

CHAPTER 3

EFFECTS OF THREE HERBICIDES ON HATCHING SUCCESS, SURVIVAL, AND DEVELOPMENT OF AMPHIBIAN LARVAE.

Abstract-- A number of factors have been suggested to explain recently observed amphibian declines, including pesticide exposure. Effects of chronic exposure to three common herbicides on hatching success and larval survival and development of amphibians were evaluated in two separate experiments. Commercial formulation Barricade® 65 WG (prodiamine), Roundup® Weed and Grass Killer (glyphosate) and Trimec® Classic Broadleaf (dimethylamine salt of 2,4-D, mecoprop, and dicamba) herbicides were used. Three test concentrations for each experiment were determined from preliminary LC50 tests with gray treefrog (*Hyla chrysoscelis*) and northern leopard frog (*Rana pipiens*) tadpoles. Wood frog (*Rana sylvatica*) and American toad (*Bufo americanus*) eggs were exposed to herbicides in static tests in 5-L aquaria. Also, American toad and gray treefrog (*Hyla chrysoscelis*) tadpoles were exposed to herbicides from approximately two weeks post-hatching to metamorphosis in 60-L aquaria. In this experiment, herbicides were reapplied once every two weeks with water renewal. Wood frog hatching success was significantly decreased in 216 mg/L Trimec® Classic. Hatching success of American toads was significantly decreased in Barricade® 65 WG concentrations ≥ 210.207 mg/L. Survival of American toad tadpoles was significantly decreased in all concentrations of Barricade® 65 WG (8.408 – 420.415 mg/L). Survival of gray treefrog tadpoles was significantly decreased in Barricade® 65 WG concentrations ≥ 84.083 mg/L. Metamorphosed American toads and gray treefrogs exposed to 84.083 mg/L Barricade® 65 WG throughout development exhibited a greater

number of deformities. Effects on hatching success, larval survival, and occurrence of deformities may contribute to amphibian population trends and will likely influence persistence of populations if exposure to these concentrations occur in natural environments.

INTRODUCTION

Amphibian species that require relatively stagnant or slow-flowing water for breeding and larval development may be at risk for pesticide exposure in agricultural and urban areas when pesticides are applied directly to or reach breeding areas via runoff [1]. In natural environments, chronic or repeated exposures to acutely sublethal levels of pesticides are more likely than single-event, short-term exposure to acutely lethal levels. Environmental levels of chemicals and principal pathways of exposure should be taken into account when designing laboratory studies [2, 3].

Traditionally, laboratory toxicity tests have used standard lab species and extrapolated results to estimate effects in natural environments. Tests on resident species may be more appropriate when using toxicity data to assess the potential for contaminants to cause adverse effects measured in field studies [4]. Because species vary in their response to different toxins [3], several species representing diverse native taxa should be tested. Wood frogs (*Rana sylvatica*) breed in shallow, often temporary, bodies of water throughout northern North America and are described as explosive breeders. All eggs are laid within a few days in early spring, after which adult frogs retreat from the breeding area [5-7]. Average development of a wood frog from egg to metamorphosis encompasses 45 days [7]. The American toad (*Bufo americanus*) also breeds in early spring in the eastern United States and southeastern Canada.

Survivorship and growth of American toad tadpoles tend to be greater in open areas than in ponds sheltered by a forest canopy [8], and American toads often breed in ponds in agricultural areas [9]. American toad eggs hatch within 3 – 10 days. Larval development lasts 40 – 60 days [10]. The gray treefrog (*Hyla chrysoscelis*) breeds throughout spring and summer in temporary pools and semi-permanent ponds in the eastern United States and southeastern Canada [5, 6, 11]. Gray treefrogs exhibit strong breeding site fidelity and rarely migrate between breeding ponds [11]. Ptacek [12] observed average larval development periods of 38 and 44 days in two experiments with laboratory-raised *H. chrysoscelis*. Spring breeding and larval development can coincide with periods of heavy pesticide application and run-off.

Prodiamine, glyphosate, 2-4-dichlorophenoxyacetic (2,4-D), mecoprop, and dicamba herbicides are used on turfgrass in large recreational areas such as golf courses, residential gardens, and agricultural crops. Effects of exposure of amphibian larvae to prodiamine, mecoprop, and mixtures of dimethylamine salt of 2,4-D, mecoprop, and dicamba have not been previously studied. Some research has been done with active ingredients glyphosate and 2,4-D. Previous studies have shown amphibians to be particularly sensitive to glyphosate formulations containing the surfactant polyoxyethyleneamine (POEA). Roundup® herbicide contains isopropylamine (ipa) salt of glyphosate formulated with approximately 15% POEA. Mann and Bidwell [13] found that Roundup® herbicide was more toxic than glyphosate alone for four species of southwestern Australian frogs in 48-h LC50 (concentration lethal to 50% of test organisms) tests. Roundup® LC50 values were 911 mg/L for western green frogs (*Litoria moorei*) and > 1000 mg/L for white-throated froglets (*Crinia insignifera*),

moaning frogs (*Heleioporus eyrei*) and western banjo frogs (*Limnodynastes dorsalis*).

