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# Toxicity of Four Surfactants to Juvenile Rainbow Trout: Implications for Use over Water

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Herbicides are frequently used to control exotic or nuisance aquatic plants. Utilization of herbicides in Integrated Pest Management (IPM) to control aquatic weeds has been hampered by concerns over the non-target toxicity of active herbicidal ingredients. However, surfactants frequently represent the most toxic component of herbicide tank mixes and can be orders of magnitude more toxic to aquatic organisms than the active ingredient (Giesy et al. 2000 and references therein). New state permitting processes within the western United States mandated by a recent Federal Court ruling (*Headwaters, Inc. v Talent Irrigation District*, 9th Circuit Court of Appeals 2001) require affected states to issue National Pollutant Discharge Elimination System permits for the use of pesticides and adjuvants in aquatic systems. Unfortunately, adequate data on the toxicity of surfactants to aquatic resources are lacking, thereby threatening permitting processes and the success of IPM strategies.

Herein, we compare the toxicity of four surfactants to juvenile rainbow trout (*Oncorhynchus mykiss*) using effects on survival and behavior as endpoints. We then compare concentrations found to be toxic in the laboratory with concentrations expected in aquatic environments.

## MATERIALS AND METHODS

The surfactants selected are either currently registered for or proposed for use in aquatic systems (Table 1). They represent several classes of surfactants commonly in use (Miller and Westra 1996). EPA classification of the active ingredients ranges from unavailable (AGRI-DEX and HASTEN) to minimal concern (R-11 and LI 700) (USDA 1997).

Static 96-hr acute toxicity tests (USEPA 1996) were conducted with the four surfactants within an environmental chamber (Bally Engineered Structures, Inc., Sparks, NV), January–March 2002. Juvenile rainbow trout, 4–6 wks old (Table 2) were obtained from Troutlodge, McMillan, WA. Upon receipt, the fish were acclimated to flowing dechlorinated city water (8±2°C, pH=6-8, DO≥5) for 5 d prior to test initiation. Fish were fed (Nutra Starter Feeds #1 crum, Moore Clark, Vancouver, BC) to satiation until Day 3 and then fasted for 48 hr. Twenty-four hr before test initiation the flow was turned off and the water in the acclimation tank (ca. 353 L [93 gal]) was allowed to warm to ambient temperature within the environmental chamber (12±1°C). A subsample of 50 fish were weighed and measured immediately before test initiation to ensure a water volume to fish weight ratio of 1.8 L/g.

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**Table 1.** Source and chemical composition of surfactants tested.

| Chemical name | Company | Aquatic label | Composition |
|---|---|---|---|
| R-11® | Wilbur-Ellis Co., Fresno, CA | Yes | 90% alkyl aryl polyethoxylates, compounded silicone and linear alcohol; 10% other (constituents ineffective as spray adjuvant) |
| LI 700® | Loveland Industries, Greeley, CO | Yes | 80% phosphatidylcholine, methyl-acetic acid, and alkyl polyoxyethylene ether, 20% other |
| HASTEN® | Wilbur-Ellis Co., Fresno, CA | Pending | 100% esterified vegetable oil and non-ionic surfactant blend |
| AGRI-DEX® | Helena Chemical Co., Memphis, TN | Pending | 99% heavy range paraffin base petroleum oil, polyol fatty acid esters, polyethoxylated derivatives thereof, 1% other |

Prior to each test, aquaria (7.6 L [2 gal], HPPI Ropak buckets) were cleaned (Argentyne, Argent Chemicals, Redmond, WA), rinsed, and conditioned with dechlorinated water for 24 hr. A random number was generated for each treatment (surfactant/concentration, negative control replicate) and each aquarium was randomly assigned a position in the environmental chamber. Stock solutions of the surfactants were prepared 24 hr before each test and stirred overnight. Approximately 3.5 hr before each test, aquaria received the appropriate amount of stock solution and dechlorinated water to achieve the desired concentration and water volume to fish weight ratio. Aquaria were allowed to warm to ambient temperature, at which time 10 trout were randomly distributed into each aquarium. Aquaria were covered with a lid propped open on one end with a vinyl-coated clip.

