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Toxicity of a glufosinate- and several glyphosate-based herbicides to juvenile amphibians from the Southern High Plains, USA

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ABSTRACT

Pesticide toxicity is often proposed as a contributing factor to the world-wide decline of amphibian populations. We assessed acute toxicity (48 h) of a glufosinate-based herbicide (Ignite® 280 SL) and several glyphosate-based herbicide formulations (Roundup WeatherMAX®, Roundup Weed and Grass Killer Super Concentrate®, Roundup Weed and Grass Killer Ready-To-Use Plus®) on two species of amphibians housed on soil or moist paper towels. Survival of juvenile Great Plains toads (*Bufo cognatus*) and New Mexico spadefoots (*Spea multiplicata*) was reduced by exposure to Roundup Weed and Grass Killer Ready-To-Use Plus® on both substrates. Great Plains toad survival was also reduced by exposure to Roundup Weed and Grass Killer Super Concentrate® on paper towels. New Mexico spadefoot and Great Plains toad survival was not affected by exposure to the two agricultural herbicides (Roundup WeatherMAX® and Ignite® 280 SL) on either substrate, suggesting that these herbicides likely do not pose an immediate risk to these species under field conditions.

1. Introduction

Amphibian populations are declining worldwide (Wyman, 1990), due in large part to the degradation of wetland and terrestrial habitats (e.g.,Wyman, 1990). Chemicals, such as insecticides, herbicides, and fertilizers used in agricultural activities may also contaminate aquatic and terrestrial habitats required by amphibians and pose a threat via direct toxicity (Semlitsch, 2003). Glyphosate (e.g., Roundup®) and glufosinate-ammonia (e.g., Ignite®) based herbicides are used worldwide (Howe et al., 2004; Lee et al., 2005) to control weeds in farmland and forests (Lee et al., 2005; Relyea 2005a). Glyphosate-based herbicides are also frequently applied in residential settings (Relyea, 2005a).

Most glyphosate-based herbicides contain two basic components: the isopropylamine (IPA) salt of glyphosate and a surfactant (the most common being a polyethoxylated tallowamine, POEA, surfactant) (Giesy et al., 2000). Glufosinate herbicides contain glufosinate-ammonium and a sodium polyoxyethylene alkylether sulfate (AES) surfactant (Koyama and Goto, 1997). Both glyphosate and glufosinate-ammonium adsorb strongly to soil (Malone et al., 2004; Lee et al., 2005), degrade rapidly via microbial activity and have limited environmental persistence (Faber et al., 1997; Giesy et

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al., 2000). In terrestrial situations, the POEA surfactant displays environmental fate similar to glyphosate (Giesy et al., 2000). Little information on the fate of the surfactant used in glufosinate herbicides is available. Since the major components of glyphosate herbicides bind tightly to soil and rapidly degrade, it is often assumed that they pose little risk to non-target organisms (Relyea, 2005a). However, recent work indicates that exposure to these chemicals can negatively affect amphibians within terrestrial (Relyea, 2005a) and aquatic habitats (Howe et al., 2004; Relyea, 2004; 2005a).

Numerous studies have investigated effects of glyphosate formulations on larval amphibians and results indicate that the surfactants, rather than the active ingredient, may be responsible for observed mortalities (Mann and Bidwell, 1999; Howe et al., 2004; Relyea, 2004; Relyea et al., 2005; Relyea, 2005a,b). Non-ionic surfactants, such as POEA, exhibit their negative effects primarily by disrupting the respiratory surfaces of aquatic organisms (Lindgren et al., 1996). Following metamorphosis, many amphibian species occupy terrestrial habitats. Yet few studies (Bidwell and Gorrie, 1995; Mann and Bidwell, 1999; Relyea, 2005a) have examined how post-metamorphic amphibians are affected by exposure to commonly applied herbicides. No work has examined whether natural environmental factors (e.g., soil) modulate the toxicity of herbicides toward post-metamorphic amphibians. Further research conducted under increasingly realistic conditions is necessary to fully understand how common agrochemicals affect amphibians (Relyea, 2005a).