In contrast, glyphosate was essentially nontoxic. No mortalities were observed in 48-h tests for glyphosate alone at concentrations between 50 - 684 mg/L. Similarly, Perkins et al. [14] used the Frog Embryo Teratogenesis Assay-Xenopus (FETAX) to determine effects of Roundup® herbicide containing POEA and glyphosate without a POEA surfactant. The 96-h LC5 and LC50 values were 5102 and 7299 mg/L, respectively, for glyphosate alone. The Roundup® herbicide 96-h LC5 and LC50 values were 19.2 and 28.2 mg/L respectively (results were reported as acid equivalent values in the Perkins et al. study and were converted to mg/L for comparison to values reported in this study).

In a genotoxicity study, Clements et al. [15] used the alkaline single-cell gel DNA electrophoresis assay to examine DNA damage in bullfrog (*Rana catesbeiana*) tadpoles exposed to various concentrations of Roundup®. Tadpoles exposed to 6.8 - 27.0 mg/L of herbicide showed significant increases in DNA and cell damage compared to control tadpoles. Sanders [16] evaluated the effects of Weeder 64 (dimethylamine salt of 2,4-D) on western chorus frog (*Pseudacris triseriata*) tadpoles. The 96-h LC50 for one-week-old western chorus frog tadpoles exposed to Weeder 64 was > 100 mg/L. Amphibian toxicological studies for herbicide mixtures of 2,4-D, mecoprop, and dicamba have not been published.

In the current study, two laboratory experiments were conducted to investigate possible effects on amphibian embryos and larvae chronically exposed to commercial formulations of herbicides containing glyphosate, prodiamine, and dimethylamine salt of 2,4-D, mecoprop, and dicamba. In the first experiment, wood frog and American toad eggs were exposed to herbicides in static tests. In the second experiment, wood

frog and gray treefrog larvae were exposed to herbicides with water renewal and repeated dosage occurring every two weeks.

The objectives of the current study were to investigate the effects of chronic exposure to three common herbicides on hatching success, and larval survival and development, of diverse amphibian taxa. Chronic, repeated fungicide exposure at levels of 1 – 50% of 48-hour LC50 concentrations (the concentration lethal to 50% of test organisms) was expected to significantly decrease survival of amphibian eggs and larvae. Sublethal effects including effects on growth, time to metamorphosis, and number of gross deformities, were expected to increase with dosage.

MATERIALS AND METHODS

Roundup® Weed and Grass Killer Concentrate (Batch 11248/5, The SOLARIS Group of Monsanto, San Ramon, California), Barricade® 65 WG Herbicide (Batch BP801014, Novartis, Greensboro, North Carolina), and Trimec® Classic Broadleaf Herbicide (Batch 8283, PBI/Gordon Corp., Kansas City, Missouri) commercial formulation herbicides were used for all experiments. Roundup® Weed and Grass Killer Concentrate (18% glyphosate) is an organophosphate herbicide. Barricade® 65 WG Herbicide (65% proflaminate) is a dinitroaniline herbicide. Trimec® Classic herbicide contains three active ingredients, 2,4-D (26%), mecoprop (14%), and dicamba (3%). All three Trimec® Classic active ingredients are formulated as dimethylamine salts. Mecoprop and 2,4-D are phenoxy acids and dicamba is an organochlorine. Herbicide solutions were made with dechlorinated water. The average (\pm 1SD) water temperature of egg exposure aquaria was $20.0 \pm 0.2^\circ\text{C}$. The average (\pm 1SD) water temperature of

larval exposure aquaria was $22.0 \pm 0.1^\circ\text{C}$. Larval aquaria pH ranged from 6.3 – 7.1.

Water conductivity of larval aquaria consistently measured $0.2 \mu\text{s/cm}$.

