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USING ACCELERATED LIFE TESTING PROCEDURES TO COMPARE THE RELATIVE SENSITIVITY OF RAINBOW TROUT AND THE FEDERALLY LISTED THREATENED BULL TROUT TO THREE COMMONLY USED RANGELAND HERBICIDES (PICLORAM, 2,4-D, AND CLOPYRALID)

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Abstract—We conducted 96-h static acute toxicity studies to evaluate the relative sensitivity of juveniles of the threatened bull trout (*Salvelinus confluentus*) and the standard cold-water surrogate rainbow trout (*Onchorhyncus mykiss*) to three rangeland herbicides commonly used for controlling invasive weeds in the northwestern United States. Relative species sensitivity was compared using three procedures: standard acute toxicity testing, fractional estimates of lethal concentrations, and accelerated life testing chronic estimation procedures. The acutely lethal concentrations (ALC) resulting in 50% mortality at 96 h (96-h ALC50s) were determined using linear regression and indicated that the three herbicides were toxic in the order of picloram acid > 2,4-D acid > clopyralid acid. The 96-h ALC50 values for rainbow trout were as follows: picloram, 41 mg/L; 2.4-D, 707 mg/L; and clopyralid, 700 mg/L. The 96-h ALC50 values for bull trout were as follows: picloram, 24 mg/L; 2.4-D, 398 mg/L; and clopyralid, 802 mg/L. Fractional estimates of safe concentrations, based on 5% of the 96-h ALC50, were conservative (overestimated toxicity) of regression-derived 96-h ALC5 values by an order of magnitude. Accelerated life testing procedures were used to estimate chronic lethal concentrations (CLC) resulting in 1% mortality at 30 d (30-d CLC1) for the three herbicides: picloram (1 mg/L rainbow trout, 5 mg/L bull trout), 2,4-D (56 mg/L rainbow trout, 84 mg/L bull trout), and clopyralid (477 mg/L rainbow trout; 552 mg/L bull trout). Collectively, the results indicated that the standard surrogate rainbow trout is similar in sensitivity to bull trout. Accelerated life testing procedures provided cost-effective, statistically defensible methods for estimating safe chronic concentrations (30-d CLC1s) of herbicides from acute toxicity data because they use statistical models based on the entire mortality:concentration:time data matrix.

Keywords—Trout Herbicides Invasive Plants Mortality

INTRODUCTION

Nonnative, invasive plants are considered one of the primary economic, environmental, and societal threats to public and federal lands in the western United States [1]. Duncan and Jachetta [2] estimated that invasive plants have infested over 44 million ha of forest and rangeland in the 17 contiguous western states west of the North Dakota/Texas longitude. Spotted knapweed (*Dentaurea solstietialis*) occupies a total of 2.1 million ha in the western United States and is expected to expand at a rate of 10 to 24% per year [2]. Leafy spurge (*Euphorbia esula*) has infested 1.5 million ha in these 17 western states and is expected to expand at a rate of 12 to 16% per year [2] with an annual adverse economic impact of $185 million (U.S. dollars) in Montana, North Dakota, South Dakota, and Wyoming [3,4] due to loss of forage, livestock sales, and costs of control efforts.

The U.S. Forest Service (USFS) has recognized the threat of invasive plant species to native plants in western forest and rangelands and has developed a National Strategy and Implementation Plan for Invasive Species Management [5]. This national strategy has four program components: prevention, early detection/rapid response, control/management, and rehabilitation/restoration. Herbicide application is a key management tool the USFS uses to control invasive and noxious plants. In 2004, the USFS applied over 10,000 kg of herbicide active ingredient (AI) to over 21,000 ha in Region 1 (Montana, North Dakota, and portions of northern Idaho and northwestern South Dakota) for control of invasive weeds (http://www.fs.fed.us/foresthealth/pesticide/reports.shtml) [6].

In many cases, herbicide applications are conducted in areas that overlap with habitats of federally listed threatened or endangered fish species. For example, the federally listed, threatened bull trout (*Salvelinus confluentus*) occurs in numerous areas of the northwestern U.S. where invasive plant infestations are spreading. Section 7 of the Endangered Species Act requires that the U.S. Fish and Wildlife Service conduct formal consultations with the USFS or other federal agencies to ensure that land management actions such as invasive plant removal do not result in adverse impacts to federally listed species. This consultation is difficult given that the only toxicity data available for cold-water species is acute toxicity information using the commonly tested, standard surrogate rainbow trout (*Onchorhyncus mykiss*). Therefore, we conducted the present study with two primary objectives: determine the relative acute toxicity of three commonly used rangeland herbicides (picloram, 2,4-D, and clopyralid) to rainbow trout and bull trout and apply these data using recently developed chronic estimation procedures to determine concentrations of herbicides that are protective of bull trout populations. The present study was conducted to assist resource managers in consultations re-

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garding use of herbicides for invasive plant control in watersheds within the range of the threatened bull trout.

