A TEST FOR ACUTE TOXIC EFFECTS OF A GLYPHOSATE-BASED HERBICIDE ON LARVAE OF THE SALAMANDER

*Ambystoma macrodactylum*

BRIANA CARMAN

SPRING, 2004

Honors Thesis

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PASS WITH DISTINCTION
TO THE UNIVERSITY HONORS COLLEGE:

As faculty advisor for Briana Carman

I have read this paper and find it satisfactory.

Faculty Advisor

1-28-04

Date
PRÉCIS

The objective of my study was to determine the toxicity of a glyphosate-based herbicide, Ortho\textsuperscript{®} GroundClear\textsuperscript{®} Triox Total Vegetation Killer, to larvae of the salamander \textit{Ambystoma macrodactylum}. I hypothesized that wild salamander larvae would be slightly more susceptible than lab-bred \textit{Xenopus laevis} that were previously tested in our laboratory. One overall purpose of this study was to increase available knowledge about the effects on amphibians exposed to pesticides. Amphibians are not widely used in toxicological assays; studies of fish are more common and most assays assume that amphibians are comparable to fish. However, research has shown that fish and amphibians have different tolerances to various chemicals and concentrations. Amphibians are highly sensitive to negative changes in their habitats, which may ultimately lead to negative effects at the level of entire communities. This makes it only logical to include amphibians when conducting toxicological assays. Due to recent amphibian declines, amphibians are now being studied more intensely, especially in the field of toxicology.

The organism I chose to study in my experiment was \textit{Ambystoma macrodactylum}, the long-toed salamander. This salamander is found in southeastern Washington; therefore, my study has special significance to this region. I used larvae instead of adult salamanders because larvae would be the life stage most affected by contamination of wetlands, and also because the survival of larvae can determine the population dynamics for the next generation.

The use of glyphosate-based products has increased exponentially over the last twenty years and has risen to number two in overall production in the agricultural sector. However, some formulations of glyphosate-products, such as GroundClear\textsuperscript{®}, have never been tested on amphibians. Since glyphosate use is so prevalent in the world, tests need to be conducted to determine how it
may affect organisms in contaminated areas. This information should then be made public so herbicide users can be informed and use the herbicide with more caution, if necessary.

In this study, I exposed larvae to various concentrations of GroundClear® based on the recommended field application (nominal), plus water controls. This experiment was modified from FETAX, a protocol often used in amphibian toxicological assays. To determine the toxicity of other chemicals, toxicologists use LC$_{50}$. This measurement is a calculation of the lethal concentration of chemical that is required to kill fifty percent of test subjects. By obtaining this measurement, my data could be used by other toxicologists. The results from the first experiment helped me to narrow my search for the LC$_{50}$ in experiment two. The results indicated a distinct dose-response curve (see figures 1 and 2 on page 17) that shows the chemical concentration has a very strong correlation to larval survival. This also helped me approximate the LC$_{50}$ at the concentration of 0.0001 of nominal. This LC$_{50}$ was a lower concentration than what was found previously for the African-clawed frog, *Xenopus laevis*, and for various juvenile fish.

While the data for *X. laevis* are valuable, it is also difficult to draw connections between that amphibian and wild amphibians found in southeastern Washington. *X. laevis* is an unusual amphibian that lives its entire life cycle in water and has been lab-bred for many generations. While testing on *X. laevis* should continue to give researchers a baseline concentration limit, there should also be assays examining animals that would be realistically exposed to the chemicals in question.