We used four replicates of 3–5 geometrically arranged concentrations and a negative control for each surfactant: R-11, n=5: 3.5–8.5 ppm, 1.25x; LI 700, n=5: 16–33 ppm, 1.2x; HASTEN, n=3: 68–90 ppm, 1.15x; AGRI-DEX, n=5: 300–525 ppm, 1.15x. To avoid cross contamination among test aquaria when taking water quality measurements, measurements were confined to three additional aquaria with 10 fish each at the low, middle and high chemical concentrations per surfactant and a negative control. Fish were monitored after 6, 24, 48, 72, and 96 hr. Number and condition of live fish and number of dead fish were recorded and dead fish removed. Because of a lack of visibility in the AGRI-DEX and HASTEN treatments, fish were gently sieved and observed at times comparable with those used for R-11 and LI 700. Water within the aquaria was slowly poured through an aquarium net into a clean aquarium. Dead fish were removed and live fish returned to the test solution. Water quality (dissolved oxygen [DO], pH, temperature, and light intensity) measurements were collected at the time fish were monitored.

**Table 2.** Lengths and weights (means ± SD, n=50) of juvenile rainbow trout tested.

| Test chemical | Length (mm) | Weight (g) |
|---|---|---|
| R-11 | 29.74 ± 1.80 | 0.22 ± 0.05 |
| LI 700 | 31.74 ± 1.84 | 0.30± 0.06 |
| AGRI-DEX and HASTEN | 32.55 ± 1.93 | 0.39 ± 0.08 |

**Table 3.** Environmental conditions during 96-hr LC50 tests with juvenile rainbow trout and four common surfactants.

| Treatment | Temperature C | | | pH | | | Dissolved oxygen (mg/L) | | |
|---|---|---|---|---|---|---|---|---|---|
| | Av | Min | Max | Av | Min | Max | Av | Min | Max |
| Control | 12.77 | 11.5 | 13.6 | 6.6 | 6.4 | 6.7 | 9.20 | 7.85 | 11.50 |
| R-11 | 12.29 | 11.4 | 13.3 | 6.6 | 6.5 | 6.8 | 8.11 | 5.95 | 9.37 |
| Control | 12.03 | 10.3 | 13.0 | 6.4 | 6.2 | 6.7 | 8.81 | 8.00 | 9.96 |
| LI 700 | 12.01 | 10.8 | 12.9 | 6.3 | 6.0 | 6.5 | 8.67 | 6.70 | 10.45 |
| Control | 11.59 | 10.3 | 12.2 | 6.5 | 6.4 | 6.6 | 9.28 | 7.53 | 11.60 |
| HASTEN | 12.05 | 11.3 | 12.8 | 6.4 | 6.1 | 6.5 | 8.11 | 2.74 | 11.60 |
| AGRI-DEX | 11.72 | 10.4 | 12.3 | 6.4 | 6.3 | 6.6 | 8.84 | 6.11 | 11.40 |

Median lethal concentrations (24- and 96-hr LC50s) and associated test statistics were determined using S-Plus (MathSoft, Inc., Cambridge, MA), DOSECOMP (Link et al. 1996), and Excel (Microsoft Corp., Redmond, WA). EC50s (24- and 96-hr) for observed behavioral effects + mortality were also calculated for R-11 and LI 700.

## RESULTS AND DISCUSSION

Values for water quality parameters during the toxicity tests (Table 3) were within test criteria (USEPA 1996): temperature 12±2°C, pH 6–8, and DO≥5.0 mg/L with few exceptions. On two separate occasions, DO in aquaria with HASTEN dropped below test criteria (DO = 4.90 mg/L at 72 hr and 2.74 at 96 hr). However, low DO may not have contributed to mortality. All fish were alive in the aquarium with the lowest DO reading; 3 of 6 were alive in the aquarium with DO at 4.90 mg/L. Light intensity varied between 4.0 and 26.0 FC with averages for the different surfactants of 7.03–17.44 FC.

