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**ACUTE TOXICITIES OF HERBICIDES USED TO CONTROL
WATER HYACINTH AND BRAZILIAN ELODEA ON LARVAL
DELTA SMELT AND SACRAMENTO SPLITTAIL**



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by

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SUMMARY

The herbicides Reward[®] (diquat), Komeen[®] (copper ethylenediamine complex) and Sonar[®] (fluridone) are used to control Brazilian elodea *Egeria densa*. The herbicides Rodeo[®] (glyphosate) and Weedar 64[®] (dimethylamine salt of 2,4-dichlorophenoxyacetic acid) and spray surfactant R-11[®] (alkylphenoethoxylates) are used to control water hyacinth *Eichhornia crassipes*. These are two invasive, exotic aquatic weeds that infest the Sacramento-San Joaquin Delta. Concern exists over possible lethal and sub-lethal effects that the herbicides and spray surfactant may have on larval Delta smelt *Hypomesus transpacificus* and Sacramento splittail *Pogonichthys macrolepidotus*, two federally-listed threatened species. Acute toxicity tests were conducted on the herbicides and surfactant using larval Delta smelt and larval Sacramento splittail. The toxicity values were compared to those for larval fathead minnow *Pimephales promelas*, a surrogate species that is used in monitoring the impacts of the herbicides and surfactant in the Sacramento-San Joaquin Delta. Based on 96-h LC₅₀ values, larval Delta smelt and larval fathead minnow were generally equally sensitive to the chemicals and larval Sacramento splittail were generally less sensitive. The surfactant R11[®] was more toxic than the herbicides, and Reward[®] and Komeen[®] were the most toxic herbicides tested. In herbicide/surfactant mixtures, acute toxicity was likely due to R-11[®]. Exposure levels of herbicides and surfactant in the Sacramento-San Joaquin Delta are several orders of magnitude less than the 96-h LC₅₀ values with the exception of Reward[®] and Komeen[®]. Larval fathead minnow sensitivity to the herbicides and surfactant suggests that this species is a good surrogate for testing toxicity to Delta smelt and Sacramento splittail.

TABLE OF CONTENTS

SUMMARY.....	i
TABLE OF CONTENTS.....	ii
LIST OF TABLES.....	iii
ACKNOWLEDGMENTS	iv
INTRODUCTION.....	1
METHODS.....	1
Test Organisms.....	1
Test Methods.....	2
Herbicide/ Surfactant Exposure.....	3
Statistics.....	3
RESULTS.....	4
DISCUSSION.....	6
LITERATURE CITED.....	9
APPENDIX A-Toxicity Test Results.....	11
APPENDIX B – Standard Operating Procedures.....	14

LIST OF TABLES

1.	LC ₅₀ values (mg/L) and confidence limits (C.L.) of herbicides and surfactant (active ingredient) to larval Delta smelt.....	4
2.	LC ₅₀ values (mg/L) and confidence limits (C.L.) of herbicides and surfactant (active ingredient) to larval fathead minnow.....	5
3.	LC ₅₀ values (mg/L) and confidence limits (C.L.) for herbicides and surfactant (active ingredient) to larval Sacramento splittail.....	5
4.	Fish sensitivity ratings to herbicides and surfactant. A unit of 1.00 is the most sensitive LC ₅₀ value, with lesser values representing a fraction of the most sensitive LC ₅₀ value.....	6
5.	Number of Toxic Units (LC _{50m} /LC _{50i}) of Rodeo [®] and R-11 [®] in mixture.....	6
6.	Number of Toxic Units (LC _{50m} /LC _{50i}) of Weedar 64 [®] and R-11 [®] in mixture.....	6
7.	Highest concentrations (mg/L) of herbicides and surfactant detected in 2002-2003 in Sacramento-San Joaquin Delta from EDCP and WHCP in comparison to LC ₅₀ Values (mg/L) for larval fish.....	7

