CALIFORNIA DEPARTMENT OF FISH AND GAME
California Fish and Game 91(1):38-46 2005

An Assessment of the Hazard of a Mixture of the Herbicide Rodeo® and the Non-Ionic Surfactant R-11® to Aquatic Invertebrates and Larval Amphibians

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This study was conducted to determine whether the aquatic herbicide Rodeo® (active ingredient: glyphosate) and the non-ionic surfactant R-11® (active ingredient nonylphenol polyethoxylate or NPE) adversely affect aquatic species including invertebrates and larval amphibians. A Rodeo®/R-11® mixture was applied directly to the surface of a pond in a manner that would produce atypically high concentrations of these compounds in water. Water samples were collected from the treated pond for chemical analyses and toxicity tests with the aquatic invertebrate *Ceriodaphnia dubia*. A toxicity test with the Rodeo®/R-11® mixture was also conducted to determine the LC_{50} value for the larval life stage of the northern leopard frog, *Rana pipiens*. Water samples collected one hour after application contained the following mean concentrations: glyphosate, 1.83 mg/L; NPE, 1.10 mg/L; and 0.02 mg/L of the NPE breakdown product nonylphenol (NP). Concentrations of glyphosate's primary breakdown product, amino methyl phosphonic acid (AMPA), were below the laboratory detection limit of 0.020 mg/L. Water samples collected from the treated pond were not acutely lethal to *Ceriodaphnia dubia*. The 96-h toxicity test with the Rodeo®/R-11® mixture using *Rana pipiens* produced LC_{50} values of 6.5 mg/L for glyphosate and 1.7 mg/L for NPE, indicating that the mixture is moderately toxic to the amphibian. A comparison of toxic units for the herbicide and surfactant in the mixture indicated that the toxicity to larval frogs was likely due to R-11® and not Rodeo®.

INTRODUCTION

The glyphosate herbicide Rodeo® is commonly mixed with the non-ionic surfactant R-11® to control vegetation growing in or near surface water. R-11® increases herbicide efficacy by improving foliar coverage and by increasing the penetration of the herbicide through the leaf's cuticle layer. Toxicity information for the active ingredient of R-11®, nonylphenol polyethoxylate (NPE), indicates that the compound is moderately toxic to fathead minnows with a 96-h LC_{50} value of approximately 4.5 mg/L (Staples et al. 1998). The NPE metabolite, nonylphenol (NP), is more toxic than its parent compound and, like NPE, exhibits estrogen-like properties.[1] NP has a 96-h LC_{50} value of approximately 0.13 mg/L, which places the compound in the highly toxic category (Brooke 1993). While the breakdown of NPE generally produces a variety of related compounds with shorter carboxylate chains, NP residues can be produced under low

[1] Bakke, D. 2003. Human and ecological risk assessment of nonylphenol polyethoxylate-based (NPE) surfactants in Forest Service herbicide applications. USFS internal report. May 2003.

water temperature, nutrient level and dissolved oxygen conditions (Brooke 1993).

The 96-h LC_{50} values for the isopropylamine salt of glyphosate for fish species range from 97 to >1,000 mg/L (Giesy et al. 2000). Based on a U.S. Environmental Protection Agency rating system, the herbicide is considered practically non-toxic to slightly toxic to aquatic species (Zucker 1985). In 1997, the California Department of Fish and Game conducted a study that investigated glyphosate toxicity to larval amphibians (Trumbo 1997). This study demonstrated that larval frogs have a sensitivity to glyphosate that is similar to that of larval fish.

This study assessed the hazard of the Rodeo®/R-11® mixture to two test species. An application of the mixture was made directly to water in order to determine worst-case impacts on non-target aquatic fauna. This study collected information on: 1) The magnitude and persistence of glyphosate, amino methyl phosphonic acid **(**AMPA), NPE and NP residues in water; 2) the toxicity of water samples collected from the Rodeo®/R-11® mixture application site to the aquatic invertebrate *Ceriodaphnia dubia*; and 3) the 96-h LC_{50} toxicity value of the Rodeo®/R-11® mixture for larval northern leopard frogs, *Rana pipiens*. *Rana pipiens* was selected for this study because of its taxonomic relationship with several other species in the genus *Rana* that have federal or state protected status in California, including the California red-legged frog, *Rana aurora draytonii*.

