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USE OF SURROGATE SPECIES IN ASSESSING CONTAMINANT RISK TO ENDANGERED AND THREATENED FISHES

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by

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NOTICE

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Abstract

Section 7 of the Endangered Species Act requires Federal agencies to insure that any action authorized, funded or carried out by them is not likely to jeopardize the continued existence of listed species or modify their critical habitat. The wide use of pesticides and other commercial chemicals potentially poses a risk to endangered and threatened species since, by definition, the distribution of listed species is limited and further adverse effects on these populations could lead to extinction. Surrogate species used in toxicity assessments must be carefully selected in order to be protective of listed species.

At present, the rainbow trout is considered to be an acceptable surrogate for coldwater fishes. Similarly, the fathead minnow is considered to be an acceptable surrogate for warmwater fishes. This research project was designed to determine the applicability of using rainbow trout and fathead minnows as surrogate species for several endangered fishes. Coldwater static acute toxicity tests were conducted with rainbow trout and three listed species of salmonids - Apache trout (Oncorhynchus apache), Lahontan cutthroat trout (Oncorhynchus clarki henshawi), and greenback cutthroat trout (Oncorhynchus clarki stomias). Warmwater static acute toxicity tests were conducted with fathead minnow and two listed species of cyprinids - bonytail chub (Gila elegans) and Colorado squawfish (Ptychocheilus lucius). In addition, warmwater static toxicity tests were conducted with the razorback sucker (Xyrauchen texanus). Chemicals used in these toxicity assessments were selected in consultation with EPA to represent different chemical classes and toxic modes of action. Chemicals used in testing were: carbaryl, copper, 4-nonylphenol, pentachlorophenol, and permethrin.

Results from the current studies indicated that the standard test organisms (rainbow trout and fathead minnows) often had a similar sensitivity to toxicant exposure as the listed salmonid and cyprinid species. The fathead minnow and the razorback sucker responses were also generally similar. However, for 30 percent (8 of 27) of the possible surrogate/listed species comparisons, the standard 96-h LC50 for the listed species was lower than the surrogate species. After 96 h of exposure, warmwater listed species were more sensitive than fathead minnows 33 percent of the time. However, the listed warmwater species were always less sensitive than rainbow trout. Hazard assessments using rainbow trout would be protective of the warmwater species tested in this study. After 96 h of exposure, the listed salmonids were more sensitive than the rainbow trout for 25 percent of the comparisons. Environmental protection procedures usually focus on protection of populations or communities and not specific species or individuals of a species as may be necessary for endangered and threatened species. These data indicate an additional margin of safety may need to be included to protect listed salmonid species when toxicity assessments utilize data obtained from studies with rainbow trout.

The tests conducted in this study were static acute toxicity tests with six listed species. Further testing should be conducted with other listed species or their FWS-identified surrogate species before definitive policy decisions concerning the protection of endangered and threatened species are made. In addition, chronic toxicity assessments should be conducted in order to compare chronic responses between listed and surrogate species.

Introduction

The Federal Insecticide, Fungicide and Rodenticide Act (FIFRA; PL 80-104) requires the U.S. Environmental Protection Agency (EPA) to register all pesticides before use in the United States. The Toxic Substances Control Act (TSCA; PL 94-469) requires regulation of commercial chemicals, other than pesticide products, that present a hazard to human health or to the environment. The Clean Water Act (CWA) specifies "it is the national policy that the discharge of toxic pollutants in toxic amounts be prohibited" (Section 101(a)(3)).

Under FIFRA, TSCA, and CWA, the EPA is charged with administering and implementing these regulations and is thus responsible for determining if the manufacture, use, or disposal of a chemical will present an unreasonable risk of injury to human health or the environment (Rand and Petrocelli, 1985). In addition to EPA, two agencies of the Department of Interior have similar interests in environmental protection, the U.S. Fish and Wildlife Service (FWS) and the National Biological Service (NBS). The FWS has responsibility to conserve, protect, and enhance the Nations' fish and wildlife resources and their habitats; the NBS mission is to gather, analyze and disseminate the information necessary for the wise stewardship of the Nation's natural resources, and to foster an understanding of biological systems.

Section 7 of the Endangered Species Act requires Federal agencies to insure that any action authorized, funded or carried out by them is not likely to jeopardize the continued existence of listed species or modify their critical habitat. The EPA and the FWS have been cooperating to determine the effects of pesticides and other chemicals on listed species under Section 7 of the Endangered Species Act. In June 1989, a Biological Opinion was completed by the FWS which listed 165 endangered and threatened species (primarily aquatic) and evaluated the hazard of 113 chemicals. Additionally, in July 1989 the EPA published its proposed Endangered Species Protection Program in the Federal Register (USFWS, 1990).

Chemicals inevitably enter the environment because of their wide use by agriculture, industry, and the public. About 70% of the 540 million kilograms of pesticides sold each year are used in agriculture. Furthermore, the American Chemical Society estimates over 63,000 chemicals are currently in use (Ramade, 1988). The TSCA Inventory lists over 70,000 chemicals that can be commercially produced and used. Some of these TSCA Inventory chemicals are produced in amounts of billions of kilograms per year. Approximately 14,000 chemicals are nonpolymers that are produced in quantities of over 4,530 kilograms per year. In 1989, the EPA Toxics Release Inventory (TRI) estimated the release of 320 chemicals from industrial facilities into various environments (USEPA, 1991a). For these 320 chemicals alone, the TRI reported releases of 2.6 billion kilograms into the environment by 22,650 facilities. The itemized summary of toxics entering the environment included: (1) 86 million kilograms released into streams, rivers, and other water bodies; (2) 250 million kilograms transferred to Publicly Owned Treatment Works; (3) 544 million kilograms injected underground; (4) 415 million kilograms transferred to treatment and disposal facilities; (5) 202 million kilograms disposed of in landfills; and (6) 1,807 million kilograms released into the air.

In order to evaluate a contaminant's potential for impact on the environment, standardized toxicity tests are conducted using standard test organisms as surrogates for other species. Under FIFRA or TSCA the aquatic risk assessment process for pesticides and other commercial chemicals includes: (1) acute toxicity tests with freshwater, estuarine, and marine fish and invertebrates; (2) embryo-larval and life cycle studies with fish and invertebrates; (3) residue studies; and (4) field testing. The freshwater fishes most often used in acute toxicity tests include one or more of the following species: rainbow trout (Oncorhynchus mykiss), brook trout (Salvelinus fontinalis), coho salmon (Oncorhynchus kisutch), bluegill (Lepomis macrochirus), channel catfish (Ictalurus punctatus), and fathead minnows (Pimephales promelas).

The CWA provides an integrated approach to protection of aquatic ecosystems through the development of water quality criteria and the control of toxic discharges (National Pollutant Discharge System - NPDES). Water quality criteria are derived to protect aquatic organisms from unacceptable adverse effects. However, there is allowance for some adverse effect including a small reduction in survival, growth, or reproduction in sensitive species (Stephan et al., 1985). The derivation of acute water quality criteria for fresh water requires a minimum data base which includes: (1) the family Salmonidae in the class Osteichthyes; (2) a second family in the class Osteichthyes, preferably a commercially or recreationally important warmwater species; (3) a third family in the phylum Chordata (may be in the class Osteichthyes or may be an amphibian etc.); (4) a planktonic crustacean; (5) a benthic crustacean; (6) an insect; (7) a family in a phylum other than Arthropoda or Chordata; and (8) a family in any order of insect or any phylum not already represented (Stephan et al., 1985). As part of the NPDES permit system, protection of freshwater aquatic environments from toxic discharges commonly includes whole effluent toxicity tests with Ceriodaphnia dubia, fathead minnows, and algae (Selenastrum capricornutum) (USEPA, 1991b).

Inherent in these programs is the assumption that the test species used for toxicity assessments are predictive of other species, including endangered and threatened species. However, the number of species tested is always limited. Mayer and Ellersieck (1986) compiled an acute toxicity data base for 410 chemicals and 66 species of freshwater animals and concluded: (1) for a given chemical, acute toxicity among species ranged over 5 orders of magnitude; (2) for a given species, acute toxicity among chemicals ranged over 9 orders of magnitude; and (3) no single species was always the most sensitive among chemicals.

Surrogate species are typically organisms that are easily tested using standardized methods. However, these species may not represent populations of endangered and threatened (listed) species. The wide use of pesticides and other commercial chemicals potentially poses a risk to endangered and threatened species since, by definition, the distribution of listed species is limited and further adverse effects on these populations could lead to extinction. Listed species may not be protected, or unnecessary regulatory programs may be implemented, if the sensitivity of listed species is not evaluated. Therefore, the EPA has expanded its role in protection of listed species to include determination of adverse effects of contaminants on survival, reproduction, and food organisms (USFWS, 1990).

At present, the rainbow trout is considered to be an acceptable surrogate for coldwater fishes. Similarly, the fathead minnow is considered to be an acceptable surrogate for warmwater fishes. This research project was designed to determine the applicability of using rainbow trout and fathead minnows as surrogate species for several endangered fishes. Coldwater static acute toxicity tests were conducted with rainbow trout and three listed species of salmonids - Apache trout (Oncorhynchus apache), Lahontan cutthroat trout (Oncorhynchus clarki henshawi), and greenback cutthroat trout (Oncorhynchus clarki stomias). Warmwater static acute toxicity tests were conducted with fathead minnow and two listed species of cyprinids - bonytail chub (Gila elegans) and Colorado squawfish (Ptychocheilus lucius). In addition, warmwater static toxicity tests were conducted with the razorback sucker (Xyrauchen texanus). Appendix 1 provides a brief listing profile for each threatened or endangered species used in these studies. Chemicals used in these toxicity assessments were selected in consultation with EPA to represent different chemical classes and toxic modes of action. Chemicals used in testing were: carbaryl, copper, 4-nonylphenol, pentachlorophenol, and permethrin.

Variation in responses between species may best be determined by the use of biochemical/physiological measurements. Radioligand binding is a technique which measures the number of receptors sites in a brain preparation and the affinity of the receptor for a specific radioligand. Exposure to either carbaryl or permethrin would be expected to increase the concentration of acetylcholine in the brain, albeit through different mechanisms. This increase could be expected to alter one or both of the binding parameters of the muscarinic cholinergic receptor (MChR) in the brain. For this reason, we also conducted a series of screening tests to evaluate the use of receptor binding as a biomonitoring technique.

Materials and Methods

Test organisms

Rainbow trout, Apache trout, Lahontan cutthroat trout, greenback cutthroat trout, bonytail chub, Colorado squawfish, and razorback suckers were obtained from various government and commercial sources (Table 1). Fish were received during the spring and summer of 1992 and 1993 as eyed-eggs at the Midwest Science Center (MSC, formerly National Fisheries Contaminant Research Center), NBS, Columbia, MO. Fathead minnows were from MSC stocks, or purchased commercially.

Once received at the MSC, fish were cultured in well water (alkalinity 258 mg/L as $CaCO_3$, hardness 286 mg/L as $CaCO_3$, pH 7.8,) until testing began. All fish were cultured at 18°C, except trout were hatched and cultured until swim-up in well water chilled to 10°C. Before the start of the toxicity tests, fish were acclimated and held for a total of 96 h (USEPA, 1975; ASTM, 1988). For the first 48 h, fish were incrementally acclimated to the test water and temperature. Fish were then moved to clean containers and held for an additional 48 h at the test temperature in 100% test water. Fish were fed during the first 48 h of acclimation but not fed during the 48 h of holding.

Chemicals

Chemicals used in testing included carbaryl, copper, 4-nonylphenol, pentachlorophenol, and permethrin. Chemicals were selected to represent different chemical classes and toxic modes of action and, with one exception, because of the existence of historical toxicity data. Carbaryl is a carbamate insecticide that inhibits cholinesterase activity. Copper occurs from mining, industrial applications, and in fungicide formulations. Copper interferes with osmoregulation. Pentachlorophenol is a chlorinated phenol and is used as a wood preservative and molluscicide. Pentachlorophenol is an uncoupler of oxidative phosphorylation. Permethrin is a pyrethroid insecticide and causes neurotoxicity. Nonylphenol was selected because of a lack of toxicity data and its widespread use in the manufacture of nonylphenol ethoxylate detergents. Nonylphenol is a monoalkyal phenol which may cause narcosis and may act as an oxidative stressor. Sources for the chemicals and percent purity are listed in Table 2. Organic chemical stocks were prepared by dissolving the chemical in reagent grade acetone, while copper was dissolved in deionized water. Complete analytical confirmation of stock concentrations is yet to be completed. Maximum volume of acetone added to any test container was 7.5 mL (0.5 mL/L).

Toxicity tests

Static acute toxicity tests were conducted in basic accordance with EPA (USEPA, 1975) and the American Society for Testing and Materials (ASTM, 1988) procedures during the summer and fall of 1992 and 1993. Fish exposures were conducted in 19.6 L glass jars containing 15 L of test solution. All tests with salmonids were conducted at 12°C and tests with cyprinids and the catastomid were conducted at 22°C. Test water was reconstituted hard water (alkalinity 110-120 mg/L as $CaCO_3$, hardness 160-180 mg/L as $CaCO_3$). Water quality (alkalinity, hardness, and pH) was measured on each batch of reconstituted water. Dissolved oxygen was measured on the control, low, medium, and high exposure concentrations at 0 h, and in those same treatments if fish survived, at 48 and 96 h of exposure. Additionally, pH was measured on the control, low, medium, and high exposure concentrations at 0 h, and in those same treatments if fish survived, at 96 h of exposure. Tests were conducted under ambient lighting.

A test series consisted of six exposure concentrations (three replicates per concentration) with a 60% dilution factor. Two years of toxicity tests were conducted with each listed species (except greenback cutthroat trout), and each listed species test had a corresponding surrogate species test. For rainbow trout and fathead minnows Runs, 1-4 were conducted in 1992 and Runs 5-6 were conducted during 1993. For all other species, Run 1 was conducted during 1992 and Run 2 was conducted during 1993. Both a solvent control and a dilution water control were included for each species (three replicates for each combination). Individual test series were randomly assigned to a waterbath and location within a waterbath (complete block design - Fig 1).

Fish were counted into groups of five with two groups pooled for each exposure replicate (10 fish/jar - 30 fish/exposure concentration). Fish mortality was the biological end-point observed at 3, 6, 9, 12, 18, 24, 48, 72, and 96 h of exposure. Mortality was defined as the lack of movement for a 5-s observation with the unaided eye. Dead fish were removed at each observational time. The study design is summarized in Table 3 and 4.

Biochemical evaluation

Muscarinic cholinergic receptor binding evaluations were done on brains from rainbow trout, Lahontan cutthroat trout, fathead minnows, and razorback suckers that survived the 96-h acute exposure to carbaryl and permethrin. Brains of fish from each treatment were removed and pooled to obtain adequate tissue. Radioligand binding was measured using [^{3}H]N-methyl scopolamine ([^{3}H]NMS) according to the method described by Bylund et al. (1982).

Statistical analysis

The LC50 and 95% confidence interval for each test was usually calculated using probit analysis. However, when probit analysis was not appropriate, LC50s and confidence intervals were calculated using moving average, untrimmed Spearman-Karber, or a non-linear interpolative procedure (Stephan, 1977).

Distribution of LC50s usually can not be tested for normality due to an insufficient number of LC50 estimates. Thus, it is suggested that a geometric mean be used to stabilize the variation (Snedecor & Cochran, 1980) for determining the central tendency of a group of LC50s. Sappington (1995) determined, from this same research, that the replicate 96-h LC50's for rainbow trout and fathead minnows were normally distributed, therefore an arithmetic mean was reported. However, there were insufficient replicates to test for normality for the listed species. For this report, the replicates were pooled within a Run and the pooled LC50s were used to calculate a geometric mean for each chemical and species. Arithmetic means are summarized in Appendix 2.