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INTRODUCTION

The Sacramento-San Joaquin Delta is a heavily utilized recreational water way for boating in the Northern California region. Control of Brazilian elodea *Egeria densa* and water hyacinth *Eichhornia crassipes* has been a concern for the California Department of Boating and Waterways (DBW) in keeping these waterways clear of vegetation for boat passage. Both species of invasive aquatic weeds form dense growths that block waterways and destroy natural habitat by slowing water flow and drastically changing water quality. Brazilian elodea is controlled using Reward[®] (diquat), Komeen[®] (copper ethylenediamine complex), and Sonar[®] (fluridone) by the DBW Elodea Densa Control Program (EDCP). Water hyacinth is controlled using Rodeo[®] (glyphosate) and Weedar 64[®] (dimethylamine salt of 2,4-dichlorophenoxyacetic acid) with spray surfactant R-11[®] (alkylphenoethoxylates) by the DBW Water Hyacinth Control Program (WHCP).

Two federally-listed threatened fish species inhabit the Sacramento-San Joaquin Delta with Brazilian elodea and water hyacinth. Protection efforts for Delta smelt (*Hypomesus transpacificus* McAllister) and Sacramento splittail (*Pogonichthys macrolepidoyus* Ayres) appeared following drastic declines in Delta smelt populations in the early 1980's (Bennett and Moyle 1996). There has been a 60% decline in Sacramento splittail in the past 60 years (Moyle 2002). The U.S. Fish and Wildlife Service concerns for these threatened species precipitated the current study to generate toxicity values for the materials used to control the invasive weeds. The toxicity values for Delta smelt and Sacramento splittail were compared to those for larval fathead minnow, a surrogate species that is used in monitoring the impacts of the EDCP and WHCP in the Sacramento-San Joaquin Delta. The toxicity data were compared to the likely environmental concentrations of these chemicals to better assess the impacts of the control programs on these threatened species.

METHODS

Test Organisms

Delta smelt larvae were spawned and hatched in Tracy, California, at the Delta Smelt Project, University of California, Davis (UC Davis), Department of Animal Science (Bridges 2003). Larvae, 5 to 10-d old, able to feed, were delivered to California Department of Fish and Game (DFG) Aquatic Toxicology Laboratory (ATL), in Elk Grove, California. The smelt were maintained in a black, flow-through circular tank (30-gallon) supplied with non-chlorinated, aerated and temperature controlled (17°C) well

water. Water quality was 68 mg/L CaCO₃ hardness, 84 mg/L CaCO₃ alkalinity, 8.2 pH and 255 µmho/cm conductivity. Smelt larvae were held for 96 h prior to testing under a simulated natural photoperiod regime (16-h light:8-h dark, 50-100 ft-c), fed (5-10/mL) rotifers (*Brachionus plicatilis*) during the holding period, and maintained in green water using algae paste (Nannochloropsis 3600- premium fresh, Reed Mariculture Inc). Due to

the algae and turbidity requirements for feeding, no feeding was done during test periods (Bridges 2003). The smelt were 0.1 mg dry weight when tested.

Sacramento splittail larvae were spawned and hatched in UC Davis Aquatic Toxicology Program (Teh 2003). Larvae, 5 to 10-d old, able to feed, were delivered to DFG ATL. The splittail were maintained in a flow-through circular tank (30-gallon) supplied with non-chlorinated, aerated and temperature controlled well water (17° C). Water quality was 69 mg/L CaCO₃ hardness, 92 mg/L CaCO₃ alkalinity, 8.1 pH and 198 µmho/cm conductivity. Splittail were held for 96 h prior to testing under a simulated natural photoperiod regime (16-h light:8-h dark, 50-100 ft-c). Fish were fed (0.2g/10 fish) brine shrimp (*Artemia*) nauplii (less than 24-h old) daily during holding time. No feeding was done during test period (Teh 2003). The splittail were 0.3 mg dry weight when tested.

