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Toxicity of R-11® Surfactant to Juvenile Rainbow Trout: Does Size Matter?

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Few studies have examined the relationship between fish size and the results of standardized toxicity tests. Available data suggest large fish may respond differently than small fish to chemicals under comparable test conditions, and that the relationship is not consistent. Anderson and Spear (1980) found that toxicity of copper to pumpkinseed sunfish (*Lepomis gibbosus*) decreased with increasing fish size, whereas toxicity to rainbow trout (*Oncorhynchus mykiss*) did not. Gupta (1987, 1988) tested industrial effluents with common carp (*Cyprinus carpio*) and spotted snakehead (*Channa puntatus*) and found that increasing size resulted in an increase in the LC50s. Person-LeRuyet et al. (1995) observed no size-dependent response to ammonia levels for seabass (*Dicentrarchus labrax*), seabream (*Sparus quarata*), and turbot (*Scophthalmus maximus*). Similar comparisons for surfactants are lacking.

Surfactants are added to herbicide tank mixes to improve the wetting, spreading, and dispersing properties of the active ingredient (Hazen 2000). They frequently represent the most toxic component of herbicide tank mixes and can be orders of magnitude more toxic than the active ingredient to aquatic species (Giesy et al. 2000 and references therein). New state permitting processes in response to a recent Federal Court ruling (*Headwaters, Inc. v Talent Irrigation District*, 9th Circuit Court of Appeals 2001) require states to issue National Pollutant Discharge Elimination System permits for the use of pesticides and adjuvants (including surfactants) in aquatic systems. Unfortunately, adequate data on the toxicity of surfactants to aquatic resources are lacking, thereby questioning the validity of the permitting process and the success of IPM strategies. The objective of the present paper was to compare the toxicity of the surfactant R-11® (Wilbur-Ellis Co., Fresno, CA; 90% akyl aryl polyethoxylates, compounded silicone and linear alcohol; 10% constituents ineffective as spray adjuvant) to two different sizes of juvenile rainbow trout (ca. 0.4 vs. 15 g) under standardized test conditions.

MATERIALS AND METHODS

We conducted static 96-hr acute toxicity tests (USEPA 1996) with R-11 and the two different sizes of juvenile rainbow trout within an environmental chamber (Bally Engineered Structures, Inc., Sparks, NV), July–August 2002. Average weights for the two size classes varied by about 40x; lengths varied 3x (Table 1). Effective concentrations (LC50s, and EC50s for mortality + behavioral effects) and associated test statistics were determined for each size class using S-Plus (MathSoft, Inc., Cambridge, MA), DOSECOMP (Link et al. 1996), and Excel (Microsoft Corp., Redmond, WA).

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Table 1. Average (±SD) weight (g) and length (mm) of juvenile rainbow trout tested. Fork length was measured except in the Range Finder in which the measurement was total length.

	N	Weight	Length
Small Juvenile	39	0.39 ± 0.07	35.69 ± 2.03
Large Juvenile			
Range Finder	15	7.75 ± 1.23	91.20 ± 5.85
Definitive	50, 39	15.46 ± 0.94	100.18 ± 6.92

Small juveniles—Small juvenile rainbow trout (Table 1) were obtained from Troutlodge, McMillan, WA. Upon receipt, the fish (ca. 500) were acclimated to flowing dechlorinated city water (12–14°C, pH = 6-8, DO ≥ 5) for at least 5 d prior to test initiation. Fish were fed (Nutra Starter Feeds #0 crum, Moore Clark, Vancouver, BC) to satiation until 2 d before test initiation.

