Bull. Environ. Contam. Toxicol. (2003) 71:912–918

DOI: 10.1007/s00128-003-8905-y

Environmental Contamination and Toxicology

Concentrations of Glyphosate and AMPA in Sediment Following Operational Applications of Rodeo® to Control Smooth Cordgrass in Willapa Bay, Washington, USA

W. W. Major III, C. E. Grue, S. C. Gardner,* J. M. Grassley

Washington Cooperative Fish and Wildlife Research Unit, School of Aquatic and Fishery Sciences, Box 355020, University of Washington, Seattle, WA 98195, USA

Received: 18 February 2003/Accepted: 4 August 2003

Smooth cordgrass (*Spartina alterniflora*) was accidentally introduced to Willapa Bay, Washington in the late 1800s during an attempt to culture Atlantic oysters (*Crassostrea virginica*). During the last decade, the distribution of the grass in the Bay increased dramatically, displacing open mudflats and outcompeting other intertidal flora. With a clonal diameter expansion rate of approximately 79 cm yr^{-1} (Feist and Simenstad 2000), the grass infests more than 6,100 ha (Murphy 2001) and threatens to occupy 75% of the Bay's mudflats by 2030 (Washington Department of Natural Reseources 2000). Affected mudflats provide critical habitat for a variety of fish, shellfish, and wildlife.

Control of *Spartina* in the Bay has been hampered by concerns over the potential non-target effects of Rodeo® (Dow AgroSciences, Indianapolis, IN; formerly Monsanto Agricultural Co., St. Louis, MO), the only herbicide currently approved for *Spartina* in Washington State. Rodeo®'s active ingredient, glyphosate (*N*-[phosphonomethyl]glycine as isopropylamine [IPA] salt), is a non-selective post-emergent herbicide commonly used in agriculture. Unlike most formulations of glyphosate, Rodeo® does not contain a surfactant. However, the manufacturer's label requires a non-ionic surfactant be added before application. The surfactants R-11® (Wilbur-Ellis Co., San Francisco, CA), X-77®, and LI 700® (Loveland Industries, Greeley, CO) have been used with Rodeo® in Washington State.

Glyphosate is known to adsorb to sediments (Giesey et al. 2000). Therefore, determining the concentrations of glyphosate and its primary breakdown product, aminomethylphosphonic acid (AMPA, Giesey et al. 2000), in Bay sediments following operational applications is a necessary first step in evaluating bioavailability and the potential for non-target effects. Previous studies in the Bay have examined the fate and persistence of glyphosate and AMPA following an aerial spray of Rodeo® to *Spartina* clones at half the recommended application rate of 8.77 L ha^{-1} (Paveglio et al. 1996), or repeated hand applications (34.3–67.4 L ha^{-1}) to *Spartina* meadow and open mudflat (Kilbride and Paveglio 2001). Here, we report concentrations of glyphosate and AMPA in sediment following operational hand and aerial applications of Rodeo® to *Spartina* in the Bay at maximum allowable rates. We then compare these concentrations to levels known to affect aquatic organisms. Efficacy and non-target effects of our treatments are reported elsewhere (Major et al. 2003; Major et al., *unpubl. ms.*).

**Present address:* Centro de Investigaciones Biologicas del Noroeste, SC, A.P. 128, La Paz, Baja California Sur, CP 23090, Mexico
Correspondence to: C. E. Grue