Northern leopard frog tadpoles were ordered from Carolina Biological Supply, Burlington, North Carolina in early spring of 2000. Wood frog and American toad eggs were collected from breeding sites near Frostburg, Maryland, in the spring of 2000. Gray treefrog eggs were ordered from Charles D. Sullivan Company, Inc., Nashville, Tennessee in late summer 1999 and 2000. To effect more natural conditions, uncontaminated sediment was collected from two nearby ponds with no history of pesticide application and where amphibians were breeding successfully. Approximately 0.25 kg sediment and 1 kg sediment (wet weight) was added to the bottom of egg exposure aquaria and larvae exposure aquaria, respectively.

Three test concentrations for herbicides for each experiment were determined from preliminary 48-h LC50 tests with gray treefrog and northern leopard frog tadpoles at approximate Gosner stage 24 [17]. In the LC50 tests, tadpoles were exposed to five different concentrations of herbicides in 5-L aquaria containing dechlorinated water. Herbicide concentrations were orders of magnitude apart, and were based loosely on those reported for fish on Material Safety Data Sheets. The LC50 tests served as range-finding tests to determine standard experiment concentrations for all species evaluated in the experiments.

Egg exposure experiments

In egg exposure experiments, three test concentrations ($0.1 \times \text{LC50}$, $0.25 \times \text{LC50}$, and $0.5 \times \text{LC50}$) and a control ($0 \times \text{LC50}$) were evaluated. Egg exposure experiments were conducted as static tests in 5-L aquaria with one initial herbicide

treatment. Wood frog and American toad eggs were tested in consecutive trials in the spring of 2000. Aggregates of approximately 40-80 eggs were counted and randomly assigned to 72 aquaria in a completely randomized block design comprised of 6 experimental blocks. All herbicides were evaluated in the same experiment. Each 12-aquaria block contained one replicate of each test concentration and a control for each herbicide.

Eggs were acclimatized for 4 h before herbicides were added. Larvae were removed from aquaria two to three days after the last observed hatching in control aquaria. Hatching rates were determined for each aquarium using consensus counts, i.e. larvae were repeatedly counted until a consensus number was achieved. Larvae and any remaining unhatched eggs were examined using a dissection microscope or magnifying glass. Numbers and descriptions of gross deformities were recorded.

Larvae exposure experiments

In larvae exposure experiments, three test concentrations (0.01 x LC50, 0.1 x LC50, 0.5 x LC50) and a control (0 x LC50) were evaluated. Larval experiments were conducted as static tests with pulsed herbicide dosage occurring with 75% water renewal every two weeks. Wood frog eggs were hatched in the laboratory in the spring of 2000. Gray treefrog tadpoles were hatched in the laboratory in late summer 2000.

A total of 10 tadpoles was randomly assigned to each aquarium in a 48-aquaria completely randomized design composed of four experimental blocks. Each 12-aquaria block contained one replicate of each test concentration and a control for each herbicide. At the beginning of the experiment, wood frog tadpoles and gray treefrog tadpoles were at approximate Gosner stage 24 [17]. Tadpoles were fed tadpole chow *ad*

libitum so that minimal food was visible in aquaria at all times. Aquaria received 12 h of artificial light daily and were filtered twice weekly. Tadpole survival was observed daily. Tadpoles were weighed at the beginning of the experiment and again when hind limbs emerged. Weights were recorded as average wet weight per aquarium. Time to metamorphosis was recorded as the number of days to front limb emergence from day 1 of the experiment.

Water analysis

Water samples were taken 24 hours after dosage from larval exposure aquaria for Roundup® and Trimec® Classic herbicides. Samples were sent to University of Guelph Laboratory Services, Guelph, Ontario, for pesticide analysis.

Statistics

LC50 values were calculated using probit analysis [2] and SAS® V7 software [18]. Data were checked for normality using probability plots. Prior to analysis, percent hatching success, percent deformities, and percent survival data were transformed using arc-sine square root transformation [19]. In most cases, data were not normal and in some cases, variances were heterogeneous. Because parametric ANOVA assumptions were violated, nonparametric analyses were used. Minitab 12.1 software [20] was used for nonparametric and chi-square analyses. Kruskal-Wallis analysis of variance by ranks was used to test for differences between different concentration exposure treatment groups ($\alpha = 0.05$) for all egg experiment data and growth and larval development period data for larvae experiments. Dunn procedure pair-wise multiple comparisons were used when results of Kruskal-Wallis tests were significant [19]. Because there were so many pair-wise comparisons, differences could

exist by chance. The experiment-wise error rate was adjusted

$(.50/k(k-1)\sqrt{N(N+1)/12(1/nu+1/ny)})$ to take into account all of the comparisons

(L. Revenaugh, personal communication). Chi-square analysis was used for larvae experiment survival and deformity data [19]. Multiple chi-square analysis comparisons were done by comparing responses in different concentrations against responses in controls for each fungicide ($\alpha = 0.05$).