Prior to each test, aquaria (7.6-L [2-gal], HPPI Ropak buckets) were cleaned (Argentyne, Argent Chemicals, Redmond, WA), then rinsed and conditioned with dechlorinated water for at least 24 hr. A random number was generated for each treatment (surfactant concentrations and negative control) and each aquaria was randomly assigned a position in the environmental chamber. Stock solution of the surfactant was prepared the morning of the test, and the appropriate amounts of stock solution and dechlorinated water were added to aquaria to achieve the desired concentration and a water volume to fish weight ratio of 1.8 L/g. Ten trout were randomly distributed into each of the aquaria. Aquaria were covered with a lid propped open on one end with a vinyl-coated clip. At test termination the control fish were weighed (g) and measured (fork length in mm).

We used four replicates of five geometrically arranged concentrations (3.5–8.5 ppm; 1.25x) and a negative control. Water quality was measured on a rotating schedule from lowest to highest concentrations such that each aquarium was tested at least twice. Number and condition of live fish and number of dead fish were recorded and dead fish removed at 6, 24, 48, 72, and 96 hr. Water quality data were collected at 0, 24, 48, 72, and 96 hr (Table 2). Light intensity within the aquaria varied from 7.0 to 23.9 FC.

Large juveniles—Large juvenile rainbow trout were obtained from Troutlodge, McMillan, WA in March 2002 and reared in our environmental chamber under flowing water conditions until testing was conducted during the summer. Fish were fed to satiation daily until 2 d before test initiation. Light intensity within the test buckets varied from 7.0 to 30.4 FC.

A range finder was conducted prior to the definitive test for large trout. For the range finder, aquaria (15.2-L [4-gal], HPPI Ropak buckets) were cleaned with Argentyne then rinsed and conditioned with dechlorinated water for at least 24 hr. A random number was generated for each treatment (surfactant concentrations and negative control) and each aquaria randomly assigned a position in the environmental chamber. Aquaria were then filled with the appropriate amount of dechlorinated water. All fish were weighed to ensure fish size of 5.5–10 g. Fish were placed singly in aquaria and left to cool to ambient temperature (12 ± 2°C) for 24 hr prior to test initiation. Aquaria were covered with lids with four ventilation holes. Fish were placed in the aquaria a day early to acclimate to the cooler ambient temperature in the environmental chamber compared with that (14–17°C) of the flowing dechlorinated water in the fish holding tank. Stock solution of the surfac-

Table 2. Environmental conditions during 96-hr LC50 tests with small (average weight = 0.39 g) and large (Range Finder: average weight = 7.75 g; Definitive Test: 15.46 g) juvenile rainbow trout exposed to the surfactant, R-11.

Test	Treatment	N	Temperature (°C) Av/Min Max	pH Av/Min Max	DO (mg/L) Av/Min Max
Small Juvenile	Control	10	13.2 12.5 14.8	6.32 6.05 6.52	8.74 7.49 9.27
	R-11	37	13.3 12.4 14.9	6.33 6.05 6.60	6.47 4.50 8.70
Large Juvenile					
Range Finder	Control	10	13.6 12.6 14.8	6.44 6.25 6.75	8.18 7.01 10.22
	R-11	22	13.6 12.7 14.5	6.45 6.27 6.56	7.48 4.78 10.23
Definitive	Control	10	13.7 12.8 14.6	6.39 6.23 6.61	7.24 6.62 7.70
	R-11	37	13.8 12.6 14.8	6.37 6.16 6.67	6.10 4.38 9.72

tant was prepared 2 wk before the test. An equivalent amount of water was removed from each aquarium before the stock solution (≤50 ml) was added to ensure correct concentrations and a water volume to fish weight ratio of 1.8 L/g. Solutions within all aquaria, including controls, were stirred with a clean pipette.

We used 10 replicates of 5 geometrically arranged concentrations (4.4–25.9 ppm; 1.56x) and a negative control. Water quality was measured on a rotating schedule from lowest to highest concentrations so each aquarium was tested once. Condition of live fish and number of dead fish were recorded and dead fish removed at 6, 24, 48, 72, and 96 hr. Water quality was measured at 0, 24, 48, 72, and 96 hr (Table 2).