This field of study has many more aspects that have not been researched sufficiently. Other end-points, such as deformities, DNA damage, and predator response tactics, have yet to be fully explored. Chemical exposure can also be part of additive or synergic affects in amphibians, causing more harmful affects than what is previously known. These topics need to be fully investigated to provide a more complete understanding of amphibian toxicology.
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ABSTRACT

The objective of this study was to determine the extent of acute toxicity of the agrochemical Ortho® GroundClear® Triox Total Vegetation Killer on larvae of the long-toed salamander, *Ambystoma macrodactylum*. This formulation of herbicide is widely available for public use and has approximately the same vegetational outcomes as other complete vegetation killers, such as Roundup®. GroundClear® contains glyphosate as its primary active ingredient; hence use of this formulation may potentially result in environmental contamination with a potent herbicidal chemical. Previous work in our laboratory has demonstrated acutely toxic effects of GroundClear® on the young stages of the frog *Xenopus laevis*, a model organism in studies of amphibian toxicology. The long-toed salamander was selected for the current study as a ‘natural’ amphibian species of regional significance to southeastern Washington. Young salamander larvae were exposed to multiple concentrations, with appropriate controls included. A dose-response curve was constructed using death as the end-point. My data suggest that acutely toxic effects of GroundClear® become apparent at exposure of larvae to approximately 0.0001 times the nominal concentration recommended for field application. Comparisons with previous work suggest that salamander larvae may be more susceptible to toxic effects than the young of *Xenopus laevis*.

INTRODUCTION

Amphibians are considered to be highly sensitive indicator species of environmental health and integrity (Pough et al., 1998); indeed, many biologists regard them as the modern-day
equivalents of 'canaries in coal mines,' the unfortunate birds that were used to detect gas leaks that could eventually be lethal to the humans working in the mine. Frogs and salamanders often are among the first vertebrate animals in natural communities to be affected negatively by changes in their habitats caused by humans (Pough et al., 1998). Together with increasing incidences of morphological deformities, recent and current declines and extinctions of amphibians on a global scale are strong evidence that negative changes are occurring within the natural habitats of these animals (Alford & Richards, 1999).

One important hypothesis is that these changes are a consequence of contamination by industrial and agricultural chemicals. However, until recently little was known about the extent to which such chemicals might be acutely and/or chronically toxic to amphibians (see Berrill et al., 1997). Chemically diverse pesticides, used to control both animal and plant pests, are readily available to the public. There is a high potential for application of such chemicals to contaminate amphibian breeding sites by run-off and drift. Exposure to such chemicals may not be toxic or lethal; however, the chemicals may impact exposed individuals by: (1) altering behavior patterns crucial for survival, such as antipredator tactics, by (2) deforming the larvae, consequently making them unable to compete for resources (see Lajmanovich et al., 2003), by (3) compromising immunocompetence (the capacity for a normal immune response), thus increasing susceptibility to pathogens (see Christin et al., 2003), or by (4) decreasing food availability, as insects are also extremely vulnerable to the acute toxic affects of glyphosate formulations (Cox, 2000). With sufficient individuals affected, negative consequences ultimately may become apparent at the population level and eventually obvious at the community level (Verrell, 2000), especially when amphibian predators become less numerous in targeted wetlands due to a lack of food.
Ortho® GroundClear® Triox Vegetation Killer is a broad-spectrum herbicide that can be purchased at most home improvement warehouses and local hardware stores. Based on information provided on the label, its primary active ingredient is glyphosate, present as an isopropylamine salt, and is 5% by mass. Imazapyr is the secondary active ingredient; it is also an isopropylamine salt and is 0.08% by mass. The remaining ingredients (94.92% by mass) are listed as ‘inert’ ingredients – so called because these ingredients do not do the killing of the plant pest. However, these inert ingredients can have a drastic effect on animal life (the non-target organisms) that are exposed for a sporadic or a continual amount of time. For example, isopropylamine, an alleged ‘inert’ ingredient, can be found in some Roundup® products. In humans, contact with this ingredient is very harmful to the respiratory tract (Cox, 2000).