MATERIALS AND METHODS

We chose sites representing a variety of substrates within the Bay for hand spraying of clones: Leadbetter Point, Lewis Unit, North River, and Nemah Beach. The Leadbetter site was located at the north end of Long Beach Peninsula on Leadbetter Point (46,40 N–124,00 W) in the shallow bay between the point and Grassy Island. Substrate was moderately firm sand. The Lewis Unit site was located at the southernmost part of the Bay on the Willapa National Wildlife Refuge (46,20 N-124,00 W) and was characterized by deep, soft, muddy substrate. The Nemah Beach site was located mid-Bay (46,35 N-123,55 W) and was characterized by hard-packed sand with underlying clay at higher tidal elevations. The North River site was at the north end of the Bay (46,45 N-124,00 W), ca. 0.5 km SW of the confluence of Smith Creek and the North River. The substrate was a mixture of sand and mud. Kaffee Meadow was aerially sprayed and was located on the NE side of Long Island (46,30 N-123,55 W) between Kaffee and Lewis sloughs. The meadow covered ca. 66 ha with >90% mature, homogeneous *Spartina*. The substrate was soft mud. *Spartina* stem densities and maximum heights across the hand-sprayed sites averaged 148 (SE 8.7) to 328 (SE 19.5) stems/m^2 and 80 (SE 3.4) to 115 (SE 4.9) cm, respectively. Comparable measurements on the aerially treated site were188 (SE 11.7) stems/m^2 and 165 (SE 5.0) cm.

Within each site, clones were selected with diameters of 5–10 m. Each clone was divided by three equidistant transects extending onto the mudflat. A single entry point to each clone was established. Travel within and around each clone was limited to the inside edge where no sampling occurred. Permanently marked sampling points were established along the three transects (excluding entry way) at 1 m from the perimeter of the clone toward the center and 1 m away from the clone on the adjacent mudflat. Points along a different transect were sampled at 0 and 30 d postspray at all sites, and 365 d postspray at Lewis Unit and Nemah Beach. Samples were pooled by clone and sampling date.

At the Lewis Unit site, Rodeo® was applied using a hovercraft equipped with a Model 60-Spotlyte® agricultural sprayer (Falkenberg, Inc., Clackamas, OR) with a hand-held wand and adjustable brass nozzle. The Nemah Beach, Leadbetter, and North River sites were sprayed using 15 L Solo® (Solo-USA, Newport News, VA) back-pack sprayers. Each clone was sprayed once between 18 and 28 July 1995. The tank mix consisted of Rodeo® at 5% solution (maximum label) with LI 700® at 2% solution, following label directions of "spray to wet." Pre-spray calibrations of equipment provided approximate application rates of 42.1 L Rodeo® ha^{-1}. All applications were made at low tides, allowing 5 to 6 hr of drying time before inundation of 50% of plant height. Air temperatures were between 19 and 29°C and wind speeds were 0–8 km h^{-1} with occasional gusts to 16 km h^{-1} at one site.

Sediment cores were collected using clean 10.2-cm PVC coring devices that had been acid washed and individually wrapped in a clean plastic bag. At each sampling point, the coring device was pushed into the sediment 1 m away from the sampling point toward the center of the clone or away from the clone on the adjacent mudflat. Approximately 10 cm of sediment were extracted from which the top 5 cm of the core were removed with a clean plastic knife, double-bagged (Ziploc™), placed on wet ice and, within a few hours, frozen at -20°C.

Rodeo® was applied by air to a 2-ha (50 x 400 m) plot parallel to the mudflat. Within the plot, we created a buffer zone 5 m from the edges and randomly selected and marked 25 points within the core area. We also established five 10-m transects at equal distances along, and perpendicular to, the water side to monitor off-target deposition onto the mudflat. Sampling points were established along the transects at 1, 3, and 10 m from the study plot.

We used glass fiber filter papers (9 cm diameter, Whatman Inc., Clifton, NJ) to monitor aerial deposition of the herbicide. We affixed the papers to the tops of the sampling point markers at a height just above the surrounding *Spartina*. Filter papers had been previously clipped (plastic) on four sides to a folded 10.2- by 15.2-cm index card, and once in the field, were mounted to a cork in the top of each pole using thumbtacks with plastic heads. Immediately after application of the herbicide, the cards were removed and placed individually inside separate Ziploc™ bags. Each of these bags was then double-bagged for protection and placed on wet ice. Filter papers were stored at -20°C.

A Soloy Bell® helicopter with a 9.1-m toe-mounted boom applied the herbicide at 0915 on 13 August 1995. The tank mix consisted of Rodeo® at 9.4% v/v and X-77 Spreader® at 0.13% v/v applied at a rate of 8.77 L ha^{-1} (maximum label) and 0.12 L ha^{-1}, respectively. Application occurred 1 hr before low tide, allowing for a minimum of 6 hr drying time before inundation of 50% of plant height. Weather conditions for the spray were optimal with winds ranging from 0-8 km h^{-1} from the south and an ambient air temperature of 14.5°C.