MATERIALS AND METHODS

Study site, source, and acclimation of fish

Studies were conducted at the Columbia Environmental Research Center (CERC), U.S. Geological Survey (Columbia, MO). Rainbow trout were obtained as eyed eggs from Aquatic Biosystems (Fort Collins, CO, USA). Eggs were shipped in chilled (5°C), oxygenated water. Once received at the CERC, rainbow trout were cultured in well water (temperature, 16°C; alkalinity, 260 mg/L as $CaCO_3$; hardness, 290 mg/L as $CaCO_3$; pH, 7.8). At swim-up, rainbow trout were fed twice daily with Ziegler #1 Finfish Diet (55% protein, 15% fat; Ziegler Brothers, Gardner, PA, USA). Prior to testing, fish were acclimated to test temperature (8°C) in well water over a 7-d period (1°C decrease/d); fish were not fed for the period of 48 h prior to testing. Bull trout were obtained from the U.S. Fish and Wildlife Service Creston National Fish Hatchery (Creston, MT, USA). Fish were shipped to the CERC as post–swim-up juveniles in chilled (6°C) oxygenated hatchery well water. On receipt at the CERC, the bull trout were acclimated to test temperature (8°C) in CERC well water over a 48-h period (1°C increase/d). Fish were fed with Bio-Vita Diet (53% protein; 18% fat; Skretting Company, Vancouver, BC, Canada) at the Creston Hatchery but were not fed during the 48-h acclimation at CERC.

Test chemicals

Three herbicides were tested: 2,4-D salt (Chemical Abstracts Service [CAS] 2008-39-1; 56.7% AI free acid; water solubility 900 mg/L at 25°C; Nufarm Americas, Burr Ridge, IL, USA), clopyralid salt (CAS 001702-17-6; 95% AI free acid; water solubility 1,000 mg/L at 25°C; Dow Agrosciences, Indianapolis, IN, USA), and picloram salt (CAS 02545-60-0; 21.1% AI free acid; water solubility 430 mg/L at 25°C; Dow Agrosciences).

Toxicity testing

Static acute toxicity tests were conducted using methods described by the American Society for Testing and Materials [7]. Fish were voided (i.e., not fed) for 48 h prior to testing. Studies were conducted in 20-L glass jars containing 15 L of CERC well water under natural lighting conditions. Tests were conducted at 8°C to simulate natural bull trout habitat conditions [8] and are equivalent to that used for previous bull trout toxicity testing with metals [9,10]. Temperature and dissolved oxygen (YSI Model 54 Meter; YSI, Yellow Springs, OH, USA) and pH (Orion Model 940, Allometrics, Boston, MA, USA) were measured at 0, 48, and 96 h in all treatments. Conductivity (YSI ATC Meter), alkalinity (titrimetry [11]) and hardness (titrimetry [11]) were measured at the beginning of the test in all treatments. Total ammonia (Technicon Autoanalyzer II System; Pulse Instrumentation, Saskatoon, SK, Canada) was measured at the beginning and end of the test in all treatments.

The test solutions were formulated on the day of testing by adding the appropriate mass of chemical to the dilution water; no solvents were used. A test series consisted of a well water control and five herbicide concentrations (two replicates per concentration) using a 50% dilution factor. Individual test series were randomly assigned to positions within the temperature-controlled water bath. Fish were exposed to the following herbicide concentrations: 0, 210, 420, 840, 1,680, and 3,360 mg/L clopyralid; 0, 250, 500, 1,001, 2,002, and 4,004 mg/L 2,4-D; and 14, 27, 54, 109, and 218 mg/L picloram. No herbicide precipitates were observed in any chemical or concentration. The technical formulation of clopyralid resulted in significant pH effects: 3,360 mg/L (pH = 2.0), 1,680 mg/L (pH = 2.7), 840 mg/L (pH = 6.6), 420 mg/L (pH = 7.0), and 210 mg/L (pH = 7.5). The 3,360-mg/L clopyralid concentration resulted in immediate mortality, and therefore this concentration of was not used in statistical analyses. We retained the 1,680-mg/L concentration, which produced mortality, in order to produce an estimate of the acutely lethal concentration resulting in 50% mortality (ALC50). However, we acknowledge that this is largely a pH effect as opposed to the herbicide mode of action. Calculated toxicity endpoints, however, were less than 840 mg/L and were associated with pH levels 6.5 or greater, which are nonlethal to salmonids. Therefore, we have confidence in the toxicity calculations for clopyralid.

Fish were similar in size between species when compared as the mean ± 1 standard deviation (n = 20): bull trout (weight, 0.55 ± 0.11 g; total length, 42 ± 3 mm) and rainbow trout (weight, 0.59 ± 0.15 g; total length, 41 ± 4 mm). Ten fish were randomly selected and added to each duplicate test chamber. Mortality was observed at 2, 6, 24, 48, 72, and 96 h of exposure, and all dead fish were removed at those times. Fish were not fed during the present study.