RESULTS

Range-finding LC50 tests

Median lethal concentrations (LC50) were determined for all commercial fungicides from range-finding toxicity tests. The 48-h LC50 for gray treefrog tadpoles exposed to Barricade® 65 WG was 0.04 mg/L. The 48-h LC50 for gray treefrog tadpoles exposed to Roundup® Weed and Grass Killer was 20.47 mg/L. The 48-h LC50 for northern leopard frog (*Rana pipiens*) tadpoles exposed to Trimec® Classic Broadleaf was 432 mg/L.

Egg exposure experiments

Hatching success of wood frogs varied in different concentrations of Trimec Classic® was significantly lower from other dosages in 216 mg/L ($p = 0.002$: Fig. 1). Hatching success of wood frogs was not affected by Barricade® 65 WG or Roundup® treatments (Appendix I, Table 3). Hatching success of American toad tadpoles varied among Barricade® 65 WG concentrations and was lower than other dosages in ≥ 210.21 mg/L ($p = 0.000$: Fig. 2). Hatching success of American toad

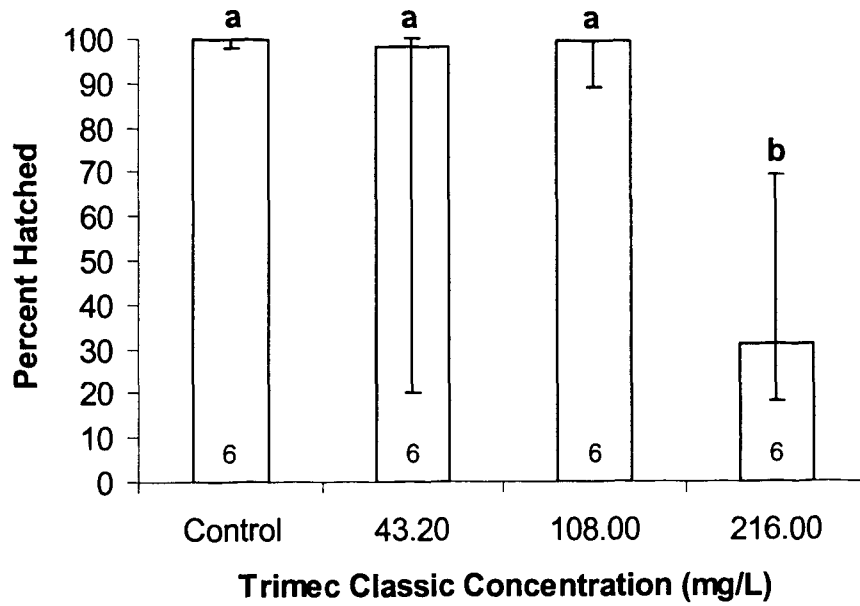


Fig. 1. Median percent of wood frog (*Rana sylvatica*) eggs hatched in Trimec[®] Classic concentrations (control is 0.0 mg/L). Value at the base of each bar is number of replicates. Treatments with the same letter at the top of the bar are not significantly different from each other at $\alpha = 0.05$. Error bars represent range among replicates.

