delays. Fry exposed to 16.8 mg NH_3-N/L continued to rest on the bottom of the exposure

chambers through the end of the test. Many fry exposed to 16.8 mg NH_3-N/L failed to convert to exogenous feeding, and high mortality occurred shortly after swim-up. Individuals that survived exposure to 16.8 mg NH_3-N/L were able to feed but exhibited edema in the abdominal area that prevented them from swimming freely for extended periods of time.

Histology

Neither the qualitative or quantitative histologic analysis of gill tissue detected the presence of any gill pathology. Mean diffusion distance was 0.002 mm for controls and all exposure groups except for the fish exposed to 16.8 mg NH_3-N/L, which exhibited a mean diffusion distance of 0.003 mm. The difference was not significant.

Discussion

This study used a vigorous, naturally reproducing strain of rainbow trout from the Colorado River. Domesticated strains have been in various hatchery programs for multiple generations and may have developed a tolerance to low levels of ammonia. Our study design used four replicates for each ammonia exposure level. Earlier chronic studies were not replicated (Calamari et al. 1981; Thurston et al. 1984; Solbe and Shurben 1989) or were replicated twice over time (Burkhalter and Kaya 1977). Survival, growth, and biomass were not significantly affected at 7.44 mg NH_3-N/L or lower concentrations but were reduced at 16.8 mg NH_3-N/L. The chronic value based on lethal and sublethal endpoints was 11.2 mg NH_3-N/L. The EC20 based on biomass at test termination (7.72 mg NH_3-N/L) was somewhat lower than the chronic value. The USEPA chronic criterion at our test pH and temperature is 3.38 mg NH_3-N/L (USEPA 1999), less than one-third of the chronic value and less than one-
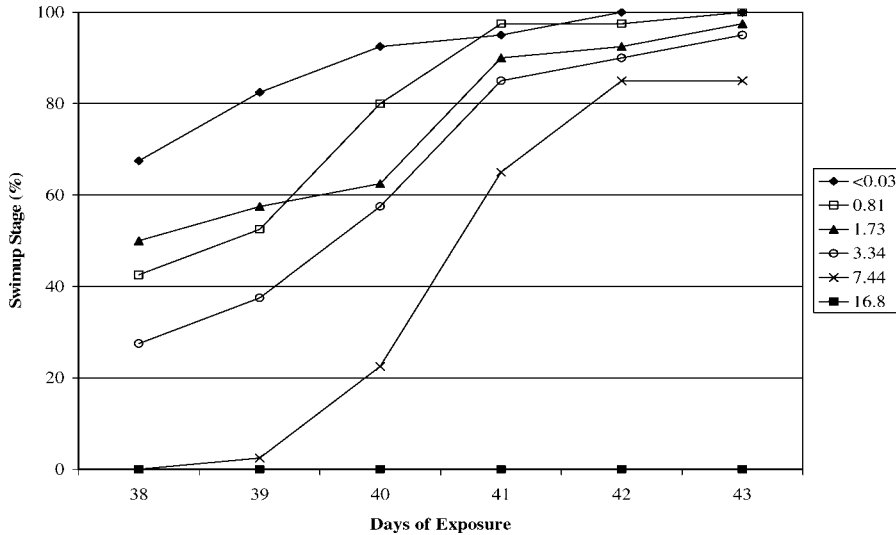
FIGURE 1.—Percentage of rainbow trout that were at the swim-up stage on days 38–43 of a 90-d ammonia exposure trial; swim-up percentages are presented as a function of ammonia concentration in exposure chambers (mg ammonia-nitrogen/L of water; mean of four replicates).

half of the EC20 determined from our test. We conclude that current USEPA chronic ammonia criteria are protective of early life stage rainbow trout.

Rainbow trout embryos and sac fry were more resistant to the lethal effects of ammonia than were swim-up fry. Hatching success and sac fry survival were unaffected by exposure to 16.8 mg NH_3-N/L, the highest concentration of ammonia used in the test. Ammonia-related mortality did not occur until shortly after absorption of the yolk sac, suggesting that the swim-up fry stage is more sensitive than the embryonic and sac fry stages. Shortly after swim-up, fry were about 20 times more sensitive to ammonia than were eggs and sac fry, as noted by Rice and Stokes (1975). Calamari et al. (1981) determined that rainbow trout swim-up fry were at least three times more sensitive than eggs and about twice as sensitive as sac fry, while Burkhalter and Kaya (1977) found no effect on hatching success at total ammonia concentrations as high as 58 mg NH_3-N/L. Only Solbe and Shurben (1989) reported high egg mortality at low ammonia levels (2.55 mg NH_3-N/L), in contrast to our results and those of Rice and Stokes (1975), Calamari et al. (1981), and Burkhalter and Kaya (1977).