Sediment cores were taken 1 m from all sampling points on the treatment plot and the adjacent mudflat immediately following filter paper retrieval and in a different cardinal direction at 30 and ca. 365 d postspray. Sampling 1-yr postspray occurred ca. 7 wk early because of an impending subsequent herbicide application to the meadow. Coring methods were identical to those for clones.

Filter papers were analyzed for glyphosate and sediment cores for glyphosate and AMPA by APT Labs, Inc., Wyomissing, PA, using the methodology described in Kilbride et al. (1995) and Paveglio et al. (1996). The detection limit (DL) for glyphosate on the filter papers was 0.05 µg. The DL for glyphosate and AMPA within sediment was 0.02 ppm or 0.05 ppm dry weight (DW) and varied among sites and dates. Average percent recovery of glyphosate from the filter papers was 99.8% (SD = 3.2%); sample residues were not corrected for percent recovery. Deposition on the filter papers was reported as µg DW (acid equivalent, a.e.) and converted to a percentage of the intended deposition of glyphosate (a.e.) based on the nominal application rate. Percent recovery for glyphosate (IPA salt) and AMPA in the sediment averaged 86.7 and 78.5%, respectively. Residues reported for sediments were corrected for recovery and expressed as ppm DW.

RESULTS AND DISCUSSION

Concentrations of glyphosate and AMPA in the sediment immediately following application (Day 0) were between 0.3–16.2 and 0.02–0.17 ppm, respectively, on hand-sprayed sites (Fig. 1), and 0.04–2.5 and 0.02–0.17 ppm, respectively, on the aerial-sprayed site. One yr later, concentrations of both were less than 1.8 and 0.5 ppm, respectively (Fig. 1). Geometric means and 95% confidence intervals are given in Table 1.

Immediately postspray, mean concentrations of glyphosate in the sediment within *Spartina* clones were between 1.4 and 5.3 ppm (Table 1). These values were similar to those in adjacent mudflats (means=1.1-5.3 ppm). The mean concentration of glyphosate

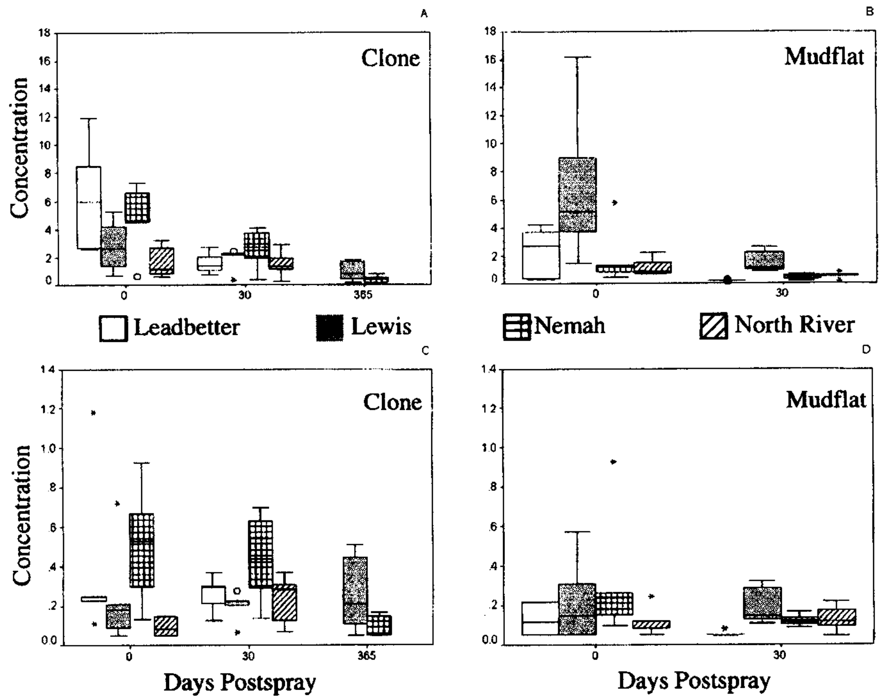

Figure 1. Box plots of actual concentrations (ppm DW) of glyphosate (IPA salt, A & B) and AMPA (C & D) within *Spartina* clones and adjacent mudflat 0, 30, and 365 d after hand application. (o) represents outliers 1.5 to 3x the box length (interquartile range); (*) represents extremes > 3x the box length.