Verification of herbicide exposure concentrations

Duplicate samples of two replicates of the high concentration of each herbicide were taken at t = 0 and analyzed by the Mississippi State Chemical Laboratory (Mississippi State, MS, USA) using high-performance liquid chromatography. Analytical recoveries were as follows: 2,4-D, 101%; clopyralid, 112%; and picloram, 108%. All toxicity endpoints were calculated using corrected concentrations based on the reported percent recoveries of the high concentrations. All references to herbicide concentrations (both within and across cited studies) refer to the free acid chemical form unless otherwise stated.

Statistical analyses

We calculated 50, 20, 10, and 5% acutely lethal concentrations (ALC50, ALC20, ALC10, and ALC5) using Toxcalc Software (Tidepool Scientific Software, McKinleyville, CA, USA) and the linear interpolation of arcsine square root–transformed mortality data using the pooled data from the two replicates of each concentration. Chronic no effect concentrations for mortality were estimated as the chronic lethal concentration calculated to cause 1% mortality in a population (CLC1) using accelerated life testing procedures (ALT) in the U.S. Environmental Protection Agency's Acute-to-Chronic Estimation (ACE) with Time-Concentration-Effect Models program, version 2.0 [12]. The ALT procedures are based on observed mortality as a covariance matrix over concentration and time using a quasi-Newton method to find the maximum likelihood estimates of model parameters [12]. The CLC1 was calculated as a no-effect concentration using Equation 1:

$$\mathrm{CLC1} = \exp\left(\{\ln[-\ln(1 - p)] - \ln(\mathrm{A}) - \mathrm{C}\ \ln(d0.24)\}/\mathrm{B}\right)$$

where d is the day of chronic effect estimation (30 d in our application), p is the proportion mortality observed at day x (i.e., 0.01 or 1% mortality at day 30), A = (1/AA)exp B, AA

Table 1. Comparative sensitivity of rainbow and bull trout to picloram (mg/L) using acute and chronic estimation procedures

Toxicity model	Endpoint or parameter	Species: Rainbow trout	Species: Bull trout
Interpolated 96-h acute[a]	96-h ALC5	29 (14–54)[b]	15 (13–20)
	96-h ALC10	30 (14–54)[b]	16 (13–26)
	96-h ALC20	34 (14–54)[b]	18 (12–37)
	96-h ALC50	41 (14–54)[b]	24 (10–68)
Fractional 96-h acute[c]	5% ALC50	2	1
Estimated 30-d chronic[d]	CLC1	0.8 (0.01–1.6)	4.9 (3.1–6.7)
	ALT 96-h ALC50	32 (23–40)	25 (22–29)
	ALT-B parameter	1.72 (1.28–2.17)	5.16 (4.38–5.94)
	ALT-C parameter	0.90 (0.82–0.98)	1.96 (1.95–1.97)
	ALT-C/B parameter	0.52 (0.39–0.65)	0.38 (0.32–0.44)
Acute:chronic ratio	96-h ALC50/CLC1	40	5

[a] Acutely lethal concentrations at x% (ALCx) from acute toxicity tests using linear interpolation at 96-h exposure (95% confidence interval [CI] in parentheses).
[b] No partial mortalities were observed. The next lower and higher exposure concentrations were used as conservative estimates of the 95% CI.
[c] Estimate of no-effect level based on 5% of the 96-h ALC50 value (95% CI in parentheses).
[d] Chronic lethal concentrations estimated to result in 1% mortality (CLC1; 95% CI in parentheses) calculated using accelerated life testing (ALT) procedures where B is the measure of mode of concentration response, C is the measure of mode of time response, and C/B is the measure of domination between concentration and response (if equal to one, then both time and concentration have equal influence).

is the measure of initial effect concentration (inferred herein as the ALT ALC50), B is the measure of mode of concentration response, C is the measure of mode of time response, and C/B is the measure of domination between concentration and time response (if equal to one, then both time and concentration have equal influence) [12].

The previously mentioned parameters are outputs of the ALT program and allow the user to compare model parameters across chemicals, species, and data sets for inferential evaluation of mode of action or difference in species response based on the mortality:concentration:time response surface. The acute:chronic ratio (ACR) was calculated on the basis of the quotient of the 96-h ALC50 and the CLC1 derived from output of the ALT procedure.

RESULTS

No mortality of either rainbow trout or bull trout in control treatments over the duration of the 96-h study was observed. Water quality of well water controls (mean ± 1 standard deviation) averaged as follows: dissolved oxygen, 8.40 ± 0.72 mg/L (n = 12) (83 ± 7% saturation at 650 mm Hg); temperature, 7.6 ± 0.8 (n = 12); pH, 8.02 ± 0.15 (n = 12); conductivity, 679 ± 1 (n = 4); alkalinity, 250 ± 0 mg/L as $CaCO_3$ (n = 4); hardness, 289 ± 1 mg/L as $CaCO_3$ (n = 4); and total ammonia, 0.18 ± 0.08 mg/L as N (n = 8). Test criteria of 90% control survival, dissolved oxygen above 60% saturation, and test temperatures maintained within 2°C of nominal target were met as described for an acceptable test [7].