Glyphosate is also found in many other formulations of broad-spectrum herbicides, including Roundup®, Rodeo®, and Kleeraway® (EXTOXNET, 1996). Glyphosate has been dubbed ‘the most important herbicidal chemical ever developed’ (Neskovic et al., 1996), and is also advertised as environmentally safe (Smith, 2001). This assertion was tested directly in a study that compared the direct toxicity of pure glyphosate versus formulations containing it to Australian frogs. While glyphosate per se appeared relatively innocuous, exposure to several glyphosate-containing formulations resulted in high levels of mortality, most likely due to the added surfactant present in commercial formulations (Mann & Bidwell, 1999). While it may be beneficial to the manufacturing company to advertise pure glyphosate as safe for the environment, most animals will be subjected to the formulations and not the pure chemical form of glyphosate.
In the United States, the use of herbicides has become a standard. According to the Environmental Protection Agency’s 1998/1999 Pesticide Sales and Usage Report (2003), approximately 40 million of 103.9 million households in the United States (38.5%) use herbicides. About 77 million (74.1%) households use some form of pesticide (insecticide, fungicide, herbicide, repellent, or disinfectant). Glyphosate is listed as the second most commonly used conventional active pesticide ingredient in the agricultural market sector, the home and garden market sector, and the industrial, commercial, and governmental sector. Of more concern is how rapidly glyphosate rose to this position. During the twelve years over which this study was conducted, glyphosate production has increased exponentially. In 1987, glyphosate was ranked 17th in production for use in the agricultural sector, and was produced in the range of six to eight million pounds. By 1993, this number had more than doubled to between 15 and 20 million pounds, and in 1997, it doubled once again. In 1999, it had yet again doubled to total a production rate between 67 and 73 million pounds, ten times the amount produced in 1987 (see Table 1).

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<td>1</td>
<td>70-75</td>
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<td>Metam Sodium</td>
<td>Fum</td>
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Table 1: The three most commonly produced conventional active pesticide ingredients, used in the agricultural sector. The range (in million pounds) produced determines rank. H indicates herbicide and Fum indicates fumigant. The production of atrazine stayed fairly constant throughout the study, yet production of both glyphosate and metam sodium both increased significantly. Glyphosate production increased to ten times the amount produced in 1987. Table has been recreated from data supplied by the EPA (2003).
At the current rate of growth for its production as an agricultural pesticide, glyphosate may soon pass the most commonly used conventional active pesticide ingredient, atrazine (which also causes notable amphibian problems: see Christin et al., 2003; Rohr et al., 2003).

Formulations containing glyphosate are applied to rid target areas of all unwanted vegetative growth. The pure compound has an average half-life of 47 days in soil (EXTOXNET, 1996), but there have been documented cases of the pure compound having a half-life of over 100 days in soil in the east United States (Cox, 2000). Glyphosate has a half-life ranging from 12 days to 10 weeks in aquatic habitats (EXTOXNET, 1996), but a much higher half-life in pond sediment, between 120 days to over one year in some studies (Cox, 2000). Contamination by glyphosate has not only been recognized in the eastern United States; it has also been found in the Pacific Northwest. Glyphosate-contaminated water has been detected in streams in the Puget Sound area and in forest streams throughout Oregon and Washington. Soil contamination was also observed at an Oregon Coast forestry site, where the persistence of the chemical in the soil was calculated at a half-life of 55 days (Cox, 2000).

The use of glyphosate-containing formulations such as GroundClear® has increased as urbanization and suburbanization pressures have changed former fields into manicured lawns. With this increase in residential land use comes the possibility of careless or ignorant application, such as in the vicinity of ponds and other wetlands used as breeding sites by amphibians. It then becomes important to determine the extent to which glyphosate-containing formulations may impact non-target frogs and salamanders, especially those life stages (embryos and larvae) whose survival is crucial for population persistence.
According to most of the chemical data sheets to which I was able to obtain access, the effects of glyphosate have not been thoroughly tested in amphibians. Tests conducted on other aquatic vertebrates, especially fishes, largely suggest that glyphosate-containing formulations are more toxic than the pure chemical because of added inert ingredients. For young fish, mortality was observed at concentrations as low as two parts per million (ppm) (Cox, 2000). While the results of studies of fishes may provide an approximation of toxicological effects on amphibians, this is not necessarily the situation (Bridges et al., 2002), and in most instances is an untested assumption. One paper found that other organisms that are not utilized in mainstream toxicological assays, such as marine copepods, are extremely vulnerable to contamination by glyphosate and its formulations (Tsui & Chu, 2003). This result could not have been hypothesized based on the current available literature. Thus, I contend that toxicological assays must be conducted using amphibians, in addition to other animals, to be more inclusive of all components of the communities that may be affected by herbicide application.