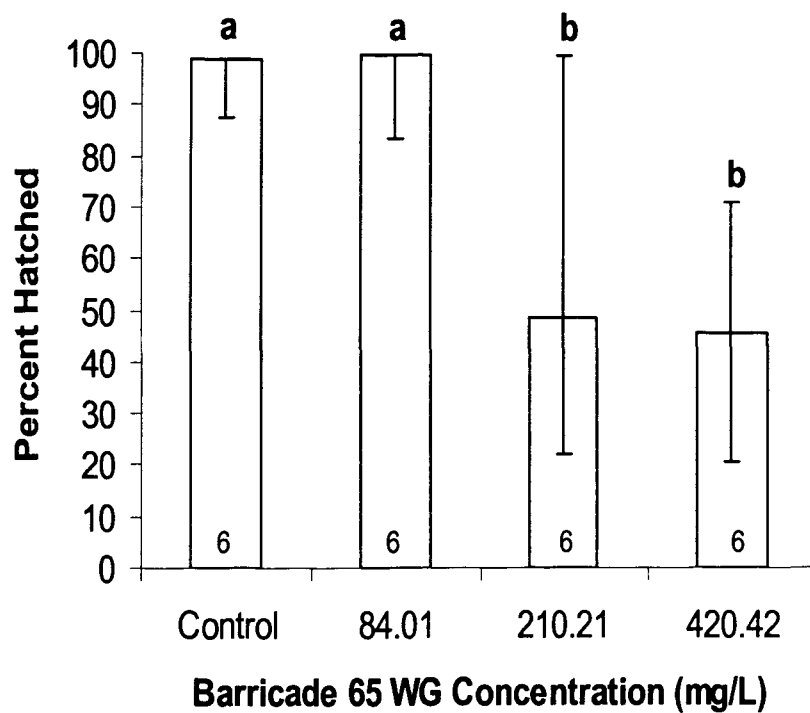


Fig. 2. Median percent of American toad (*Bufo americanus*) eggs hatched in Barricade[®] 65 WG concentrations (control is 0.00 mg/L). Value at the base of each bar is number of replicates. Treatments with the same letter at the top of the bar are not significantly different from each other at $\alpha = 0.05$. Error bars represent range among replicates.

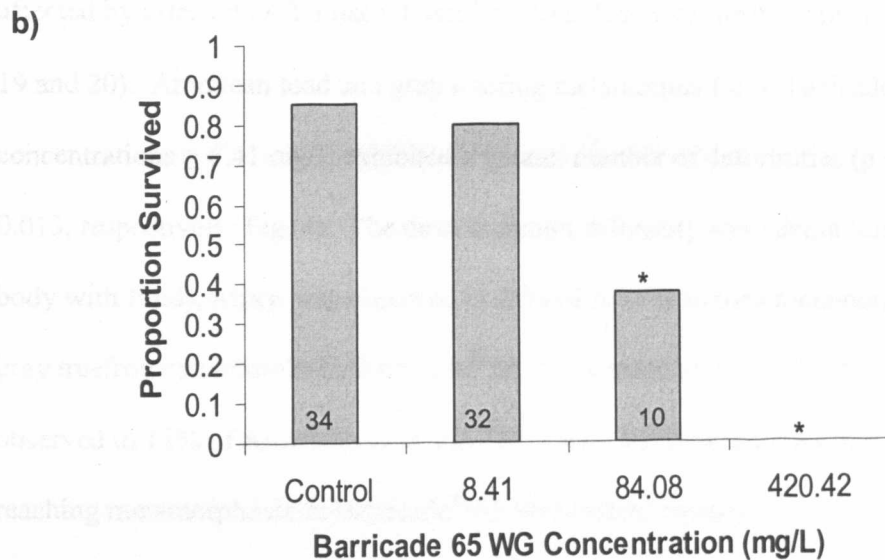
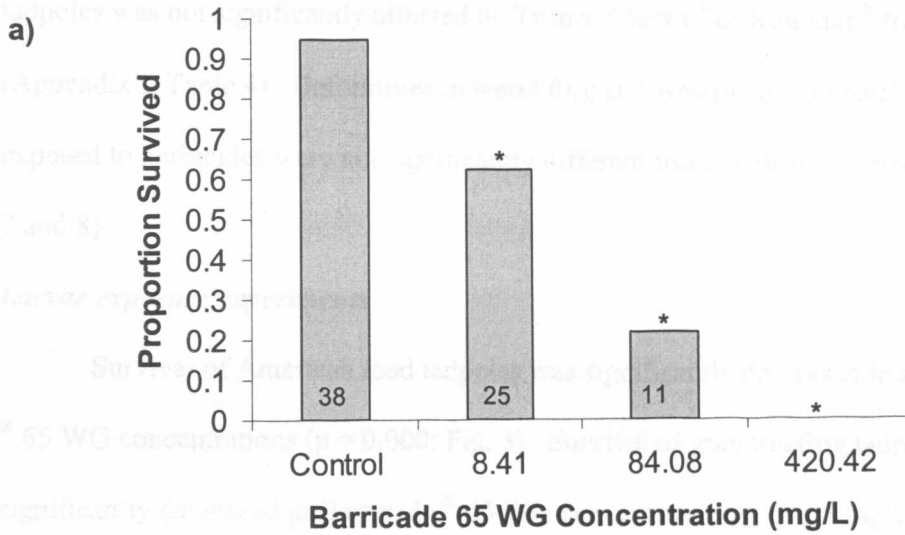


Fig. 3. Survival of a) American toad (*Bufo americanus*) tadpoles and b) gray treefrog (*Hyla chrysoscelis*) tadpoles in Barricade[®] 65 WG concentrations ranging from 0.00 mg/L (control) to 420.42 mg/L. Value at the base of each bar is number of individuals that survived in that treatment. * denotes proportions that differed significantly from expected proportions (controls).

tadpoles was not significantly affected by Trimec Classic[®] or Roundup[®] treatments (Appendix I, Table 4). Deformities in wood frog and American toad hatchlings exposed to herbicides were not significantly different from controls (Appendix I, Tables 7 and 8).

