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Relative toxicity of the components of the original formulation of Roundup® to five North American anurans

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ABSTRACT

The responses of five North American frog species that were exposed in an aqueous system to the original formulation of Roundup® were compared. Carefully designed and un-confounded laboratory toxicity tests are crucial for accurate assessment of potential risks from the original formulation of Roundup® to North American amphibians in aquatic environments. The formulated mixture of this herbicide as well as its components, isopropylamine (IPA) salt of glyphosate and the surfactant MON 0818 (containing polyethoxylated tallowamine (POEA)) were separately tested in 96h acute toxicity tests with Gosner stage 25 larval anurans. Rana pipiens, R. clamitans, R. catesbeiana, Bufo fowleri, and Hyla chrysoscelis were reared from egg masses and exposed to a series of 11 concentrations of the original formulation of Roundup ® herbicide, nine concentrations of MON 0818 and three concentrations of IPA salt of glyphosate in static (non-renewal) aqueous laboratory tests. LC50 values are expressed as glyphosate acid equivalents (ae) or as mg/L for MON 0818 concentrations for comparison between the formulation and components. R. pipiens was the most sensitive of five species with 96h-LC50 values for formulation tests, for the five species, ranging from 1.80 to 4.22 mg ae/L, and MON 0818 exposures with 96h-LC50 values ranging from 0.68 to 1.32 mg/L. No significant mortality was observed during exposures of 96 h for any of the five species exposed to glyphosate IPA salt at concentrations up to 100 times the predicted environmental concentration (PEC). These results agree with previous studies which have noted that the surfactant MON 0818 containing POEA contributes the majority of the toxicity to the herbicide formulations for fish, aquatic invertebrates, and amphibians. These study results suggest that anurans are among the most sensitive species, and emphasize the importance of testing the herbicide formulation in addition to its separate components to accurately characterize the toxicity and potential risk of the formulation.

1. Introduction

Roundup® brand herbicides contain the active ingredient glyphosate, which is the most extensively used herbicide in the United States (Kiely et al., 2004). The original formulation of Roundup® contains the isopropylamine (IPA) salt of glyphosate and MON 0818 containing a polyethoxylated tallow amine (POEA) surfactant. Glyphosate is a broad spectrum, post-emergent herbicide, which works by inhibiting 5-enolpyruvyl shikimate-3-phosphate synthetase, an enzyme essential for production of aromatic amino acids in plants and some microorganisms (Franz et al., 1997). Animals obtain these aromatic amino acids from their diet and lack this enzyme; therefore, glyphosate is relatively nontoxic to animals (Giesy et al., 2000; Solomon and Thompson, 2003). POEA is a common adjuvant in glyphosate formulations, which enables the aqueous herbicide to stick to the surface of vegetation and aides the herbicide in penetrating the waxy cuticle on plant leaves (Giesy et al., 2000; Solomon and Thompson, 2003). Previous studies have indicated that the toxicity manifested by Roundup® herbicides to aquatic organisms is largely due to the surfactant in the mixture (Folmar et al., 1979; Mann and Bidwell, 1999; Edginton et al., 2004). In the United States, Roundup® branded herbicides are prohibited for direct application to water, however, these herbicides can enter aquatic systems through spray drift, unintended overspray, and to a limited extent through runoff from treated sites, incidentally exposing aquatic and semi-aquatic organisms (Giesy et al., 2000; Solomon and Thompson, 2003).

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Larval anurans have been identified as relatively sensitive organisms to glyphosate based herbicide exposures in laboratory and field studies compared to other aquatic species with acute LC_{50} values ranging from approximately 1–12 mg ae/L (Mann and Bidwell, 1999; Howe et al., 2004; Wojtaszek et al., 2004; Relyea, 2005a, 2005b, 2005c). Since Roundup® herbicides are used for both agricultural and silvicultural applications and relatively sensitive organisms such as larval anurans can be exposed, questions have arisen regarding the toxicity of these exposures (Mann and Bidwell, 1999; Howe et al., 2004; Relyea, 2005a, 2005b, 2005c). Therefore it is important to understand responses of these organisms to exposures of Roundup® formulations as well as the formulation components. Un-confounded laboratory tests using North American anuran species will help to discern the potential risks to these species from incidental exposures as well as the relative contribution of the components of Roundup® to the observed toxicity.