Fathead minnow larvae *Pimephales promelas* came from Aquatic Bio Systems, Inc, Fort Collins, Colorado, and were shipped to the DFG ATL within 48 h of hatching. Fathead minnow larvae were utilized for testing upon arrival at the laboratory and no pretest maintenance was required. Fish were examined upon arrival to determine that no more than 20% mortality had occurred. Water temperature was adjusted to 25° C at a rate of no more than 1° C per hour and no more than 4° C per day, and testing was performed under a simulated natural photoperiod regime (16-h light:8-h dark, 50-100 ft-c). Water quality was 78 mg/L CaCO₃ hardness, 81 mg/L CaCO₃ alkalinity, 8.2 pH and 206 µmho/cm conductivity. Fish were fed (0.1g/10 fish) brine shrimp nauplii (less than 24-h old) daily (U.S. EPA 1993). The minnow were 0.1 mg dry weight when tested.

Test Methods

The tests generally followed United States Environmental Protection Agency (USEPA) guidelines for larval fish testing (USEPA 1993; 1994). Delta smelt, Sacramento splittail and fathead minnow larvae were exposed to 5 concentrations of the chemicals in a dilution series (factor of 0.5) and a control (laboratory water).

Delta smelt and splittail were tested for 96-h using approved protocols (Appendix A). Forty fish were exposed per concentration, with four replicate test chambers per concentration (10 fish per chamber). Test temperatures were maintained at $17 \pm 1^{\circ} \text{C}$, and no feeding was done during the test. Test solutions were renewed at 48 h (USEPA 1993).

Fathead minnow were tested for 7-d using standard methods (Appendix A). Forty fish were exposed per concentration, with four replicate test chambers per each concentration (10 fish per chamber). Test temperatures were maintained at $25 \pm 1^{\circ} \text{C}$, and minnows were fed two to three times per day newly hatched *Artemia* nauplii. Test solutions were renewed daily (USEPA 1994).

Fish survival was recorded daily. Daily water quality (conductivity, dissolved oxygen, pH, and temperature) was measured for each treatment. Alkalinity and hardness

were measured for each batch of test solution. At the start of a test, fish dry weight was determined. After completion of the test, all surviving fish (fathead minnows only) were weighed to determine average dry weight per test chamber. The difference in weight was used to determine growth in the fathead minnow tests.

Herbicide and Surfactant Exposure

Fish were exposed to the individual herbicides and surfactant and to mixtures of Weedar 64[®] and R-11[®] and Rodeo[®] and R-11[®]. All materials tested were commercially available products used in the EDCP and WHCP:

Reward[®] (EPA Reg. No. 10182-353) produced by Zeneca Incorporated (37.3 % diquat dibromide).

Komeen[®] (EPA Reg. No. 1812-312) produced by Griffin Corporation (8 % copper from copper-ethylenediamine complex and copper sulfate pentahydrate).

Sonar[®] (EPA Reg. No. 67690-4) produced by SePRO Corporation (41.7 % fluridone).

Rodeo[®] (EPA Reg. No. 524-343) produced by Monsanto (53.8 % glyphosate).

Weedar 64[®] (EPA Reg. No. 71368-1-264) produced by Nufarm Incorporated (46.8 % 2,4-Dichlorophenoxyacetic acid).

R-11[®] (California Reg. No. 2935-50142-AA) produced by Wilbur-Ellis Company (90 % alkylphenolethoxylates as 80 % nonylphenol polyethoxylate [NPE], compounded silicon and linear alcohol).

Exposure levels of each chemical were confirmed by analyses at the DFG Water Pollution Control Laboratory. Samples were analyzed by high performance liquid chromatography and mass spectrometry except that copper was analyzed by atomic absorption spectrophotometry. The LC₅₀ values were based on concentrations of active ingredients in the commercial products. The active ingredient in R-11[®] was represented by the total concentration of nonylphenol polyethoxylate (NPE) and nonylphenol (NP). Percent recovery of spikes averaged 103 % for glyphosate, 100 % for NPE and NP, 104 % for 2, 4-D, 89 % for diquat, 99 % for fluridone, and 97 % for copper.