MATERIALS AND METHODS

Rodeo® /R-11® Mixture Application

The Rodeo®/R-11® mixture was applied to a 0.72 acre-feet pond at the Sacramento-Yolo Mosquito and Vector Control District facility in Elk Grove, California. A similar pond, located nearby, was used as an untreated control. Neither the control or treatment pond had any outflow. The ponds were not connected at the time of the application. Water temperatures during the project ranged from 15º to 18º C. Both ponds had minimal growth of aquatic vegetation and supported populations of mosquitofish, *Gambusia sp.*

The Rodeo®/R-11® mixture application to the pond was made with a vehicle-mounted hose-gun sprayer using typical herbicide and surfactant tankmix concentrations of 1% and 0.5%, respectively. The herbicide use rate was 5 pints/surface acre. In a departure from the standard application technique, the mixture was applied directly to the water surface rather than to the foliage of emersed aquatic weeds. This was done to provide atypically high herbicide and surfactant concentrations in water. Additionally, the lack of outlet flow from the treated pond assured the maximum residency time for chemical residues. All other aspects of the application were made according to the herbicide and surfactant product labels.

Sample Collection

Water samples were collected from three locations in the treated pond and from one location in the non-treated control pond. Each sampling location consisted of a transverse transect with three sampling subsites. The three subsamples collected along each transect were composited into a single sample.

Samples were collected in 500-ml amber glass containers at the water surface by hand or with a polyvinyl chloride (PVC) sampling cup with a handle extension. The sampling cup was cleaned with petroleum ether solvent and deionized water between transects. After collection, samples were immediately stored at a temperature of 4°C, protected from sunlight and then transported directly to the laboratory. One field blank, one matrix spike, and one matrix spike duplicate were collected for quality assurance purposes for every twenty samples collected in the field.

Water samples were collected for chemical analyses and toxicity tests according to the following schedule: one day prior to treatment (Pre-treatment); less than one hour after treatment (Day 0); one day after treatment(1 DAT);two days after treatment (2 DAT); four days after treatment (4 DAT); and eight days after treatment (8 DAT).

Chemical Analyses

Chemical analyses were conducted by the California Department of Fish and Game (DFG) Water Pollution Control Laboratory (WPCL). NPE and NP samples were analyzed using analytical methods developed by Thiele et al. (1997). Glyphosate and AMPA samples were analyzed using high performance liquid chromatography (HPLC) following an accepted analytical method (USEPA 1990).

Acute Toxicity Tests

Toxicity tests were conducted by the DFG Aquatic Toxicology Laboratory (ATL). All laboratory procedures were done in compliance with Good Laboratory Practices, in strict observance of the ATL Quality Assurance Manual.

An acute toxicity test was conducted to determine the 96-h LC_{50} value for the Rodeo®/R-11® mixture using larval northern leopard frogs, *Rana pipiens*. Test frogs were purchased from Carolina Biological Supply and were < 7-d old during the test. Four replicates per treatment and a negative control (laboratory well-water) were used. The larvae were exposed in 1000-ml beakers, each containing 250 ml of test solution. The test was conducted at a temperature of 22ºC, and there were 10 larvae per replicate (40 larvae per treatment). The tests were conducted in environmental chambers under constant temperature, humidity and photoperiod conditions. Test solutions were renewed at 48 hours. Samples of test solutions were collected and analyzed at the beginning of the test to verify the Rodeo® and R-11® exposure levels.

Water samples were collected from one location in the treated pond and from one location in the non-treated control pond prior to the application and on Day 0 and 4 DAT to test for toxicity to *Ceriodaphnia dubia*. Standard 96-h toxicity tests with 48-h renewals were completed using accepted methods (USEPA 1993).

RESULTS

Environmental Exposure Levels

Rodeo®

Analysis of water samples collected prior to the herbicide/surfactant application did not reveal any detectable glyphosate residues in the test ponds. One hour after the Rodeo®/R-11® mixture application, the mean glyphosate concentration in the treated pond was 1.83 mg/L (Table 1), with a maximum concentration of 3.1 mg/L. Glyphosate concentrations in the treated pond declined rapidly during the first 24 hours after the application. At 1 DAT, mean glyphosate residues had declined by more than 84%. Glyphosate residues in samples collected 4 and 8 DAT remained stable at 0.2 mg/L. AMPA residues were never detected in the treated pond above the laboratory minimum detection limit of 0.02 mg/L. The untreated control pond contained no detectable residues of either glyphosate or AMPA.