Larvae exposure experiments

Survival of American toad tadpoles was significantly decreased in all Barricade[®] 65 WG concentrations ($p = 0.000$; Fig. 3). Survival of gray treefrog tadpoles was significantly decreased in Barricade[®] 65 WG concentrations ≥ 84.08 mg/L ($p = 0.004$; Fig. 3). Survival of American toad and gray treefrog tadpoles was not significantly affected by affected by Trimec Classic[®] or Roundup[®] treatments (Appendix II, Tables 19 and 20). American toad and gray treefrog metamorphs from Barricade[®] 65 WG concentrations ≥ 8.41 mg/L exhibited a greater number of deformities ($p = 0.001$, $p = 0.013$, respectively; Fig. 4). The most common deformity was edema (distension of the body with fluid), which was observed in 28% of American toad metamorphs and 9% of gray treefrog metamorphs in Barricade[®] 65 WG-treated aquaria. Edema was also observed in 12% of American toad and 7% of gray treefrog tadpoles that died before reaching metamorphosis in Barricade[®] 65 WG-treated aquaria.

A small percentage (4 %) of gray treefrog metamorphs from Barricade[®] 65 WG concentrations exhibited a hind limb deformity where the motility of one hind limb was restricted by a locked femur-tibiofibula joint (knee joint). One gray treefrog metamorph from an aquarium with a 8.41 mg/L Barricade[®] 65 WG treatment had an abnormally short fore limb. A small percentage (4 %) of gray treefrog metamorphs from Roundup[®] concentrations exhibited abnormal rotations of hind limbs at femur-pelvic girdle joints.

Observations of deformities attributable to Roundup[®] were not significant, however femur-pelvic girdle joint rotations were not observed in metamorphs from other treatments.

The median growth of American toad tadpoles in different treatment groups ranged from 1.2 – 1.3 g (Appendix II, Table 11). The median growth of gray treefrog tadpoles in different treatment groups ranged from 0.4 – 0.9 g (Appendix II, Table 12). Median periods of larval development in different treatments were 26.8 – 50.0 days for American toads (Appendix II, Table 15) and 40.1 – 49.5 (Appendix II, Table 16) for gray treefrogs. Growth and larval development periods of wood frogs and gray treefrogs were not significantly affected by treatments.

Water analysis

Experimental concentrations of Roundup[®] herbicide ranged from 0.20 - 10.24 mg/L. Experimental concentrations contained approximately 0.04 - 1.84 mg/L glyphosate, and approximately 0.03 - 1.54 mg/L POEA. Water samples were taken 24-h after Roundup[®] treatments to larvae aquaria. Samples from 0.20 mg/L (n = 1), 2.05 mg/L (n = 1) and 10.24 mg/L (n = 2) treatments were sent to University of Guelph Laboratory Services for analysis. Analysis revealed glyphosate concentrations of 0.07 mg/L, 0.17 mg/L, 0.35 mg/L, and 0.88 mg/L respectively for dosage samples listed above. Samples were expected to contain concentrations lower than dosage because glyphosate degrades rapidly in water [21]. POEA measurements were not taken post-dosage for any treated aquaria. Glyphosate concentrations were expected to be slightly higher in sediment bottoms of aquaria than in water columns because glyphosate