Ammonia toxicity is greatly affected by pH. As pH increases, ammonia toxicity increases. To facilitate comparison of toxicity data among different studies, toxicity values can be normalized to pH 8 using the following equation (USEPA 1999):

$$CV_{pH} = CV_8 \times \left[\left(\frac{0.0676}{1 + 10^{7.688 - pH}} \right) + \left(\frac{2.91}{1 + 10^{pH - 7.688}} \right) \right],$$

where CV_{pH} is the chronic value at the test pH, CV_8 is the chronic value normalized to pH 8, and pH is the test pH.

The chronic value and EC20 from our test are 8.06 and 5.56 mg NH_3-N/L, respectively, when normalized to pH 8. A previous study demonstrated that growth and mortality of rainbow trout larvae exposed for 42 d posthatch were significantly affected at 16.7 mg NH_3-N/L but not at 8.8 mg NH_3-N/L (Burkhalter and Kaya 1977; concentrations normalized to pH 8). The geometric mean of the two concentrations in that study was 12.1 mg NH_3-N/L, which is near the chronic value from the present study. A 5-year, multigeneration exposure study (Thurston et al. 1984) did not find effects on survival or growth up to 5.4 mg NH_3-N/L (at pH 8), the highest concentration tested, which is consistent with toxicity thresholds from the present study and the study by Burkhalter and Kaya (1977). Contrasting data come from two early life stage tests where toxicity thresholds were much lower. Calamari et al. (1981) estimated that the concentration lethal to 50% of test organisms over 72 d (LC50) was 4.2 mg NH_3-N/L, and an early life stage test found high mortality (71%) at the lowest ammonia level tested (1.44 mg NH_3-N/L at pH 8; Solbe and Shurben 1989).

Growth of rainbow trout fry was significantly reduced (50%) at 16.8 mg NH_3-N/L (equivalent to 12.1 mg NH_3-N/L at pH 8). Similarly, reduced growth

was observed at 16.7 mg NH_3-N/L (at pH 8) by Burkhalter and Kaya (1977). No effect on growth was detected in fry exposed to 7.44 mg NH_3-N/L (5.36 mg NH_3-N/L at pH 8), a level similar to those in previous chronic studies that did not find growth effects at 8.8 NH_3-N/L (Burkhalter and Kaya 1977), 5.4 mg NH_3-N/L (Thurston et al. 1984), or 14.6 mg NH_3-N/L (Solbe and Shurben 1989; all concentrations normalized to pH 8).

Development of sac fry to the swim-up stage was delayed by exposure to ammonia. The delay in development was considerable at 16.8 mg NH_3-N/L but was otherwise relatively modest. Significantly reduced length and weight of fry at test termination were observed at 16.8 mg NH_3-N/L (12.1 mg NH_3-N/L at pH 8) but not at ammonia concentrations of 7.44 mg NH_3-N/L or less (≤ 5.36 mg NH_3-N/L at pH 8), indicating that fry were able to compensate for delayed swim-up within the next 52 d before the test was terminated. Growth and developmental retardation and failure to absorb the yolk sac have been previously observed in fry of rainbow trout (Burkhalter and Kaya 1977) and pink salmon *O. gorbuscha* (Rice and Bailey 1980). Both tests were terminated before the swim-up stage; therefore, no determination can be made as to whether exposed fry would have recovered from early retardation of development.

We did not detect any changes in gills of rainbow trout fry up to the highest concentration of 16.8 mg NH_3-N/L (12.1 mg NH_3-N/L at pH 8). Similarly, rainbow trout fingerlings exposed to 11.4 and 22.8 mg NH_3-N/L (at pH 8) for 90 d did not develop changes in gill structure (Daoust and Ferguson 1984). Epithelial hypertrophy occurred in gills of rainbow trout exposed to 16.7–32.6 mg NH_3-N/L (at pH 8; Burkhalter and Kaya 1977), which are generally higher than the concentrations used in the present study. Chronic exposure of rainbow trout to ammonia concentrations less than those used in this study has often been associated with histological changes in the gills, including hyperplasia that progresses to fusion of lamellae and filaments, epithelial hypertrophy, aneurysms, and edema (Smith and Piper 1975; Smart 1976; Thurston et al. 1984). Mild hyperplasia, fusion of lamellae, and epithelial hypertrophy were "sometimes" observed in gills of rainbow trout exposed to 0.85 mg NH_3-N/L (at pH 8; Smith and Piper 1975). Thurston et al. (1984) found that rainbow trout developed hyperplasia, fusion of lamellae, epithelial hypertrophy, and aneurysms, which increased in severity as concentrations increased from 0.77 to 5.40 mg NH_3-N/L (at pH 8). The effects noted by Smith and Piper (1975) and Thurston et al. (1984) occurred at ammonia concentrations that did not affect growth, reproduction, or mortality, which contrasts with our results that did not