Our purpose was to estimate juvenile survival of two of the most abundant amphibian species (*Spea multiplicata*, New Mexico spadefoot; *Bufo cognatus*, Great Plains toad) from playa wetlands of the Southern High Plains (SHP) following exposure to common herbicides at environmentally relevant levels. The SHP of Texas and New Mexico is one of the most heavily cultivated regions in the world (Bolen et al., 1989). It is therefore not surprising that the total volume of pesticides applied in Texas is among the greatest in the United States (Gianessi and Marcelli, 2000). Application to cotton represents one of the most prevalent uses of glyphosate-based herbicides (National Pesticide Use Database, 2004).

Because the nearly 25,000 SHP playas are principally embedded throughout an intensively farmed region, terrestrial margins of many playas likely receive overspray during applications of agrochemicals. Following metamorphosis, juvenile amphibians inhabit areas near playas while the soil remains moist (Voss, 1961; Graves and Kruppa, 2005; Morey, 2005). New Mexico spadefoots and Great Plains toads often occupy shallow burrows (Degenhardt et al., 1996) and emerge primarily for nocturnal foraging (Bragg, 1944; Garrett and Barker, 1987). However, recently metamorphosed individuals may also disperse away from drying playas (Degenhardt et al., 1996). Due to this behavior and the fact that herbicides are applied to cotton at various times throughout the spring and summer (National Research Council, 1975; Bayer CropScience LP, 2005; Monsanto Company, 2005), juvenile SHP amphibians may be exposed to common herbicides. During our study, juvenile amphibians were exposed to environmentally relevant concentrations of a glufosinate-ammonium based herbicide [Ignite® 280 SL (IG)] and several glyphosate-based herbicide formulations [Roundup WeatherMAX® (WM), Roundup Weed and Grass Killer Super Concentrate® (WGKC), and Roundup Weed and Grass Killer Ready-To-Use Plus® (WGKP)] while housed on moist paper towels or natural soil and survival was monitored for 48 h following application.

2. Materials and methods

Recently metamorphosed Plains and New Mexico spadefoot toads were collected on 27 June 2007 adjacent to a cropland playa wetland in Hale County, TX, USA. A mixture of the two species was collected because at a young age the two are difficult to distinguish (Degenhardt et al., 1996). Great Plains toad juveniles were collected near a cropland playa in Hale County, TX on 8 July 2007. Similar sized individuals were collected to ensure that animals used for subsequent toxicity testing were of similar developmental stage. The specific exposure history of the populations from which animals used in this study were drawn is unknown. However, these amphibian populations likely experienced previous pesticide exposure because they inhabit wetlands surrounded by agriculture. All subsequent animal care and experimental procedures (with exceptions noted) were the same for both spadefoot and Great Plains toads. This research was completed under a Texas Tech University Institutional Animal Care and Use Committee approved protocol (No. 06018-06). After collection, animals were transported to The Institute of Environmental and Human Health at Texas Tech University in Lubbock, TX. They were held in 37.9 L glass aquaria containing 6 cm of moistened natural soil obtained from Terry County, TX. The physiochemical characteristics of this sandy loam soil were previously determined by A&L Midwest Laboratories (Omaha, NE). The soil displayed the following properties: 74% sand, 10% silt, and 16% clay, 1.3% organic matter, and pH of 8.3 (Zhang et al., 2006). Though this soil was not tested for glyphosate- or glufosinate-based herbicide residues, significant chemical contamination is unlikely because the soil was obtained from an area where no pesticides have been applied for at least five years. Small crickets were provided *ad libitum* to juveniles throughout the following experiments. Fluker's Orange Cube Complete Diet (Fluker's Cricket Farm, Inc., Port Allen, LA) was provided to all crickets for at least 6 h.

Spadefoot and Great Plains toads were allowed to acclimate to laboratory conditions for three and four days, respectively. The spadefoot toad experiment commenced on 30 June 2007, while that with Great Plains toads began 13 July 2007. Experimental compartments were 11.4 L (31.5 cm long by 20.1 cm wide) plastic tubs lined with either paper towel or the previously described natural soil (260 g – dry weight). The soil covered the bottom of each tub evenly without allowing metamorphs to bury themselves. A 946.4 mL (32 oz) garden spray bottle was used to spray both substrates with aged well water until they were visibly moist. Paper towel lined containers received 14 g of evenly dispersed water, while soil lined containers received 28 g of water. Ten randomly selected juveniles were then added to each tub and allowed to acclimate for 6 h prior to herbicide application. Due to a counting error, a single tub received only nine spadefoot juveniles.