The protocol for the definitive test for the large trout was the same as the range finder and only differed in that the stock solution of the surfactant was prepared the morning of the test and placed into clean, 22.7-L aquaria (6-gal HPPI Ropak buckets). An amount of water equivalent to the stock solution added was not removed due to the small amount of stock solution (< 10 ml, 0.04%) needed to achieve the desired concentrations. Ten replicates of 4 geometrically arranged concentrations (4.4–6.4 ppm; 1.13x) and a negative control were used. Fish weighed 13–16 g resulting in maximum water volume to fish weight ratio of 1.6 L/g, given the average size of the fish and the aquaria.

RESULTS AND DISCUSSION

The 96-hr LC50 for the large juvenile trout was statistically greater than that of their smaller counterparts (Table 3). Slopes of the dose response curves were similar (Table 3; Fig. 1). Mortality through time is shown in Figure 2. The majority of the small fish succumbed within 24 hr; no mortality of large fish had occurred by that time. LC50s for R-11 in juvenile rainbow trout reported by others are 3.8 ppm (Ebasco Environmental

Table 3. LC50s and EC50s (erratic swimming [ES], on-bottom gilling [OBG], both with mortality) for small (average weight = 0.39 g) and large (Definitive Test: 15.46 g) juvenile rainbow trout exposed to the surfactant, R-11. LC50s and EC50s are in ppm.

	24 h			96 h		
	LC50	95% CI	Slope ± SE	LC50	95% CI	Slope ± SE
Small Juvenile						
LC50	5.53	0.24	5.63 ± 2.31	5.18	0.28	3.93 ± 1.30
EC50 (OBG + Mortality)	3.59	0.23	6.13 ± 1.23	4.70	0.30	3.61 ± 1.02
EC50 (ES + Mortality)	4.97	0.35	2.80 ± 1.46	4.93	0.29	3.84 ± 1.05
EC50 (All Inclusive)	3.26	1.08	--- [a]	4.44	0.30	3.72 ± 0.89
Large Juvenile- Definitive						
LC50				6.57	0.93	5.07 ± 2.28
EC50 (OBG + Mortality)				5.61	0.37	4.04 ± 1.92
EC50 (ES + Mortality)				5.81	0.47	3.28 ± 2.00
EC50 (All Inclusive)				5.10	0.23	5.41 ± 1.79

[a] Slope could not be determined using DOSECOMP

1993:52) and 6.0 ppm (95% CI = 5.7–6.2; Smith et al., *unpubl. ms*); the latter was conducted under very similar test conditions.

Two behavioral effects were observed during the tests: erratic swimming (ES) and on-bottom gilling (OBG). ES was characterized by an inability to maintain correct horizontal or vertical orientation to the line of movement and included fish that were "gulping" or coming to the surface and extending their heads above the water line. Fish displaying OBG lay on the bottom of the test chamber on their side or back with the only observable movement being the opening and closing of the mouth and opercular (gill) covering. Some of these fish were able to swim when disturbed, but quickly returned to the bottom. The onset of ES and OBG was dose-related. For example, only 20% of the small fish exposed to 3.5 ppm R-11 were overtly affected at 6 hr, whereas all fish exposed to 4.4 ppm were affected at that time. Sixty percent of the large fish in the highest dose level displayed behavioral effects at 6 hr, whereas similar effects were not observed at lower chemical concentrations until 24 hr (Fig. 2).

ES was just as likely as OBG to be the initial overt effect in the small trout. However, affected large fish initially showed erratic swimming more often than OBG (Fig. 2). Fifty-eight percent of the fish suffering OBG displayed ES first. All large fish that died displayed OBG. However, OBG was not necessarily a precursor to death; some continued OBG for the duration of the test (31%); others recovered to ES (24%) or appeared normal (3%). The experimental design for the small fish prevented a similar comparison. The proportion of affected fish (small and large) showing recovery was also dose related. Some recovery to normal was observed in the small fish at the three lowest concentrations and in the large fish at the two lowest concentrations. In most cases, recovery from OBG to normal was preceded by ES. A good example of recovery can be seen in Figure 2 (5 ppm R-11). The only case where fish appeared to recover directly from OBG to normal was in the small fish exposed to 4.4 ppm.