Median lethal concentrations (24- and 96-hr LC50s) and associated test statistics for the four surfactants are given in Table 4. At both 24- and 96-hr, the toxicity of R-11>LI 700>HASTEN>AGRI-DEX. LC50s differed by more than tenfold. Slopes for the dose response curves shown in Figure 1 were not statistically different (p >0.05, Table 4). Most fish exposed to the surfactants succumbed within the first 24 hr (Fig. 2). This was particularly true for R-11 and is reflected in the ratio of the 96- and 24-hr LC50s (1.02, Table 4).

Our 96-hr LC50 for R-11 (6.0 ppm) is 13% and 37% greater than those reported by others (5.2 ppm; Curran et al. 2004; 3.8 ppm; USDA 1997). In contrast, our values for LI 700 (17 ppm) and AGRI-DEX (271 ppm) are only 13% and <27% of those previously reported (130 and >1000 ppm; USDA 1997). Without published information on how some of the earlier tests were conducted, it is not possible to explain these differences. A number of factors can affect test results (for review, see Mayer and Ellersieck 1986:5-28). However, irrespective of the source of the data, the relative rankings of R-11, LI 700 and AGRI-DEX remained the same. Comparable data for HASTEN are lacking.

Two behavioral effects were observed during the tests: erratic swimming (ES) and on-bottom gilling (OBG), although only the latter was consistently recorded, and only in the R-11 and LI 700 treatments. ES was characterized by an inability to maintain cor-

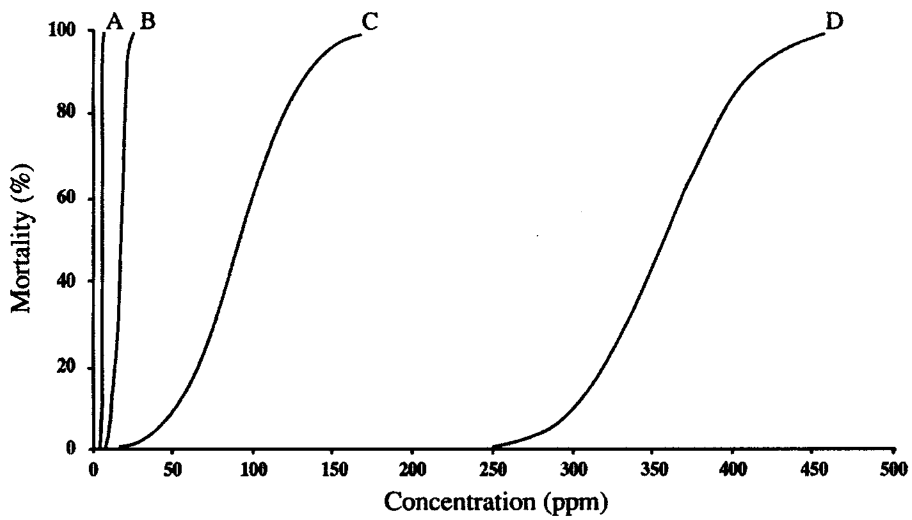


**Figure 1.** Dose response curves for juvenile rainbow trout exposed to four common surfactants: R-11 (A), LI 700 (B), HASTEN (C), and AGRI-DEX (D).