detect changes in gill structure at a concentration that reduced survival and growth. Histopathological endpoints were not used to develop ammonia criteria due to a lack of a clear relationship between histological and population effects (USEPA 1999). Quantifying and correlating ammonia-induced gill lesions with exposure concentrations and with decreases in organism fitness (e.g., reduced oxygen uptake) should be the subject of additional research.

Our test conditions were based on established guidelines (ASTM 1997) to enable comparison of results with criteria and other published results. However, the conditions are artificial and minimize stress and internal ammonia levels (Ip et al. 2001). Consequently, toxicity tests may underestimate toxicity of ammonia in natural environments. In addition to pH, several factors affect the toxicity of ammonia; these include temperature, ionic composition of exposure water, and combinations of biotic factors. The effect of temperature is minor when ammonia levels are expressed as total ammonia (USEPA 1999); however, ionic composition can affect acute toxicity of ammonia (Soderberg and Meade 1992; Ankley et al. 1995; Borgmann and Borgmann 1997). In general, increasing the concentrations of some cations reduces ammonia toxicity. The dilution water used in our test was relatively soft (hardness = 44.6 mg/L); thus, these toxicity values should be conservative for waters with higher hardness levels. A lack of understanding and information on the effects of different ions on ammonia toxicity was cited as a reason for their exclusion from consideration of ammonia criteria (USEPA 1999).

Biotic factors that may influence ammonia toxicity include quiescent water in exposure tanks, feeding of test organisms, and other stressors. Toxicity test exposures are typically conducted in quiescent solutions, whereas salmonids usually must swim to maintain position in a flowing stream. Forced swimming elevated ammonia levels in the plasma and muscle of rainbow trout and reduced the 96-h LC50 by a factor of more than six (Wicks et al. 2002). Also, studies conducted since development of the USEPA criteria in 1999 have related decreased swimming performance of salmonids to ammonia exposure (Shingles et al. 2001; Wicks et al. 2002; McKenzie et al. 2003). A combination of reduced swimming performance and increased ammonia toxicity will have adverse effects on survival in the wild.

Fish in natural environments are often exposed to multiple contaminants that may interact with external ammonia to increase toxicity. A combination of copper and low pH increased concentrations of ammonia in the plasma of brown trout *Salmo trutta* (Beaumont et al. 1995, 2003). Other contaminants and stressors raise

circulating cortisol levels, which leads to increased ammonia production in fish (Mommsen et al. 1999). Stress-induced ammonia production may increase toxicity of external ammonia (Ip et al. 2001; Wicks and Randall 2002), and stressed fish are generally more sensitive to ammonia (Eddy 2005).

Conducting tests in quiescent solutions and in the absence of other stressors may underestimate the effect of ammonia in natural environments. However, results from the previous studies are based on relatively short exposures (96 h), and caution should be exercised before making conclusions about chronic exposures.

In summary, our results indicate that current USEPA chronic ammonia criteria are protective of early life stage rainbow trout under test conditions. Sublethal effects of ammonia on growth and development observed in this study are consistent with results from other published reports. Lethal thresholds were in reasonable agreement with those given by Burkhalter and Kaya (1977) and Thurston et al. (1984) but were much greater than those reported by Solbe and Shurben (1989) and Calamari et al. (1981). Our study did not explain possible reasons for the discrepancy in toxicity values among studies. The USEPA criteria document suggests that the use of different strains of rainbow trout in the tests may account for some of the observed differences in ammonia sensitivity. Rainbow trout from Ennis National Fish Hatchery, Ennis, Montana, were used by Burkhalter and Kaya (1977) and Thurston et al. (1984), and strains used by Calamari et al. (1981) and Solbe and Shurben (1989) were unreported. This study used a wild strain of rainbow trout from the Colorado River. Additional research is warranted to investigate (1) the USEPA statement that different strains may account for differences in ammonia sensitivity and (2) influences of other factors (e.g., quiescent exposure waters and combinations of other stressors) that may lead to underestimation of ammonia toxicity to fish in the wild.

Acknowledgments

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