*Ambystoma macrodactylum* (subspecies *columbianum*), the central long-toed salamander, is a common ambystomatid salamander in the Palouse region of southeastern Washington. Adults breed in small wetlands such as ponds in late winter to early spring. Embryos in eggs and free-swimming larvae remain in water until as late as September (Pough et al., 1998; Verrell, 2000). Thus, use of GroundClear® in the vicinity of a breeding site has the potential to have long-term exposure on these young life history stages. An earlier study conducted in our laboratory found that exposure to concentrations of GroundClear® between, but not including, 0.0001 and 0.001 of nominal (the recommended concentration for field application) is directly
and acutely toxic to embryos and larvae of the South African clawed frog, *Xenopus laevis* (VanBuskirk, 2003). While this species has become a model laboratory species, the so-called ‘white rat’ of the amphibian world, and has also become a model for studies of amphibian toxicology (e.g., as in the development of the Frog Embryo Teratogenesis Assay-Xenopus, FETAX, a standard guide to test amphibian toxicology), it also has rather unique characteristics because it is wholly aquatic, has been bred in captivity for many generations, and is not native to areas outside of Africa. Therefore, we may ask how well the results obtained from a lab-bred animal such as *Xenopus laevis* compare with those from wild amphibians (Bridges et al., 2002). In the experiments that do test on wild amphibians, there is a lack of toxicological research utilizing caudate amphibians as test subjects. Observing this, I decided to test for acutely toxic effects of GroundClear® on larvae of the long-toed salamander. In addition, because GroundClear® could be used in residential areas near ponds and subsequently run-off into local wetlands, my data for this species have relevance for public use of this formulation on southeastern Washington.

**MATERIALS AND METHODS**

I exposed larvae of the salamander *Ambystoma macrodactylum* to a range of concentrations of Ortho® GroundClear® Triox Total Vegetation Killer and determined mortality as an end-point over a 96 hour period. I conducted two separate experiments in order to determine a dose-response curve, a graphical representation of the quantitative relationship between a dose of a substance (in this case, GroundClear®) and the specific biological effects (in this case, the end-point of mortality of the salamander larvae). This procedure was adapted from the FETAX (American Society for Testing and Materials, 1998).
Female *Ambystoma macrodactylum* swollen with eggs were captured using minnow traps in a pond near Pullman, Washington in February and March during the breeding season of 2003. They were placed in plastic boxes containing aged tap water and rocks, and allowed to lay eggs. The eggs were then removed and placed into larger aquaria containing aerated and aged tap water at approximately 15° C. Hatched larvae were removed and placed into similar aquaria until the first trial began when they had developed distinct forelimbs, a characteristic that indicates that the larvae had progressed to between developmental stages 6 to 9, as determined according to Watson and Russell (2000), a salamander larvae staging guide.

I set the recommended nominal concentration of GroundClear® (one part formulation to four parts water) as '1'; all other concentrations were dilutions of this. These were 0.1, 0.01, 0.001, 0.0004, 0.0002, 0.00013, 0.0001, and 0.00001 for my first experiment. These concentrations were chosen based on a previous study using *Xenopus laevis* (VanBuskirk, 2003). In addition, I included a control treatment in which larvae were exposed to aged tap water only. Two replicates, each consisting of five larvae, were formed for each of these ten treatments.