in sediment decreased 14–74% within the clones by 30 d postspray and 55–85% in the adjacent mudflats. Mean concentrations of AMPA within clones and adjacent mudflats immediately postspray were between 0.02 (DL) and 0.4 ppm. Concentrations 30 d later either were unchanged, decreased (33–100%), or increased (100%).

Within the *Spartina* meadow, deposition of glyphosate (mean = 3.84 kg a.e. ha^{-1}, SD = 1.1 kg ha^{-1}) represented 91.2% of that intended. Immediately postspray, the mean concentrations of glyphosate and AMPA in the sediment and adjacent mudflat were 0.4 and 0.1 ppm, respectively. Glyphosate concentrations within the meadow and adjacent mudflat decreased 25 and 75%, respectively by 30 d postspray; AMPA concentrations were unchanged. By 1-yr postspray, concentrations had decreased 75%, whereas AMPA levels remained at 0.1 ppm. Comparable samples were not collected within adjacent mudflats.

In natural freshwater environments, glyphosate is rapidly adsorbed to suspended particulates and sediment where it is biodegraded to AMPA and CO_2. AMPA also undergoes rapid degradation in water and soil, but in the latter at a slower rate than glyphosate. Concentrations of AMPA in sediments may fluctuate depending on its rate of biodegradation relative to that of glyphosate. In their recent review, Giesey et al. (2000) suggested that a "conservative range" of half-life values for glyphosate and AMPA in freshwater aquatic environments would be 7–14 d.

Table 1. Geometric means (95% confidence intervals) for sediment concentrations (ppm DW) of glyphosate (IPA salt) and AMPA within *Spartina* clones and meadows and adjacent mudflats.[1]

Location		*SPARTINA* Days Postspray			MUDFLAT Days Postspray	
		0	30	365	0	30
		HAND SPRAY[2]				
Lewis	Glyphosate	2.2 (0.8-6.3)	1.6 (0.6-4.5)	0.7 (0.2-2.6)	5.3 (1.7-6.2)	1.4 (0.8-2.7)
	AMPA	0.2 (0.1-0.6)	0.2 (0.1-0.4)	0.2 (0.1-0.6)	0.3 (0.0-0.6)	0.2 (0.1-0.3)
Nemah	Glyphosate	3.6 (1.0-12.6)	2.1 (0.7-6.3)	0.2 (0.1-0.8)	1.3 (0.4-4.1)	0.4 (0.3-0.7)
	AMPA	0.4 (0.2-1.1)	0.4 (0.2-1.2)	0.1 (0.0-0.2)	0.2 (0.1-0.7)	0.1 (0.0-0.2)
North River	Glyphosate	1.4 (0.6-3.5)	1.2 (0.4-3.6)		1.1 (0.6-2.1)	0.5 (0.2-1.0)
	AMPA	0.1 (0.0-0.2)	0.2 (0.1-0.5)		0.1 (0.0-0.2)	0.1 (0.0-0.2)
Leadbetter	Glyphosate	5.3 (2.3-12.3)	1.4 (0.8-2.8)		1.3 (0.3-6.9)	0.2 (0.1-0.3)
	AMPA	0.3 (0.1-0.8)	0.2 (0.1-0.4)		0.1 (0.0-0.3)	≤0.1 (0.04-0.07)
		AERIAL SPRAY[3]				
Kaffee Meadow	Glyphosate	0.4 (0.2-0.7)	0.3 (0.2-0.4)	0.1 (0.0-0.5)	0.4 (0.0-4.1)	0.1 (0.0-0.2)
	AMPA	0.1 (0.0-0.2)	0.1 (0.0-0.2)	0.1 (0.0-0.2)	0.1 (0.0-0.2)	≤0.05 (—)

[1]Detection limits were standardized at 0.05 for both glyphosate and AMPA. Means were rounded to the nearest 0.1 ppm.
[2]N = 5
[3]N = 24 for 0 and 30 days and 5 for 365 days.