R-11®

Within one hour after the Rodeo®/R-11® mixture application, the mean NPE and NP concentrations in the treated pond were 1.1 and 0.020 mg/L respectively (Table 1). The maximum concentrations of these compounds were 1.8 and 0.03 mg/L, respectively. Chemical analyses prior to the application indicated the water in both the treatment and control ponds contained low concentrations of NPE and NP. The minimum detection limit (MDL) for both compounds was 0.0002 mg/L.

NPE concentrations in the treated pond declined rapidly from those detected one hour after application. After 24 hours, NPE concentrations had been reduced by 64%, and by 4 DAT the peak concentration had been reduced by more than 98%. By 8 DAT the NPE concentration in the pond had been reduced to nearly the pretreatment level.

Residues of the NPE metabolite, NP, were markedly lower than those of its parent compound one hour after the Rodeo®/R-11® mixture application (0.020 mg/L vs 1.1 mg/L). By 1 DAT, NP residues had been reduced by 75%, and by 4 DAT they had been reduced by 95%. By 8 DAT, the NP concentration had been reduced to nearly the pretreatment level.

Table 1. Mean concentrations of glyphosate, AMPA, NPE and NP, after application of a Rodeo®/R-11® mixture.

	Glyphosate (mg/L)	AMPA (mg/L)	NPE (mg/L)	NP (mg/L)
Pretreatment	ND[a]	ND	0.003	0.001
1-H POST	1.830	ND	1.100	0.020
1 DAT[b]	0.300	ND	0.400	0.005
2 DAT	0.300	ND	0.040	0.002
4 DAT	0.200	ND	0.020	0.001
8 DAT	0.200	ND	0.004	0.001

[a] ND indicates no detectable residues ≥ the minimum detection limit (MDL). MDL values are as follows: NPE, 0.0002 mg/L; NP, 0.0002 mg/L; glyphosate, 0.02 mg/L; and AMPA, 0.02 mg/L;
[b] DAT = Days after treatment

Acute Toxicity Tests

Rodeo®/R-11® Mixture - The calculated 96-h LC_{50} values for larval northern leopard frogs were 6.5 mg/L glyphosate and 1.7 mg/L NPE (Table 2).

Table 2. Calculated LC_{50} values and mean characteristics of chemical dilutions and survival of larval *Rana pipiens* in 96-h acute toxicity test.

Dilution No.	Glyphosate (mg/L)	NPE (mg/L)	NP (mg/L)	Survival (%)
1	17.6	4.5	<MDL	0*
2	8.7	2.5	<MDL	0*
3	4.5	1.0	<MDL	92.5
4	2.4	0.6	<MDL	100
5	1.3	0.3	<MDL	100
96-h LC_{50}	6.5	1.7	NA	NA

* Indicates survival significantly less than control group (P<0.05).

Treated Pond Water - There was no significant mortality of *Ceriodaphnia dubia* in any of the water samples collected from the treated pond. Cladoceran survival ranged from 90 to 100% in the pond water samples and from 95 to 100% in the laboratory controls.

DISCUSSION

Glyphosate, NPE and NP Environmental Exposure Levels

Initial concentrations of glyphosate and NPE were relatively high, particularly when compared to residue levels that result from more typical application scenarios (i.e., applications made to emersed aquatic plants and not directly to water). For example, monitoring conducted by the California Department of Boating and Waterways (DBW) as part of their water hyacinth control program generally failed to detect glyphosate and NPE residues in samples collected within 24 hours after Rodeo®/R-11® applications (California Department of Boating and Waterways 2003).

It is difficult to assess the relative magnitude of the NPE and NP residues that were observed during this study because little historical data are available on the residues of these compounds after they have been applied directly to water. However, the glyphosate residues that were observed are consistent with those seen in a previous study conducted by Horner[2]. In that study, 1.7 mg/L glyphosate was detected in pond water after an herbicide application.

The persistence of glyphosate, NPE and NP was in general agreement with what had been demonstrated by previous investigations. Research by Trumbo (2002) showed that peak concentrations of the three compounds in water were achieved relatively soon after application and declined rapidly during the first 24 hours. While glyphosate residues were still detectable in the pond eight days after treatment, this is not inconsistent with what is known about the herbicide. Environmental fate information for glyphosate indicates that the compound's half-life in water can range from 12 days to 10 weeks (USEPA 1992). Residues of NPE and NP were more transitory than the herbicide residues with concentrations for both compounds approaching pretreatment levels by eight days after the herbicide/surfactant application. While the source of the pretreatment detections of NPE and NP is unknown, it may be related to previous uses of surfactant products for terrestrial weed control near the ponds.