Five species of North American anurans, northern leopard frog (*Rana pipiens* Schreber), green frog (*Rana clamitans* Latreille), American bullfrog (*Rana catesbeiana* Shaw), Fowler's toad (*Bufo fowleri* Hinckley), and Cope's gray tree frog (*Hyla chrysoscelis* Laurenti) were chosen to determine the toxicity of the original formulation of Roundup® and its components to larval anurans. This research is intended to contribute to the accurate assessment of potential aquatic risks of the original formulation of Roundup® to North American amphibians. In order to predict responses to potential exposures and partition the toxicity of the components, the formulated mixture of this herbicide as well as its components were separately tested in 96 h acute toxicity tests with sensitive Gosner stage 25 (Gosner, 1960) larval anurans. The results of these acute toxicity tests were used to determine the relative contribution of the components, the IPA salt of glyphosate and the surfactant containing POEA, to the toxicity of the original formulation of Roundup®.

2. Materials and Methods

2.1. Test Substances

The original formulation of Roundup® (MON 2139) and components were supplied by Monsanto Company (St. Louis, MO, USA). The original formulation of Roundup® as supplied was a mixture of the IPA salt of glyphosate at 29.7% acid equivalent (ae) by weight and 15% MON 0818 containing POEA by weight. Separate components of the original formulation of Roundup®, IPA salt of glyphosate (CAS no. 38461-94-0) at 46.0% ae by weight and MON 0818 containing 69.4% POEA by weight (CAS no. 61791-26-2), were also tested individually. Stock solutions of the Roundup® formulation and the components used for toxicity tests were prepared at a nominal concentration of 1000 mg ae/L for the formulation and the glyphosate IPA salt, and 1000 mg/L for MON 0818 using NANOpure™ water.

2.2. Culture water

Water used for holding tanks, controls, and treatments was moderately hard water formulated to simulate general water characteristics of US lakes and streams (Sawyer et al. 1994; Wetzel, 2001). This water was comprised of 2.5 mg $CaCO_3$, 50.9 mg $NaHCO_3$, 24 mg $MgSO_4$, 16.5 mg $CaSO_4$, 32.5 mg $CaCl_2$, 1.05 mg KCl, 0.41 mg KNO_3, 0.009 mg K_2PO_4, 0.22 mL of 1000 ppm Cu reference standard, 0.11 mL of 1000 ppm Se reference standard, 0.22 mL of 1000 ppm Zn reference standard per liter of reverse osmosis filtered water. Water temperature was maintained at 20 ± 1 °C, pH ranged from 6.7 to 7.7, and dissolved oxygen was greater than 4.0 mg O_2/L.

2.3. Experimental design

Bioassays were performed according to the published US EPA method EPA-821-R-02–012 (U.S. Environmental Protection Agency, 2002). Chemical and physical measurements of testing conditions, dilution water, and test solutions were conducted according to the published American Society for Testing and Materials (ASTM) methods (ASTM, 2003). The aqueous tests were 96 h static non-renewal. Concentrations for definitive testing were determined from range-finding tests for the formulation as well as MON 0818. Concentrations tested included 0.3, 0.7, 1.0, 1.4, 1.7, 2.0, 2.4, 2.7, 3.2, 3.8, 5.0, and 7.0 mg ae/L for the Roundup® formulation. The large number and close spacing of testing concentrations in formulation tests enabled the NOEC and LC_{50} values to be calculated precisely. Concentrations for MON 0818 tests included 0.06, 0.18, 0.26, 0.37, 0.44, 0.59, 0.92, 1.25, and 2.00 mg/L. Stock solutions were diluted to definitive test concentrations in 3 L of water for each replicate.