The acute toxicity of non-ionic surfactants that are polyethoxylated derivatives of

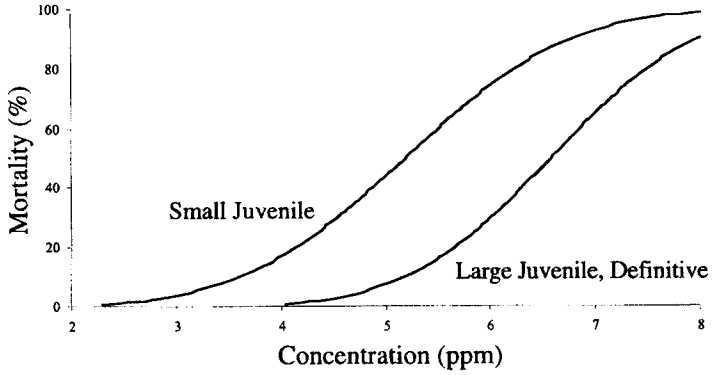

Figure 1. Predicted dose response curves for small (average weight = 0.39 g) and large (Definitive Test: average weight = 15.46 g) juvenile rainbow trout exposed to the surfactant, R-11.

alkylphenols and polyalkoxylated fatty alcohols, such as R-11, to aquatic organisms appears to be related to chemical-induced non-specific narcosis (reviewed by Mann and Bidwell 2001) defined as a "reversible state of arrested activity ..." (Veith et al. 1983) characterized by lethargy and unconsciousness (Hecht and Boese 2002). Calamari and Marchetti (1973) observed a response similar to OBG they described as "a condition of apparent death persisting with overturning for a very long time..." in juvenile rainbow trout exposed to nonylphenol. Similar narcoses have been reported in other taxa including amphipods (Hecht and Boese 2002) and snails (Talmage 1994) exposed to nonylphenol, and tadpoles (Mann and Bidwell 2001) exposed to nonylphenol ethoxylate and alkoxylate surfactants. Recovery during chemical exposure, such as that seen in our study and that of Mann and Bidwell (2001), may be the result of adaptive changes in the composition of membranes within poikilotherms similar to those in fish associated with varying environmental temperatures (reviewed by Mann and Bidwell 2001).

EC50s for OBG, ES, and OBG+ES with mortality are given in Table 2. The addition of behavioral effects resulted in a 14–41% reduction in the median effective concentrations. Recovery is illustrated in a comparison of 24-hr and 96-hr EC50s for OBG and both behavioral effects+mortality in the small trout. The 24-hr and 96-hr EC50s for ES+mortality in these fish were similar because of recovery of OBG fish to ES status. The small fish that were ES at 24 hr were most likely not the same fish that were ES at the end of the test. This was more obvious in our large trout in which no fish displaying only ES continued to do so through the test; all recovered by 96 hr. The survival implications of ES, OBG, and the similar narcoses observed by others are not known, but may not necessarily translate to death. The duration of effects, the extent to which avoidance strategies are impaired, and the probability of encounters with predators likely govern the outcome (for review, see Grue et al. 2002).