Statistics

The 96-h LC₅₀ values were derived from survival counts during the 96-h tests with Delta smelt and Sacramento splittail. The herbicide concentration and mortality data were analyzed by the Comprehensive Environmental Toxicity Information System (CETIS) statistical package (Tidepool 2002). A variety of techniques were utilized to estimate LC₅₀ values including Fisher's Exact T-test, two-point interpolation and linear interpolation. Herbicide and surfactant concentrations in the mixtures at the LC₅₀ values were interpolated from least-squares regressions (mixture concentration versus herbicide and surfactant concentration). The toxicity of herbicides and surfactant in mixtures were

expressed as toxic units (1 toxic unit of a chemical = 96-h LC₅₀ concentration of that chemical):

$$H_m/H_i + S_m/S_i$$

Where H is the herbicide, S the surfactant, i is the LC₅₀ value of an individual chemical tested separately, m is the LC₅₀ value of an individual chemical tested in a herbicide/surfactant mixture (Marking 1977). The chemical with the highest toxic unit (TU) was likely responsible for causing toxicity.

Both 96-h and 7-d LC₅₀ values were determined for the fathead minnow tests. Growth data from the 7-d tests were analyzed by unequal variance t (including Bartlett and Shapiro-Wilk W) to determine if significant effects occurred from the herbicides and the surfactant (Tidepool 2002).

The relative sensitivities of the three larval species to the herbicides and surfactant were assessed using fish sensitivity units. The lowest 96-h LC₅₀ value for each chemical was assigned the value of 1.00 and the higher LC₅₀ values were normalized as fractions (< 1.00) of the lowest LC₅₀ value.

RESULTS

The surfactant R-11[®] was the most toxic and the herbicide Rodeo[®] the least toxic material to larval Delta smelt (Table 1).

Table 1. LC₅₀ values (mg/L) and confidence limits (C.L.) of herbicides and surfactant (active ingredient) to larval Delta smelt.

Herbicides and Surfactant		LC ₅₀ (95 % lower and upper C.L.)
R-11 [®] (NP & NPE)		0.7 (0.57-0.80)
Reward [®] (diquat)		1.1 (1.0-1.2)
Komeen [®] (copper)		1.4 (1.4-1.5)
Sonar [®] (fluridone)		6.1 (3.8-9.6)
Weedar 64 [®] (2,4-D)		149 (72.1-185.6)
Rodeo [®] (glyphosate)		270 (186-324)
Rodeo [®] /R-11 [®]	Rodeo [®] (glyphosate)	5.5 (5.3-5.7)
	R-11 [®] (NP & NPE)	2.2 (2.17-2.3)
Weedar 64 [®] /R-11 [®]	Weedar 64 [®] (2,4-D)	3.5 (2.5-4.0)
	R-11 [®] (NP & NPE)	1.7 (1.3-1.9)

The herbicide Komeen[®] was the most toxic and the herbicide Rodeo[®] the least toxic material to larval fathead minnows (Table 2).

Table 2. LC₅₀ values (mg/L) and confidence limits (C.L.) of herbicides and surfactant (active ingredient) to larval fathead minnow.

Herbicides and Surfactant	96-h LC ₅₀ (95 % lower and upper C.L.)	7-d LC ₅₀ (95 % lower and upper C.L.)
Komeen [®] (copper)	0.31(0.18-0.53)	0.19(0.16-0.23)
Reward [®] (diquat)	0.43(0.38-0.49)	0.40(0.38-0.42)
R-11 [®] (NP & NPE)	1.1(0.99-1.2)	1.1(0.97-1.2)
Sonar [®] (fluridone)	5.7(5.0-6.1)	3.6(3.0-4.3)
Weedar 64 [®] (2,4-D)	216(163-304)	211(163-293)
Rodeo [®] (glyphosate)	1154(903-1432)	652(484-967)
Rodeo [®] /R-11 [®]	Rodeo [®] (glyphosate)	3.9(2.5-4.9)
	R-11 [®] (NP & NPE)	1.3(0.82-1.6)
Weedar-64 [®] / R-11 [®]	Weedar 64 [®] (2,4-D)	3.4(3.3-3.5)
	R-11 [®] (NP & NPE)	1.3(1.25-1.31)