Glyphosate IPA salt concentrations were based on the predicted environmental concentration (PEC) immediately following an application of herbicide at a recommended label application rate 0.84 kg ae/ha (0.75 lb ae/acre) (personal communication, Joy Honegger 2008) into a body of water with a depth of 20.3 cm. This depth corresponds to the depth proposed for a field study to be completed after the laboratory portion of this study, which would create ponds 20.3 cm deep including the worst case scenario depth of 15.2 cm from Solomon and Thompson, (2003) and 5.1 cm to account for evaporation calculated according to the evaporation rates for South Carolina and time of year corresponding to herbicide treatment to the ponds. Concentrations for glyphosate IPA salt tests included 0.42, 4.15, and 41.48 mg ae/L, which correspond to the PEC, ten times the PEC and 100 times the PEC.

Test vessels were 3.8 L glass jars filled with 3 L of test solution. In tests with the original formulation of Roundup®, there were four replicates per concentration and untreated control with 10 tadpoles per replicate. In MON 0818 and IPA salt of glyphosate tests, there were three replicates of each concentration and untreated control with 10 tadpoles per replicate. Tadpoles were not fed for the duration of the test to preserve water quality. Jars were gently aerated with single bubble aeration (ASTM, 2003). Frog eggs were field collected as available and tests were completed as eggs became available. All standard operating procedures were approved by Clemson University's Institutional Animal Care and Use Committee.

2.4. Animals

Egg masses were collected (*B. fowleri*, *R. catesbeiana*, *H. chrysoscelis*, *R. clamitans*) in Pickens and Greenwood Counties, South Carolina, or purchased from vendors (*R. pipiens* from Wards Natural Science, Rochester NY, Nasco, Fort Atkinson WI, and Carolina Biological Supply Co., Burlington NC, *R. catesbeiana* from Sullivan Co., Nashville TN and Carolina Biological Supply Co., Burlington NC). During holding, tadpoles were fed twice daily *ad libitum* a mixture of ground goldfish fish flakes (Tetra™) in water (Nace, 1974). Eggs and tadpoles were maintained in glass aquaria with a 16:8 light:dark cycle and single bubble aeration. Holding tanks were cleaned twice daily and up to 50% water changes were completed every other day to maintain good water quality. Tadpoles were reared to Gosner stage 25 prior to testing. Previous research has shown that this stage in amphibian development is more sensitive to exposures of contaminants than either embryo, earlier larval stages, later larval stages or adults (Berrill et al., 1994; Mann and Bidwell, 1999; Edginton et al., 2004; Howe et al., 2004).

2.5. Observed endpoint

The endpoint observed was mortality. Mortality was determined when an organism did not appear to have any respiratory functions or movement and did not respond to gentle prodding stimuli using a glass stir rod or removal from water (ASTM, 2003). Mortality was assessed and dead animals removed daily for four days.

2.6. Analytical

Analytical concentration verification was completed at Clemson University, Clemson South Carolina. Test solution samples were collected for glyphosate verification from dilution water, stock solution, and every replicate at all concentrations and controls immediately prior to adding animals to test chambers. Samples were stored in silanized glass vials at 3 °C prior to analysis. Glyphosate concentrations (reported as glyphosate acid equivalents, ae) were determined using Dionex Ultra-Mate-3000 High Performance Liquid Chromatography with autosampler and Variable Wavelength Detector system with Dionex Chromeleon software (Dionex Corp., Sunnyvale CA), and a YMC-Pack ODS-AM column with a 40 uL injection volume and 500 nm as the primary wavelength. Methods used for derivatization and analysis of glyphosate in water samples were supplied by Monsanto Company (Powell et al., 1990). A glyphosate analytical standard (99.8% purity) was used to create calibration standards. The range of recovery was 85–115% according to external standards.

