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Effects of Acute Exposure to a Commercial Formulation of Glyphosate on the Tadpoles of Two Species of Anurans

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Glyphosate is one of the most widely used herbicides in the world, and its use is continuing to increase (Baylis 2000). Glyphosate itself is purportedly environmentally safe (e.g., Caffrey 1996; Baylis 2000). However, glyphosate is a common active ingredient in several commercial, household herbicidal products (e.g., Round-Up®, Kleeraway®, Touchdown®). With increasing urbanization and suburbinization of many regions of the world, the potential for accidental or intentional introductions of glyphosate into aquatic systems (e.g., spills, improper disposal, use to clear aquatic vegetation) is likely to increase as household use of its commercial formulations expands. Amphibians are one group of aquatic organisms that is potentially affected by the introduction of commercial glyphosate formulations into aquatic systems. They use a wide range of aquatic habitats for their breeding sites, including many that are being altered by changes in water use patterns (i.e., increased urbanization and changes in agricultural practices; see Wear et al. 1998). We know relatively little about the potential toxicity of the various commercial glyphosate formulations for amphibians (e.g., Berrill et al. 1997; Glaser 1998; Mann and Bidwell 1999; Perkins et al. 2000).

MATERIALS AND METHODS

The acute toxicity of a commercial formulation of glyphosate (Kleeraway® Grass and Weed Killer RTU (Monsanto); active ingredient: Glyphosate, isopropylamine salt 0.75%; surfactant: Ethoxylated tallowamine) to the tadpoles of the western chorus frog (*Pseudacris triseriata*) and plains leopard frog (*Rana blairi*) was investigated. This study also investigated whether acute exposure to

this formulation affected post-exposure tadpole growth and survivorship.

A single *R. blairi* egg mass and several *P. triseriata* egg masses (the small number of *P. triseriata* eggs per egg mass necessitated using more than one egg mass) were collected on 20 May 2000. Egg masses were collected from a temporary pond located in Liberty, Clay Co., Missouri, and brought back to the laboratory where they were incubated in a mixture of pond water and deionized water at 19°C. Upon hatching, tadpoles were maintained in plastic containers and fed daily. The first acute toxicity experiment began on 15 June 2000 (approximately 1 week post-hatching) and included both species. All tadpoles used in the first experiment were Gosner stage 25. The mean body mass of 10 randomly selected *P. triseriata* tadpoles at the time of the first experiment was 0.018 ± 0.001 g, and the mean body mass of 10 randomly selected *R. blairi* tadpoles was 0.021 ± 0.001 g. The second acute toxicity experiment began on 4 July 2000 and included only *R. blairi*. For the second experiment, all tadpoles were Gosner stage 26-30, and the mean body mass of 10 randomly selected tadpoles at the time of the second experiment was 0.076 ± 0.008 g.

Tadpoles were exposed to one of five treatments: control (deionized water), 0.1 concentration (i.e., 1 part Kleeraway®: 9 parts deionized water), 0.01 concentration, 0.001 concentration, and 0.0001 concentration. Mixtures for each treatment were made up in 2000 mL stock solutions for the first experiment, and 1000 mL stock solutions for the second experiment.

The same basic experimental protocol was used for the first and second acute toxicity experiments. Four randomly selected tadpoles were placed into 200 mL of the test solution. All tadpoles were treated in the same fashion while being introduced into the test containers. Each treatment was replicated 5 times for each species. Tadpoles were exposed for 24 h and the number of surviving tadpoles at the end of the 24 h counted. The experiment was run at 19°C. Tadpoles were not fed during the experiment.

In addition to the acute toxicity experiment, an experiment designed to assess if surviving tadpoles exhibited sublethal effects from their acute exposure, such as reduced growth or developmental rate, was

performed. For this experiment, surviving tadpoles from the first acute exposure experiment were used (9 tadpoles for *P. triseriata*; 10 tadpoles for *R. blairi*). Since mortality was complete except for the control and 0.0001 concentration treatments for both species, only tadpoles from these two treatments were used. At the beginning of the experiment tadpoles were removed from their acute exposure containers and placed into a common holding container (one for each treatment and species combination) filled with deionized water. This served to decrease the amount of any residual herbicide on the tadpoles or potentially introduced into the new experimental containers. Randomly chosen tadpoles were then transferred individually into new containers containing 200 mL of deionized water. Tadpoles were fed, feces and excess food removed, and evaporated water replaced every third day. After 2 weeks, each tadpole was weighed to the nearest 0.001 g, and staged using Gosner (1960).

Prior to analysis all survival proportions were transformed using arcsin square root transformation. The results of the acute toxicity experiments were analyzed for each species separately, using a one-way ANOVA for *P. triseriata*, and a two-way ANOVA with concentration and experiment (first vs. second) as factors for *R. blairi*. The results of the growth and development experiment were analyzed using Mann-Whitney U tests with each species analyzed separately. Means are given $\pm$ 1 SE.