Our data and that of others suggest degradation of glyphosate is slower in estuarine habitats than freshwater environments, and more similar to that reported for agricultural (including forest) soils (mean half life = 32 d, 90th percentile = 95 d; Giesey et al. 2000). Concentrations of glyphosate declined 88% in sediments of Maryland tidal marshes by 56 d after application of Rodeo® (Kroll 1991 *in* Kilbride and Paveglio 2001). Paveglio et al. (1996) reported changes in glyphosate concentrations of -25 to +3% 14 d after aerial application of Rodeo® to mudflats in Willapa Bay, -10 to -63% 28 d postspray, and -41 to -72% 119 d postspray. Changes in AMPA concentrations through time are more variable and degradation rates for AMPA more difficult to determine in the field because of the continued contribution of AMPA associated with the breakdown of glyphosate (e.g., see

Paveglio et al. 1996). Additionally, because values are so low, small changes in concentrations appear relatively large when expressed as a percentage.

Concentrations of glyphosate we detected in sediments are a fraction of those known to be toxic to aquatic invertebrates. Sediment LC50s for juvenile amphipods (*Eohaustorius estuarius*) and oyster (*C. gigas*) pediveliger larvae were 13,368 and 3,988 ppm DW, respectively (Kubena 1998). Studies in which oysters and clams were exposed to sediment concentrations of 6 ppm glyphosate DW and water concentrations ≤2.0 ppm reported maximum tissue concentrations of glyphosate of ≤0.4 ppm wet weight (Smith et al. 2000), ca. one-tenth of the current human health tolerance for edible tissues. Comparable data for AMPA are lacking.

On the basis of these results, we would not expect direct toxic effects on aquatic organisms, even at the highest concentration of glyphosate detected in sediment during our study (16.2 ppm). Use of Rodeo® has not been associated with decreases in abundance of aquatic invertebrates when applied to purple loosestrife (*Lythrum salicaria*) within freshwater wetlands (Gardner et al. 1996) or directly to tidal mudflats (Simenstad et al. 1996). A more recent study of the fate of glyphosate following repeated multi-year Rodeo® applications to mudflats in the Bay (using LI 700® as the surfactant) also concluded that long-term detrimental effects of the active ingredient to aquatic biota were unlikely (Kilbride and Paveglio 2001).

However, studies that have tested the toxicity of surfactants or tank mixes separately from glyphosate have shown that the former are the primary toxic agents, and that surfactants can be orders or magnitude more toxic to aquatic invertebrates and fish than the active ingredient (Kubena 1998; Giesey at al. 2000; Smith et al., *unpubl. ms.*). In our study, LI 700® was used as the surfactant for the hand spray because of its lower toxicity compared with other surfactants approved for over-water use in Washington State (Smith et al., *unpubl. ms.*). However, for reasons of efficacy, we used X-77 Spreader® as the surfactant for the aerial sprays. Studies have shown that the acute toxicity of Rodeo® to fish and aquatic invertebrates is significantly greater with the addition of X-77. For example, Henry et al. (1994) found that X-77 was about 100 times more toxic to aquatic invertebrates than Rodeo® alone, and that their effects were additive. However, investigations conducted in Willapa Bay with Rodeo®and X-77 did not detect any short or long-term effects on the mudflat community following an aerial application directly to the mudflat (Simenstad et al. 1996).

Studies of the efficacy of Rodeo® indicate that the current maximum aerial application rate of the herbicide is not effective in controlling *Spartina* meadows (Major et al. 2003), and that an increase in the aerial application rate is warranted (currently in review by the USEPA). Our study and others suggest that a proposed doubling of the aerial application rate will pose little hazard to non-targets, and that concerns over non-target effects should be directed at the selection of surfactants rather than the quantity of active ingredient.