Analysis of variance and least square difference mean separations were determined on ranked LC50s at 12, 24, and 96 h of exposure. In order to summarize the 12, 24, and 96 h responses, the geometric mean LC50 was determined for each particular chemical/species/time combination. Differences between the surrogate species and listed species were evaluated separately for coldwater and warmwater species. Only those tests for which an LC50 could be calculated were used for statistical analysis. Biological Opinions written as a result of consultations held under Section 7 of the Endangered Species Act, usually estimate the number of individuals likely to be "taken" as a result of an action (USFWS, 1994). However, most environmental assessment procedures estimate effects on populations rather than individuals of a population. Since a greater degree of protection is required for endangered species (protection of individuals rather than a population), it is necessary to minimize Type 2 errors (acceptance of the null hypothesis that LC50s are the same, when they are different). For this reason, differences were considered significant at $p \leq 0.1$. However, the differences at $p \leq 0.05$ are also presented.

Results and Discussion

<u>Quality control</u>

Water quality for each batch of reconstituted hard water was within acceptable ranges for alkalinity and hardness, but average pH was above 8.0 (Table 5). Test chemicals spiked into the test water did not affect the pH. Appendix 3 lists the 0 and 96 h pH range, which represents the lowest and highest pH measured for all exposure concentrations and replicates within a test. Appendix 4 is a list of the exposure replicates and concentrations for which dissolved oxygen was below 60% saturation at 48 h of exposure or below 40% saturation at 96 h of exposure. Overall, dissolved oxygen drops were isolated and interspersed throughout the exposures. However, for Run 2 and 4 of the rainbow trout exposures there was a trend for dissolved oxygen to fall below the percent saturation limits. These two Runs of fish had the largest average weight. The LC50s did not appear to be different for these two Runs or for any other test in which low dissolved oxygen was measured. Therefore, no tests were eliminated from the statistical analysis due to dissolved oxygen falling below percent saturation limits.

Organic chemical stocks from tests conducted during 1992 were analyzed to confirm nominal concentrations. Chemical analysis was conducted at Mississippi State Chemical Laboratory (Mississippi State, MS) by gas chromatography. The average percent of nominal concentration and spike recovery were: carbaryl - 93% (n=5), spike recovery 91%; 4-nonylphenol - 99% (n=7), spike recovery 74%; pentachlorophenol - 79% (n=7), spike recovery 91%; and permethrin 93% (n=5), spike recovery 91%. One permethrin sample had a percent nominal concentration of 308%, however the biological results from the tests using this stock were no different than the tests conducted with the other permethrin stocks used in toxicity tests during 1992 and 1993 (n=8). For this reason we believe the reported value for this sample was incorrect. Therefore, it was not included in the average percent of nominal calculation.

Organic chemical stocks from tests conducted during 1993 were also analyzed at Mississippi State Chemical Laboratory to confirm nominal concentrations. The average percent of nominal concentration and spike recovery were: carbaryl - 119% (n=3), spike recovery 83%; 4-nonylphenol - 160% (n=3), spike recovery 103%; pentachlorophenol - 174% (n=2), spike recovery 58%; and permethrin 128% (n=3), spike recovery 88%. One pentachlorophenol sample had a percent nominal concentration of 572%, however the biological results from the tests using this stock were no different than the tests conducted with the other pentachlorophenol stocks used in either 1992 or 1993 (n=9). For this reason we believe the reported value for this sample was incorrect. Therefore, it was not included in the average percent of nominal calculation.

Copper stocks were confirmed at the MSC by atomic absorption spectrophotometry. The average percent of nominal concentration for copper was: 1992 - 100% (n=7); and 1993 - 96% (n=4). Spike recovery was 98%.

Control survival, regardless of species, with and without solvent, was always greater than 90% (Table 6).

Toxicity

All LC50 calculations are based on nominal concentrations. Appendix 5 is a complete listing of all the toxicity data (LC50, confidence interval, and slope) for each chemical, species, Run (pooled replicates), and time period.

In general, after 96 h of exposure, permethrin was the most toxic and carbaryl was the least toxic. The two phenolic compounds (4-nonylphenol and pentachlorophenol) exhibited similar toxicity. For the coldwater species, the order of toxicity from most to least toxic was permethrin > copper > pentachlorophenol $\geq$ 4-nonylphenol > carbaryl. For the warmwater, species the order of toxicity from most to least toxic was permethrin > pentachlorophenol $\geq$ 4-nonylphenol > copper > carbaryl. The rainbow trout 96-h LC50 values were lower than the fathead minnow LC50s for the five chemicals tested (Table 7 - 11). Macek and McAllister (1970) exposed 12 species of fish (five families) to nine insecticides and determined that salmonids were the most sensitive and that cyprinids and ictalurids were the least sensitive. Mayer and Ellersieck (1986) compared the sensitivity of four fish families to 65 chemicals and found that salmonids were the most sensitive and cyprinids the least sensitive. Results from our study are consistent with their findings.

Carbaryl

Fish exposed to higher concentrations of carbaryl were quickly immobilized. Fish dying from carbaryl exposure generally exhibited arched backs, gaping mouths, and flared gills and fins.

The Apache trout and Lahontan cutthroat trout were significantly more sensitive to carbaryl exposure (LC50s - 3.29 and 4.38 mg/L, respectively) than the rainbow trout at 12 h of exposure (LC50 - 6.76 mg/L; Table 7). The greenback cutthroat trout was less sensitive (LC50 - 8.50 mg/L) than the rainbow trout at 12 h of exposure. The Apache trout was also more sensitive (LC50 - 2.50 mg/L) than the rainbow trout (LC50 - 4.04 mg/L) at 24 h of exposure. At 96 h of exposure, there was no significant difference in sensitivity between any of the coldwater species with LC50s ranging from 1.54 mg/L for the Apache trout to 2.25 mg/L for the Lahontan cutthroat trout.

The warmwater fishes exhibited a different time relationship (Table 7). While there were no significant differences in sensitivity at 12 and 24 h of exposure between the warmwater species, at 96 h of exposure the LC50s for the bonytail chub (3.49 mg/L) and Colorado squawfish (3.07 mg/L) were significantly lower than the surrogate fathead minnow (5.21 mg/L). Observational analysis indicates that while the bonytail chub and Colorado squawfish were more sensitive than fathead minnows, they were no more sensitive than the rainbow trout.

Beyers et al. (1994) exposed bonytail chub and Colorado squawfish to carbaryl in static renewal tests using 26 d old squawfish and 6-d-old chub. The median lethal concentration in their tests was 1.31 mg/L for the Colorado squawfish and 2.02 mg/L for the bonytail chub. Their results are similar to our findings under static conditions with larger fish.

The LC50s for rainbow trout and fathead minnows from the current studies with carbaryl (Table 7) are similar to reported values calculated from static toxicity tests. Mayer and Ellersieck (1986) reported 96-h LC50s for rainbow trout exposed to carbaryl ranging between <0.32 to 3.5 mg/L with a geometric mean of 1.60 mg/L (n=15). Marking et al. (1984) reported LC50s (n=5) ranging between 0.94 and 1.74 mg/L for rainbow trout exposed to carbaryl. Reported 96-h LC50s for fathead minnows range from 7.7 to 14.6 mg/L (Mayer and Ellersieck, 1986; n=3) and 6.7 to 12.0 mg/L (Henderson et al., 1960; n=2).

Copper

The sensitivity of the listed coldwater species to copper was not significantly different than the surrogate rainbow trout at 12, 24, or 96 h of exposure. The LC50s at 96 h ranged from 0.07 mg/L for the Apache and Lahontan trout to 0.08 mg/L for the rainbow trout (Table 8).

At 96 h of exposure, both the bonytail chub (LC50 - 0.22 mg/L) and the razorback sucker (LC50 - 0.27 mg/L) were more sensitive than the fathead minnow (LC50 - 0.47 mg/L). The bonytail was also more sensitive than the fathead minnow at 12 and 24 h of exposure. LC50s for the bonytail were 0.30 (12 h) and 0.24 mg/L (24 h) compared to LC50s for the fathead minnow of 1.30 (12 h) and 0.73 mg/L (24 h). However, as was the case for carbaryl, both the surrogate and listed warmwater species were less sensitive than the rainbow trout.

The 96-h LC50s for copper in the current studies are similar to reported values. Mayer and Ellersieck (1986) report a copper LC50 of 135 μg/L for rainbow trout (hardness - 44 mg/L as $CaCO_3$) and 838 μg/L for fathead minnows (hardness - 272 mg/L as $CaCO_3$). Calamari and Marchetti (1973) report a copper LC50 of 890 μg/L for rainbow trout (290 mg/L as $CaCO_3$). Fathead minnows tested under static conditions had LC50 ranges of 22 to 1,760 μg/L (Pickering and Henderson, 1966; hardness 20 to 360 mg/L as $CaCO_3$). In the Ambient Water Quality Criteria for Copper (USEPA, 1985), the Species Mean Acute Value for rainbow trout is 42.5 μg/L and 115.5 μg/L for fathead minnows (hardness of 50 mg/L as $CaCO_3$).

4-Nonylphenol

During the toxicity tests with 4-nonylphenol, fish exposed to higher concentrations often exhibited increased mucus production and a white particulate material which formed on the fins and gills.

At 24 h of exposure, the Apache trout and Lahontan cutthroat trout were significantly more sensitive (LC50 of 0.24 and 0.25 mg/L, respectively) to 4-nonylphenol exposure than were the rainbow trout (LC50 of 0.30 mg/L; Table 9). However, after 96 h of exposure, all four coldwater species had similar LC50s (range 0.15 mg/L - greenback cutthroat trout, to 0.19 mg/L - rainbow trout). There was no significant difference between the LC50 values for listed warmwater species and the fathead minnow at 12, 24, or 96 h of exposure. The 96-h LC50s ranged from 0.17 mg/L for the razorback sucker to 0.29 mg/L for the bonytail chub.

Little information is available concerning the acute toxicity of 4-nonylphenol. However, the U.S. EPA Draft Report - Chemical Hazard Information Profile for Nonylphenol (Etnier, 1985) - reports toxicity information from various sources. For rainbow trout, one 96-h LC50 was reported as 230 μg/L and another 96-h LC50 was reported in the range of 560 to 920 μg/L. The 96-h LC50 for fathead minnows was reported as 135 μg/L and 300 μg/L. The reported LC50s are greater than the LC50s calculated for rainbow trout in this study, but similar to the LC50 values that we derived for fathead minnows.

Pentachlorophenol

The Apache trout was significantly more sensitive to pentachlorophenol than was the surrogate rainbow trout at 96 h of exposure. The 96-h LC50 for Apache trout was 0.11 mg/L while the LC50 for rainbow trout was 0.16 mg/L (Table 10). There were no significant differences between the LC50s at 12 and 24 h of exposure for any of the coldwater species.

None of the LC50s for the warmwater listed species were significantly different from those for fathead minnows, regardless of time period. The 96-h LC50s for the warmwater fish ranged from 0.23 mg/L for the bonytail chub to 0.28 mg/L for the razorback sucker.

In the current studies, LC50s for the rainbow trout are about 3 times greater than those previously reported by other authors, while the results with the fathead minnow are similar to reported values. Davis and Hoos (1975) reported that LC50s for rainbow trout exposed to pentachlorophenol ranged between 44 and 92 μg/l with a geometric mean of 68 (n=6) for rainbow trout. Mayer and Ellersieck (1986) reported LC50s for rainbow trout ranging between 34 and 115 μg/L with a geometric mean of 59 μg/L (n=3). These authors also reported the acute toxicity of pentachlorophenol to fathead minnows as 205 μg/L. The Ambient Water Quality Criteria for Pentachlorophenol (USEPA, 1986) reports a species mean acute value of 35.34 μg/L for rainbow trout and 63.11 μg/L for fathead minnows.

Permethrin

The LC50s for Apache trout and Lahontan cutthroat trout were significantly less than the LC50 for the surrogate rainbow trout at 24 and 96 h (Table 11). After 24 h of exposure, the LC50 for Apache trout and Lahontan cutthroat trout were 2.27 and 1.93 μg/L, respectively. The 24-h LC50 for rainbow trout was 3.78 μg/L. At 96 h of exposure, the LC50 for Apache trout (1.71 μg/L) and Lahontan cutthroat trout (1.58 μg/L) was about half that for rainbow trout (3.31 μg/L). There was no significant difference in the 12-h LC50s for the coldwater fishes.

The razorback sucker was significantly more sensitive to permethrin at 96 h of exposure than was the fathead minnow. While not statistically confirmed (the LC50 was greater than highest concentration tested), the bonytail chub was much less sensitive to permethrin exposure than was the fathead minnow at 12, 24, and 96 h of exposure. The Colorado squawfish was also less sensitive to permethrin than was the fathead minnow. Permethrin-exposed fish were often hyperactive - darting throughout the water column, tremoring, and skimming the water surface.

Mayer and Ellersieck (1986) reported LC50s for rainbow trout exposed to permethrin ranging from 2.9 to 8.2 μg/L with a geometric mean of 5.1 μg/L (n=9). The LC50 for fathead minnows was 5.7 μg/L. These LC50s are similar to those calculated in the current study for rainbow trout and fathead minnows (Table 11).

Comparisons to other studies

Few studies have evaluated the sensitivity of endangered species to contaminant exposures. Beleau and Bartosz (1982) conducted toxicity tests with Colorado squawfish, humpback chub, and northern squawfish. They exposed these fish to 13 inorganic and eight organic toxicants. The authors stated that there was a margin of safety associated with the use of channel catfish and fathead minnows as surrogates since the listed species they tested were more tolerant to the contaminants used in their toxicity assessments. However, they made their comparison based on information reported in the literature for fathead minnows and channel catfish. In addition, their tests were conducted in water different than that used in the fathead minnow and channel catfish studies. We conducted Colorado squawfish and fathead minnow tests concurrently and in the same standardized water. These reasons possibly explain differences between the current studies and those of Beleau and Bartosz (1982).

The current research indicated that bonytail chub and Colorado squawfish were significantly more sensitive to carbaryl exposure than were fathead minnows, but less sensitive than rainbow trout. This finding is supported by work done by Beyers et al. (1994). They conducted 4-d renewal acute tests and 32-d early life-stage toxicity tests with carbaryl, Sevin 4-oil, and malathion. These authors compared their results from the 4-d renewal acute test to acute data summarized by Mayer and Ellersieck (1986). They determined that for carbaryl exposures, the Colorado squawfish and bonytail chub were similar in response to the cutthroat trout (Oncorhynchus clarki), Atlantic salmon (Salmo salar), rainbow trout and brook trout. The listed species were more sensitive (2 to 10 times) than fathead minnows, bluegill, and channel catfish. Conversely, Colorado squawfish and bonytail chub exposed to malathion were similar in sensitivity to fathead minnows and channel catfish and less sensitive than the trout and bluegill.

Biochemical evaluation

The number of muscarinic cholinergic receptors (MChR) decreased in the fathead minnow and the razorback sucker brains at carbaryl exposures of 3.6 mg/L and higher. Lahontan cutthroat trout brain receptor number decreased at a carbaryl concentration of 2.2 mg/L and higher while those for rainbow trout decreased at 1.3 mg/L and higher. Exposure to permethrin caused a reduction in receptor number in all four species relative to the control. There was no change in affinity in any of the samples.