Picloram

Rainbow trout and bull trout were similar in acute sensitivity (i.e., overlapping confidence intervals) to picloram based on comparable 96-h ALCx values (Table 1). Fractional estimates of the ALC5 overestimated toxicity compared to statistically interpolated values of the ALC5 level by an order of magnitude. The estimated CLC1 was approximately fivefold lower for rainbow trout (0.8 mg/L) compared to bull trout (4.9 mg/L). Differences in the calculated CLC1 value between the two species was explained by the differences in the ALT-B and ALT-C parameters in Equation 1 (Table 1), which were twofold higher for the bull trout because of high mortality in the higher concentrations at the 2-h observation interval (Fig. 1). Rainbow trout, however, exhibited delayed mortality in the high treatments at the 6-h period of observation (Fig. 1). These differences in the effect of time and concentration were also reflected in the ALT-C/B ratio from Equation 1 (Table 1), which was higher for rainbow trout than bull trout (Fig. 1). Acute:chronic ratios were accordingly higher for rainbow trout compared to bull trout because of the steeper slope of the concentration–response relationship at 96 h in bull trout.

2,4-D

Bull trout were slightly more sensitive to 2,4-D than rainbow trout based on the 96-h ALC50 (Table 2). However, the ALC20, ALC10, ALC5, and CLC1 for rainbow trout and bull trout were similar based on overlapping confidence intervals. Fractional acute values (5% of ALC50) overestimated toxicity compared to the interpolated 96-h ALC5 levels by an order of magnitude and overestimated toxicity predicted by the CLC1 values for rainbow trout and bull trout by factors of 1.6 and 4.2, respectively. The ALT-B and ALT-C parameters (Table 2) were higher for bull trout because they tended to die earlier in the highest concentration with continued mortality through 96 h (Fig. 2); in contrast, the majority of mortality in rainbow trout occurred at 6 and 24 h (Fig. 2). Therefore, concentration effects were similar in the two species, but time had a greater impact on bull trout. Subsequently, the ACRs for 2,4-D were about twofold higher for rainbow trout compared to bull trout.

Clopyralid

Rainbow trout and bull trout were similar in acute and chronic sensitivity to clopyralid regardless of the toxicity model used (Table 3). The CLC1 values for the two species were similar and approximated the 96-h ALC5. Fractional acute values overestimated toxicity compared to calculated 96-h ALC5 levels and the CLC1 by an order of magnitude. Comparison of the ALT-B and ALT-C parameters indicated that they were similar for the two species; thus, the ALT-C/B parameter was similar for both species (Table 3), and this is visually apparent in Figure 3, which demonstrates the similarity in the mortality:concentration:time matrix. The ALT-B parameter was threefold higher for clopyralid compared to that

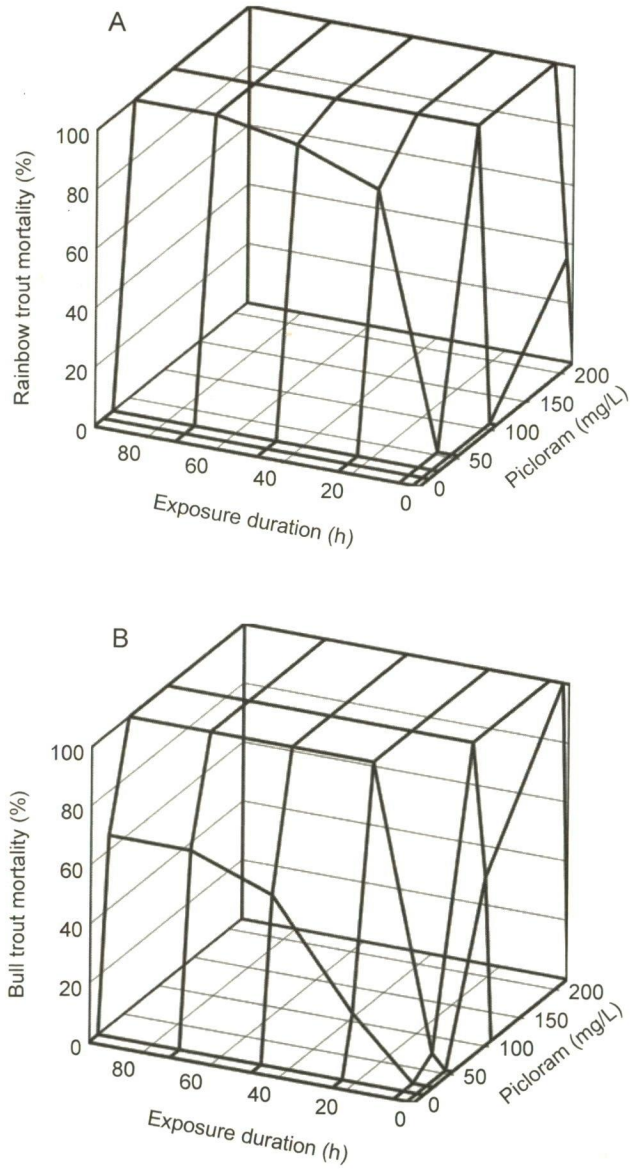

Fig. 1. Surface response model of rainbow trout (**A**) and bull trout (**B**) exposed to picloram.