Aquatic Toxicity Hazard

The hazard of the Rodeo®/R-11® mixture to aquatic life is largely determined by the concentration of R-11® (NPE and NP) because it is the more toxic compound in the tankmix. Although glyphosate can be toxic at levels in excess of 500 mg/L, R-11® can be toxic at approximately 1 to 6 mg/L (Table 4). When the two products are tested together in a 2:1 mixture, the toxicity of R-11® changes little (mean LC_{50} value decreases by 1.8x), but the toxicity of glyphosate changes dramatically

[2] Horner LM. 1990. Dissipation of glyphosate and aminomethyl phosphonic acid in forestry sites. Unpublished report MSL-9940. Monsanto Company, St Louis, MO.

(mean LC_{50} value decreases by 208x). When toxic units (LC_{50} mixture/LC_{50} individual chemical) for each compound are calculated, the values for NPE are greater than glyphosate's.

It is not surprising that there was no significant mortality to cladocerans in the water samples collected from the treated pond. The concentrations of NPE and NP one hour after application were about 50% of their LC_{50} values for this species, and concentrations of both compounds decreased significantly by one day after the herbicide/surfactant application.

Table 4. Comparison of LC_{50} values(mg/L) and toxic units for various invertebrate and vertebrate species to Rodeo®, R-11® and a Rodeo®/R-11® mixture.

Species	Rodeo® [a]	R-11® [b]	Rodeo®/R-11® Mixture	Toxic Units [c]
Ceriodaphnia dubia[d]	586	5.7	3.1/2.8	Rodeo: <0.01 R-11: 0.49
Fathead minnow[e]	652	1.1	2.8/0.9	Rodeo: <0.01 R-11: 0.82
Sacramento splittail[f]	1132	3.9	5.5/2.1	Rodeo: <0.01 R-11: 0.54
Leopard frog[g]	--	--	6.5/1.7	--

[a] As glyphosate.
[b] As sum of NPE and NP.
[c] Toxic Units = LC_{50} mixture/LC_{50} individual chemical.
[d] California Department of Fish and Game, Aquatic Toxicology Laboratory Report P-2376, Elk Grove California.
[e] California Department of Fish and Game, Aquatic Toxicology Laboratory Report P-2365, Elk Grove California.
[f] California Department of Fish and Game, Aquatic Toxicology Laboratory Report P-2369, Elk Grove California.
[g] From this study.

CONCLUSIONS

The results of this study demonstrate that the application of a Rodeo®/R-11® mixture directly to water at labeled rates can produce relatively high glyphosate, NPE and NP residues that decline rapidly within 24 hours after application. While undiluted Day 0 samples collected from the treated pond did not produce significant mortality of the aquatic invertebrate *Ceriodaphnia dubia* in laboratory tests, the initial concentration of NPE in the pond was approximately equal to the 96-h LC_{50} value for larval northern leopard frogs that was determined during this study. Glyphosate and NP residues, even at their highest concentrations immediately after application, never exceeded 29% and 15%, respectively, of their established acute toxicity values for larval frogs.

Future areas of research could include testing the toxicity of recently treated water to larval frogs. Also, increasing the frequency of sample collection on Day 0 would be useful for determining the persistence of the chemicals. This information would also prove valuable in establishing if NPE toxicity to non-target aquatic species occurs primarily during the first 24 hour period as other researchers have suggested.

Finally, additional monitoring studies for the NPE metabolite NP would also be important, particularly in light of the compound's relatively high toxicity and its identification by the U.S. Department of Agriculture as a potential endocrine disruptor.[3]

ACKNOWLEDGEMENTS

The author would like to thank Frank Riley and his staff at the California Department of Fish and Game (DFG) Aquatic Toxicology Laboratory for their assistance with the toxicity tests and Abdou Mekebri of the DFG Water Pollution Control Laboratory for conducting the chemical analyses. Thanks also to David Brown and his staff at the Sacramento–Yolo Mosquito and Vector Control District for allowing us to use their fish ponds. Finally, thanks to Dave Bakke of the U.S. Forest Service for his encouragement and assistance in developing this project.

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