2.7. Data analysis

Data were analyzed using SAS® Version 9.1 (SAS, 2007). 96-h LC_{50} values were considered significantly different from each other when their 95% confidence intervals did not overlap. Where appropriate, probit analysis was used to determine the no observed effect concentration (NOEC), LC_{50} values, and 95%

confidence intervals (CI). Under the probit analysis, the NOEC value was estimated to be the highest concentration that was not significantly different from the control response at the 95% level of confidence. When the concentration response data did not meet the assumptions of probit analysis, the USEPA MS-DOS application for trimmed Spearman-Karber analysis was used to estimate LC_{50} values and 95% confidence intervals. Non-parametric rank converted ANOVA's, equivalent to Kruskal–Wallis and Wilcoxon Rank Sum with Dunnett's test were used to determine NOEC values, here the highest concentration that was not significantly different from the control was considered the NOEC. The relative contribution (RC) of the components, MON 0818 and glyphosate IPA salt, to the toxicity of the formulation was calculated according to the method of Tsui and Chu (2003).

Regression analyses were used to generate potency equations for each test. For regression analyses, the data points creating the linear portion of the potency curve were used to calculate the potency equation. The linear equation calculated from this portion of the potency curve contains a key piece of information, namely the rate at which mortality for a population of organisms increases with increasing concentration of a toxicant, which can be shown by the slope of the concentration-response line (Perkins et al., 2000). A two way ANCOVA was used to look for significant differences in potency curve slopes among both formulations and species. Where significant differences among the slopes were detected, an one way ANCOVA was used to compare species within each formulation and to compare formulations within each species. Specific comparisons of slopes between species and/or formulations were performed using t-tests.

2.8. Quality control

In analytical concentration verification, recovery of glyphosate was between 85 and 115% for all tests. The concentration of glyphosate was analytically verified in each replicate at all concentrations tested, and the arithmetic mean concentration of the four replicates at each concentration was used for statistical analyses and reporting results. Control mortality was less than 5% in all tests, consequently, a correction for the natural rate of mortality was not made for LC_{50} estimations.

3. Results

R. pipiens was the most sensitive species tested in this study to exposures of the original formulation of Roundup® followed by *H. chrysoscelis, R. catesbeiana, B. fowleri*, and *R. clamitans* with 96-h LC_{50} values ranging from 1.80 to 4.22 mg ae/L (Table 1). *R. pipiens* was 2.3 times more sensitive than *R. clamitans* to the original formulation of Roundup®. In tests of the original formulation, 96-h LC_{50} values were significantly different from each other for all species except when comparing *B. fowleri* and *R. clamitans*, for which LC_{50} values were not significantly different. Potency slopes for the original formulation of Roundup® tests for the five species ranged from 24.3 to 92.5. *R. pipiens* slope was statistically different from *H. chrysoscelis*, *R. catesbeiana*, and *R. clamitans*, and, *B. fowleri* slope was statistically different from *R. clamitans* and *R. catesbeiana* ($F=6.77$, $df=4, 98$, $p<0.05$). All other potency slope comparisons for the original formulation of Roundup® were not statistically different ($p>0.05$) (Fig. 1).

Consistent with the results for the formulation, in tests with MON 0818, *R. pipiens* was the most sensitive species. *R. clamitans* was the least sensitive species for which a definitive LC_{50} value could be determined. The 96-h LC_{50} values for MON 0818 ranged from 0.68 to > 1.32 mg/L (Table 2). *R. pipiens* was two times more sensitive to MON 0818 than *R. clamitans*. Only 40% mortality was observed after 96 h in the highest concentration of MON 0818 tested for *H. chrysoscelis,* so a 96-h LC_{50} value was not calculated for this species. The potency equation for the MON 0818 test of *H. chrysoscelis* was estimated with mortality points from zero to 40% mortality since the 100% mortality rate was not attained. 96-h LC_{50} values for the tests of MON 0818 were significantly different from each other, except when comparing *B. fowleri* and *R. catesbeiana*, which were not. Potency slopes for MON 0818 for all five species ranged from 66.5 to 158.2. *H. chrysoscelis* potency slope was statistically different from *R. pipiens*, *B. fowleri*, and *R. catesbeiana*, and, *R. clamitans* potency slope was statistically different from *R. pipiens*, *B. fowleri*, and *R. catesbeiana* ($F=7.49$, $df=4, 45$, $p<0.05$). All other potency slope comparisons for MON 0818 were not statistically different ($p>0.05$) (Fig. 1). No significant mortality was observed during exposures of 96 h for any of the five species exposed to glyphosate IPA salt at the three concentrations tested (Fig. 1).