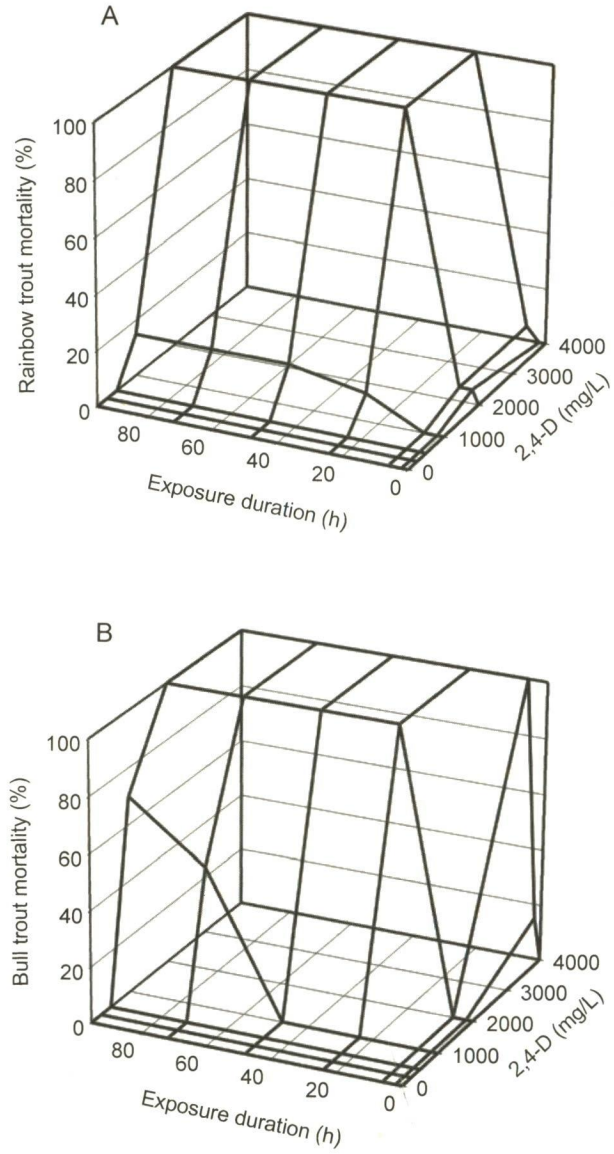

Fig. 2. Surface response model of rainbow trout (**A**) and bull trout (**B**) exposed to 2,4-D.

Table 2. Comparative sensitivity of rainbow and bull trout to 2,4-D (mg/L) using acute and chronic estimation procedures

Toxicity model	Endpoint or parameter	Species: Rainbow trout		Species: Bull trout	
Interpolated 96-h acute[a]	ALC5	334	(250–500)	265	(256–277)
	ALC10	417	(250–751)	280	(262–304)
	ALC20	530	(412–612)	309	(274–360)
	ALC50	707	(633–772)	398	(308–524)
Fractional 96-h acute[b]	5% ALC50	35		20	
Estimated 30-d chronic[c]	CLC1	56	(39–74)	84	(67–100)
	ALT 96-h ALC50	516	(448–584)	449	(408–492)
	ALT-B parameter	3.8	(3.5–4.2)	6.3	(5.9–6.8)
	ALT-C parameter	1.98	(1.97–2.00)	3.1	(3.1–3.1)
	ALT-C/B parameter	0.51	(0.47–0.56)	0.48	(0.45–0.52)
Acute:chronic ratio	96-h ALC50/CLC1	9.2		5.3	

[a] Acutely lethal concentrations at *x*% (ALC*x*) from acute toxicity tests using linear interpolation at 96-h exposure (95% confidence interval [CI] in parentheses).

[b] Estimate of no-effect level based on 5% of the 96-h ALC50 value (95% CI in parentheses).

[c] Chronic lethal concentrations estimated to result in 1% mortality (CLC1; 95% CI in parentheses) calculated using accelerated life testing (ALT) procedures where B is the measure of mode of concentration response, C is the measure of mode of time response, and C/B is the measure of domination between concentration and response (if equal to one, then both time and concentration have equal influence).