Dissolved oxygen (DO) levels dropped below the EPA criterion of 5 mg/L eight times (17%) (mean = 4.70, SD = 0.17, extremes = 4.38–4.95) during the large definitive test. Three of the low values occurred at 48 hr, 2 at 72 hr, and 3 at 96 hr. Low DO did not appear to be related to location in the environmental chamber or water temperature ($R^2_{\text{water temp}} = 0.00$, P = 0.80), but generally decreased during the test ($R^2_{\text{time}} = 0.33$, P =

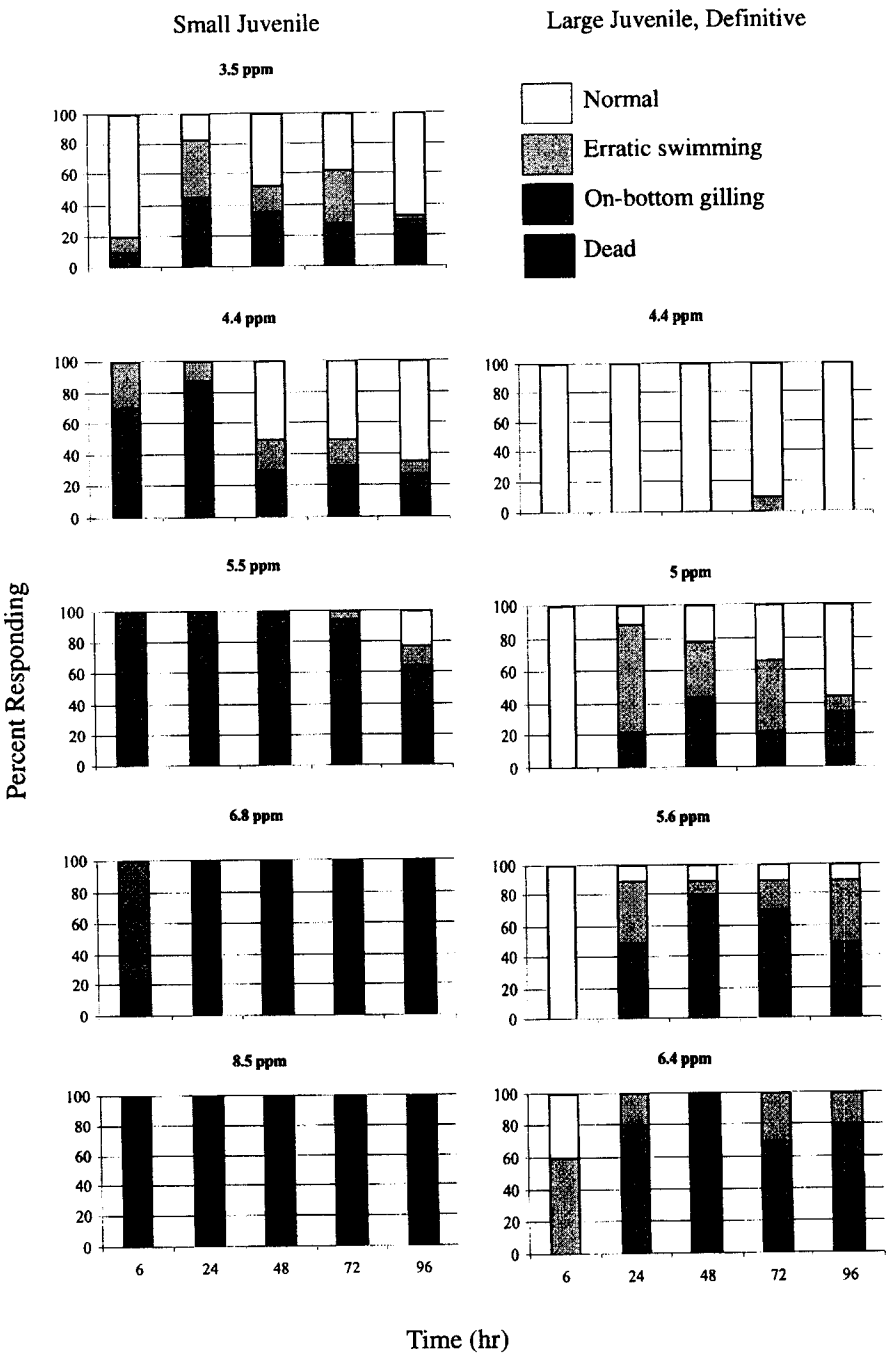

Figure 2. Occurrence of erratic swimming (ES) and on-bottom gilling (OBG) in small (average weight = 0.39 g) and large (Definitive Test: 15.46 g) juvenile rainbow trout exposed to the surfactant, R-11.