This preliminary study indicated a downregulation or decrease in receptor number in brains from fish exposed to each of these contaminants as well as a difference in MChR binding between cold and warmwater species and between listed and surrogate species. These results reflect a compensatory response to the increase in acetylcholine resulting from exposure to these contaminants. Further studies utilizing lower concentrations of contaminant, longer exposure periods and a recovery period would enhance our ability to evaluate species sensitivity differences.

Management Implications

Environmental protection procedures usually focus on protection of populations or communities and not specific species or individuals of a species as may be necessary for endangered and threatened species. For this reason, additional margins of safety may be required.

At 12, 24, and 96 h of exposure, there are a total of 81 possible comparisons of LC50s between a listed species and surrogate species. Exposures of the greenback cutthroat trout to copper, pentachlorophenol, and permethrin are not included in this total since the LC50s for these chemicals could not be estimated.

For the warmwater species at 12 and 24 h of exposure (30 possible comparisons), the listed species were more sensitive twice (12 and 24-h exposure of bonytail chub to copper) and less sensitive four times (12 and 24-h exposure of bonytail chub and Colorado squawfish to permethrin). This would seem to indicate that for these listed species, evaluations of risk for short (pulse) exposures (e.g. water quality criteria exceedance, agricultural pesticide spraying, or runoff events) could be estimated using surrogate species. However, at 96 h of exposure (15 possible comparisons) the listed warmwater species were more sensitive 5 times and less sensitive only once.

At 12 and 24 h of exposure, the coldwater listed species were more sensitive than the rainbow trout eight times (22 possible comparisons). This would indicate the need for careful evaluation of short-term exposure risk. At 96 h of exposure the listed species were more sensitive three times (12 possible comparisons).

The 96-h LC50 is the endpoint typically used in hazard assessment. When deriving water quality criteria, the Criterion Maximum Concentration can be determined for important species by dividing the Species Mean Acute Value by two (Stephan et al., 1985). Dividing the fathead minnow LC50 by two may not be protective of the more sensitive warmwater listed species we tested. However, the LC50s for the rainbow trout were always less than the LC50 for the listed warmwater species we tested. National criteria typically include rainbow trout as a test species. These criteria would probably be protective of the listed warmwater species used in this study. Caution must be exercised when national water quality criteria are modified by states in setting state water quality standards. This is of particular concern when toxicity data for sensitive species, such as rainbow trout, are eliminated.

As was the case with the listed warmwater species, dividing the rainbow trout Species Mean Acute Value by two would not necessarily be protective of the coldwater listed species tested in these studies. If a national water quality criterion is close to half the rainbow trout Species Mean Acute Value, an additional margin of safety may need to be included.

Ecological risk procedures for endangered species and pesticides (Urban and Cook, 1985) require formal consultation if expected environmental concentrations exceed 1/20 the LC50 (when slopes are not available) or 1/10 the LC10 (when slopes are available). Results from this study with warmwater and coldwater species support the validity of this assessment procedure.

Summary and Recommendations

Federal agencies need to assure that chemical use regulations comply with the Endangered Species Act and that these regulations are protective of threatened and endangered (listed) aquatic species. Surrogate species used in toxicity assessments must be carefully selected in order to be protective of listed species.

Results from the current studies indicated that the standard test organisms (rainbow trout and fathead minnows) often had a similar sensitivity to toxicant exposure as the listed salmonid and cyprinid species. The fathead minnow and the razorback sucker responses were also generally similar. However, for 30 percent (8 of 27) of the of the possible surrogate/listed species comparisons, the standard 96-h LC50 for listed species was lower than for the surrogate species. After 96 h of exposure, warmwater listed species were more sensitive than the fathead minnow 33 percent of the time. However, the listed warmwater species were always less sensitive than rainbow trout. Hazard assessments using rainbow trout would be protective of the warmwater species tested in this study. After 96 h of exposure, the listed salmonids were more sensitive than the rainbow trout for 25 percent of the comparisons. Environmental protection procedures usually focus on protection of populations or communities and not specific species or individuals of a species as may be necessary for endangered and threatened species. These data indicate an additional margin of safety may need to be included in order to protect listed salmonid species when toxicity assessments utilize data obtained from studies with rainbow trout.

The tests conducted in this study were static acute toxicity tests with six listed species. Further testing should be conducted with other listed species or their FWS-identified surrogate species before definitive policy decisions concerning the protection of endangered and threatened species are made. In addition, chronic toxicity assessments should be conducted in order to compare chronic responses between listed and surrogate species.

Acknowledgments

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Table 1. Test organisms and sources.

Species	Source	Strain
rainbow trout (Oncorhynchus mykiss)	Beity's Enterprise Valley, WA	Beity's
	Ennis National Fish Hatchery (NFH) Ennis, MT	Backcross, Erwin, and Shasta
Apache trout (Oncorhynchus apache)	Williams Creek NFH White River, AZ	East Fork
greenback cutthroat trout (Oncorhynchus clarki stomias)	Saratoga NFH Saratoga, WY	GCD greenback
Lahontan cutthroat trout (Oncorhynchus clarki henshawi)	Lahontan NFH Gardnerville, NV	Pyramid lake
fathead minnow (Pimephales promelas)	MSC cultures	NA[1]
	Osage Fisheries Osage Beach, MO	NA
bonytail chub (Gila elegans)	Dexter NFH Dexter, NM	NA
Colorado squawfish (Ptychocheilus lucius)	Dexter NFH Dexter, NM	NA
razorback sucker (Xyrauchen texanus)	Dexter NFH Dexter, NM	NA

[1]NA - not applicable

Table 2. Sources and percent active ingredient of chemicals used in acute toxicity tests with rainbow trout and listed salmonids.

CHEMICAL	SOURCE	ACTIVE INGREDIENT (%)
Carbaryl	Donated by Rhone-Pôulenc Agricultural Co., Research Triangle Park, NC	99.7
Copper sulfate	Fisher Chemical, St. Louis, MO	25.5
4-Nonylphenol	Fluka Chemical, New York, NY	85.0
Pentachlorophenol	Aldrich Chemical, Milwaukee, WI	99.0
Permethrin	Donated by ICI Americas Inc., Richmond, CA	95.2

Table 3. Summary of study design for the comparative toxicity of selected chemicals to rainbow trout and coldwater species.

Test type:	Static acute
Test volume:	15 L
Test temperature:	12°C
Water Quality:	Reconstituted ASTM hard
Chemicals:	Carbaryl, copper, 4-nonylphenol, pentachlorophenol, permethrin
Species:	rainbow trout - 6 Runs Run 1 - 0.67 ± 0.35 g Run 2 - 1.25 ± 0.57 g Run 3 - 0.27 ± 0.07 g Run 4 - 1.09 ± 0.38 g Run 5 - 0.48 ± 0.08 g Run 6 - 0.50 ± 0.21 g Apache trout - 2 runs Run 1 - 0.85 ± 0.49 g Run 2 - 0.38 ± 0.18 g greenback cutthroat trout - 1 run Run 1 - 0.31 ± 0.17 g Lahontan cutthroat trout - 2 runs Run 1 - 0.34 ± 0.08 g Run 2 - 0.57 ± 0.23 g
Exposure design:	Dilution series - 60% Replicates - 3/concentration Organisms - 10/replicate
Observations:	Mortality at 3, 6, 9, 12, 18, 24, 48, 72, and 96 h of exposure

Table 4. Summary of study design for the comparative toxicity of selected chemicals to fathead minnows and listed warmwater species.

Test type:	Static acute
Test volume:	15 L
Test temperature:	22°C
Water Quality:	Reconstituted ASTM hard
Chemicals:	Carbaryl, copper, 4-nonylphenol, pentachlorophenol, permethrin
Species:	fathead minnows - 5 Runs Run 1 - 0.32 $\pm$ 0.16 g Run 2 - 0.56 $\pm$ 0.19 g Run 3 - 0.45 $\pm$ 0.35 g Run 4 - 0.40 $\pm$ 0.21 g Run 5 - 0.34 $\pm$ 0.24 g Run 6 - 0.39 $\pm$ 0.14 g bonytail chub - 2 runs Run 1 - 0.29 $\pm$ 0.08 g Run 2 - 0.52 $\pm$ 0.09 g Colorado squawfish - 2 runs Run 1 - 0.32 $\pm$ 0.05 g Run 2 - 0.34 $\pm$ 0.05 g razorback sucker - 2 runs Run 1 - 0.31 $\pm$ 0.04 g Run 2 - 0.32 $\pm$ 0.07 g
Exposure design:	Dilution series - 60% Replicates - 3/concentration Organisms - 10/replicate
Observations:	Mortality at 3, 6, 9, 12, 18, 24, 48, 72, and 96 h of exposure

Table 5. Average measured water quality characteristics of reconstituted water used in the acute toxicity tests with coldwater and warmwater fishes.

Water Quality Parameter	Nominal value	Coldwater Measured (n = 7)	Warmwater Measured (n = 6)
Alkalinity[1]	110 - 120	117 ± 8	117 ± 4
Hardness[1]	160 - 180	169 ± 10	173 ± 9
pH	7.8 - 8.0	8.24 ± 0.29	8.35 ± 0.29

[1]mg/L as $CaC0_3$

Table 6. Average control survival (standard deviation in parentheses) for coldwater and warmwater species.

Species	Without Solvent	With Solvent
rainbow trout		
Run 1	100	100
Run 2	100	100
Run 3	100	100
Run 4	100	100
Run 5	100	100
Run 6	100	100
Apache trout		
Run 1	100	96.7 (5.8)
Run 2	96.8 (5.2)	100
Lahontan cutthroat		
Run 1	100	100
Run 2	100	100
greenback cutthroat		
Run 1	96.7 (5.8)	100
fathead minnow		
Run 1	100	100
Run 2	100	100
Run 3	100	100
Run 4	100	100
Run 5	100	100
Run 6	100	100
bonytail chub		
Run 1	100	100
Run 2	100	100
Colorado squawfish		
Run 1	100	100
Run 2	100	100
razorback sucker		
Run 1	100	100
Run 2	100	100

Table 7. Acute toxicity of carbaryl (mg/L) to 8 species of fish (2 surrogate and 6 listed) at 12, 24, and 96 h of exposure. Toxicity values are the geometric mean of the LC50s (number of LC50s in parentheses) used in the rank analysis. When significant, the probability associated with determining whether a listed species is different than the surrogate is also listed (results from analysis of variance). An asterisk (*) indicates a least square difference mean separation of $p \leq 0.1$ and (**) indicates a $p \leq 0.05$.

Species	12-h LC50 (n)	24-h LC50 (n)	96-h LC50 (n)
rainbow trout	6.76 (4) p<.01	4.04 (6) p=.07	1.88 (6) n.s.[1]
Apache trout	3.29** (2)	2.50** (2)	1.54 (2)
greenback cutthroat	8.50* (1)	3.59 (1)	1.55 (1)
Lahontan cutthroat	4.38** (2)	3.60 (2)	2.25 (2)
fathead minnow	12.0 (1) ns[1]	8.25 (3) ns[1]	5.21 (5) p=.06
bonytail chub	7.93 (2)	6.13 (2)	3.49** (2)
Colorado squawfish	>10.0 (NA)[2]	6.31 (1)	3.07** (2)
razorback sucker	8.88 (1)	6.67 (2)	4.35 (2)

[1]ns = not significant
[2]NA = not applicable

Table 8. Acute toxicity of copper (mg/L) to 8 species of fish (2 surrogate and 6 listed) at 12, 24, and 96 h of exposure. Toxicity values are the geometric mean of the LC50s (number of LC50s in parentheses) used in the rank analysis. When significant, the probability associated with determining whether a listed species is different than the surrogate is also listed (results from analysis of variance). An asterisk (*) indicates a least square difference mean separation of $p \leq 0.1$ and (**) indicates a $p \leq 0.05$.

Species	12-h LC50 (n)	24-h LC50 (n)	96-h LC50 (n)
rainbow trout	0.40 (3) ns[1]	0.12 (4) ns[1]	0.08 (4) ns[1]
Apache trout	0.18 (2)	0.09 (2)	0.07 (1)
greenback cutthroat	>0.03 (NA)[2]	>0.03 (NA)	>0.03 (NA)
Lahontan cutthroat	0.39 (2)	0.11 (2)	0.07 (2)
fathead minnow	1.30 (4) p=.04	0.73 (4) p=.08	0.47 (6) p=.05
bonytail chub	0.30** (2)	0.24** (2)	0.22** (2)
Colorado squawfish	>1.00 (NA)	0.64 (2)	0.43 (2)
razorback sucker	>1.00 (NA)	0.39 (1)	0.27** (2)

[1]ns = not significant
[2]NA = not applicable

Table 9. Acute toxicity of 4-nonylphenol (mg/L) to 8 species of fish (2 surrogate and 6 listed) at 12, 24, and 96 h of exposure. Toxicity values are the geometric mean of the LC50s (number of LC50s in parentheses) used in the rank analysis. When significant, the probability associated with determining whether a listed species is different than the surrogate is also listed (results from analysis of variance). An asterisk (*) indicates a least square difference mean separation of $p \leq 0.1$ and (**) indicates a $p \leq 0.05$.

Species	12-h LC50 (n)	24-h LC50 (n)	96-h LC50 (n)
rainbow trout	0.35 (6) ns[1]	0.30 (6) p=.04	0.19 (6) ns[1]
Apache trout	0.30 (2)	0.24** (2)	0.17 (2)
greenback cutthroat	0.38 (1)	0.30 (1)	0.15 (1)
Lahontan cutthroat	0.29 (2)	0.25** (2)	0.18 (2)
fathead minnow	0.38 (6) ns[1]	0.33 (6) ns[1]	0.27 (6) ns[1]
bonytail chub	0.56 (2)	0.49 (2)	0.29 (2)
Colorado squawfish	0.45 (2)	0.28 (2)	0.26 (2)
razorback sucker	0.29 (2)	0.22 (2)	0.17 (2)

[1]ns = not significant

Table 10. Acute toxicity of pentachlorophenol (mg/L) to 8 species of fish (2 surrogate and 6 listed) at 12, 24, and 96 h of exposure. Toxicity values are the geometric mean of the LC50s (number of LC50s in parentheses) used in the rank analysis. When significant, the probability associated with determining whether a listed species is different than the surrogate is also listed (results from analysis of variance). An asterisk (*) indicates a least square difference mean separation of $p \leq 0.1$ and (**) indicates a $p \leq 0.05$.

Species	12-h LC50 (n)	24-h LC50 (n)	96-h LC50 (n)
rainbow trout	0.22 (6) ns[1]	0.17 (6) ns[1]	0.16 (6) p=.06
Apache trout	0.21 (2)	0.21 (2)	0.11** (2)
greenback cutthroat	>0.01 (NA)[1]	>0.01 (NA)	>0.01 (NA)
Lahontan cutthroat	0.27 (1)	0.23 (2)	0.17 (2)
fathead minnow	0.33 (6) ns[1]	0.30 (6) ns[1]	0.25 (6) ns[1]
bonytail chub	0.42 (2)	0.26 (2)	0.23 (2)
Colorado squawfish	0.23 (2)	0.16 (2)	0.24 (2)
razorback sucker	0.53 (2)	0.29 (2)	0.28 (2)

[1]ns = not significant
[2]NA = not applicable

Table 11. Acute toxicity of permethrin (μg/L) to 8 species of fish (2 surrogate and 6 listed) at 12, 24, and 96 h of exposure. Toxicity values are the geometric mean of the LC50s (number of LC50s in parentheses) used in the rank analysis. When significant, the probability associated with determining whether a listed species is different than the surrogate is also listed (results from analysis of variance). An asterisk (*) indicates a least square difference mean separation of $p \leq 0.1$ and (**) indicates a $p \leq 0.05$.