Table 3. Comparative sensitivity of rainbow and bull trout to clopyralid (mg/L) using acute and chronic estimation procedures

Toxicity model	Endpoint or parameter	Species: Rainbow trout		Species: Bull Trout	
Interpolated 96-h acute[a]	ALC5	448	(441–456)	458	(445–473)
	ALC10	476	(462–392)	496	(471–527)
	ALC20	532	(504–564)	582	(523–633)
	ALC50	700	(630–780)	802	(674–955)
Fractional acute[b]	5% ALC50	35		40	
Estimated 30-d chronic[c]	CLC1	477	(53–900)	552	(330–775)
	ALT 96-h ALC50	810	(736–884)	845	(837–852)
	ALT B parameter	11.5	(0.0–34.8)	16.2	(0–38)
	ALT C parameter	0.57	(0.15–1.00)	1.17	(0.00–3.23)
	ALT C/B parameter	0.05	(0.00–0.13)	0.07	(0.00–0.18)
Acute:chronic ratio	ALT 96-h ALC50/CLC1	1.7		1.5	

[a] Acutely lethal concentrations at x% (ALCx) from acute toxicity tests using linear interpolation at 96-h exposure (95% confidence interval [CI] in parentheses).
[b] Estimate of no-effect level based on 5% of the 96-h ALC50 value (95% CI in parentheses).
[c] Chronic lethal concentrations estimated to result in 1% mortality (CLC1; 95% CI in parentheses) calculated using accelerated life testing (ALT) procedures where B is the measure of mode of concentration response, C is the measure of mode of time response, and C/B is the measure of domination between concentration and response (if equal to one, then both time and concentration have equal influence).

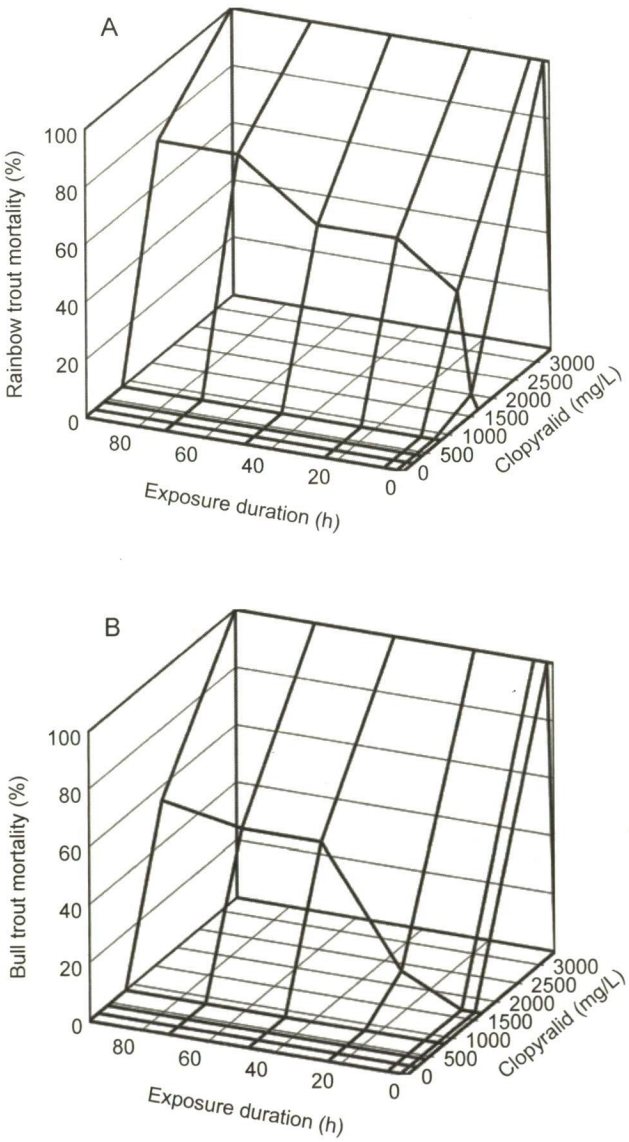

Fig. 3. Surface response model of rainbow trout (**A**) and bull trout (**B**) exposed to clopyralid.

for picloram and 2,4-D, indicating that concentration was more important than time in the response to clopyralid. The ACRs were similar for rainbow trout and bull trout exposed to clopyralid.

DISCUSSION

Comparative acute toxicity of herbicides to rainbow trout

Our results indicated that picloram was more acutely toxic to rainbow trout than either 2,4-D or clopyralid (Table 4). This relative toxicity ranking of these herbicides for rainbow trout are similar to that reported in the literature (Table 4). In addition, our 96-h ALC50 data for these herbicides (and associated 95% confidence interval [CI]) are within the range of data published by other researchers (Table 4) with the exception of 2,4-D; our 96-h ALC50 (707 mg/L; 673–772 mg/L 95% CI) was sixfold higher than reported by Mayer and Ellersieck [13] (110 mg/L; 77–157 mg/L 95% CI). Although Mayer and Ellersieck [13] tested the 98.7% technical formulation of 2,4-D, it is possible that higher observed toxicity of 2,4-D manufactured in the early 1980s was related to small amounts of manufacturing by-products such as dioxin [14]. Differences in water quality conditions are not known to affect the toxicity of these herbicides. Variation among published acute toxicity values is not unusual given the nature of such studies and the variation in statistical methods used to calculate such endpoints.