Potency slopes for the original formulation of Roundup® and MON 0818 were compared for each of the five species tested. For *H. chrysoscelis*, potency slopes for the formulation and MON 0818 were not statistically different ($F=0.49$, $df=1, 29$, $p=0.49$), this may be an artifact of having only partial mortality in the MON 0818 test. For all other species, the slopes of the formulation and MON 0818 were statistically different ($p<0.05$). *R. catesbeiana* ($F=21.48$, $df=1, 24$, $p=0.0001$), *R. clamitans* ($F=16.62$, $df=1, 21$, $p=0.0005$), *R. pipiens* ($F=6.48$, $df=1, 28$, $p=0.0142$), *B. fowleri* ($F=28.45$, $df=1, 21$, $p<0.0001$).

RC values are based on toxic units (TU), which are calculated from 96-h LC_{50} values for the formulation and both components of the formulation. In this study, 96-h LC_{50} values were not obtained for the glyphosate IPA salt component because it was nontoxic to tadpoles at all concentrations tested. At tested concentrations of 100 times the PEC, there was no significant mortality. Perkins et al. (2000) published a 96-h LC_{50} value of 7296.8 mg ae/L for *Xenopus laevis*. This value was used to estimate toxic units. For *R. pipiens*, *R. catesbeiana*, *B. fowleri*, and *R. clamitans*, MON 0818 contributed 100% of the toxicity to the formulation. For *H. chrysoscelis*, only 40% mortality was observed after 96 h in the highest concentration of MON 0818 tested, as such, neither the 96-h LC_{50} value nor the RC for this species could be calculated due to lack of sufficient mortality.

4. Discussion

The 96-h LC_{50} values determined in this study, ranging from 1.80 to 4.22 mg ae/L, are in agreement with previous research showing that invertebrates, fish, and anurans have 48–96-h LC_{50} values ranging from 0.8 to 18.0 mg ae/L for exposures to the

Table 1
Responses of five North American species of Gosner stage 25 larval anurans to 96 h exposures of the original formulation of Roundup®.

Species	NOEC[a]	96-h LC_{50}[a]	(95% CI)	Slope of Potency Equation	Margin of Safety[b]
R. pipiens	1.29	1.80	(1.73, 1.88)	92.5	0.46/9.2
H. chrysoscelis	1.74	2.50	(2.38, 2.63)	44.7	0.63/12.4
R. catesbeiana	2.02	2.77	(2.66, 2.89)	66.9	0.73/14.4
B. fowleri	3.40	4.21	(4.08, 4.33)	47.2	1.23/24.3
R. clamitans	3.27	4.22	(4.02, 4.42)	24.3	1.18/23.4

[a] Measured in mg ae/L.
[b] Based on predicted environmental concentrations for the highest label application rate assuming direct overspray/5% drift, and a water depth of 15.2 cm (6 in).