Species	12-h LC50 (n)	24-h LC50 (n)	96-h LC50 (n)
rainbow trout	5.75 (6) ns[1]	3.78 (6) p=.08	3.31 (6) p=.08
Apache trout	3.88 (2)	2.27* (2)	1.71* (2)
greenback cutthroat	>1.0 (NA)[1]	>1.0 (NA)	>1.0 (NA)
Lahontan cutthroat	3.33 (2)	1.93** (2)	1.58* (2)
fathead minnow	13.43 (4) ns[1]	9.73 (5) ns[1]	9.38 (6) p=.08
bonytail chub	>25.0 (NA)	>25.0 (NA)	>25.0 (NA)
Colorado squawfish	>25.0 (NA)	>25.0 (NA)	24.4 (1)
razorback sucker	13.05 (1)	8.87 (1)	5.95* (2)

[1]ns = not significant
[2]NA = not applicable

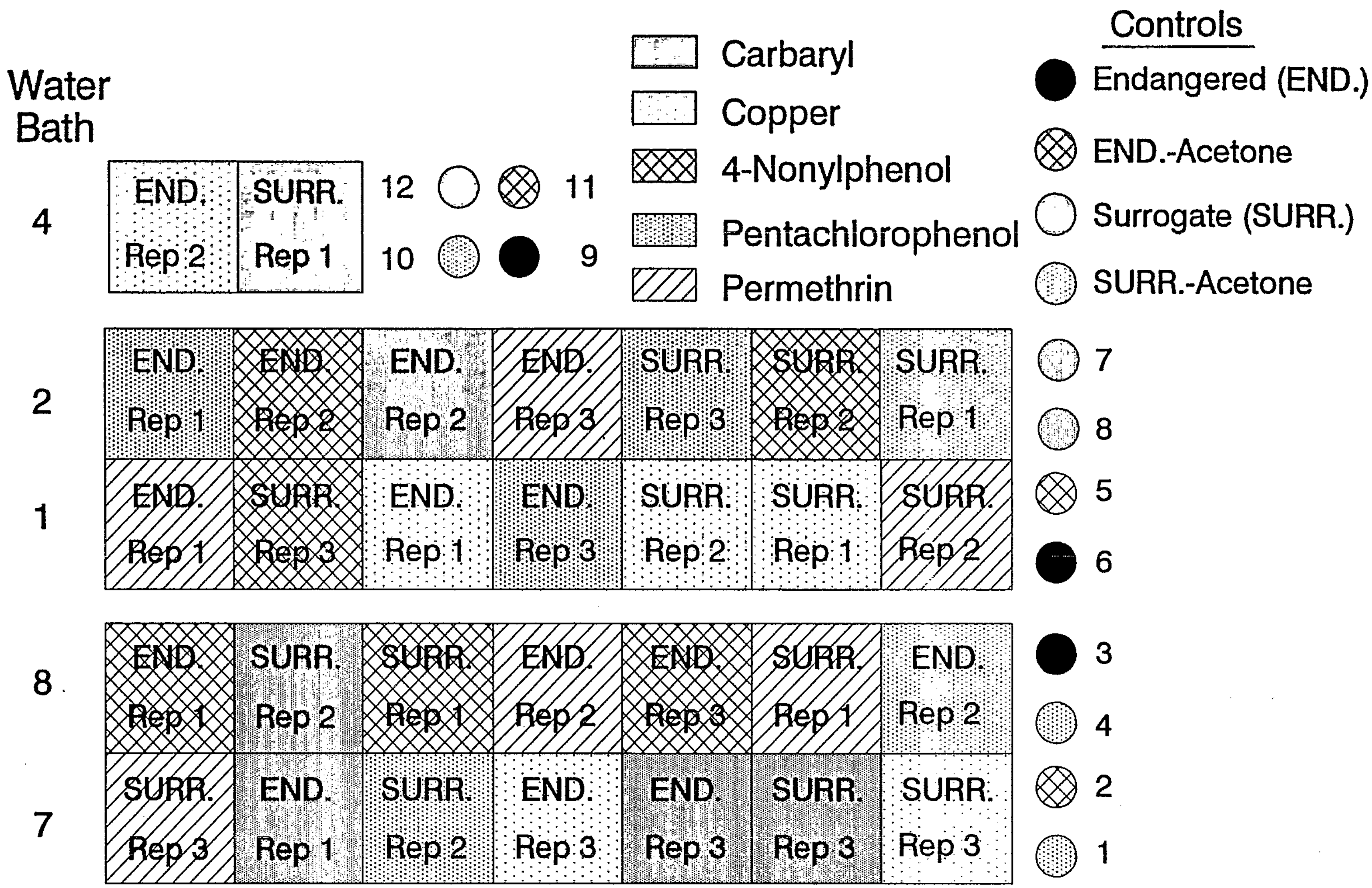

Figure 1. Test series assignment to a waterbath and location within a waterbath (complete block design).

APPENDIX 1. SPECIES PROFILES

Appendix 1. Species profile - Apache Trout (Oncorhynchus apache)

Historic range: The historic range is difficult to determine because by the time they were positively identified pure populations were diminished. The apparent range included the upper Salt River system of Arizona and headwater streams of the little Colorado River and Blue River of the San Francisco drainage.

Current range: It is estimated that pure strains of Apache trout occupy 5% of their former range. Those areas include Boggy Creek, Crooked Creek, South Fork Diamond Creek, East Fork of the White River, Centerfire Creek, and Soldier Creek. These streams are located on the Fort Apache Indian Reservation and The Apache-Sitgreaves National Forest in Arizona.

Reason for decline: The reasons for population decline include; reduction in habitat, detrimental interactions with introduced species, competition, predation and hybridization with cutthroat trout (Oncorhynchus gila).

Status: Threatened

References

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Appendix 1. Species profile - greenback cutthroat trout (Oncorhynchus clarki stomias)

Historic range: The historic range of the greenback cutthroat trout includes the head waters of South Platte basin and Arkansas River basins in Colorado.

Current range: Only three pure populations of greenback cutthroat trout remain including Como Creek, South Fork - Cache La Poudre River, and Cascade Creek in Colorado.

Reason for decline: Declines in population were due to irrigation, mining pollution, logging, grazing and introduction of exotics (brown and brook trout) that displaced or hybridized with greenbacks.

Status: Threatened

References

Behnke, R.J. and M. Zarn. 1976. Biology and management of threatened and endangered western trouts. USDA For. Serv. Gen. Tech. Rep. RM-28, 45 p. Rocky Mt. For. and Range Exp. Stn. Fort Collins, CO.

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Appendix 1. Species profile - Lahontan cutthroat trout (Oncorhynchus clarki henshawi)

Historic range: The historic range of the Lahontan cutthroat trout included the Lahontan basin of California and Nevada, major lacustrine populations in Pyramid Lake, Walker Lake, Independence Lake and Lake Tahoe in California and Summit Lake in Nevada, and fluvial populations in the Carson, Walker and Truckee River systems in Nevada.

Current range: All populations are believed to be lost except Summit Lake, Nevada and Independence Lake, California.

Reasons for decline: The Pyramid Lake stock was lost due to construction of Derby Dam in 1905 and Lahontan Dam (Reservoir) in 1915 inhibiting migration from Pyramid Lake to Truckee River. Thus, there has not been any successful reproduction since 1938. The Lake Tahoe populations declined due to damming, pollution and siltation. The population in Walker Lake was reduced due to irrigation, diversion and reduction of stream flows which interfered in reproduction.

Status: Threatened

References

Behnke, R.J. and M. Zarn. 1976. Biology and management of threatened and endangered western trouts. USDA For. Serv. Gen. Tech. Rep. RM-28, 45 p. Rocky Mt. For. and Range Exp. Stn. Fort Collins, CO.

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Appendix 1. Species profile - Bonytail chub (Gila elegans)

Historic range: The historic range of the bonytail chub included the upper and lower basins of the Colorado River, the Green River (WY, UT), Yampa and Gunnison River (CO), and the Gila and Salt River (AZ).

Current range: The current range for the bonytail includes Lake Mojave and Desolation and Cataract Canyon of the Upper Colorado River basin.

Reason for decline: The reasons for species decline include building of impoundments, water withdrawal and the introduction of non-native species.

Status: Endangered

References

Carter, J.G., R.A. Valdez, R.J. Ryel and V.A. Lamarra. 1985. Fisheries habitat dynamics in the upper Colorado River. J. Freshwater Ecology. 3:249.

Minckley, W.L. 1991. Native fishes of the grand Canyon Region: An Obituary? Colorado River Ecology and Dam Management, Proceedings of a symposium. National Academy Press, Washington, D.C. p. 124.

U.S. Fish and Wildlife Service. 1990. Bonytail chub recovery plan. Denver, CO. 35 p.

Appendix 1. Species profile - Colorado squawfish (Ptychochelius lucius)

Historic range: Colorado squawfish were common in the lower Colorado River basin in Arizona and Colorado until the 1930s.

Current range: The largest populations occur in the Green River sub-basin and upper Colorado River basin.

Reason for decline: Reasons for decline of the Colorado squawfish include the construction of dams and reservoirs, water withdrawals, and competition and predation by non-native species.

Status: Endangered

References

Carter, J.G., R.A. Valdez, R.J. Ryel and V.A. Lamarra. 1985. Fisheries habitat dynamics in the upper Colorado River. J. Freshwater Ecology. 3:249.

Tyus, H.M. 1991. Ecology and Management of Colorado Squawfish. in Battle against extinction: Native fish management in the American West. eds. W.L. Minckley and J.E. Deacon 517 pp.

U.S. Fish and Wildlife Service. 1990. Colorado squawfish recovery plan. Denver, CO. 56 p.

Appendix 1. Species profile - Razorback sucker (Xyrauchen texanus)

Historic range: The historic range of the razorback sucker included the upper and lower Colorado River basin and the Gila River.

Current range: The razorback sucker is abundant only in Lake Mohave and about 1000 razorbacks occur in the upper Green River.

Reasons for decline: Causes for declines in razorback sucker populations are similar to those for Colorado squawfish; alterations of the river environment including the construction of dams and potential competition and predation by non-native species.

Status: Endangered

References

Carter, J.G., R.A. Valdez, R.J. Ryel and V.A. Lamarra. 1985. Fisheries habitat dynamics in the upper Colorado River. J. Freshwater Ecology. 3:249.

McAda, C.W. and R.S. Wydoski. 1980. The razorback sucker, Xyrauchen texanus, in the upper Colorado River Basin, 1974-1976. Technical papers of the USFWS/FWS, Washington, DC 15 p.

Minckley, W.L., P.C. Marsh, J.E. Brooks, J.E. Johnson and B.L. Jensen. 1991. Management toward recovery of the razorback sucker. in Battle against extinction: Native fish management in the American West. eds. W.L. Minckley and J.E. Deacon 517 pp.

APPENDIX 2. ARITHMETIC MEANS

Appendix 2. Acute toxicity of carbaryl (mg/L) to 8 species of fish (2 surrogate and 6 listed) at 12, 24, and 96 h of exposure. Toxicity values are the arithmetic means for the individual replicate LC50s (Sappington, 1995). The number of LC50s used in the calculation is listed in parentheses.

Species	12-h LC50 (n)	24-h LC50 (n)	96-h LC50 (n)
rainbow trout	6.87 (11)	4.23 (16)	2.00 (18)
Apache trout	3.62 (5)	2.73 (5)	1.53 (4)
greenback cutthroat	7.40 (2)	3.63 (3)	1.59 (2)
Lahontan cutthroat	4.65 (6)	3.45 (6)	2.18 (5)
fathead minnow	10.9 (2)	9.10 (2)	5.20 (16)
bonytail chub	7.00 (2)	6.52 (5)	3.36 (5)
Colorado squawfish	> 10.0 (NA)[1]	6.51 (3)	3.23 (6)
razorback sucker	> 10.0 (NA)[1]	6.87 (5)	4.39 (5)

[1]NA = not applicable

Appendix 2. Acute toxicity of copper (mg/L) to 8 species of fish (2 surrogate and 6 listed) at 12, 24, and 96 h of exposure. Toxicity values are the arithmetic means for the individual replicate LC50s (Sappington, 1995). The number of LC50s used in the calculation is listed in parentheses.

Species	12-h LC50 (n)	24-h LC50 (n)	96-h LC50 (n)
rainbow trout	0.39 (12)	0.13 (14)	0.08 (12)
Apache trout	0.17 (4)	0.08 (2)	0.068 (2)
greenback cutthroat	>0.03 (NA)[1]	>0.03 (NA)	>0.03 (NA)
Lahontan cutthroat	0.32 (5)	0.15 (6)	0.072 (4)
fathead minnow	1.53 (11)	0.85 (12)	0.49 (15)
bonytail chub	0.31 (6)	0.24 (6)	0.22 (6)
Colorado squawfish	>1.00 (NA)	0.52 (4)	0.43 (6)
razorback sucker	>1.00 (NA)	0.41 (3)	0.28 (6)

[1]NA = not applicable

Appendix 2. Acute toxicity of 4-nonylphenol (mg/L) to 8 species of fish (2 surrogate and 6 listed) at 12, 24, and 96 h of exposure. Toxicity values are the arithmetic means for the individual replicate LC50s (Sappington, 1995). The number of LC50s used in the calculation is listed in parentheses.

Species	12-h LC50 (n)	24-h LC50 (n)	96-h LC50 (n)
rainbow trout	0.36 (15)	0.32 (13)	0.25 (14)
Apache trout	0.31 (6)	0.23 (6)	0.17 (5)
greenback cutthroat	0.37 (3)	0.30 (3)	0.156 (3)
Lahontan cutthroat	0.27 (3)	0.23 (3)	0.17 (5)
fathead minnow	0.36 (12)	0.35 (17)	0.32 (11)
bonytail chub	0.57 (4)	0.48 (5)	0.29 (4)
Colorado squawfish	0.44 (3)	0.28 (3)	0.24 (4)
razorback sucker	0.31 (6)	0.23 (5)	0.20 (2)

Appendix 2. Acute toxicity of pentachlorophenol (mg/L) to 8 species of fish (2 surrogate and 6 listed) at 12, 24, and 96 h of exposure. Toxicity values are the arithmetic means for the individual replicate LC50s (Sappington, 1995). The number of LC50s used in the calculation is listed in parentheses.

Species	12-h LC50 (n)	24-h LC50 (n)	96-h LC50 (n)
rainbow trout	0.22 (12)	0.19 (13)	0.15 (17)
Apache trout	0.21 (5)	0.14 (5)	0.104 (5)
greenback cutthroat	>0.01 (NA)[1]	>0.01 (NA)	>0.01 (NA)
Lahontan cutthroat	0.26 (1)	0.20 (3)	0.17 (5)
fathead minnow	0.35 (17)	0.32 (17)	0.27 (17)
bonytail chub	0.44 (6)	0.40 (3)	0.23 (6)
Colorado squawfish	0.23 (5)	0.14 (2)	0.13 (3)
razorback sucker	0.52 (3)	0.27 (3)	0.27 (3)

[1]NA = not applicable

Appendix 2. Acute toxicity of permethrin (μg/L) to 8 species of fish (2 surrogate and 6 listed) at 12, 24, and 96 h of exposure. Toxicity values are the arithmetic means for the individual replicate LC50s (Sappington, 1995). The number of LC50s used in the calculation is listed in parentheses.