Larvae of the appropriate developmental stage were randomly placed into plastic boxes that contained the respective solutions used in experiment one. They were then left for 96 hours of exposure, during which time they were not fed and their media were not aerated. Survivors at 96 hours were counted and then euthanized in a saturated solution of chloretone (1,1,1-tricholor-2methyl-propanolol).

My second experiment was conducted later in the breeding season of 2003, but used larvae of *Ambystoma macrodactylum* that were of the same range of developmental stages as in the
first. Based on the results from that first experiment, I sought to obtain a finer resolution on the relationship between concentration and mortality. Specifically, I wanted to determine the LC$_{50}$, the lethal concentration of GroundClear$^\text{®}$ required to kill 50% of exposed test subjects. Herbicide concentrations were adjusted to the following dilutions of nominal: 0.00013, 0.00011, 0.0001, 0.00004, 0.00002, and 0.000013. In addition, I included a control treatment in which larvae were exposed to aged tap water only. Two replicates, each consisting of five larvae, were formed for each of the GroundClear$^\text{®}$ treatments; a single replicate of three larvae was formed for the control. All subsequent methods were the same as those used in my first experiment.

**RESULTS**

Larval mortality was complete in my first experiment for all concentrations of GroundClear$^\text{®}$ greater than and inclusive of concentration 0.00013. Indeed, observation of the larvae as they were placed into the highest concentrations of these solutions shows that they ceased to move, indicating death, most within minutes of exposure to the solution. At the lower concentrations, death occurred within hours to days. More than half of larvae, seven of ten, died on exposure to a concentration of GroundClear$^\text{®}$ of 0.0001 of nominal. In contrast, all larvae survived at a concentration of 0.00001 of nominal and in the water control (see Table 2).

My second experiment was designed to provide a finer resolution to the dose-response relationship established in the first experiment. I included a range of concentrations between 0.00013 and 0.00001 that was previously unexplored, and also included a water control. At
Table 2: Mortality of *A. macrodactylum* larvae exposed to GroundClear\textsuperscript{®} dilutions in experiment one. Each dilution of nominal contained ten randomly chosen larvae, equally separated into two separate plastic boxes, as was the water control. The numbers recorded are number of total larvae dead after each 24 hour interval. Complete mortality was seen in concentrations as low as 0.00013 of nominal. The 0.0001 of nominal concentration surpassed LC\textsubscript{50} rates. Complete survival was seen in the water control and in the dilution of 0.00001 of nominal.

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Casting these data into the form of a dose-response curve (Figure 1) indicates that the threshold concentration for acute toxic effects of GroundClear\textsuperscript{®} on salamander larvae lies between concentrations of 0.00013 and 0.0001 of nominal (see also Figure 2).

Table 3: Mortality of *A. macrodactylum* larvae exposed to GroundClear\textsuperscript{®} dilutions in experiment two. Each dilution of nominal contained ten randomly chosen larvae equally separated into two plastic boxes. The numbers reported are the total larvae that were dead after each 24 hour interval. The water control contained three larvae (N=3) in one plastic box. Complete mortality was not seen in this experiment. The LC\textsubscript{50} rate was seen in 0.0001 of nominal concentration. Complete survival was seen in 0.00004 and lower concentrations, as in the water control.

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the end of the trial there was complete survival at a concentration of 0.00004 and in all lower concentrations than this, plus the water control. However, as shown in Table 3, levels of mortality were variable at higher concentrations within the range included.
Figure 1: Overall dose-response curve for both experiments exposing *A. macrodactylum* to different dilutions of GroundClear®. Total mortality numbers are the number dead after 96 hours. This graph indicates that the threshold concentration (shown at closer detail in figure 2) is between 0.00013 and 0.0001 of nominal. There is a basic inverse relationship between survival rate and concentration strength.