**Table 4.** LC50s in ppm (24-, 96-hr, 95% CI) and slopes (± SE) of dose response curves for rainbow trout exposed to four common surfactants. EC50s in ppm (behavioral effects [OBG] + mortality) and associated statistics are given for R-11 and LI 700. In some cases (—) slopes could not be calculated because of a lack of incremental response (Fig. 2).

| Surfactant | Test | 24 h | 96 h | 96 h:24h |
|---|---|---|---|---|
| R-11 | LC50 | 5.9 | 6.0 | 1.02 |
| | | (4.1-7.7) | (5.7-6.2) | |
| | | — | 6.7 ± 9.9 | |
| | EC50 | 2.3 | 4.4 | |
| | | (1.8-2.8) | (4.2-4.6) | |
| | | — | — | |
| LI 700 | LC50 | 22 | 17 | 0.77 |
| | | (21-23) | (14-21) | |
| | | 4.5 ± 0.8 | 5.2 ± 0.8 | |
| | EC50 | 17 | 17 | |
| | | (16.5-17.5) | (16-18) | |
| | | 12.2 ± 1.94 | 5.1 ± 1.36 | |
| HASTEN | LC50 | 98 | 74 | 0.76 |
| | | (87-110) | (71-76) | |
| | | 4.2 ± 1.3 | 7.7 ± 3.9 | |
| AGRI-DEX | LC50 | 386 | 271 | 0.70 |
| | | (359-413) | (237-304) | |
| | | 2.4 ± 2.2 | 3.0 ± 1.4 | |

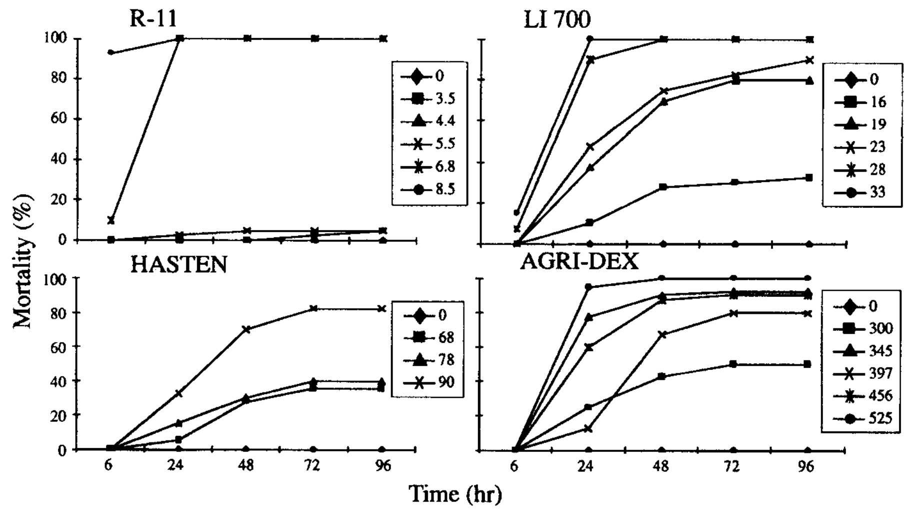

**Figure 2.** Cumulative mortality of juvenile rainbow trout exposed to four common surfactants. Symbols denote ppm.

rect horizontal or vertical orientation to the line of movement. 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 OBG was dose-related. For example, only 10% of the fish exposed to 3.5 ppm of R-11 were overtly affected at 6 hr, whereas 88% of the fish exposed to 4.4 ppm of R-11 were affected at 6 hr (Fig. 3).

EC50s (OBG + mortality) for R-11 and LI 700 are given in Table 4. The 24-hr EC50 for R-11 was statistically lower (48%) than the 96-hr EC50, whereas comparable values for LI 700 did not differ. The former reflects recovery in some fish suffering OBG in the R-11 treatments (Fig. 3). The 24- and 96-hr EC50s for LI 700 were more similar to the respective LC50s (79% and 100%) than comparable values for R-11 (39% and 73%). The proportion of affected fish showing recovery was dose-related. Some recovery to normal was observed at the three lowest concentrations of R-11 (e.g., 4.4 ppm, Fig. 3). In a subsequent, more comprehensive study of behavioral responses, similar effects were observed in two sizes of juvenile rainbow trout exposed to R-11 (Curran et al. 2004).