Species	12-h LC50 (n)	24-h LC50 (n)	96-h LC50 (n)
rainbow trout	5.4 (15)	3.9 (16)	3.8 (18)
Apache trout	3.9 (4)	2.5 (4)	1.97 (4)
greenback cutthroat	>1.0 (NA)[1]	>1.0 (NA)	>1.0 (NA)
Lahontan cutthroat	3.5 (6)	2.0 (6)	1.80 (7)
fathead minnow	13.6 (11)	9.7 (14)	9.7 (17)
bonytail chub	>25.0 (NA)	22.2 (1)	22.2 (1)
Colorado squawfish	>25.0 (NA)	8.2 (1)	17.6 (3)
razorback sucker	13.1 (3)	7.0 (3)	6.3 (5)

[1]NA = not applicable

APPENDIX 3. EXPOSURE pH

Appendix 3. Exposure water pH for each test (species/Run/chemical) at 0 and 96 h. The pH range represents the lowest and highest pH measured for all exposure concentrations and replicates within a test.

Species	Chemical	0 h	96 h
rainbow trout			
Run 1	control	8.6-8.7	7.7-7.9
	carbaryl	8.5-8.7	7.7-7.9
	copper	8.3-8.6	7.8-7.9
	4-nonylphenol	8.5-8.6	7.8-7.9
	pentachlorophenol	8.4-8.6	7.7-7.7
	permethrin	8.4-8.7	7.7-7.9
Run 2	control	8.4-8.5	7.6-8.0
	carbaryl	8.3-8.5	7.6-7.8
	copper	8.3-8.4	7.6-8.0
	4-nonylphenol	8.4-8.5	7.5-7.7
	pentachlorophenol	8.3-8.4	7.5-7.6
	permethrin	8.2-8.5	7.4-7.7
Run 3	control	8.4-8.7	7.9-8.1
	carbaryl	7.4-8.5	7.9-8.1
	copper	8.1-8.4	
	4-nonylphenol	8.2-8.7	7.9-7.9
	pentachlorophenol	8.4-8.7	7.8-7.9
	permethrin	8.4-8.6	7.8-8.1
Run 4	control	8.4-8.7	7.9-8.1
	carbaryl	8.2-8.4	7.7-7.8
	copper	8.3-8.4	
	4-nonylphenol	8.4-8.5	7.7-7.9
	pentachlorophenol	7.8-8.5	7.8-7.9
	permethrin	6.5-8.5	7.8-7.9
Run 5	control	7.7	7.9-8.0
	carbaryl	7.9-8.0	7.8-8.0
	copper	7.9-8.0	7.9
	4-nonylphenol	7.7-8.0	7.5-7.9
	pentachlorophenol	7.9	7.6-8.0
	permethrin	7.8-8.0	7.8-8.1

Appendix 3. continued

Species	Chemical	0 h	96 h
Run 6	control	8.3-8.4	7.6-7.9
	carbaryl	8.2-8.4	7.5-7.9
	copper	8.1-8.4	7.5-7.9
	4-nonylphenol	7.8-8.4	6.5-7.9
	pentachlorophenol	8.1-8.3	6.8-8.2
	permethrin	8.0-8.4	7.1-7.9
Apache trout			
Run 1	control	8.6-8.7	7.7-7.9
	carbaryl	8.4-8.6	7.8-8.0
	copper	8.3-8.7	7.9-8.2
	4-nonylphenol	8.4-8.6	7.8-7.9
	pentachlorophenol	8.5-8.6	7.6-7.7
	permethrin	8.5-8.6	7.8-7.8
Run 2	control	8.2-8.4	7.7-7.8
	carbaryl	8.2-8.3	7.7-8.0
	copper	8.2-8.3	7.7-7.9
	4-nonylphenol	7.3-8.3	7.3-7.7
	pentachlorophenol	8.1-8.3	7.6
	permethrin	8.2	7.7
greenback cutthroat trout			
Run 1	control	8.5-8.6	7.8-7.9
	carbaryl	8.5-8.6	7.6-8.0
	copper	8.5-8.7	7.6-7.9
	4-nonylphenol	8.5-8.7	7.5-7.6
	pentachlorophenol	8.4-8.5	7.6-8.1
	permethrin	8.4-8.7	7.6-8.1

Appendix 3. continued

Species	Chemical	0 h	96 h
Lahontan cutthroat trout			
Run 1	control	8.4-8.5	7.6-8.0
	carbaryl	8.3-8.4	7.9-8.2
	copper	8.3-8.4	7.8-8.0
	4-nonylphenol	8.4	7.9
	pentachlorophenol	8.4	7.7-7.9
	permethrin	8.4	7.9-8.0
Run 2	control	8.2-8.4	7.7-7.8
	carbaryl	8.0-8.4	7.5-7.9
	copper	8.2-8.3	7.7-7.8
	4-nonylphenol	8.2-8.3	7.6-7.7
	pentachlorophenol	8.2-8.3	7.6-7.9
	permethrin	8.1-8.3	7.6-7.9
fathead minnow			
Run 1	control	8.4-8.5	7.9-8.2
	carbaryl	8.4-8.5	8.0-8.1
	copper	8.2-8.5	8.0-8.3
	4-nonylphenol	8.3-8.4	7.7-8.1
	pentachlorophenol	8.4-8.5	7.8-7.9
	permethrin	8.4-8.5	7.7-8.2
Run 2	control	8.4-8.5	7.9-8.2
	carbaryl	8.4-8.5	7.9-8.1
	copper	7.8-8.4	8.0-8.2
	4-nonylphenol	8.4-8.5	7.8-8.1
	pentachlorophenol	8.3-8.5	7.8-8.0
	permethrin	7.9-8.5	7.8-8.1

Appendix 3. continued

Species	Chemical	0 h	96 h
Run 3	control	8.5-8.6	7.8-8.0
	carbaryl	8.2-8.6	7.5-8.0
	copper	8.1-8.4	7.9
	4-nonylphenol	8.3-8.6	7.6-7.8
	pentachlorophenol	8.0-8.6	7.6-7.9
	permethrin	7.5-8.5	7.7-7.8
Run 4	control	8.3-8.4	7.7-8.0
	carbaryl	8.1-8.4	7.6-7.9
	copper	8.0-8.3	7.6-8.2
	4-nonylphenol	8.1-8.3	7.5-7.9
	pentachlorophenol	7.9-8.3	7.4-7.6
	permethrin	7.9-8.3	7.4-7.9
Run 5	control	9.5-9.8	7.2-7.4
	carbaryl	9.2-9.5	7.4-7.5
	copper	8.8-9.5	7.5-7.7
	4-nonylphenol	8.2-9.2	7.5-7.6
	pentachlorophenol	8.8-9.3	7.3-7.6
	permethrin	8.4-9.7	7.3-7.6
Run 6	control	7.1	6.7-7.9
	carbaryl	6.9-7.2	7.7-7.8
	copper	6.7-7.1	7.8-7.9
	4-nonylphenol	5.5-6.9	7.8-8.2
	pentachlorophenol	6.6-6.9	7.8-7.9
	permethrin	6.5-6.9	7.7-8.0
bonytail chub			
Run 1	control	8.5-8.6	7.8-8.0
	carbaryl	7.9-8.5	7.7-8.0
	copper	8.0-8.5	7.6-7.8
	4-nonylphenol	8.0-8.6	7.7-7.9
	pentachlorophenol	8.3-8.6	7.5-7.9
	permethrin	8.3-8.6	7.6-7.9

Appendix 3. continued

Species	Chemical	0 h	96 h
Run 2	control	9.5-9.8	7.2-7.4
	carbaryl	8.4-9.5	7.5-7.6
	copper	8.9-9.4	7.5
	4-nonylphenol	8.3-9.5	7.4-7.6
	pentachlorophenol	9.3-9.5	7.4-7.5
	permethrin	8.7-9.3	7.4-7.6
Colorado squawfish			
Run 1	control	8.4-8.5	7.9-8.2
	carbaryl	8.2-8.5	8.1-8.3
	copper	8.3-8.4	8.0-8.6
	4-nonylphenol	8.4-8.5	8.1-8.2
	pentachlorophenol	8.4-8.5	8.0-8.1
	permethrin	8.4-8.5	8.0-8.3
Run 2	control	7.1	6.7-7.9
	carbaryl	6.2-7.2	7.7-8.1
	copper	6.7-7.1	7.8
	4-nonylphenol	6.4-6.9	7.8-8.0
	pentachlorophenol	6.9-7.1	7.7-7.8
	permethrin	6.7-7.1	7.9-8.0
Razorback sucker			
Run 1	control	8.4-8.5	7.9-8.2
	carbaryl	8.3-8.5	8.0-8.1
	copper	8.3-8.4	7.9-8.1
	4-nonylphenol	8.3-8.5	7.8-8.1
	pentachlorophenol	8.3-8.6	7.9-8.1
	permethrin	8.3-8.5	7.6-8.1

Appendix 3. continued

Species	Chemical	0 h	96 h
Run 2	control	8.3-8.4	7.7-8.0
	carbaryl	8.0-8.3	7.7-8.0
	copper	7.9-8.2	7.9-8.0
	4-nonylphenol	8.1-8.3	7.9-8.0
	pentachlorophenol	8.2-8.5	7.6-8.0
	permethrin	8.2-8.3	7.6-8.2

APPENDIX 4. DISSOLVED OXYGEN CONCENTRATIONS

Appendix 4. Exposure replicates (rep) and concentrations (conc) for which dissolved oxygen was below 40% saturation at 48 h of exposure or below 60% saturation at 96 h of exposure. Dissolved oxygen concentration limits for the coldwater (12°C) studies are: 48 h - 6.4 mg/L; 96 h - 4.3 mg/L. Dissolved oxygen concentration limits for the warmwater (22°C) studies are: 48 h - 5.2 mg/L; 96 h - 3.5 mg/L.

Species	Chemical	rep	conc	48h	96h
rainbow trout					
Run 1	carbaryl	1	low	4.2	
		2	low	3.8	
	pentachlorophenol	2	low	4.2	
		3	low	4.0	
Run 2	control	1	water	5.9	3.1
		2	water	4.9	
		3	water	4.5	2.6
		1	acetone	6.1	
	carbaryl	1	low	3.1	3.2
			medium	3.8	3.5
		2	low	3.2	
			medium	4.9	
		3	low	3.6	3.4
			medium	4.8	
	copper	1	low	4.8	
			medium	5.0	
		2	low	4.4	
			medium	4.8	
	4-nonylphenol	1	low	4.5	
			medium	5.0	
		2	low	6.1	4.1
			medium	5.0	2.1
		3	low	5.5	
			medium	4.1	2.8

Appendix 4. continued

Species	Chemical	rep	conc	48h	96h
Run 2 cont.					
	pentachlorophenol	1	low	4.1	2.8
			medium	4.6	3.4
		2	low	4.7	3.2
			medium	3.9	3.3
		3	low	6.0	
			medium	4.5	3.3
	permethrin	1	low	6.3	
			medium	5.5	3.3
		2	low	5.2	
		3	medium		3.1
Run 4	control	1	water	6.2	
		2	water	5.4	
		3	acetone	5.7	
	carbaryl	1	low	3.7	3.5
			medium	5.1	
		2	low	3.3	3.0
			medium	5.2	
		3	low	3.5	2.6
			medium	4.7	
	4-nonylphenol	1	low	4.7	
			medium	4.8	
		2	low	4.9	
			medium	4.2	
		3	low	5.4	
			medium	4.7	
	pentachlorophenol	1	low	5.4	
			medium	4.6	
		2	low	5.7	
			medium	4.3	
		3	low	5.5	
			medium	3.9	
		3	low		3.2

Appendix 4. continued

Species	Chemical	rep	conc	48h	96h
Run 4 cont.					
	permethrin	1	low	5.4	
			medium	5.6	
		2	low	5.5	
		3	low	5.4	
Run 5	carbaryl	1	medium		3.2
		2	medium		3.4
		3	medium		3.9
Apache trout					
Run 1	pentachlorophenol	1	low		3.4
		2	low		3.6
Run 2	pentachlorophenol	1	medium		4.2
greenback cutthroat trout					
Run 1	carbaryl	2	low	6.0	
			medium	5.4	
		3	low	6.0	
			medium	5.2	
	copper	3	medium	6.2	
	4-nonylphenol	1	low	5.4	
		2	low	6.2	
		3	low	6.2	
	pentachlorophenol	1	low	5.6	
			med	6.2	
			high	4.8	
		2	high	5.9	
		3	high	6.1	3
			medium	2.9	2.4

Appendix 4. continued

Species	Chemical	rep	conc	48h	96h
Run 1 cont.					
	permethrin	3	low	6.1	
			medium	6.3	
Lahontan cutthroat trout					
Run 1	pentachlorophenol	1	medium	5.0	3.4
Run 2	pentachlorophenol	1	medium	3.4	
		2	medium	3.7	
		3	medium	4.0	
fathead minnow					
Run 1	4-nonylphenol	3	medium		2.0
Run 2	control		acetone	4.8	
	4-nonylphenol	1	medium	3.7	2.8
		2	low	5.1	
			medium	3.1	2.4
		3	medium	2.9	2.4
	pentachlorophenol	1	low	3.8	3.2
			medium	4.6	
		2	low	4.4	3.1
		3	low	3.9	3.0
			medium	4.9	
	permethrin	1	high	4.7	2.3
		2	high	5.2	
		3	high		2.8
Run 3	4-nonylphenol	2	medium		2.7
		3	medium	4.0	1.5
	pentachlorophenol	2	medium		2.7

Appendix 4. continued

Species	Chemical	rep	conc	48h	96h
Run 3 cont.					
	permethrin	1	medium		1.7
		2	medium		1.2
Run 4	carbaryl	1	low	5.1	
		3	low	4.9	
	copper	3	low	4.7	
	4-nonylphenol	1	medium	3.4	2.5
		3	medium	4.0	3.2
	pentachlorophenol	1	low	4.9	
			medium	4.8	
		2	low	5.2	
			medium	4.5	3.0
		3	low	4.6	
Run 5	carbaryl	1	medium		2.8
	pentachlorophenol	1	medium	4.1	3.2
		2	low	4.4	3.4
		3	low		3.0
			medium	4.2	2.0
	permethrin	2	medium		3.5
Run 6	control	1	water	5.2	
		2	water	5.0	
	4-nonylphenol	3	medium	4.6	3.5
	pentachlorophenol	1	low	2.7	2.9
		2	low	4.0	3.3
		3	low	3.7	2.8
	permethrin	2	high	4.6	2.8
		3	high	4.9	

Appendix 4. continued

Species	Chemical	rep	conc	48	96
bonytail chub					
Run 2	4-nonylphenol	1	medium	4.9	
		2	medium	4.1	3.0
	pentachlorophenol	2	low	5.1	4.2
			medium	4.9	
	permethrin	2	medium	4.7	
Colorado squawfish					
Run 2	pentachlorophenol	1	low	5.0	
		3	low	5.0	
razorback sucker					
Run 1	permethrin	1	medium	5.0	1.6
			high		1.8
Run 2	pentachlorophenol	2	medium	5.2	
		3	medium	4.7	

APPENDIX 5. ACUTE TOXICITY DATA

Appendix 5. Calculated LC50, 95% confidence interval and slope for each chemical, species, Run and time period. LC50's and associated parameters were calculated using probit analysis unless otherwise noted.