The herbicide Komeen[®] was the most toxic and the herbicide Rodeo[®] the least toxic material to larval Sacramento splittail (Table 3).

Table 3. LC₅₀ values (mg/L) and confidence limits (C.L.) for herbicides and surfactant (active ingredient) to larval Sacramento splittail.

Herbicides and Surfactant	LC ₅₀ (95 % lower and upper C.L.)	
Komeen [®] (copper)	0.51 (0.45-0.60)	
Reward [®] (diquat)	3.7 (3.3-4.3)	
R-11 [®] (NP & NPE)	3.9 (3.0-4.4)	
Sonar [®] (fluridone)	4.8 (3.8-5.9)	
Weedar 64 [®] (2,4-D)	446 (431-453)	
Rodeo [®] (glyphosate)	1132 (814-1450)	
Rodeo [®] /R-11 [®]	Rodeo [®] (glyphosate)	5.5(5.3-5.8)
	R-11 [®] (NP & NPE)	2.1(2.0-2.2)
Weedar-64 [®] / R-11 [®]	Weedar 64 [®] (2,4-D)	3.0(3.0-3.0)
	R-11 [®] (NP & NPE)	2.2(2.1-2.2)

Using a sensitivity unit of 1.00 to indicate the most sensitive species during a 96-h exposure, Delta smelt larvae had a mean rating of 0.82 followed by fathead minnow larvae with a rating of 0.73, and Sacramento splittail larvae with a rating of 0.36 (Table 4).

Table 4. Fish sensitivity units to herbicides and surfactant. A unit of 1.00 is the most sensitive LC₅₀ value, with lesser values representing a fraction of the most sensitive LC₅₀ value.

Herbicides and Surfactant	Delta Smelt	Fathead Minnow	Sacramento Splittail
Reward [®] (diquat)	0.28	1.00	0.08
Komeen [®] (copper)	0.84	1.00	0.31
R-11 [®] (NP & NPE)	1.00	0.64	0.18
Sonar [®] (fluridone)	0.79	0.84	1.00
Weedar 64 [®] (2,4-D)	1.00	0.69	0.33
Rodeo [®] (glyphosate)	1.00	0.23	0.24
Mean	0.82	0.73	0.36

The toxicity of the Rodeo[®]/R-11[®] and Weedar 64[®]/R-11[®] mixtures to aquatic life are largely determined by the concentration of R-11[®] (NPE and NP) present. Both herbicides are individually toxic at concentrations > 100 mg/L, and R-11[®] is toxic at approximately 0.7 to 4.0 mg/L (Table 1, 2 and 3). When the herbicides are tested with R-11[®] in mixtures, the LC₅₀ values of R-11[®] change little while the LC₅₀ values toxicity of Rodeo[®] and Weedar 64[®] are dramatically reduced. The surfactant R-11[®] comprises ≥ 99% of the Toxic Units in the mixtures (Tables 5 and 6).

Table 5. Number of Toxic Units (LC_{50m}/LC_{50i}) of Rodeo[®] and R-11[®] in mixture.

Fish Species	Rodeo [®] T.U. (% of total T.U.)	R-11 [®] T.U. (% of total T.U.)
Delta smelt	0.02 (1%)	3.1 (99%)
Fathead minnow	0.003 (0%)	1.2 (100%)
Sacramento splittail	0.005 (1%)	0.54 (99%)

Table 6. Number of Toxic Units (LC_{50m}/LC_{50i}) of Weedar 64[®] and R-11[®] in mixture.