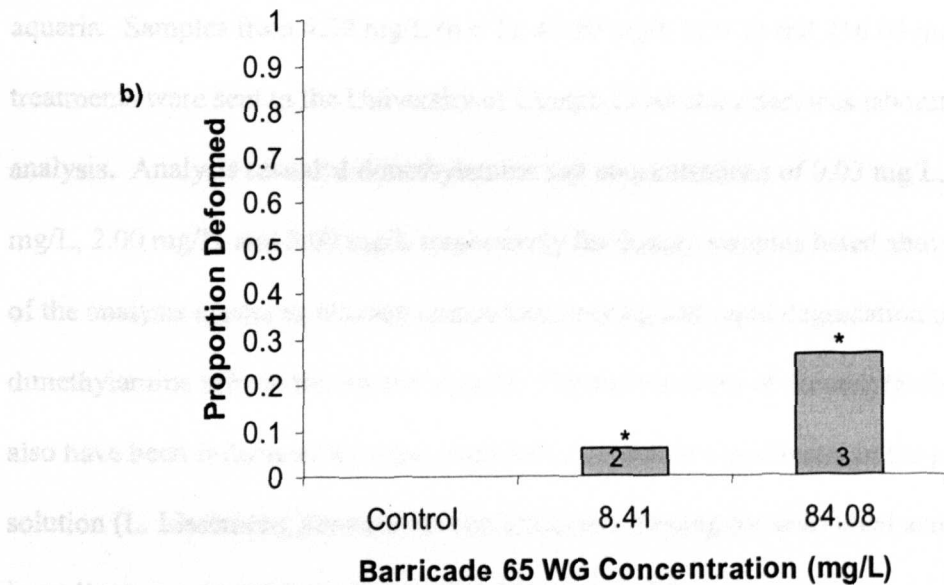
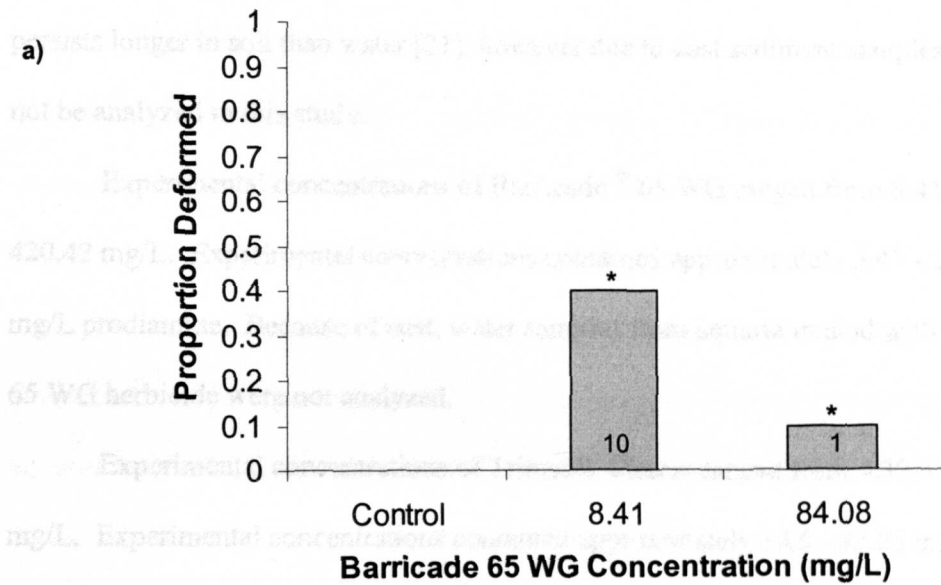


Fig. 4. Deformities in a) American toad (*Bufo americanus*) and b) gray treefrog (*Hyla chrysoscelis*) metamorphs exposed to Barricade[®] 65 WG throughout larval development. Control aquaria contained 0.00 mg/L of Barricade[®] 65 WG. Value at the base of each bar is number of deformed metamorphs in that treatment. * denotes proportions that differed significantly from expected proportions (controls).

persists longer in soil than water [21], however due to cost sediment samples could not be analyzed in this study.

Experimental concentrations of Barricade® 65 WG ranged from 8.41 mg/L - 420.42 mg/L. Experimental concentrations contained approximately 5.47 - 273.27 mg/L prodiamine. Because of cost, water samples from aquaria treated with Barricade® 65 WG herbicide were not analyzed.

Experimental concentrations of Trimec® Classic ranged from 4.32 - 216.00 mg/L. Experimental concentrations contained approximately 1.86 - 92.88 mg/L active ingredient. Water samples were taken 24-h after Trimec® Classic treatments to larvae aquaria. Samples from 4.32 mg/L (n = 1), 43.20 mg/L (n = 1) and 216.00 mg/L (n = 2) treatments were sent to the University of Guelph Laboratory Services laboratory for analysis. Analysis revealed dimethylamine salt concentrations of 0.03 mg/L, 0.03 mg/L, 2.00 mg/L, and 8.00 mg/L respectively for dosage samples listed above. Results of the analysis appear to indicate nonuniform mixing and rapid degradation of the dimethylamine salts in the treated aquaria. Normal recovery of dimethylamine may also have been influenced by other chemicals (i.e. active ingredients) in the pesticide solution (L. Lissemore, personal communication). Testing for individual active ingredients may provide more accurate degradation rates.