CARBARYL (mg/L)

SPECIES AVG. WEIGHT (grams)	HOURS								
	3	6	9	12	18	24	48	72	96
RAINBOW TROUT 1 (0.67 ± 0.35)	10.53 (8.8-15.7) 4.63	9.07 (7.4-12.6) 3.56	8.0 (6.8-10.1) 4.04	7.0 (6.1-8.3) 4.81	5.24 (4.6-6.0) 5.61	4.51 (3.2-6.6) 4.75	2.89 (2.5-3.3) 5.15	2.20 (1.9-2.5) 5.31	2.00 (1.8-2.3) 5.88
RAINBOW TROUT 2 (1.25 ± 0.57)	>10	>10	10.0 (8.3-14.2) 4.37	7.5 (6.5-8.7) 5.81	5.1 (4.5-5.9) 5.97	3.7 (3.2-4.4) 3.78	1.98 (1.7-2.3) 3.64	1.24 (1.0-1.5) 3.65	1.24 (1.0-1.5) 3.65
RAINBOW TROUT 3 (0.27 ± 0.07)	8.2 [c] (7.2-9.2)	7.11 [c] (6.2-8.1)	6.67 (5.5-8.7) 2.98	5.5 (4.7-6.7) 3.49	4.62 (4.0-5.4) 3.94	3.65 (3.2-4.2) 4.65	2.60 (2.3-3.0) 5.44	2.31 (2.1-2.6) 6.47	2.26 (2.0-2.5) 6.87
RAINBOW TROUT 4 (1.09 ± 0.38)	>10	>10	8.27 (7.1-10.4) 4.35	7.22 (6.3-8.6) 4.88	4.39 (3.8-5.1) 4.62	3.43 (3.0-3.9) 4.79	1.27 (1.1-1.4) 5.98	1.22 (1.1-1.4) 5.34	1.22 (1.1-1.4) 5.43
RAINBOW TROUT 5 (0.48 ± 0.08)	>6.0	>6.0	>6.0	>6.0	>6.0	4.75 [c] (4.2-5.4)	3.30 (2.9-3.8) 5.19	2.7 (2.4-3.0) 6.79	2.07 (1.9-2.3) 7.58
RAINBOW TROUT 6 (0.50 ± 0.21)	>6.0	>6.0	>6.0	>6.0	>6.0	4.41 (4.0-4.9) 11.10	3.5 (3.2-3.8) 10.04	3.14 (2.8-3.5) 8.70	3.11 [a] (2.6-3.6)

[a]--LC50 and 95% CI calculated using moving average
[b]--LC50 calculated using nonlinear interpolation; CI calculated using binomial procedure
[c]--LC50 and 95% CI calculated using Spearman-Karber

[a] -- LC50 and 95% CI calculated using moving average
[b] -- LC50 calculated using nonlinear interpolation; CI calculated using binomial test (Stephan)
[c] -- LC50 calculated using Spearman-Karber

Appendix 5. (cont.)

CARBARYL (mg/L)

SPECIES AVG. WEIGHT (grams)	HOURS								
	3	6	9	12	18	24	48	72	96
APACHE TROUT 1 (0.85 ± 0.49)	5.59 (4.9-6.4) 5.56	4.96 (4.3-5.8) 4.15	4.64 (4.0-5.4) 4.20	4.16 (3.6-4.8) 4.11	3.55 (3.1-4.1) 4.65	3.12 (2.7-3.6) 4.46	2.02 (1.7-2.3) 4.20	1.74 (1.5-2.0) 4.88	1.65 (1.5-1.9) 5.73
APACHE TROUT 2 (0.38 ± 0.18)	5.60 (4.2-10.0) 2.83	3.75 (2.9-5.7) 2.42	2.99 (2.4-3.9) 3.02	2.60 (2.1-3.3) 3.22	2.12 (1.8-2.5) 4.99	2.00 (1.7-2.4) 4.71	1.66 (0.8-4.4) 4.77	1.45 (0.6-4.2) 5.20	1.43 [b] (1.3-2.2)
GREENBACK CUTTHROAT TROUT (0.31 ± 0.17)	>10.0	10.00 (7.5-17.7) 2.25	9.89 (6.8-20.2) 1.66	8.50 (6.1-14.9) 1.80	5.08 (4.1-6.6) 2.39	3.59 (3.0-4.3) 3.05	2.30 (2.0-2.6) 4.61	2.19 (1.9-2.5) 4.47	1.55 (1.4-1.8) 5.67
LAHONTAN CUTTHROAT TROUT 1 (0.34 ± 0.08)	5.20 (3.0-11.5) 4.01	4.91 (4.2-5.8) 3.93	4.59 (3.9-5.4) 3.73	4.48 (2.5-9.2) 3.94	4.26 (3.7-5.0) 4.08	3.56 (3.1-4.1) 4.93	2.53 (2.3-2.8) 7.08	2.19 (1.96-2.4) 8.32	2.00 (1.8-2.2) 8.73
LAHONTAN CUTTHROAT TROUT 2 (0.57 ± 0.23)	4.60 (4.0-5.5) 5.11	4.6 (4.0-5.5) 5.11	4.35 (3.8-5.1) 5.26	4.28 (3.7-5.0) 5.21	4.0 (3.5-4.6) 6.13	3.62 (3.3-4.0) 8.48	3.06 (2.8-3.4) 10.56	2.6 [b] (2.2-3.6)	2.53 [b] (2.2-3.6)

Appendix 5. (cont.)

CARBARYL (mg/L)

SPECIES AVG. WEIGHT (grams)	HOURS								
	3	6	9	12	18	24	48	72	96
FATHEAD MINNOW 1 (0.32 ± 0.16)	>10	>10	>10	>10	>10	9.57 [b] (6.0-10.0)	7.02 [b] (6.0-10.0)	4.85 (4.4-5.4) 9.74	4.38 (3.95-4.86) 8.69
FATHEAD MINNOW 2 (0.56 ± 0.19)	>10	>10	>10	>10	>10	>10	7.90 (7.1-8.8) 8.36	7.1 (6.5-7.8) 10.20	5.86 (5.3-6.5) 9.69
FATHEAD MINNOW 3 (0.45 ± 0.35)	>16.7	15.90 (14.1-18.8) 7.59	12.0 (10.7-13.2) 8.91	12.0 (10.7-13.2) 8.91	12.0 (11.4-13.6) 14.0	10.0 [b] (6.0-16.7)	8.66 [b] (6.0-10.0)	7.55 [b] (6.0-10.0)	7.43 [b] (6.0-10.0)
FATHEAD MINNOW 4 (0.40 ± 0.21)	>10	>10	>10	>10	>10	>10	6.88 [c] (6.1-7.7)	4.69 (4.1-5.4) 4.53	3.94 (3.4-4.5) 4.69
FATHEAD MINNOW 5 (0.34 ± 0.24)	>10	>10	>10	>10	>10	>10	7.22 [b] (6.0-10.0)	5.86 (5.3-6.5) 9.69	5.24 (4.7-5.8) 9.52
FATHEAD MINNOW 6 (0.39 ± 0.14)	>10	>10	>10	>10	>10	>10	>10	7.36 (6.5-8.5) 6.01	5.72 (5.1-6.5) 6.45

Appendix 5. (cont.)

CARBARYL (mg/L)

SPECIES AVG. WEIGHT (grams)	HOURS								
	3	6	9	12	18	24	48	72	96
BONYTAIL CHUB 1 (0.29 ± 0.08)	>16.7	0.90 [b] (10-16.7)	8.45 (7.5-9.6) 5.98	7.56 [a] (6.8-8.4)	5.44 [b] (3.6-6)	5.27 [b] (3.6-6)	5.21 [a] (4.6-5.9)	4.33 [b] (3.6-6)	3.58 (3.3-4.0) 9.86
BONYTAIL CHUB 2 (0.52 ± 0.09)	>10	>10	8.42 [b] (6.0-10.0)	8.31 [b] (6.0-10.0)	8.10 (7.4-8.8) 14.04	7.12 [b] (6.0-10.0)	5.53 [b] (3.6-6)	4.22 [a] (3.7-4.9)	3.40 [c] (3.0-3.7)
COLORADO SQUAWFISH 1 (0.32 ± 0.05)	>10	>10	>10	>10	7.22 [b] (6.0-10.0)	6.31 [c] (5.7-7.0)	4.46 [b] (3.6-6.0)	2.91 (2.6-3.2) 9.36	2.32 (2.1-2.6) 8.72
COLORADO SQUAWFISH 2 (0.34 ± 0.05)	>10	>10	>10	>10	>10	>10	7.36 [c] (6.9-7.8)	5.42 [c] (4.9-6.0)	4.06 [c] (3.7-4.4)
RAZORBACK SUCKER 1 (0.31 ± 0.04)	>10	>10	>10	8.88 [c] (8.1-9.7)	7.19 [b] (6.0-10.0)	5.83 (5.2-6.5) 7.49	4.87 (4.4-5.3) 11.17	4.46 [b] (3.6-6)	4.42 [b] (3.6-6.0)
RAZORBACK SUCKER 2 (0.32 ± 0.07)	>10	>10	>10	>10	9.13 (8.3-10.2) 10.07	7.64 (6.7-8.9) 6.03	5.06 (4.6-5.6) 8.99	4.81 (4.3-5.3) 9.09	4.29 (4.0-4.8) 12.65

Appendix 4. (cont.)

COPPER (mg/L)

SPECIES AVG. WEIGHT (grams)	HOURS								
	3	6	9	12	18	24	48	72	96
RAINBOW TROUT 1 (0.67 ± 0.35)	>1.0	>1.0	>1.0	1.03 (0.8-1.6) 3.33	0.33 (0.29-0.39) 3.97	0.19 (0.17-0.21) 5.79	0.13 (0.12-0.15) 6.96	0.11 (0.10-0.13) 5.62	0.11 (0.09-0.12) 6.02
RAINBOW TROUT 2 (1.25 ± 0.57)	>0.6	>0.6	>0.6	>0.6	0.35 (0.31-0.39) 5.78	0.23 (0.20-0.27) 4.65	0.17 (0.14-0.19) 3.69	0.13 (0.001-2.5) 2.41	0.10 (0.00003-0.32) 2.26
RAINBOW TROUT 3 (0.27 ± 0.07)	>0.91	>0.91	0.33 (0.17-0.86) 2.19	0.15 (0.12-0.18) 2.69	0.44 (0.01-0.07) 2.83	<0.07	<0.07	<0.07	<0.07
RAINBOW TROUT 4 (1.09 ± 0.38)	>0.91	0.91 (0.73-1.35) 3.55	0.49 [b] (0.33-0.55)	0.42 (0.36-0.49) 3.77	0.14 (0.12-0.17) 3.44	0.07 (0.04-0.09) 2.57	<0.07	<0.07	<0.07
RAINBOW TROUT 5 (0.48 ± 0.08)	>0.36	>0.36	>0.36	>0.36	0.09 (0.08-0.10) 4.68	0.07 (0.06-0.07) 6.11	0.06 (0.05-0.06) 9.65	0.05 (0.05-0.06) 9.92	0.05 [b] (0.05-0.09)
RAINBOW TROUT 6 (0.50 ± 0.21)	>0.36	>0.36	>0.36	>0.36	0.10 (0.09-0.12) 5.14	0.07 (0.06-0.08) 7.80	0.06 (0.06-0.07) 9.71	0.06 [b] (0.05-0.06) 9.28	0.06 [b] (0.05-0.09)

Appendix 4. (cont.)

COPPER (mg/L)

SPECIES AVG. WEIGHT (grams)	HOURS								
	3	6	9	12	18	24	48	72	96
APACHE TROUT 1 (0.85 ± 0.49)	>1.0	0.76 (0.06-1.07) 2.64	0.34 (0.29-0.40) 3.43	0.21 (0.18-0.24) 3.41	0.12 (0.01-0.13) 4.94	0.10 (0.08-0.11) 4.67	<0.08	<0.08	<0.08
APACHE TROUT 2 (0.38 ± 0.18)	>0.36	>0.36	0.26 (0.21-0.39) 2.91	0.16 (0.14-0.20) 4.10	0.11 (0.09-0.12) 4.80	0.08 (0.07-0.10) 5.95	0.07 (0.06-0.08) 6.08	0.07 (0.06-0.08) 6.08	0.07 (0.06-0.08) 6.08
GREENBACK CUTTHROAT TROUT (0.31 ± 0.17)	>0.03	>0.03	>0.03	>0.03	>0.03	>0.03	>0.03	>0.03	>0.03
LAHONTAN CUTTHROAT TROUT 1 (0.34 ± 0.08)	>0.06	>0.06	>0.06	0.55 (0.47-0.68) 5.51	0.23 (0.13-0.44) 3.62	0.14 (0.12-0.16) 4.22	0.10 (0.06-0.16) 7.10	0.08 [b] (0.08-0.13)	0.08 (0.07-0.09) 10.42
LAHONTAN CUTTHROAT TROUT 2 (0.57 ± 0.23)	>0.36	>0.36	>0.36	0.28 (0.25-0.32) 7.04	0.15 (0.13-0.16) 8.01	0.09 (0.08-0.10) 11.82	0.06 [b] (0.05-0.08)	0.06 [b] (0.05-0.08)	0.06 [b] (0.05-0.08)

Appendix 4. (cont.)

COPPER (mg/L)

SPECIES AVG. WEIGHT (grams)	HOURS								
	3	6	9	12	18	24	48	72	96
FATHEAD MINNOW 1 (0.32 ± 0.16)	>1.0	>1.0	>1.0	0.91 (0.8-1.1) 7.31	0.63 (0.6-0.7) 6.36	0.58 (0.5-0.7) 6.83	0.52 (0.5-0.6) 6.42	0.35 (0.2-0.6) 2.94	0.29 (0.1-0.6) 2.73
FATHEAD MINNOW 2 (0.56 ± 0.19)	>7.7	4.43 (3.9-5.0) 5.99	3.22 (2.9-3.6) 6.57	2.75 (2.5-3.1) 7.44	2.10 (1.1-4.9) 4.0	1.70 (1.4-2.0) 3.43	1.16 (0.9-1.4) 2.68	0.76 (0.4-1.0) 1.87	0.63 (0.3-0.9) 1.77
FATHEAD MINNOW 3 (0.45 ± 0.35)	>4.6	3.58 (3.2-4.0) 8.86	1.83 (1.6-2.1) 3.68	1.28 (1.1-1.5) 4.24	0.73 (0.6-0.8) 4.78	0.53 [a] (0.5-0.6)	0.50 [a] (0.4-0.6)	0.44 (0.4-0.5) 4.55	0.40 (0.3-0.5) 3.95
FATHEAD MINNOW 4 (0.40 ± 0.21)	>1.0	>1.0	>1.0	>1.0	>1.0	>1.0	0.58 [b] (0.4-0.6)	0.56 (0.3-3.7) 2.79	0.46 (0.4-0.6) 2.97
FATHEAD MINNOW 5 (0.34 ± 0.24)	>1.0	>1.0	>1.0	>1.0	>1.0	>1.0	1.0 (0.8-1.7) 2.68	0.91 (0.7-1.5) 2.31	0.81 (0.6-1.3) 2.08
FATHEAD MINNOW 6 (0.39 ± 0.14)	>1.0	>1.0	>1.0	0.9 (0.8-1.2) 4.0	0.68 [c] (0.6-0.8)	0.55 [c] (0.5-0.6)	0.48 (0.4-0.6) 3.1	0.39 (0.3-0.5) 3.0	0.39 (0.3-0.5) 3.0

Appendix 4. (cont.)