Figure 2: A finer resolution for the concentration that is lethal to at least 50% of exposed test subjects, the LC$_{50}$. Data were combined from the two exposures of *A. macrodactylum* larvae to GroundClear®, and the total mortality count is the number dead after 96 hours. The LC$_{50}$ is variable and appears to be between 0.00013 and 0.0001.
DISCUSSION

My results clearly demonstrate that exposure to GroundClear® results in complete mortality to salamander larvae at concentrations that likely are realistic in terms of those that might be obtained in the field. Only at concentrations 0.00004 lower than nominal does exposure to GroundClear® appear innocuous — at least, and perhaps only, in terms of the end-point considered here.

Death at high concentrations of GroundClear® was both complete and rapid (most within 48 hours or less, sometimes within seconds). Since Ortho® GroundClear® Triox Total Vegetation Killer is a formulation that contains a ‘cocktail’ of chemicals, I cannot state that the effects I observed on salamander larvae were due to glyphosate per se. Herbicidal formulations contain so-called ‘inert ingredients’ that, while they may not have effects on target organisms (plants), nevertheless may have effects on aquatic or terrestrial non-target animals (perhaps including earthworms: Verrell & VanBuskirk, 2004). Evidence indicates that surfactants added to glyphosate-containing formulations as ‘wetting agents’ and aiding in the biological uptake of the pesticides by the plants may show approximately the same end-point effects as the formulations themselves (Perkins et al., 2000). Reductions of these chemicals have been shown to reduce harmful effects in fish (Wan et al., 1989), but are currently untested in amphibians. Further studies are required in which the separate components of formulations such as GroundClear® are examined for toxic effects, both singly and in combination.

Formulations of glyphosate such as Roundup® have been shown to compromise the morphological integrity of the gills of juvenile fish, resulting in death from asphyxiation and/or osmotic imbalance (Mann & Bidwell, 1999; Jiraungkoorskul et al., 2003). Similar
problems may have affected the gills of my salamander larvae, although additional work is needed to test this hypothesis of mechanism. Recently metamorphosed and adult amphibians appear to be less vulnerable to the negative effects exerted by agrochemicals than are larvae. This may be due to the heavy reliance placed by larvae on gills in the twin function of respiration and osmotic balance. While adults do exchange gases cutaneously (via the skin), the availability of pulmonary gas exchange may compensate if cutaneous function is compromised (Mann & Bidwell, 1999). While adult amphibians in water may be relatively 'safe,' those on land may be more vulnerable than larvae if, as is probable, direct exposure to much higher concentrations of contaminants is more likely while in terrestrial habitats. The need for data on contaminant concentrations in different variations of habitats, and for pharmacokinetic (how drugs are absorbed, distributed, metabolized, and excreted from the body) data in organisms possibly at risk, clearly is indicated.

This is not to say that the exposure of larvae in freshwater wetlands is either unlikely or of no concern. Amphibians tend to breed in slow-moving or stationary water, such as impermanent pools, wetlands, and ponds, and these often are temporary due to changes in water flow patterns near residential, commercial, and agricultural areas. It has proven easy to increase concentrations of glyphosate formulations to lethal toxic levels using normal application rates in shallow, stationary water (Mann & Bidwell, 1999). Although the manufacturer of Ortho® GroundClear® Triox Total Vegetation Killer states that most glyphosate-containing formulations are not for aquatic use (Monsanto, 1999), it does not specify how far from aquatic areas use should occur.
Populations in the wild are unlikely to experience continuous exposure to glyphosate or its formulations since glyphosate and its formulations are complete vegetation killers and highly effective, eliminating the need for an immediate reapplication. However, since these herbicides are used both in gardens and agricultural areas, they are most likely to be applied in the spring and early summer during the growing season, at the precisely the same times when the embryos and larvae of many amphibians may be in adjacent bodies of water. Thus, I cannot dismiss the possibility that, at least on a local scale, application of formulations such as GroundClear® in the vicinity of breeding ponds could have catastrophic effects on the survivorship of young life history stages. Glyphosate and its formulations could also exert long-term and perhaps additive and/or synergistic effects with other environmental factors that only become apparent after several years of use and could not be tested until it has deeply hurt the infected amphibians (Mann & Bidwell, 1999).