RESULTS AND DISCUSSION

Pseudacris triseriata survival in the acute toxicity experiment was greatly affected by the concentration of Kleeraway® to which they were exposed ($F_{4,20} = 76.0$, $P < 0.0001$). All the control tadpoles survived whereas no tadpoles in the 0.1, 0.01, and 0.001 concentrations survived. Survival in the 0.0001 concentration treatment was intermediate with 45 $\pm$ 12% on average surviving. In the subsequent growth and development study, the exposure the tadpoles experienced in the acute exposure experiment had no effect on tadpole final mass or final Gosner stage (Table 1). During the growth and development experiment, four of the nine 0.0001 concentration treatment tadpoles died within the first 2 days of the experiment, but none of the control treatment tadpoles died.

The responses of *R. blairi* to acute exposure to Kleeraway® differed between the first or second experiment (i.e., the interaction term was significant in the two-way ANOVA: $F_{4,40} = 218.5$, $P < 0.0001$, as well as the main effects: both $P < 0.0001$). In the first experiment, all the tadpoles in the control and 0.0001 concentration treatments survived, whereas no tadpoles in the other concentrations survived. In the second treatment, the only tadpoles to survive were those in the control treatment (mean survival = 95 ± 5%). In the subsequent growth and development study, the exposure the tadpoles experienced in the acute exposure experiment had no effect on tadpole final mass or final Gosner stage (Table 1). All the tadpoles in the growth and development study survived the experiment.

These results suggest that both *P. triseriata* and *R. blairi* may be at risk from exposure to commercial formulations of glyphosate. Even exposure to relatively low concentrations for a short period of time, as might be experienced in an accidental spill scenario or with an application to clear aquatic vegetation, appears to induce high mortality in tadpoles of both species. Berrill et al. (1997) found high mortality in *Rana clamitans* tadpoles exposed to 8 ppm of Roundup® but not at 4 ppm. Mann and Bidwell (1999) showed that some commercial formulations of glyphosate (e.g., Roundup® Herbicide and Touchdown® glyphosate acid) were toxic to four species of frogs, but that other formulations (e.g., Roundup® Biactive) were not

Table 1. Acute exposure of *Pseudacris triseriata* and *Rana blairi* tadpoles and subsequent tadpole growth (final mass in g) and development (Gosner stage). In all cases, the Mann-Whitney U test resulted in a $P \geq 0.18$.

	Control	Exposed
P. triseriata	(N = 9)	(N = 5)
Final mass	0.155 ± 0.004	0.147 ± 0.013
Gosner stage	33.3 ± 0.02	32.8 ± 0.02
R. blairi	(N = 10)	(N = 10)
Final mass	0.186 ± 0.022	0.179 ± 0.014
Gosner stage	30.5 ± 0.2	30.3 ± 0.3

very toxic, probably due to the surfactant used rather than the active ingredient (Mann and Bidwell 1999).

Mortality was slightly higher in western chorus frogs than in plains leopard frogs. Toxicity of glyphosate commercial formulations differed among the four species studied by Mann and Bidwell (1999). These results suggest the possibility that exposure to commercial glyphosate formulations alter not only population dynamics, but also community dynamics via differences in species-specific mortality rates. Differential sublethal effects, while not seen in my study, may also have consequences for anuran communities. For example, other agricultural chemicals have been shown to alter or potentially alter competitive or predator-prey interactions in anuran tadpoles (e.g., Bridges 1999; Verrell 2000; including *R. blairi* Bridges 1997), thus potentially altering community trajectories.

Ontogenetic differences in the toxicity of this glyphosate formulation were also found. *Rana blairi* tadpoles in the first experiment had lower mortality than in the second experiment. This was actually the opposite of what might have been predicted; that older, larger tadpoles tolerate exposure better. Indeed, Mann and Bidwell (1999) suggested that size differences among species might explain differences in susceptibility to glyphosate formulations. One possible explanation was that the tadpoles in the second experiment were more stressed prior to the experiment, having lived in the relatively high density holding container for longer than those in the first experiment. Such conditions may be slightly unrealistic, however, tadpoles that live in small, temporary ponds may experience high densities (pers. observ.) and might experience similar stress levels.

No effect of acute exposure on subsequent tadpole growth was found in either species. This conclusion is somewhat limited by the total or nearly total mortality in every exposure treatment except for the control and the 0.0001 concentration. However, it appears that acute exposure to low levels of this commercial formulation does not affect future performance of these tadpoles. Longer-term studies (e.g., following tadpoles to metamorphosis) are needed..

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