The acute toxicity of non-ionic surfactants that are polyethoxylated derivatives of 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 others (Mann and Bidwell 2001; Curran et al. 2004), may be the result of adaptive

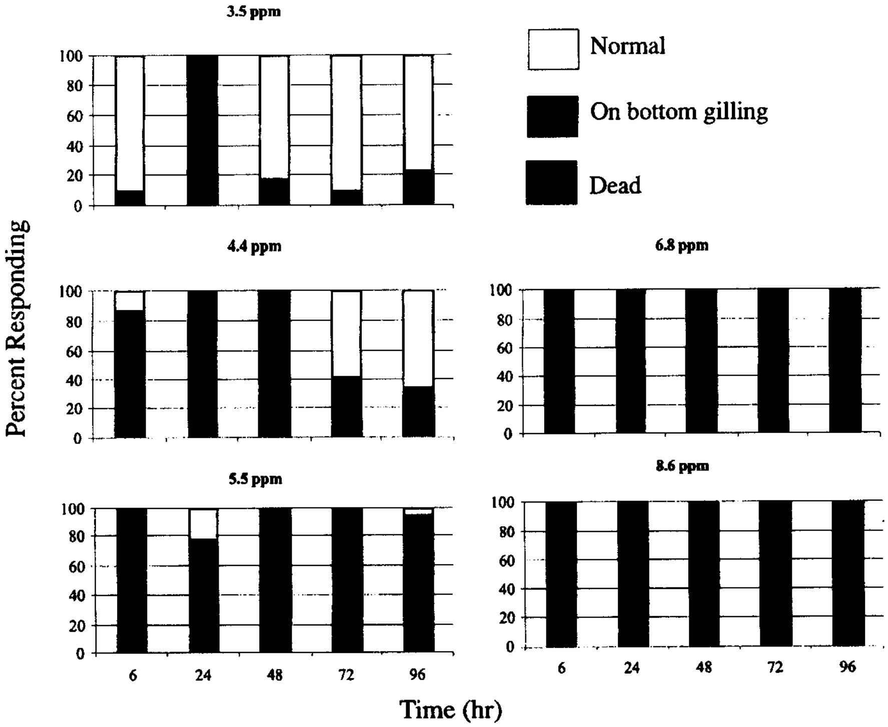


**Figure 3.** Occurrence of on-bottom gilling (OBG) in juvenile rainbow trout exposed to the surfactant, R-11.

changes in the composition of membranes within poikilotherms similar to those in fish associated with varying environmental temperatures (reviewed by Mann and Bidwell 2001). In the case of LI 700, fish suffering OBG did not recover and therefore the behavioral effect shown in these fish does not fit the definition and conditions described above for polyethoxylated derivatives of alkylphenols and polyalkoxylated fatty alcohols.

The survival implications of ES, OBG, and the similar narcoses observed by others are not known. Duration of effects, predator to which avoidance strategies are impaired, and probability of encounters with predators will likely govern the outcome (Grue et al. 2002).

Labels of all four surfactants specify a surfactant concentration as a fixed percentage of the tank mix (0.5–5%). However, delivery volumes of tank mixes per unit area may vary within and among herbicide labels. To compare the expected concentrations of the four surfactants relative to water depth (Fig. 4), we used the maximum tank mix application volume (187 L /ha [20 gal/A]) for the aquatic herbicide, Rodeo® (Dow AgroSciences, Indianapolis, IN) and the minimum and maximum application rates from the surfactant labels such that ppm surfactant within the water column = $[[(TM/A) * (S/TM)] / [(TM/A) + W]] * 10^6$, where S = L surfactant within TM (total volume of tank mix in L]), A = total area treated ($m^2$), and W = total depth of standing water (mm [L/$m^2$]) within A. We chose Rodeo because two of the surfactants have been used operationally with the herbicide to control smooth cordgrass (*Spartina alterniflora*) and purple loosestrife (*Lythrum salicaria*) in Washington State and elsewhere.