Table 4. Comparison of 96-h acutely lethal concentration resulting in 50% mortality (ALC50; 95% confidence interval [CI] in parentheses) from the present study to other published data for rainbow trout. All concentrations are in mg/L

Herbicide	96-h ALC50	Reference
Picloram	41 (14–54)	Present study
	19 (16–22)	[24]
	12 (10–15)	[13]
	16 (14–17)	[22]
2,4-D	707 (673–772)	Present study
	358 (320–400)	[25]
	110 (77–157)	[13]
Clopyralid	700 (630–780)	Present study
	104 (94–110)	[26]
	1,968 (1,445–2,802)	[26]

Comparative sensitivity of rainbow and bull trout to herbicides

Our acute toxicity data indicated that the rainbow trout and bull trout were similar in sensitivity to picloram and clopyralid; however, bull trout were slightly more sensitive to 2,4-D compared to rainbow trout. These are the only published data for the sensitivity of bull trout to herbicides. Hansen et al. [9,10] examined the acute sensitivity of rainbow trout and bull trout to cadmium, zinc, and copper and also found the two species similar in sensitivity. Any apparent differences in sensitivity in the present study were likely due to the nature of the individual data sets (e.g., number of partial mortalities observed) and the method used to calculate the effects endpoints. In the present study, we limited our statistical analysis of the 96-h ALC50 to the most robust methods which fit all data sets. Statistical bias is commonly observed in acute toxicity testing, as discussed later. These acute toxicity results add to the emerging recognition that federally listed threatened and endangered species are similar in contaminant sensitivity to most commonly tested surrogates when using similar test protocols and ALC50 endpoint [15,16].

Comparison of models used to estimate acute and chronic toxicity

The ALC50 is the most commonly measured endpoint used for pesticide registration and the development of water quality criteria in the United States, Canada, and Europe. Acute toxicity tests are used for several reasons including the availability of standardized test protocols, low cost, and perceived repeatability, replicability, and reproducibility. The ALC50 is usually presented along with associated confidence intervals as a standard estimate of toxicity. In addition, knowledge of the slope of the concentration–response curve can be used as we have done to calculate other effect levels. However, frequently the slope values are not reported in data readily accessible to managers using toxicity data. Therefore, a safety factor of 10 or even 100 is often used. Urban and Cook [17] proposed the use of 10 or 5% of the ALC50 (or a safety factor of 10 or 20) as an indication of a safe or no-effect level for an endangered species. The U.S. Environmental Protection Agency [18] re-evaluated that assumption and has indicated that the degree of safety of this approach depends on the slope of the concentration–response relationship and is probably overprotective in practical application. For example, in a review of the acute toxicity database for the insecticide carbofuran, the U.S. Environmental Protection Agency calculated the probability of a level of concern of 0.1(ALCx) over a range of slope of two to nine and found that this assumption could result in individual mortality ranges from 0.02 (slope = 2) to less than 1×10^{-16} (slope = 9) (http://www.epa.gov/espp/consultation/ecorisk-overview.pdf) [18]. Our results, using a factor of 0.05 of the 96-h ALC50 for herbicides, clearly indicated that the use of such a fractional model is overprotective by an order of magnitude for picloram, 2,4-D, and clopyralid because of the steep response of the dose–response curve, which is less than two for these species and chemicals. Therefore, application of the 5% fractional model in herbicide consultations in habitats occupied by federally listed fishes may result in overestimates of risk and therefore hinder agency efforts in using herbicides to control invasive plants.

Chronic toxicity data is sometimes generated in aquatic toxicity testing to determine the effects of prolonged exposure on survival, growth, and reproduction. Such studies traditionally use an analysis of variance (ANOVA) model to determine a chronic no-observed-effect concentration (NOEC) and a lowest-observed-effect concentration (LOEC). The chronic value, or concentration presumed to be protective of a population, is calculated as the geometric mean of the NOEC and LOEC values and has often been referred to as the maximum acceptable toxicant concentration (MATC). Traditionally, the 96-h ALC50 is divided by the MATC to derive the application factor or ACR, which is used to extrapolate acute data across species or chemicals to get an estimated safe chronic value for a species and chemical when no chronic data exist. This approach has been widely applied since originally proposed by Kenaga [19]. However, use of ANOVA to calculate safe levels such as the NOEC, LOEC, chronic value, and MATC has been increasingly criticized because of the inherent biases associated with experimental design and statistical power. For example, calculated MATCs based on ANOVA have been shown to result in up to 50% mortality in regression models [20,21]. Various forms of regression that allow calculation of various effect levels (e.g., ALCx, CLCx) along with associated confidence intervals are preferred.