Fish Species	Weedar 64 [®] T.U. (% of total T.U.)	R-11 [®] T.U. (% of total T.U.)
Delta smelt	0.02 (1%)	2.4 (99%)
Fathead minnow	0.016 (1%)	1.2 (99%)
Sacramento splittail	0.008 (1%)	0.56 (99%)

DISCUSSION

Environmental monitoring for the WHCP and EDCP utilize water samples collected for herbicide and surfactant analyses and toxicity tests. To assess potential toxicity impacts that the WHCP and EDCP might have on fish, the maximum detected residue concentrations were compared to larval fish LC₅₀ values (Table 7).

Table 7. Highest concentrations (mg/L) of herbicides and surfactant detected in 2002-2003 in the Sacramento-San Joaquin Delta from EDCP and WHCP and 96-h LC₅₀ values (mg/L) for larval fish.

Herbicides and Surfactant	Highest Detected Concentration	Smelt LC ₅₀	Fathead LC ₅₀	Splittail LC ₅₀
Weedar 64 [®] (2,4-D)	0.260	149	216	446
Rodeo [®] (glyphosate)	0.037	270	1154	1132
R-11 [®] (NP & NPE)	0.167	0.7	1.1	3.9
Sonar [®] (fluridone)	0.012	6.1	5.7	4.8
Reward [®] (diquat)	0.110	1.1	0.43	3.7
Komeen [®] (copper)	0.800	1.4	0.31	0.51

Rodeo[®], Weedar 64[®] and Sonar[®] 96-h LC₅₀ values for the three fish species are several orders of magnitude higher than detected concentrations in the environment. However, the LC₅₀ values for Komeen[®], Reward[®], and R-11[®] are lower and approach the environmental concentrations.

Trial applications of Komeen[®] were made in the Sacramento-San Joaquin Delta with the highest concentration of copper detected of 0.8 mg/L at Frank's Tract. At Sandmound Slough, 0.2 mg/L copper was detected. Target application rates were 1.0 mg/L copper at Frank's Tract and 0.75 mg/L copper at Sandmound Slough; copper levels declined to background levels with in 24 hours (Anderson 2003). Highest concentration of copper detected was above the LC₅₀ levels for larval fathead minnow and larval Sacramento splittail.

Reward[®] (diquat) LC₅₀ values for the three larval fish species approximate the highest detected concentrations in the environment or the target application rate. Reward is used in the EDCP. Maximum application rate for diquat from the product label is 0.50 mg/L, and target application rate for the EDCP 2002-2003 season was 0.47 mg/L (Owens 2003). These rates are greater than the LC₅₀ value for fathead minnow (Table 2) and approach the LC₅₀ values for Delta smelt and Sacramento splittail larvae (Table 1 and 3). There have been several indications that Reward[®] is causing toxicity. It is very likely that Reward[®] cannot be used at these application rates without killing larval fish. If larval fish are in the application area, they likely will be killed. A possible mitigation measure would be to limit Reward[®] (diquat) use when larval fish are present during spring time. Applications could be made later in the year when juvenile fish can move away from application areas.

The WHCP uses R-11[®] as a surfactant for both Rodeo[®] and Weedar 64[®]. Throughout the WHCP for 2002-2003 season, R-11[®] was not detected in the Sacramento-San Joaquin Delta with one exception when it was detected at 0.167 mg/L NP and NPE. Applicators should be careful when applying mixtures containing R-11[®] so that the spray is on the emergent plants and not in the water column.

With the exception of Reward[®] and Komeen[®], it is unlikely that acute toxicity from EDCP and WHCP is a problem to these larval fish. Sublethal effects from the

WHCP are unlikely since the exposure levels are so less than acute toxic levels and the materials are relatively nonpersistent in the environment. Sonar[®] should be further examined for sub-lethal effects due to its slow break down in the environment and repeated treatments in the same location.

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