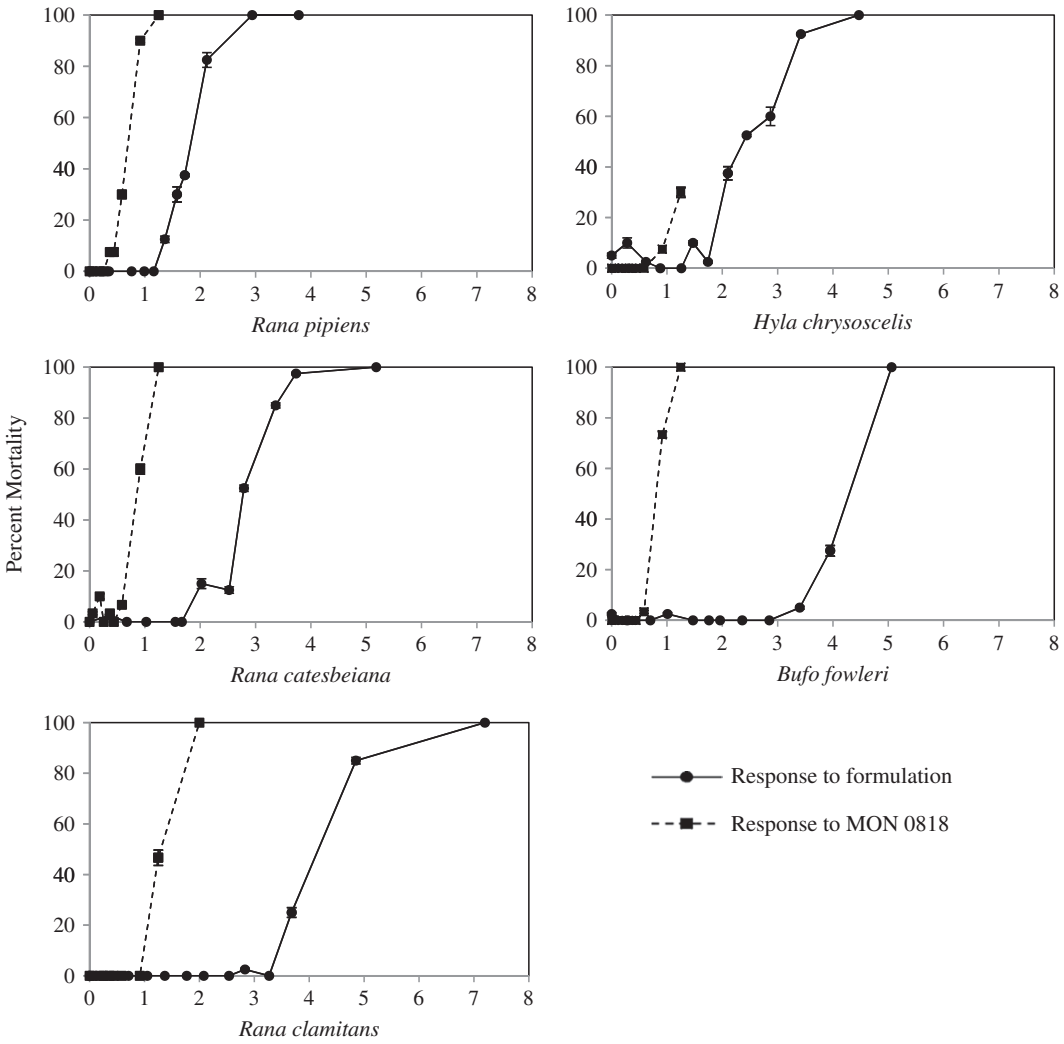

Fig. 1. Responses of five North American species of larval anurans to the original formulation of Roundup® and MON 0818 in 96 h aqueous toxicity test. Means ± standard deviation at each concentration are based on four replicates.

Table 2
Responses of five North American species of Gosner stage 25 larval anurans to 96 h exposures of MON 0818.