COPPER (mg/L)

SPECIES AVG. WEIGHT (grams)	HOURS								
	3	6	9	12	18	24	48	72	96
BONYTAIL CHUB 1 (0.29 ± 0.08)	1.33 (1.16-1.61) 5.03	0.41 (0.37-0.45) 8.32	0.29 (0.27-0.32) 10.59	0.25 (0.22-0.27) 9.49	0.23 (0.20-0.25) 7.57	0.22 (0.19-0.24) 8.31	0.20 (0.18-0.22) 7.64	0.20 (0.18-0.22) 7.64	0.20 (0.18-0.22) 7.64
BONYTAIL CHUB 2 (0.52 ± 0.09)	>1.0	>1.0	0.57 (0.48-0.68) 3.84	0.35 [b] (0.22-0.36)	0.28 (0.25-0.31) 8.81	0.27 (0.24-0.29) 10.05	0.25 [b] (0.22-0.36)	0.25 [b] (0.22-0.36)	0.25 [b] (0.22-0.36)
COLORADO SQUAWFISH 1 (0.32 ± 0.05)	>1.0	>1.0	>1.0	>1.0	0.69 (0.57-0.91) 2.91	0.46 (0.39-0.55) 3.53	0.40 (0.35-0.46) 4.40	0.39 (0.33-0.45) 4.25	0.38 (0.33-0.44) 4.24
COLORADO SQUAWFISH 2 (0.34 ± 0.05)	>1.0	>1.0	>1.0	>1.0	>1.0	0.89 (0.79-1.04) 6.9	0.57 [c] (0.51-0.64)	0.50 (0.23-4.7) 2.7	0.48 (0.22-4.2) 2.6
RAZORBACK SUCKER 1 (0.31 ± 0.04)	>1.0	>1.0	>1.0	>1.0	0.55 (0.47-0.64) 4.37	0.39 (0.34-0.43) 7.06	0.25 (0.22-0.29) 5.88	0.24 (0.21-0.27) 6.25	0.22 (0.19-0.25) 5.52
RAZORBACK SUCKER 2 (0.32 ± 0.07)	>1.0	>1.0	>1.0	>1.0	>1.0	>1.0	0.52 (0.46-0.60) 5.45	0.38 (0.33-0.43) 6.23	0.34 [a] (0.29-0.40)

Appendix 4. (cont.)

4-NONYLPHENOL (mg/L)

SPECIES AVG. WEIGHT (grams)	HOURS								
	3	6	9	12	18	24	48	72	96
RAINBOW TROUT 1 (0.67 ± 0.35)	0.73 (0.67-0.80) 13.28	0.56 (0.51-0.62) 10.09	0.43 (0.39-0.47) 12.65	0.40 [b] (0.22-0.6)	0.33 [b] (0.22-0.36)	0.31 (0.29- 0.34) 12.04	0.22 (0.20-0.24) 9.76	0.19 (0.17-0.21) 9.10	0.19 (0.17-0.21) 9.34
RAINBOW TROUT 2 (1.25 ± 0.57)	>0.6 [b]	0.54 [b] (0.36-0.6)	0.45 [b] (0.36-0.6)	0.44 [b] (0.36-0.6)	0.37 (0.34-0.41) 9.79	0.35 (0.32-0.39) 8.56	0.27 (0.24-0.30) 11.32	0.26 [b] (0.22-0.36)	0.26 [b] (0.22-0.36)
RAINBOW TROUT 3 (0.27 ± 0.07)	>0.36 [b]	0.39 [b] (0.22-0.6)	0.28 (0.26-0.31) 17.08	0.27 [b] (0.22-0.36)	0.27 [b] (0.22-0.36)	0.27 [b] (0.22-0.36)	0.17 [b] (0.13-0.22)	0.14 [b] (0.08-0.22)	0.14 [b] (0.08-0.22)
RAINBOW TROUT 4 (1.09 ± 0.38)	>0.6 [b]	0.59 [b] (0.36-0.6)	0.49 [b] (0.36-0.6)	0.39 [b] (0.22-0.6)	0.34 (0.31-0.37) 10.38	0.32 (0.29-0.35) 9.82	0.27 [b] (0.22-0.36)	0.27 [b] (0.22-0.36)	0.27 [b] (0.22-0.36)
RAINBOW TROUT 5 (0.48 ± 0.08)	>0.6	0.45 [b] (0.36-0.6)	0.37 [b] (0.36-0.6)	0.31 [b] (0.22-0.6)	0.29 (0.26-0.31) 15.60	0.29 (0.26-0.31) 15.60	0.18 [b] (0.13-0.22)	0.16 [b] (0.13-0.22)	0.16 [b] (0.13-0.22)
RAINBOW TROUT 6 (0.50 ± 0.21)	>0.6	0.46 [b] (0.36-0.6)	0.41 [b] (0.36-0.6)	0.32 [b] (0.22-0.36)	0.30 [b] (0.22-0.36)	0.29 [b] (0.22-0.36)	0.22 [b] (0.13-0.36)	0.19 [b] (0.13-0.22)	0.18 [b] (0.13-0.22)

Appendix 4. (cont.)

4-NONYLPHENOL (mg/L)

SPECIES AVG. WEIGHT (grams)	HOURS								
	3	6	9	12	18	24	48	72	96
APACHE TROUT 1 (0.85 ± 0.49)	0.67 [b] (0.6-1)	0.44 (0.40-0.48) 13.28	0.40 [b] (0.36-0.6)	0.34 [b] (0.22-0.36)	0.26 (0.24-0.29) 13.77	0.25 (0.22-0.27) 11.18	0.21 (0.19-0.23) 9.76	0.19 [a] (0.17-0.21)	0.18 [a] (0.16-0.02)
APACHE TROUT 2 (0.38 ± 0.18)	>0.6	0.41 [c] (0.38-0.45)	0.32 [c] (0.29-0.35)	0.27 [c] (0.25-0.30)	0.23 [c] (0.21-0.25)	0.22 [a] (0.19-0.25)	0.19 [a] (0.17-0.21)	0.16 [a] (0.14-0.18)	0.16 [b] (0.13-0.22)
GREENBACK CUTTHROAT TROUT (0.31 ± 0.17)	0.55 [b] (0.36-0.6)	0.47 (0.42-0.51) 16.54	0.40 [b] (0.22-0.6)	0.38 [b] (0.22-0.6)	0.31 (0.28-0.34 10.16	0.30 (0.27-0.33) 10.07	0.19 (0.17-0.21) 11.33	0.17 (0.15-0.18) 12.19	0.15 (0.14-0.17) 11.78
LAHONTAN CUTTHROAT TROUT 1 (0.34 ± 0.08)	0.43 [b] (0.36-0.6)	0.30 [b] (0.22-0.36)	0.28 [b] (0.22-0.36)	0.27 [b] (0.22-0.36)	0.24 [b] (0.13-0.36)	0.23 [b] (0.13-0.36)	0.18 (0.16-0.19) 11.47	0.16 [b] (0.13-0.22)	0.14 [b] (0.08-0.22)
LAHONTAN CUTTHROAT TROUT 2 (0.57 ± 0.23)	0.56 (0.49-0.68) 8.60	0.41 [b] (0.36-0.6)	0.31 [b] (0.22-0.36)	0.30 [b] (0.22-0.36)	0.27 [b] (0.22-0.36)	0.27 [b] (0.22-0.36)	0.26 [b] (0.22-0.36)	0.23 [b] (0.22-0.36)	0.22 [b] (0.13-0.36)

Appendix 4. (cont.)

4-NONYLPHENOL (mg/L)

SPECIES AVG. WEIGHT (grams)	HOURS								
	3	6	9	12	18	24	48	72	96
FATHEAD MINNOW 1 (0.32 ± 0.16)	0.72 (0.66-0.79) 12.64	0.42 [b] (0.36-0.6)	0.34 (0.30-0.37) 8.92	0.30 (0.27-0.34) 7.64	0.27 (0.24-0.30) 7.72	0.25 (0.22-0.27) 8.44	0.24 (0.22-0.27) 8.09	0.23 (0.21-0.25) 9.96	0.21 [b] (0.13-0.22)
FATHEAD MINNOW 2 (0.56 ± 0.19)	0.75 [b] (0.6-1)	0.48 (0.44-0.53) 11.87	0.43 [b] (0.36-0.6)	0.42 [b] (0.36-0.6)	0.42 [b] (0.36-0.6)	0.41 [b] (0.36-0.6)	0.40 [b] (0.22-0.6)	0.36 [b] (0.22-0.6)	0.36 [b] (0.22-0.6)
FATHEAD MINNOW 3 (0.45 ± 0.35)	0.74 [b] (0.6-1)	0.55 [b] (0.36-0.6)	0.48 [b] (0.36-0.6)	0.45 [b] (0.36-0.6)	0.40 [b] (0.36-0.6)	0.38 [b] (0.22-0.6)	0.34 [b] (0.22-0.36)	0.31 [b] (0.22-0.36)	0.31 [b] (0.22-0.36)
FATHEAD MINNOW 4 (0.40 ± 0.21)	0.82 [b] (0.6-1)	0.61 [b] (0.6-1)	0.44 [b] (0.36-0.6)	0.42 [b] (0.36-0.6)	0.39 [b] (0.36-0.6)	0.38 [b] (0.36-0.6)	0.36 [b] (0.22-0.6)	0.35 [b] (0.22-0.36)	0.33 [b] (0.22-0.36)
FATHEAD MINNOW 5 (0.34 ± 0.24)	0.71 (0.65-078) 10.20	0.40 [b] (0.36-0.6)	0.31 (0.29-0.34) 12.04	0.28 (0.26-0.31) 14.04	0.25 [b] (0.22-0.36)	0.21 [b] (0.13-0.22)	0.17 [b] (0.13-0.22)	0.17 [b] (0.13-0.22)	0.17 [b] (0.13-0.22)
FATHEAD MINNOW 6 (0.39 ± 0.14)	0.87 [b] (0.6-1)	0.58 (0.52-0.64) 9.8	0.48 (0.44-0.53) 11.8	0.45 (0.42-0.50) 15.0	0.43 [b] (0.36-0.6)	0.39 [b] (0.36-0.6)	0.31 [b] (0.22-0.36)	0.30 [b] (0.22-0.36)	0.29 [b] (0.22-0.36)

Appendix 4. (cont.)

4-NONYLPHENOL (mg/L)

SPECIES AVG. WEIGHT (grams)	HOURS								
	3	6	9	12	18	24	48	72	96
BONYTAIL CHUB 1 (0.29 ± 0.08)	0.63 [b] (0.36-1)	0.46 [b] (0.36-0.6)	0.45 [b] (0.36-0.6)	0.44 [b] (0.36-0.6)	0.38 [b] (0.22-0.6)	0.38 [b] (0.22-0.6)	0.34 (0.31-0.38) 10.19	0.27 [b] (0.22-0.36)	0.27 [b] (0.22-0.36)
BONYTAIL CHUB 2 (0.52 ± 0.09)	>1.0	.78 [b] (0.6-1)	0.74 [b] (0.6-1)	0.72 [b] (0.6-1)	0.68 [b] (0.6-1)	0.62 [b] (0.6-1)	0.38 [b] (0.36-0.6)	0.35 [b] (0.22-0.36)	0.31 [b] (0.26-0.37)
COLORADO SQUAWFISH 1 (0.32 ± 0.05)	0.77 [b] (0.6-1)	0.63 [b] (0.36-1)	0.48 [b] (0.36-0.6)	0.44 (0.40-0.48) 13.28	0.31 [b] (0.22-0.36)	0.28 (0.26-0.31) 14.04	0.26 [b] (0.22-0.36)	0.25 [b] (0.22-0.36)	0.24 [b] (0.13-0.36)
COLORADO SQUAWFISH 2 (0.34 ± 0.05)	0.80 [b] (0.6-1)	0.63 [b] (0.6-1)	0.47 [b] (0.36-0.6)	0.45 [b] (0.36-0.6)	0.31 [b] (0.22-0.36)	0.28 [b] (0.22-0.36)	0.27 [b] (0.22-0.36)	0.27 [b] (0.22-0.36)	0.27 [b] (0.22-0.36)
RAZORBACK SUCKER 1 (0.31 ± 0.04)	0.73 [b] (0.6-1)	0.41 (0.37-0.46) 8.24	0.29 (0.26-0.33) 7.11	0.27 (0.24-0.30) 7.41	0.23 (0.21-0.26) 7.80	0.21 (0.19-0.23) 9.65	0.19 [b] (0.13-0.22)	0.18 [b] (0.13-0.22)	0.16 [b] (0.13-0.22)
RAZORBACK SUCKER 2 (0.32 ± 0.07)	>1.0	0.63 (0.57-0.70) 4.18	0.44 (0.40-0.49) 3.45	0.31 (0.28-0.34) 3.89	0.25 [b] (0.22-0.36)	0.23 [b] (0.22-0.36)	0.22 [b] (0.13-0.22)	0.20 [b] (0.13-0.22)	0.19 [b] (0.13-0.22)

Appendix 4. (cont.)

PENTACHLOROPHENOL (mg/L)

SPECIES AVG. WEIGHT (grams)	HOURS								
	3	6	9	12	18	24	48	72	96
RAINBOW TROUT 1 (0.67 ± 0.35)	0.43 [b] (0.36-0.06)	0.30 [b] (0.22-0.36)	0.21 (0.19-0.23) 9.92	0.18 (0.17-0.20) 12.10	0.20 (0.18-0.22) 10.35	0.13 [b] (0.08-0.22)	0.11 (0.10-0.12) 11.63	0.11 (0.95-0.12) 8.61	0.15 [b] (0.13-0.22)
RAINBOW TROUT 2 (1.25 ± 0.57)	0.27 (0.25-0.30) 15.60	0.21 [b] (0.13-0.22)	0.18 [b] (0.13-0.22)	0.17 [b] (0.13-0.22)	0.15 [b] (0.13-0.22)	0.13 [b] (0.13-0.22)	0.12 (0.11-0.13) 11.04	0.12 (0.11-0.13) 11.04	0.12 [b] (0.08-0.13)
RAINBOW TROUT 3 (0.27 ± 0.07)	>0.60	0.29 [b] (0.22-0.36)	0.26 [c] (0.24-0.28)	0.23 (0.20-0.25) 8.16	0.18 [b] (0.016-0.20)	0.16 [a] (0.14-0.18)	0.15 [a] (0.14-0.17)	0.14 (0.13-0.16) 6.6	0.15 [b] (0.13-0.22)
RAINBOW TROUT 4 (1.09 ± 0.38)	>0.60	0.31 [b] (0.22-0.36)	0.27 (0.25-0.29) 14.57	0.21 [b] (0.13-0.22)	0.17 [b] (0.13-0.22)	0.17 [b] (0.13-0.22)	0.15 [b] (0.13-0.22)	0.15 [b] (0.13-0.22)	0.15 [b] (0.13-0.22)
RAINBOW TROUT 5 (0.48 ± 0.08)	>0.36	>0.36	0.29 (0.27-0.32) 14.57	0.26 [b] (0.22-0.36)	0.25 [b] (0.22-0.36)	0.22 [b] (0.13-0.36)	0.19 [b] (0.13-0.22)	0.19 [b] (0.13-0.22)	0.18 [b] (0.13-0.22)
RAINBOW TROUT 6 (0.50 ± 0.21)	>0.36	>0.36	>0.36	0.29 [b] (0.22-0.36)	0.27 [b] (0.22-0.36)	0.25 [b] (0.22-0.36)	0.19 [b] (0.13-0.22)	0.19 [b] (0.13-0.22)	0.19 [b] (0.13-0.22)

Appendix 4. (cont.)

