Chapter 9
Amphibians Are Not Ready for Roundup®

Rick A. Relyea

As I peered into the water, I recall being shocked at the sight. This tank was one of hundreds we were using as experimental ponds, each simulating a real pond by containing leaf litter, algae, zooplankton, and tadpoles. However, this particular tank did not contain very many tadpoles - at least not many live ones. Instead, the bodies of dead tadpoles were littered across the bottom. This tank and a few others of similar macabre appearance had been exposed to the most popular herbicide in the world. It was at that moment that we learned that the herbicide could be highly lethal to amphibians. As I think back upon that day, I am struck by the fact that one simple experiment led us to a discovery that would take me into years of debate.

Abstract The herbicide glyphosate, sold under a variety of commercial names including Roundup® and Vision®, has long been viewed as an environmentally friendly herbicide. In the 1990s, however, after nearly 20 years of use, the first tests were conducted on the herbicide's effects on amphibians in Australia. The researchers found that the herbicide was moderately toxic to Australian amphibians. The leading manufacturer of glyphosate-based herbicides, Monsanto, declared that the researchers were wrong. Nearly 10 years later, my research group began examining the effects of the herbicides on North American amphibians. Based on an extensive series of experiments, we demonstrated that glyphosate-based herbicides can be highly toxic to larval amphibians. Monsanto declared that we were also wrong. These experiments have formed the basis of a spirited debate between independent, academic researchers, and scientists that either work as consultants for Monsanto or have a vested interest in promoting the application of the herbicide to control undesirable plants in forests and agriculture. The debate also moved into unexpected

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arenas, including the use of glyphosate-based herbicides in the Colombian drug war in South America where a version of Roundup is being used to kill illegal coca plantations. In 2008, the US EPA completed a risk assessment for the effects of glyphosate-based herbicides on the endangered California red-legged frog (*Rana aurora draytonii*) and concluded that it could adversely affect the long-term persistence of the species. More recent data from Colombia have confirmed that the herbicides not only pose a risk to tadpoles in shallow wetlands, but that typical applications rates also can kill up to 30% of adult frogs. As one reflects over the past decade, it becomes clear that our understanding of the possible effects of glyphosate-based herbicides on amphibians has moved from a position of knowing very little and assuming no harm to a position of more precise understanding of which concentrations and conditions pose a serious risk.

Introduction

As a community ecologist studying aquatic organisms, my research for many years focused on understanding how animals respond to natural stressors including predation and competition. I spent my doctoral years focusing on how tadpoles responded to these natural stressors by changing their behavior, morphology, and life history. I certainly had no interest in toxicology and no goal of working in this field of research. I never had and still do not have a personal antipesticide agenda. I grew up in a rural community and spent a good deal of my younger life working on a farm and spraying a lot of different pesticides. As a result, I recognized the benefits of pesticides for feeding the world and protecting human health. So years later, why did I find myself debating toxicology with a multinational corporation like Monsanto and their consultants?

After completing my Ph.D. and before moving to a faculty position at the University of Pittsburgh, I spent the summer of 1999 collaborating with Ray Semlitsch at the University of Missouri-Columbia. Ray's research group not only shared my interest in studying natural stressors, but also was developing an exciting research program in amphibian toxicology, primarily with the insecticide carbaryl (commercial name: Sevin®). At the time, few amphibian biologists were interested in venturing into this realm, but the University of Missouri was located just a few miles away from the U.S.G.S. Toxicology Lab in Columbia, making it an ideal collaborative effort. An impromptu conversation with one of Ray's graduate students, Nathan Mills (now a professor at Harding University), raised an interesting question: If tadpoles could respond to predators by altering their behavior and morphology, how might they respond to the smell of predators in the presence of an insecticide like carbaryl that is designed to interfere with an animal's nervous system? Could sublethal insecticide concentrations interfere with a tadpole's ability to smell or respond to a predator in the water? We decided to conduct an experiment to find the answer. We placed predators in small cages so that the smells of preda-

tors could scare the tadpoles but the predators could not actually eat the tadpoles. In regard to that particular question, the experiment was a complete failure – but not for lack of an adequate experimental design. It turned out that sublethal concentrations of carbaryl, when combined with the smell of predators in the water, became quite lethal to tadpoles (Relyea and Mills 2001). Instead of the tadpoles changing their behavior or morphology, they simply died.

For reasons that we still do not understand, adding just one element of the tadpole's natural world – the smell of a predator – made the pesticide deadlier than anyone had ever suspected. In the years that followed, we found that several commercial pesticide formulations including carbaryl, malathion, and Roundup had a similar effect on a diverse group of amphibians (Relyea 2003, 2004a, 2005c). No one had previously described such a synergistic interaction. As I began to learn about the field of toxicology, I realized that most toxicology research is conducted on species in the laboratory that are isolated from all other species with which they coexist in nature. I realized that as a community ecologist, I could approach toxicology from a different perspective than traditionally trained toxicologists. A community ecologist asking toxicology questions would focus more on how pesticides affect complex communities rather than a single species. Not necessarily a better perspective, just different. It was this opportunity to offer a unique perspective that led me to pursue research into community ecotoxicology and brought me face to face with a tank full of dead tadpoles that had been treated with the popular herbicide Roundup®.