Acknowledgments. We thank the Washington Departments of Fish and Wildlife (WDFW) and Natural Resources (WDNR) for funding; and both of these agencies and the US Fish and Wildlife Service's Willapa NWR and Vancouver, Washington Field Office, and the Washington Cooperative Fish and Wildlife Research Unit for in-kind support. The Unit is supported by the US Geological Survey (USGS), University of Washington, and the Washington State Department of Ecology, WDFW, and WDNR. C.E. Grue is employed by the USGS. Janie Civille, Brett Dumbauld, Jim Hidy, Kevin Kilbride, Tom Mumford, Mike Norman, Kim Patten, Marty Peebles, Fred Paveglio, Steve Ratchford, Kathleen Sayce, and Kevin Sittauer contributed to project design. Field assistance was provided by Chris Bonsignore, Richard Brocksmith, Kevin Kilbride, Nathaniel Overman, Fred Paveglio, Mark Tagal, and Mariana Tamayo. Ron Crockett, Brett Dumbauld, Kevin Kilbride, Kim Patten, Fred Paveglio, Charles Stenvall, and Steve Stupp reviewed earlier drafts.

REFERENCES

Giesey JP, Dobson S, Solomon KR (2000) Ecotoxicological risk assessment for Roundup® herbicide. Rev Environ Contam Toxicol 167:35-120

Feist BE, Simentad CA (2000) Expansion rates and recruitment frequency of exotic smooth cordgrass, *Spartina alterniflora* (Loisel), colonizing unvegetated littoral flats in Willapa Bay, Washington. Estuaries 23:267-274

Gardner SC, Grue CE (1996) Effects of Rodeo® and Garlon® 3A on nontarget wetland species in central Washington. Environ Toxicol Chem 15:441-451

Henry CJ, Higgins KF, Buhl KJ (1994) Acute toxicity and hazard assessment of Rodeo®, X-77® Spreader, and Chem-trol® to aquatic invertebrates. Arch Environ Contam Toxicol 27:394-399

Kilbride, KM, Paveglio FL, Grue CE (1995) Control of smooth cordgrass with Rodeo® in a southwestern Washington estuary. Wildl Soc Bull 23:520-524

Kilbride KM, Paveglio FL (2001) Long-term fate of glyphosate associated with repeated Rodeo applications to control smooth cordgrass (*Spartina alterniflora*) in Willapa, Bay, Washington. Arch Environ Contam Toxicol 40:179-183

Kubena KM (1998) Rounding up the facts about Rodeo®: An evaluation of non-target effects on estuarine invertebrates and juvenile salmonids. MS thesis, University of Washington, Seattle, WA USA

Major WW III, Grue CE, Grassley JM, Conquest LL (2003) Mechanical and chemical control of smooth cordgrass in Willapa Bay, Washington. J Aquatic Plant Manage 41:6-12

Murphy, KC (2001) Report to the Legistlature - Progress of the *Spartina* eradication and control programs. Washington State Department of Agriculture, Olympia, WA USA

Paveglio FL, Kilbride KM, Grue CE, Simenstad CA, Fresh KL (1996) Use of Rodeo and X-77 Spreader to control smooth cordgrass (*Spartina alterniflora*) in a southwestern Washington estuary: 1. Environmental fate. Environ Toxicol Chem 15:961-968

Simenstad CA, Cordell JR, Tear L, Weitkamp LA, Paveglio FL, Kilbride KM, Fresh KL, Grue CE (1996) Use of Rodeo® and X-77® Spreader to control smooth cordgrass (*Spartina alterniflora*) in a southwestern Washington estuary: 2. Effects on benthic macroflora and invertebrates. Environ Toxicol Chem 15:969-978

Smith BC, Grue CE, Kohn NP (2000) Tissue residues of glyphosate and aminomethyl-phosphonic acid (AMPA) in shellfish associated with the application of Rodeo® to control *Spartina alterniflora*. Report submitted to the Washington Department of Agriculture by the Washington Cooperative Fish and Wildlife Research Unit, University of Washington, Seattle, WA USA

Washington State Department of Natural Resources (2000) Changing our waterways—Trends in Washington's water systems. Washington State Department of Natural Resources, Olympia, WA USA