Species	NOEC[a]	96-h LC_{50}[a]	(95% CI)	Slope of Potency Equation
R. pipiens	0.38	0.68	(0.63, 0.74)	158.2
B. fowleri	0.59	0.80	(0.75, 0.85)	135.6
R. catesbeiana	0.59	0.83	(0.77, 0.90)	97.4
H. chrysoscelis	0.59	> 1.25[b]	[b]	60.2
R. clamitans	0.92	1.32	(1.23, 1.41)	66.5

[a] measured in mg/L.
[b] insufficient mortality to calculate LC_{50} and 95% confidence intervals.

original formulation of Roundup® (Table 3). LC_{50} values for aquatic animals including fish, invertebrates, and anurans are all within the same order of magnitude, which demonstrates the consistency of the response to Roundup® despite these studies being performed with a wide variety of aquatic organisms in different laboratories using different methodology. However, Table 3 also shows that different laboratories produced 96-h LC_{50} values, which varied greatly even within a single anuran species. 96-h LC50 values for *R. catesbeiana* range from 0.8 mg ae/L in Relyea and Jones (2009) to 2.8 mg ae/L in this study. *R. pipiens* 96-h LC50 values ranged from 1.1 mg ae/L in Edginton et al. (2004) to 2.9 mg ae/L in Howe et al. (2004). R. *clamitans* 96-h LC50 values ranged from 1.4 mg ae/L in Edginton et al. (2004) to 4.6 mg ae/L in this study. These differences in values could be due to different methods of collection, animals from different populations, different holding conditions, and/or testing procedures.

Less information is available regarding the effects of exposures of POEA on aquatic animals (Folmar et al., 1979; Wan et al., 1989; Giesy et al., 2000; Solomon and Thompson, 2003; Edginton et al., 2004). The results from this study agree with previous studies, which have noted that POEA contributes the majority of the toxicity to the herbicide formulations for fish, invertebrates, and amphibians, and again these study results suggest that anurans are among the most sensitive species (Folmar et al., 1979; Mitchell et al., 1987; Wan et al., 1989; Perkins et al., 2000; Howe et al., 2004).

MON 0818 contributed essentially 100% of the toxicity to the original formulation of Roundup®. No interaction was evident between the toxicity of MON 0818 and glyphosate IPA salt components in the formulation. When comparing the formulation tests and POEA component tests, 96-h LC_{50} values for the MON 0818 component tests were approximately equivalent to the values that would be expected if the toxicity was additive and POEA was contributing 100% of the toxicity. For example, in the

Table 3
Responses of test organisms to the original formulation of Roundup® and POEA in acute aqueous toxicity tests with 96-h LC_{50} glyphosate values originally published in mg/L converted to mg ae/L for comparison.

Species	Roundup® mg ae/L	MON 0818 mg/L	Citation
North American anurans			
Rana catesbeiana	0.8		Relyea and Jones, 2009
Rana catesbeiana	2.8	0.8	This research
Rana pipiens	1.1		Edginton et al., 2004
Rana pipiens	1.5		Relyea and Jones, 2009
Rana pipiens	1.8	0.7	This research
Rana pipiens	2.9		Howe et al., 2004
Rana clamitans	1.4		Edginton et al., 2004
Rana clamitans	1.4		Relyea and Jones, 2009
Rana clamitans	2.0	1.1	Howe et al., 2004
Rana clamitans	4.6	1.3	This research
Bufo americanus	1.6		Relyea and Jones, 2009
Bufo americanus	1.7		Edginton et al., 2004
Bufo americanus	< 4.0		Howe et al., 2004
Bufo fowleri	4.2	0.8	This research
Hyla chrysoscelis	2.5		This research
Hyla versicolor	1.7		Relyea and Jones, 2009
Australian anurans			
Litoria moorei	2.9–11.6[a]		Mann and Bidwell, 1999
Lymnodynastes dorsalis	3.0[a]		Mann and Bidwell, 1999
Crinia insignifera	3.6[a]		Mann and Bidwell, 1999
Heleioporus eyrei	6.3[a]		Mann and Bidwell, 1999
Fish and invertebrates			
Oncorhynchus mykiss	1.3–8.3	2.0	Folmar et al., 1979
Pimephales promelas	2.3	1.0	Folmar et al., 1979
Daphnia magna	3.0[a]		Folmar et al., 1979
Chironomous plumosus	18	13[a]	Folmar et al., 1979

[a] 48-h LC_{50}.