2.44E-05) and only occurred in the R-11 treatments. DO in the controls was ≥ 6 mg/L. DO levels in the R-11 treatments were significantly different from controls but not from each other (ANOVA: df = 4, F = 4.03, P = 0.007 with Dunnett's Test). Although water volume/g fish in this test was 11% less than that in the small fish test, it was much greater (1.3x) than the EPA test criterion of 1 L/0.8 g. None of the fish exposed to the low DO levels succumbed during the test, and ES and OBG were observed irrespective of O_2 levels. Our results suggest the low DO observed was the result of chemical-induced reduction in O_2 within the test water, chemical-induced changes in the respiratory processes of the fish, or a combination thereof. Bradbury et al. (1989) examined the respiratory-cardiovascular responses of rainbow trout exposed to phenol, a polar narcotic, and found the chemical increased both the cough frequency and total oxygen consumption of fish. Others (for recent review, see Wood 2001) suggest that observed increases in ventilation rates in fish exposed to anionic and non-ionic detergents are a result of irritant effects on the ventilatory control system or respiratory blockade.

The absolute difference in the toxicity of R-11 to the two sizes of trout we tested was small (27%) in comparison to the difference in the size of the fish (ca. 40x). Statistically significant differences reported by others for 96-hr static, static-renewal, or flow-through tests and a variety of fish species vary greatly. However, none showed an increase in toxicity with fish size under comparable test conditions. Anderson and Spear (1980) reported no difference in toxicity of copper to juvenile rainbow trout despite a 45x difference in weight (3.9–176 g). In the same study, however, a 3.5x increase in size of pumpkinseed sunfish (1.2–7.6 g) resulted in a 37% increase in the LC50. A 5x increase in size (6.0–28 cm) was equated with a 95–289% increase in tolerance of common carp and 78–290% increase in that of spotted snakehead when exposed to six effluents (Gupta 1987, 1988). Person-LeRuyet et al. (1995) found that a 26x increase in size (6–160 g) did not affect the toxicity of ammonia to seabass, seabream and turbot. Within the compilation by Mayer and Ellersieck (1986), we reviewed data for test conditions and sizes of rainbow trout comparable to those in our study. Chlordane (29x, 0.8–23.5 g, 60%) 2,4-DB (3.25x, 0.8–2.6 g, 214%) and temephos (20x, 1.2–23.5 g, 375%) showed a decrease in toxicity with increasing fish size, whereas aldicarb (5.4x, 0.5–2.7 g) and antimycin A (15x, 0.7–10.8 g) did not show a difference. Results suggest that within the EPA test criterion (< 3 g), differences in 96-hr LC50s due to fish size may be as great as 200% and exceed that attributed to variability among comparable tests (e.g., 16% between our test and that of Smith et al., *unpubl. ms*).

R-11 is used operationally with the herbicide Rodeo® (ai: glyphosate, Dow AgroSciences Indianapolis, IN) in aquatic systems that support salmonids of the sizes we tested. Irrespective of the size of fish we tested, the toxicity of the surfactant was 1-2 orders of magnitude greater than the active herbicidial ingredient ($LC50_{IPA\ salt}$=140->1000; Geisy et al. 2000). However, when R-11 is used at the recommended percentage of the tank mix (1.25%: 4.7 L [1.25 gal]/379 L [100 gal]) and maximum application volume (187 L/ha, 20 gal/ac), the total water depths toxic to small and large juveniles (LC10) would be ≤65 mm (after Smith et al., *unpubl. ms*).

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