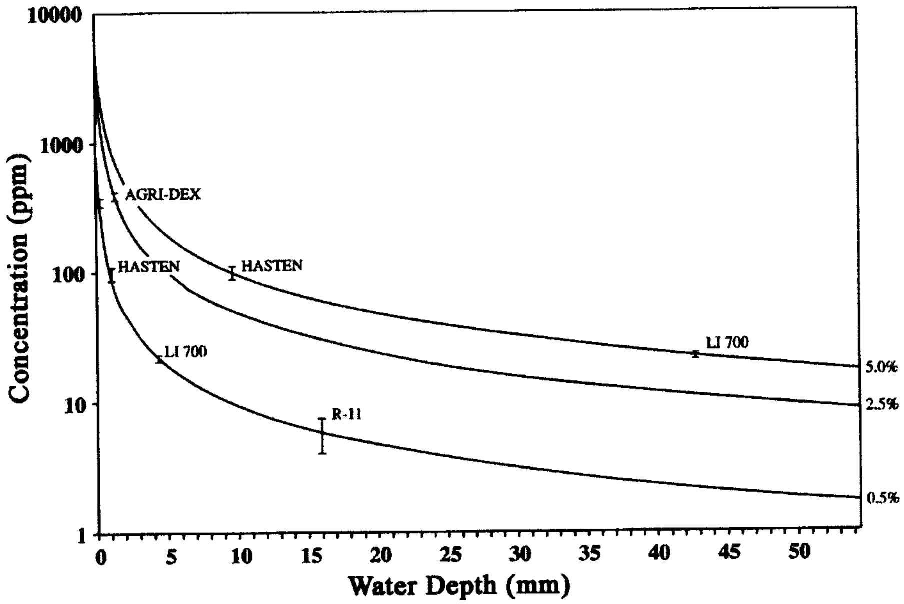


**Figure 4.** Expected concentrations of four common surfactants relative to total water depth based on their respective manufacturer's labels (0.5-5.0% of tank mix volume) and a tank mix application volume of 187 L/ha (20 gal/A). Vertical bars denote 95% CI of LC50s.

At the minimum recommended percentage of surfactant in the tank mix (0.5%: 1.9 L [2 qts]/380 L [100 gal]), water depths at which 50% of exposed trout would be expected to die would be <16 mm for R-11 and <5 mm for the other three surfactants. If the maximum recommended value for AGRI-DEX (2.5% of tank mix) were used, the effective water depth would remain <5 mm. If the maximum recommended value for HASTEN and LI 700 (5.0%) were used, the effective water depth for HASTEN would increase to ca. 10 mm and that for LI 700 would increase to ca. 43 mm. In comparison with the maximum recommended value on the R-11 label, LI 700 would then pose the greater environmental hazard. Each of the surfactant labels recommends that the label for the herbicide also be consulted. In the case of Rodeo, the current label does not specify a maximum volume for a surfactant and recommends 0.5% or more of the tank mix.

LI 700 has been considered the safest of the surfactants currently approved for over-water use. However, our results indicate that it is more toxic than HASTEN and AGRI-DEX, and depending on concentrations in the tank mix, LI 700 may pose the greatest environmental hazard to fish. To minimize non-target effects and assuming equivalent efficacy, we recommend AGRI-DEX over the other three surfactants. In addition, the toxicity to rainbow trout of each of the surfactants is greater than or equal to that of Rodeo (LC50=60–1100; Mitchell et al. 1987, Dow AgroSciences 2000) and its active ingredient, glyphosate ($LC50_{acid}$=22–197; $LC50_{IPA\ salt}$=140->1000; Giesy et al. 2000). Finally, we note that LC50s were designed to rank chemicals based on their relative toxicity under standardized conditions, not as a measure of environmental hazard (Mayer and Ellersieck 1986, Grue et al. 2002). More sensitive endpoints (e.g., endocrine disruption associated with exposure to nonylphenols, Servos 1999) may be necessary to fully appreciate the potential effects of surfactants in aquatic systems.

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