formulation test with *R. pipiens* the 96-h LC_{50} value was 1.80 mg ae/L. Since POEA is 10.41% of the original formulation of Roundup®, the expected 96-h LC_{50} value for a MON 0818 test, with this species, would be 0.63 mg/L (95% confidence interval 0.61–0.65 mg/L). The actual 96-h LC_{50} value for the *R. pipiens* MON 0818 component test was 0.68 mg/L (95% confidence interval 0.63–0.74 mg/L), which is not significantly different from the value for the formulation normalized to POEA content. These results demonstrate concordance between the predicted and observed LC_{50} values for these two treatments when comparisons are based on POEA content. Because the surfactants included in many herbicide formulations are proprietary information and surfactants are mixtures of polyethoxylated long-chain aliphatic amines is it difficult to measure the actual concentration of surfactant (Wang et al., 2005; Mann et al., 2009). As shown above, the concentration of glyphosate can be used to calculate the surfactant concentration in these acute toxicity tests.

The experimental design of this study, with close spacing of test concentrations, enables the NOEC and LC_{50} values to be determined with greater precision than in most acute toxicity tests. These NOEC values can be used to assess the risk to anurans from exposures to the original Roundup® formulation. NOEC values ranged from 1.29 to 3.40 mg ae/L for the original formulation of Roundup® (Table 1). By comparing the NOEC values and the PEC values, the margin of safety (NOEC/PEC) can be determined. For the original formulation of Roundup®, three recommended one-time application rates are 0.84 kg ae/ha, 1.68 kg ae/ha, and 4.21 kg ae/ha (0.75 lb ae/acre, 1.5 lb ae/acre, and 3.75 lb ae/acre), with the latter being the maximum applied amount allowed in a single application on crops (personal communication, Joy Honegger 2008). The PEC for these label rates immediately after direct application into a 15.2 cm deep water body would be 0.55, 1.11, and 2.76 mg ae/L, respectively. These calculated PECs are in agreement with the results of Thompson et al., 2004, which found observed glyphosate concentration ranging from 0.01 to 1.95 mg ae/L in oversprayed wetlands from aerial applications of a glyphosate formulation. Along with these worst case scenario PECs, which assume direct application to water, which is expressly prohibited on the Roundup® label, PEC values derived assuming 5% drift, which is the screening level assumption for risk assessment for terrestrial applications, would be 0.03, 0.06, and 0.14 mg ae/L.A margin of safety value greater than one signifies a NOEC value greater than the PEC, suggesting that at this application rate no toxic effects on larval amphibians would be expected. The MOS values for the worst case scenario at the highest application rate range from 0.46 to 1.23 and MOS values using the 5% drift of the highest application rate range from 9.2 to 24.3 (Table 1).

With this in mind, it is important to remember that the NOEC values calculated in this research are based on conservative aqueous laboratory tests and do not take into account the strong affinity both glyphosate and POEA have for binding with soil and sediment (Giesy et al., 2000; Solomon and Thompson, 2003) and dissipation of POEA from the water column (Wang et al., 2005). Thus, in a field environment, the bioavailability of glyphosate and POEA would be substantially less, thereby reducing the actual exposure and increasing the margins of safety.

5. Conclusions

North American anurans are among the most sensitive species to exposures of glyphosate containing herbicides but are within the same order of magnitude as other tested species including fish, invertebrates, and Australian anurans. Published literature on the toxicity of glyphosate containing herbicides is not in agreement as to the risk from exposures in the environment. This study was designed with rigorous testing protocols to accurately pinpoint NOEC and LC_{50} values without the complication of confounding factors. The conservative NOEC values derived from this data can be used to help predict the risk of adverse effects from exposures of herbicides. This study also contributes to the relatively small amount of data on the toxicity of the surfactants used in herbicide formulations. More work on the effects of glyphosate containing herbicides in the environment should focus on the surfactant components, which are currently unregulated and contribute 100% of the toxicity to the glyphosate formulations.

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