The Rise of Roundup

In 1974, Monsanto Corp. (St. Louis, MO, USA) first commercially produced the chemical glyphosate after discovering that the chemical could prevent plants from making essential amino acids, thereby causing the plants to die. However, most herbicides are not good at penetrating the waxy outer coating of plant leaves. This coating, known as the cuticle, acts as a natural barrier to prevent foreign organisms and compounds, including herbicides, from entering the plant's tissues. To penetrate the cuticle, manufacturers frequently include additional chemicals to help the herbicide to penetrate into the leaf. These chemicals, called surfactants, are good at dissolving in water and at cutting through wax and grease (e.g., dish soap is a surfactant). In the case of Roundup, the most common surfactant is polyethoxylated tallowamine (POEA), a derivative of animal fat. Together, glyphosate and POEA make a very effective herbicide. Some glyphosate formulations use different surfactants or blends of surfactants. Others contain no surfactants, but applicators are encouraged to add an after-market surfactant if glyphosate alone proves to be ineffective at killing weeds.

In the early years, glyphosate had a relatively small share of the herbicide markets. In the late 1980s and early 1990s, however, glyphosate sales began to grow rapidly for a variety of uses including homes, gardens, no-till agriculture, and forestry

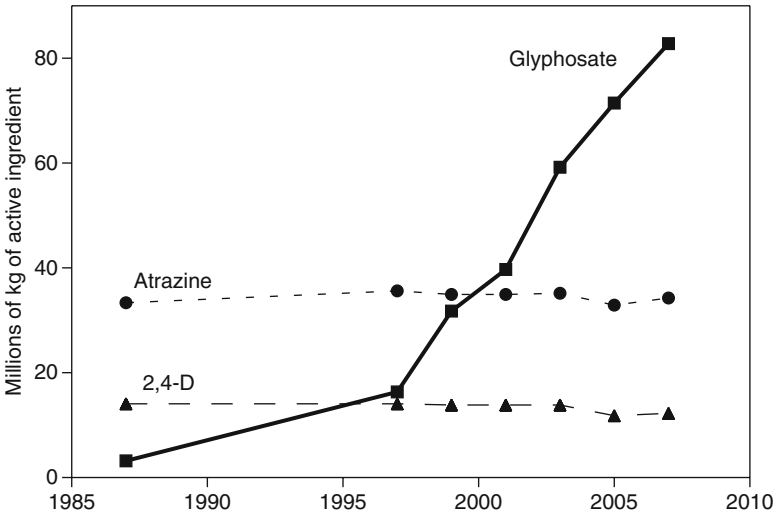

Fig. 9.1 The growth of glyphosate-based herbicides relative to other globally common herbicides (Kiely et al. 2004; Grube et al. 2011)

(where herbicides are used to kill broadleaf trees and favor the growth of conifer trees). In 1996, however, Monsanto took advantage of new genetic techno-logies which allowed the insertion of glyphosate-resistant genes into crop plants, thereby making the plant much more resistant to the herbicide than surrounding weeds. This discovery allowed Roundup to kill weeds that compete with crops without harming the crops. This marked the birth of Roundup-Ready® crops that currently span a variety of common crops including corn, soybeans, cotton, canola, and sugar beets. The genetically modified seeds were patented and farmers, eager to plant a seed variety that would tolerate a powerful herbicide, signed contracts with Monsanto. The pairing of Roundup herbicide and Roundup-Ready seeds produced an exponential increase in the use of glyphosate-based herbicides around the world (Fig. 9.1) and it is now the top selling herbicide in the world (Baylis 2000). Although Monsanto is one of the largest manufacturers of glyphosate-based herbicides, many other companies now produce them as well.

How Do We Assess the Risk of Pesticides?

Whenever a new pesticide is developed, it must be tested to determine its impact on nontarget organisms before it can be registered. Amphibians, however, have never been part of the testing process for the vast majority of pesticides used today, including glyphosate. Instead, the standard protocol of the US Environmental Protection Agency (EPA) requires testing of each pesticide on mammals, birds, fish, and tiny crustaceans such as water fleas (e.g., *Daphnia* spp.).

In traditional toxicology studies, individuals are exposed to a range of concentrations for 1–4 days under laboratory conditions (i.e., acute toxicity tests). Providing that mortality in the control (i.e., no pesticide) is less than 10%, one can use these experiments to determine the lethal concentration that will kill 50% of the population (i.e., the LC50 value). The LC50 values for a new pesticide can be compared to a large amount of past data from these four animal groups to assess its relative toxicity. Using US EPA classifications,[1] the toxicity of a pesticide is then categorized as either *highly toxic* (0.1 mg/L < LC50 < 1 mg/L), *moderately toxic* (1 mg/L < LC50 < 10 mg/L), or *slightly toxic* (10 mg/L < LC50 < 100 mg/L).[2] In addition to these acute toxicity tests, some organisms are also tested over longer time intervals to examine potential reproductive impacts (i.e., chronic toxicity tests).