Sun et al. [20] has proposed the use of the ALT model as a statistically based multiple regression approach to calculate chronic no-effect levels for use in risk assessments when chronic testing is not feasible (e.g., availability of adequate test organisms such as threatened or endangered species). We applied the ALT model, using the entire mortality:concentration:time data matrix, to estimate safe concentrations (CLC1) of herbicides for rainbow and bull trout. The ALT-based CLC1 in all cases were equal to or lower than the 96-h ALC5 levels for all herbicides and allowed us to calculate ACRs for rainbow and bull trout without the time or expense of chronic testing.

Comparison of ALT-based chronic effect levels to published chronic data

Surprisingly little chronic toxicity data has been published for picloram or 2,4-D and none for clopyralid. Mayes et al. [22] conducted 60-d early life-stage toxicity tests with rainbow trout exposed to picloram free acid and determined a LOEC of 2.02 mg/L picloram (resulting in 20% reduction in survival compared to controls) and a NOEC of 1.34 mg/L; in addition, they calculated a 96-h ALC50 of 16 mg/L. The Mayes et al. [22] data yield an ACR of 12 for picloram. Dividing our 96-h ALC50 value by the ACR would give us an estimated safe concentration for rainbow trout of 3.4 mg/L. This value underestimated the CLC1 for rainbow trout (0.8 mg/L) by fourfold but accurately predicted our CLC1 for bull trout (4.9 mg/L). We determined an ACR by dividing our ALT-derived 96-h ALC50 by our CLC1, resulting in an ACR of 40 for rainbow trout and five for bull trout, which are threefold higher and twofold lower than predicted using the ACR of Mayes et al. [22]. These disparities are not surprising given that the calculated no-effect level of Mayes et al. [22] was based on an ANOVA as opposed to our approach, which used an interpolated value from the ALT program. While not unequivocal, these data illustrate the advantage of using the ALT approach to estimate chronic effect levels when actual chronic data are not available. However, even the ALT approach can be sensitive to individual data sets given the difference in calculated CLC1 and resultant ACRs observed for rainbow trout and bull trout exposed to picloram in the present study.

No chronic toxicity data exist in the literature that evaluate the sensitivity of rainbow trout or bull trout exposed to

2,4-D. Holcombe et al. [23] exposed Japanese medaka (*Oryzias latipes*) to 2,4-D and reported a 96-h ALC50 of 2,780 mg/L and an average 28-d chronic value (two tests) of 41 mg/L; the calculated average ACR was 68. Holcombe et al. [23] indicated that Japanese medaka are far less sensitive to both 2,4-D and phenol compared to rainbow trout on an acute basis. However, Japanese medaka were similar in chronic sensitivity to 2,4-D compared to rainbow trout and bull trout used in the present study. As a result, our calculated ACRs for rainbow trout (9) and bull trout (5) are lower by a factor of 7 to 14 compared to Japanese medaka. Chronic values for Japanese medaka were based on the results of ANOVA and are therefore limited in interpretation as expressed previously. Probable interspecies differences preclude further comparison of these data.

No chronic data exist for clopyralid in the literature for any fish species. This is most likely due to the low acute toxicity of the herbicide compared to other commonly used herbicides such as 2,4-D and picloram. Our data for rainbow trout and bull trout were nearly identical for all acute and chronic endpoints largely because of the strong effect of concentration (e.g., B parameter of the ALT model), which was similar for both species.

CONCLUSIONS

The threatened bull trout and standard surrogate rainbow trout were similar in acute sensitivity (within a factor of two) when exposed to picloram, 2,4-D, and clopyralid for 96 h. Relative herbicide toxicity decreased in the order of picloram, 2,4-D, and clopyralid. These results indicate that rainbow trout is a good surrogate test species for the threatened bull trout. The fractional acute model overestimated herbicide toxicity compared to the statistically interpolated 96-h ALC5 by an order of magnitude because of the steep slope of the concentration–response curve. The ALT model (30-d CLC1 endpoint) provided estimates of safe concentrations of herbicides that appear conservative (range 1–30) compared to the statistically interpolated 96-h ACL5. Comparisons of predicted no-effect levels derived from the ALT model to the literature were limited by two factors: lack of actual published data and the difficulties in comparison of data due to statistical methodology (ANOVA) most commonly used in the literature. Accelerated life testing procedures made maximum use of acute toxicity data by using the entire mortality:concentration:time matrix and provided cost-effective and statistically defensible estimates of the potential chronic toxicity of picloram, 2,4-D, and clopyralid to rainbow trout and the federally listed threatened bull trout. We have conducted additional flow-through acute and chronic studies with these herbicides to further examine effects on growth and survival. The results of these studies are currently in preparation for publication and will further examine the utility and accuracy of the ALT model in prediction of actual chronic effects of these herbicides on salmonids.

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