Short-term mortality is just one end-point – additional work should examine other end-points that were not considered here. One possible endpoint is increased predation, which might affect larvae that can survive short-term and directly toxic effects. For example, salamander larvae exposed to the insecticide methoxychlor do not die directly. However, they exhibit attenuated startle responses and paralysis, both of which increase their vulnerability to dragonfly predators (Verrell, 2000; Ingermann et al., 2002). Another possibility is that exposure to chemicals could increase the incidence of malformations of the limbs or other body areas (see Lajmanovich et al., 2003), and perhaps also increase the risk of predation on such affected animals. Other end-points that deserve further consideration include DNA damage (Clements et al., 1997), a decrease of availability of food due to insect susceptibility to the glyphosate formulations, and the affects on the immune system that increase
susceptibility to pathogens. For example, a decrease in immunocompetence could provide a window of opportunity for the trematode parasite *Riberioa ondatrae*, a distant relative of the tapeworm. This parasite infects larvae around their hind limbs and causes deformities, mostly seen as multiple limbs, as the larvae transforms into adults. These limbs are not vascularized; this lack of the blood supply to these limbs tends to slow the adult amphibian when feeding or fleeing, which in turn leads to increased predation (Blaustein & Johnson, 2003). If future studies show that glyphosate-based chemicals negatively impact the immune system, then the frequency of deformed amphibians presently seen may have increased as a negative consequence of past careless use of glyphosate-based formulations.

The complete mortality seen in this study was much higher than expected based on VanBuskirk’s (2003) previous data obtained for *Xenopus laevis*. It was observed that the $LC_{50}$ for *Xenopus laevis* lies between, but not including, 0.001 and 0.0001 of nominal concentration, while the $LC_{50}$ for *Ambystoma macrodactylum* was obtained at about 0.0001 of nominal concentration. This information has implications for many gardening chemicals on the market today. If the only amphibian species that is tested for susceptibility to chemicals is *Xenopus laevis*, then permissible concentrations of chemicals may be much higher than what are realistic for species in local communities.

**CONCLUSION**

While it is easy for chemical researchers to use one species to test toxicological affects, a thorough testing of all categories of animals that could potentially be affected is needed. This can be shown through the differences between data obtained for amphibians and fish, and the differences seen between a lab-bred amphibian and a wild amphibian (see Bridges et al.,
2002). Amphibian larvae should also be used for testing due to their likely increased susceptibility to chemicals and the potential capability that their survival (or lack of) can have on future population dynamics. My study indicated that there was significant mortality for amphibians that were exposed to an herbicide at concentrations much lower than the nominal concentration (i.e., the one recommended for field application). While the precise LC$_{50}$ is still not known, it is at a much lower concentration than what was previously hypothesized by VanBuskirk (2003). Much research remains to be done on this subject, such as exposing eggs to glyphosate formulations, and then immediately to the parasite *Riberioa ondatrae* to determine if there is an increase in parasitic infections. With amphibian declines occurring around the world, this is a topic that should be researched heavily until the culprit, or the synergism of culprits, is found.

**ACKNOWLEDGMENTS**

First of all, I’d like to say thank you to my advisor, Dr. Paul Verrell. He shared his expertise about everything ‘salamander’, helped me establish the protocol for the experiments, edited and reedited, and partook in my enthusiasm (and most of the time, in my disgust) about the topic at hand. Cynthia White was also a tremendous help in this study. She allowed me to use the eggs from the female salamanders that she collected for her own study, and her editing skills were also extremely helpful. Finally, I’d like to thank Jeremy and Michael Faxon for their time in editing this paper and showing me the places I needed to further clarify.
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Thesis Author
Carman, Briana

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