PENTACHLOROPHENOL (mg/L)

SPECIES AVG. WEIGHT (grams)	HOURS								
	3	6	9	12	18	24	48	72	96
APACHE TROUT 1 (0.85 ± 0.49)	0.35 [b] (0.22-0.36)	0.28 (0.25-0.30) 13.02	0.21 (0.19-0.23) 9.80	0.19 (0.17-0.20) 11.77	0.16 [b] (0.13-0.22)	0.30 [b] (0.08-0.22)	0.11 (0.10-0.12) 12.19	0.11 (0.10-0.12) 9.70	0.11 (0.10-0.12) 8.97
APACHE TROUT 2 (0.38 ± 0.18)	>0.36	0.32 [b] (0.22-0.36)	0.27 [b] (0.25-0.31) 13.69	0.23 (0.20-0.26) 8.81	0.17' (0.15-0.19) 11.25	0.15 (0.13-0.17) 8.07	0.12 (0.10-0.13) 6.55	0.10 (0.09-0.12) 5.67	0.10 (0.08-0.12) 5.05
GREENBACK CUTTHROAT TROUT (0.31 ± 0.17)	>0.01	>0.01	>0.01	>0.01	>0.01	>0.01	>0.01	>0.01	>0.01
LAHONTAN CUTTHROAT TROUT 1 (0.34 ± 0.08)	>0.36	0.30 [b] (0.22-0.36)	0.28 [b] (0.22-0.36)	0.27 [b] (0.22-0.36)	0.23 [b] (0.22-0.36)	0.19 [b] (0.13-0.22)	0.18 (0.17-0.20) 12.29	0.18 [a] (0.16-0.20)	0.16 (0.08-0.36) 6.21
LAHONTAN CUTTHROAT TROUT 2 (0.57 ± 0.23)	>0.36	>0.36	>0.36	>0.36	0.29 [b] (0.22-0.36)	0.28 [b] (0.22-0.36)	0.18 (0.17-0.20) 12.29	0.18 (0.17-0.20) 12.29	0.18 [b] (0.13-0.22)

Appendix 4. (cont.)

PENTACHLOROPHENOL (mg/L)

SPECIES AVG. WEIGHT (grams)	HOURS								
	3	6	9	12	18	24	48	72	96
FATHEAD MINNOW 1 (0.32 ± 0.16)	0.74[b] (0.6-1)	0.52 (47-56) 11.73	0.38 (0.34-0.42) 6.94	0.34 (0.31-0.39) 7.06	0.31 (0.27-0.35) 6.39	0.29 (0.26-0.33) 5.80	0.29 (0.25-0.33) 5.91	0.29 (0.25-0.33) 5.91	0.27 (0.24-0.31) 5.89
FATHEAD MINNOW 2 (0.56 ± 0.19)	0.84 (0.76-0.91) 12.63	0.51[b] (0.36-0.6)	0.38[b] (0.36-0.6)	0.32[b] (0.22-0.36	0.30[b] (0.22-0.36)	0.28 (0.26-0.31) 12.0	0.26 (0.23-0.29) 9.24	0.23 (0.21-0.26) 7.75	0.22 (0.20-0.24) 8.39
FATHEAD MINNOW 3 (0.45 ± 0.35)	0.72 (0.66-0.79) 12.64	0.50 (0.46-0.55) 12.64	0.40[b] (0.36-0.6)	0.34[b] (0.22-0.36)	0.34[b] (0.22-0.36)	0.34[b] (0.22-0.36)	0.33 (0.30-0.36) 10.89	0.29 (0.13-0.67) 5.68	0.23 (0.11-0.49) 4.15
FATHEAD MINNOW 4 (0.40 ± 0.21)	>1.0	0.64[b] (0.6-1)	0.47 (0.42-0.51) 16.54	0.44 (0.41-0.49) 14.05	0.36 (0.33-0.40) 9.74	0.33[a] (0.30-0.36)	0.31 (0.28-0.34) 8.72	0.31[a] (0.27-0.34)	0.28 (0.16-0.48) 7.94
FATHEAD MINNOW 5 (0.34 ± 0.24)	0.98[b] (0.6-1)	0.68[b] (0.6-1)	0.55[b] (0.36-0.6)	0.54[b] (0.36-0.6)	0.54[b] (0.36-0.6)	0.53 (0.48-0.59) 9.26	0.50 (0.45-0.55) 7.69	0.46 (0.41-0.51) 7.27	0.44[a] (0.39-0.50)
FATHEAD MINNOW 6 (0.39 ± 0.14)	0.34[b] (0.22-0.36)	0.21[b] (0.13-0.22)	0.16 (0.15-0.18) 9.99	0.15 (0.13-0.16) 10.6	0.14 (0.13-0.16) 10.4	0.14 (0.13-0.16) 10.4	0.14 (0.13-0.16) 10.4	0.14 (0.13-0.16) 10.4	0.14 (0.13-0.16) 10.2

Appendix 4. (cont.)

PENTACHLOROPHENOL (mg/L)

SPECIES AVG. WEIGHT (grams)	HOURS								
	3	6	9	12	18	24	48	72	96
BONYTAIL CHUB 1 (0.29 ± 0.08)	0.76 [b] (0.6-1)	0.51 [b] (0.36-0.6)	0.44 [b] (0.36-0.6)	0.33 [b] (0.22-0.36)	0.28 [b] (0.22-0.36)	0.28 [b] (0.22-0.36)	0.24 [b] (0.22-0.36)	0.22 [b] (0.13-0.22)	0.20 [b] (0.13-0.22)
BONYTAIL CHUB 2 (0.52 ± 0.09)	>1.0	0.78 (0.71-0.85) 16.54	0.66 [b] (0.6-1)	0.54 [b] (0.36-0.6)	0.43 [b] (0.36-0.6)	0.40 [b] (0.36-0.6)	0.28 (0.25-0.30) 15.60	0.27 [b] (0.22-0.36)	0.26 [b] (0.22-0.36)
COLORADO SQUAWFISH 1 (0.32 ± 0.05)	0.78 [b] (0.6-1)	0.51 [b] (0.36-0.6)	0.47 [b] (0.36-0.6)	0.40 [b] (0.36-0.6)	0.28 [b] (0.22-0.36)	0.27 [b] (0.22-0.36)	0.23 [b] (0.22-0.36)	0.18 [b] (0.13-0.22)	0.18 [b] (0.13-0.22)
COLORADO SQUAWFISH 2 (0.34 ± 0.05)	0.28 [b] (0.22-0.36)	0.17 [b] (0.13-0.22)	0.17 [b] (0.13-0.22)	0.13 [b] (0.08-0.13)	0.10 [b] (0.08-0.13)	0.10 [b] (0.08-0.13)	0.10 [b] (0.08-0.13)	0.10 [b] (0.08-0.13)	0.10 [b] (0.08-0.13)
RAZORBACK SUCKER 1 (0.31 ± 0.04)	0.79 (0.72-0.86) 16.75	0.56 [b] (0.36-0.6)	0.45 [b] (0.36-0.6)	0.45 [b] (0.36-0.6)	0.34 (0.31-0.38) 8.71	0.27 [b] (0.22-0.36)	0.27 [b] (0.22-0.36)	0.27 [b] (0.22-0.36)	0.27 [b] (0.22-0.36)
RAZORBACK SUCKER 2 (0.32 ± 0.07)	>1.0	0.84 [b] (0.6-1)	0.73 [b] (0.6-1)	0.63 [b] (0.6-1)	0.47 [b] (0.36-0.6)	0.47 [b] (0.36-0.6)	0.32 [b] (0.22-0.36)	0.29 [b] (0.22-0.36)	0.28 [b] (0.22-0.36)

Appendix 4. (cont.)

PERMETHRIN (μg/L)

SPECIES AVG. WEIGHT (grams)	HOURS								
	3	6	9	12	18	24	48	72	96
RAINBOW TROUT 1 (0.67 $\pm$ 0.35)	>10	>10	7.16 (6.6-7.9) 12.64	5.96 (5.4-6.6) 9.58	2.97 (2.7-3.2) 11.58	4.20 (2.4-7.6) 5.82	4.00 (3.5-4.5) 5.95	3.87 (3.4-4.4) 6.20	3.90 (3.4-4.4) 6.20
RAINBOW TROUT 2 (1.25 $\pm$ 0.57)	>10	>10	9.57 [b] (6-10)	8.31 [b] (6-10)	6.39 [b] (6-10)	6.00 [b] (3.6-10)	6.00 [b] (3.6-10)	6.00 [b] (3.6-10)	4.80 [a] (4.3-5.3)
RAINBOW TROUT 3 (0.27 $\pm$ 0.07)	>10	7.62 [c] (6.9-8.5)	4.42 (4.0-4.9) 8.73	3.39 [b] (2.2-3.6)	2.64 (2.3-3.0) 6.42	2.18 (1.9-2.4) 6.92	1.65 (1.5-1.9) 6.30	1.65 (1.5-1.9) 6.30	1.65 (1.5-1.9) 6.30
RAINBOW TROUT 4 (1.09 $\pm$ 0.38)	>10	>10	8.47 (7.6-9.5) 8.66	6.37 (5.7-7.1) 8.55	5.00 (4.6-5.5) 12.64	4.60 (4.2-5.0) 12.55	4.47 (4.1-4.9) 11.80	4.40 (4.0-4.8) 11.18	4.40 (4.0-4.8) 11.18
RAINBOW TROUT 5 (0.48 $\pm$ 0.08)	>10	>10	6.94 (6.35-7.64) 11.62	5.13 [b] (3.6-6)	3.39 [b] (2.2-3.6)	3.08 (2.8-3.4) 12.54	2.86 (2.6-3.2) 9.85	2.67 (2.4-2.9) 11.57	2.67 (2.4-2.9) 11.57
RAINBOW TROUT 6 (0.50 $\pm$ 0.21)	>10	>10	9.72 (8.6-11.8) 7.26	6.62 [b] (6-10)	4.16 [b] (3.6-6)	3.74 (3.4-4.1) 9.79	3.58 (3.2-3.9) 9.86	3.58 (3.2-3.9) 9.86	3.60 (3.2-3.9) 9.86

Appendix 4. (cont.)

PERMETHRIN (μg/L)

SPECIES AVG. WEIGHT (grams)	HOURS								
	3	6	9	12	18	24	48	72	96
APACHE TROUT 1 (0.85 ± 0.49)	>10	8.07 (7.3-8.8) 11.68	5.07 (4.6-5.6) 10.21	4.07 (3.7-4.5) 9.0	2.97 (2.7-3.2) 11.58	2.63 (2.4-2.9) 9.61	2.21 (2.0-2.4) 9.56	2.17 (2.0-2.4) 9.59	2.2 (1.96-2.4) 9.59
APACHE TROUT 2 (0.38 ± 0.18)	>10	>10	5.90 (4.5-8.2) 4.42	3.70 (2.8-4.8) 4.44	2.06 [b] (1.3-2.2)	1.96 (1.6-2.4) 7.99	1.61 (1.3-2.0) 7.23	1.39 (1.1-1.7) 7.66	1.33 (1.1-1.7) 7.46
GREENBACK CUTTHROAT TROUT (0.31 ± 0.17)	>1.0	>1.0	>1.0	>1.0	>1.0	>1.0	>1.0	>1.0	>1.0
LAHONTAN CUTTHROAT TROUT 1 (0.34 ± 0.08)	>10	4.42 (4.0-4.9) 8.73	2.81 (2.6-3.1) 14.04	2.38 (2.2-2.6) 10.55	1.75 (1.6-1.9) 8.83	1.42 (1.3-1.6) 8.62	1.16 (1.0-1.3) 7.37	1.14 (1.0-1.3) 7.48	1.14 (1.0-1.3) 7.48
LAHONTAN CUTTHROAT TROUT 2 (0.57 ± 0.23)	>10	>10	5.71 (5.3-6.2) 10.16	4.65 (4.3-5.0) 14.83	3.06 [b] (2.2-3.6)	2.61 [b] (2.2-3.6)	2.23 (2.0-2.4) 10.05	2.20 (2.0-2.4) 10.05	2.20 (2.0-2.4) 10.05

Appendix 4. (cont.)

PERMETHRIN (μg/L)

SPECIES AVG. WEIGHT (grams)	HOURS								
	3	6	9	12	18	24	48	72	96
FATHEAD MINNOW 1 (0.32 ± 0.16)	>25	>25	13.91 [b] (9-15)	13.43 [a] (11.5-16.0)	10.02 (8.5-11.9) 3.55	9.24 (7.9-11.0) 3.41	8.55 (7.4-10.0) 3.89	8.09 (7.0-9.4) 4.31	8.09 (7.0-9.4) 4.31
FATHEAD MINNOW 2 (0.56 ± 0.19)	>10	>10	>10	>10	9.94 (8.7-12.8) 6.13	9.48 (8.2-12.1) 5.51	9.21 (8.0-11.4) 5.80	9.21 (8.0-11.4) 5.80	8.97 (7.9-10.9) 6.10
FATHEAD MINNOW 3 (0.45 ± 0.35)	>25	>25	17.49 (15.0-21.1) 4.3	13.64 (11.9-15.7) 5.03	11.36 (9.9-13.2) 4.48	11.11 (9.7-12.8) 4.77	10.34 (9.1-11.7) 5.57	10.0 (8.7-11.4) 5.00	10.0 (9.1-11.7) 5.57
FATHEAD MINNOW 4 (0.40 ± 0.21)	>25	>25	>25	17.32 (14.6-21.8) 3.58	10.04 (5.6-18.8) 4.42	9.69 (5.65-16.8) 4.72	8.74 [a] (7.6-10.0)	8.74 [a] (7.6-10.0)	8.96 [a] (7.7-10.7)
FATHEAD MINNOW 5 (0.34 ± 0.24)	>25	>25	12.53 (10.5-15.3) 3.14	10.25 (8.7-12.3) 3.23	9.75 (8.2-11.8) 3.01	9.26 (7.7-11.3) 2.72	8.93 (7.4-10.9) 2.74	8.27 (6.9-9.9) 2.99	6.68 (5.8-7.7) 4.50
FATHEAD MINNOW 6 (0.39 ± 0.14)	>25	>25	>25	>25	>25	>25	16.8 (14.2-20.9) 3.6	15.9 (13.6-19.3) 3.9	15.7 (13.4-18.9) 3.9

Appendix 4. (cont.)

PERMETHRIN (μg/L)

SPECIES AVG. WEIGHT (grams)	HOURS								
	3	6	9	12	18	24	48	72	96
BONYTAIL CHUB 1 (0.29 ± 0.08)	>25	>25	>25	>25	>25	>25	>25	>25	>25
BONYTAIL CHUB 2 (0.52 ± 0.09)	>25	>25	>25	>25	>25	>25	>25	>25	>25
COLORADO SQUAWFISH 1 (0.32 ± 0.05)	>10	>10	>10	>10	>10	>10	>10	>10	>10
COLORADO SQUAWFISH 2 (0.34 ± 0.05)	>25	>25	>25	>25	>25	>25	>25	>25	24.4 [b] (15-25)
RAZORBACK SUCKER 1 (0.31 ± 0.04)	>25	>25	>25	>25	>25	>25	17.43 (13.1-28.4) 1.86	4.97 [b] (3.2-5.4)	4.6 [b] (3.2-5.4)
RAZORBACK SUCKER 2 (0.32 ± 0.07)	>25	>25	>25	13.05 (11.2-15.4) 4.05	9.04 (3.6-22.9) 3.34	8.87 (3.6-21.5) 3.26	8.23 (1.8-26.4) 3.03	7.95 (3.0-17.3) 3.00	7.70 (3.1-15.9) 3.49

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