DISCUSSION

Barricade® 65 WG demonstrated toxic effects in American toad egg and larval development and in larval development of gray treefrogs. Specific effects were reduced hatching success in egg experiments and reduced survival and increased occurrence of

deformities in tadpole experiments. Differences existed in the responses of individual organisms to Barricade[®] 65 WG exposure. Differences in responses are common in toxicology studies and likely reflect natural variation that is attributable to different genetic makeup and condition of individuals [2]. A greater proportion of American toad metamorphs from tadpoles in aquaria dosed with 8.41 mg/L of Barricade[®] 65 WG were deformed when compared to metamorphs from tadpoles in aquaria dosed with 84.08 mg/L of Barricade[®] 65 WG. Differences could be due to natural variation, or premature death of tadpoles in the aquaria dosed with 84.08 mg/L of Barricade[®] 65 WG. A fewer number of tadpoles survived to metamorphosis in the aquaria dosed with 84.08 mg/L of Barricade[®] 65 WG and tadpoles that may have survived in the lower concentration likely died before teratogenic effects could be observed.

The LC50 for northern leopard frog tadpoles exposed to Barricade[®] 65 WG herbicide was 840.83 mg/L. Because Barricade[®] 65 WG herbicide contains approximately 65% prodiamine by weight, the approximate amount of prodiamine contributing to the LC50 was 546.54 mg/L. Toxic effects of chronic exposure were observed at Barricade[®] 65 WG concentrations \geq 8.41 mg/L. A Barricade[®] 65 WG concentration of 8.41 mg/L contains approximately 5.47 mg/L prodiamine. The rat LD50 (dose lethal to 50% of test organisms) for prodiamine is > 5000 mg/kg, and the herbicide is considered practically nontoxic to mammals if ingested [22]. Rainbow trout (*Oncorhynchus mykiss*), and bluegill sunfish (*Lepomis macrochirus*) 96-h LC50 values are > 0.83 mg/L and > 0.55 mg/L respectively [22]. No previous studies of prodiamine amphibian toxicity have been published. Observations from this study

indicate that prodiamine may be more toxic to amphibians than mammals, but less toxic to amphibians than fish. However, the amphibian species tested in this study demonstrated species-specific responses to Barricade® 65 WG concentrations and further testing should be done before extrapolating findings to all amphibian species.

Prodiamine is a root inhibitor belonging to the dinitroaniline chemical class. Water solubility of dinitroaniline compounds is quite low and is roughly estimated to be < 1 part per billion (<http://www.agcom.purdue.edu/AgCom/Pubs/WS/WS-23.htm>). Barricade® 65 WG herbicide contains a yellow dye and visual observations of aquaria treated with Barricade® 65 WG indicated that the herbicide had low water solubility. Toxicological effects on tadpoles were assumed to have occurred either through ingestion of the herbicide or close contact with the herbicide in the sediment bottoms of the aquaria. Because dinitroaniline compounds have low water solubility, the potential for motility through groundwater to ponds is low [2]. Effects observed in this study would likely only occur in natural environments with direct application. In amphibian breeding areas where prodiamine herbicides are used, it is advised that pond sediment levels be monitored to determine exposure levels.

Test concentrations in this study were fractions of gray treefrog and northern leopard frog LC50 values. Without specific monitoring, it is often difficult to assess what herbicide concentrations, if any, amphibians are being exposed to in natural breeding areas. Because the 216.00 mg/L Trimec Classic® concentration that affected gray treefrog tadpole survival is relatively high, it is unlikely that similar levels are found in natural waters, except in spill situations. Roundup® concentrations ranged from 0.20 - 10.24 mg/L. Clements et al. [15] observed genotoxic effects in bullfrog

tadpoles exposed to ≥ 6.75 mg/L Roundup[®]. No toxic effects were observed with chronic Roundup[®] exposure in this study. Abnormal rotations of femur-pelvic girdle joints were observed only in gray treefrog metamorphs exposed to Roundup[®] treatments, but this deformity was infrequent (< 5% of exposed individuals) and not statistically significant. Experimental concentrations of Roundup[®] were likely similar to ecologically relevant levels. Newton et al. [21] evaluated glyphosate herbicide residues in forest brush field ecosystems that were aerially treated with 3.3 kg/ha glyphosate in the Oregon Coast Range, USA. The half-life of glyphosate ranged from 10 to 27 days in foliage and litter and 29 to 40 days in soil. Soil measurements peaked at 0.8 mg/L. The treated stream peaked at 0.27 mg/L and decreased rapidly.

This study attempted to bridge the gap between field conditions and laboratory environments by including natural pond sediment in test aquaria and using native test species. The extrapolation of lab results to field observations also requires that ecologically relevant levels of pesticides be used in laboratory tests. Field monitoring is needed to determine if herbicides are present at toxic levels in natural areas. Species-specific responses were observed in this study. If herbicides occur at measurable levels in areas experiencing amphibian declines, laboratory experiments should be conducted with native species of these areas to establish or provide evidence against links between herbicide application and amphibian declines.

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