When the pesticide registration rules were designed long ago, there was little concern about amphibians and no sense that amphibians were experiencing the massive global population declines that are well documented today (Stuart et al. 2004). For aquatic stages of amphibians (e.g., tadpoles and larval salamanders), the EPA currently assumes that fish can serve as a surrogate group. This is based on the assumption that the sensitivity of tadpoles is similar to that of fish. This is often a reasonable assumption. When the assumption is correct, regulating pesticides at levels that protect the most sensitive species of fish simultaneously protects the aquatic stages of all amphibians. If amphibian data on aquatic stages of amphibians are available, these data can also be considered by the EPA.

For terrestrial stages of amphibians, the EPA assumes that birds can serve as a surrogate group. These are not studies of birds being over sprayed, but birds ingesting food that has been contaminated by a given chemical (Jones et al. 2004). Thus, exposure via the skin (i.e., *dermal exposure*) or respiratory system is currently not part of the risk assessment process. Unfortunately, it is unknown whether regulating pesticides at levels that protect birds (based on ingestion studies) is also protective of terrestrial amphibians that can be exposed to pesticides via ingestion, dermal exposure, and respiration.

In assessing risk, we need to know the concentrations of the chemical that cause harm (based on LC50 studies) and then compare these values to the concentrations of the chemical that occur in nature. To set a safe upper limit for the concentrations of a chemical in nature, the EPA uses an index known as the "Risk Quotient." The Risk Quotient is calculated by dividing the LC50 value for the most sensitive species in a group (e.g., the most sensitive fish) by the expected concentration of the chemical in the nature. To not harm wildlife, this ratio should not exceed 0.05 (Jones et al. 2004). More simply put, the concentration that we expect to see in nature should not exceed 5% of the most sensitive species' LC50 value. The logic is that if the LC50 concentration kills 50% of the animals, 5% of this number should provide a concentration that kills few or none of the animals.

[1] http://www.epa.gov/espp/litstatus/effects/redleg-frog/.

[2] In the case of glyphosate-based herbicides, the most common reported units are milligrams of acid equivalents per liter (mg a.e./L).

When a pesticide is being considered for approval by the EPA, the agency also can require data from toxicity data on inert ingredients if there is evidence that the inert ingredients pose a risk (Jones et al. 2004). Inert ingredients are not designed to kill the target pest, but are added to the commercial formulation to make the active ingredient more effective. Inert ingredients are considered trade secrets and therefore do not have to be listed on the container's label. Many people assume that the "inert ingredient" category implies that these chemicals are not toxic to any organism. For surfactants such as POEA, this is not the case.

The First Studies of Roundup's Impact on Amphibians

Because the EPA and the regulatory agencies in other countries have not required amphibian testing as part of their process for registering most pesticides, little was known about the effect of Roundup formulations on amphibians. Indeed, the herbicide had been on the market for nearly 20 years before the first amphibian study was ever conducted. In the 1990s, this began to change.

In the early 1990s, the East Kimberly shire council submitted a proposal to the Western Australia Department of Environmental Protection to aerially spray an emergent weed in Lake Kunnunurra (Mann et al. 2003). At the time, Roundup with POEA could be applied to control aquatic plants (this was not the case in the USA). However, there had been numerous reports of dead amphibians following the spraying of herbicides, so the government funded Joe Bidwell and John Gorrie (then at the Curtin University of Technology) to conduct acute toxicity tests using both the active ingredient and the commercial formulation (which contained the POEA surfactant). Their first experiments found that the commercial formulation (Roundup Herbicide®) was much more toxic to amphibians than the active ingredient alone, likely because of the POEA surfactant (Bidwell and Gorrie 1995; Fig. 9.2). Similar results had previously been reported in fish (Folmar et al. 1979). Bidwell and Gorrie's research prompted a special review of how glyphosate-based products were being used over water. Eighty-four products were deemed no longer safe for application to plants growing in and around water. These products now were required to carry a new label in Australia, "Do NOT apply to weeds growing in or over water. Do NOT spray across open water bodies, and do NOT allow spray to enter the water."

A series of follow-up studies (Mann and Bidwell 1999) demonstrated for the first time that Roundup containing the POEA surfactant had $LC50_{2-d}$ values (measured in units of acid equivalents; a.e.) that ranged from 2.9 to 11.6 mg a.e./L for four species of tadpoles: sign-bearing froglet (*Crinia insignifera*), moaning frog (*Heleioporus eyrei*), western bullfrogs (*Limnodynastes dorsalis*), and golden bell frogs (*Litoria moorei*; Fig. 9.2). This meant that Roundup with POEA could be classified as slightly to moderately toxic to amphibians. Because the commercial formulation was moderately toxic but the active ingredient was not, there was the suggestion that aquatic plants could be sprayed with glyphosate that was combined with a separately purchased surfactant. Subsequent studies found that, similar to the POEA

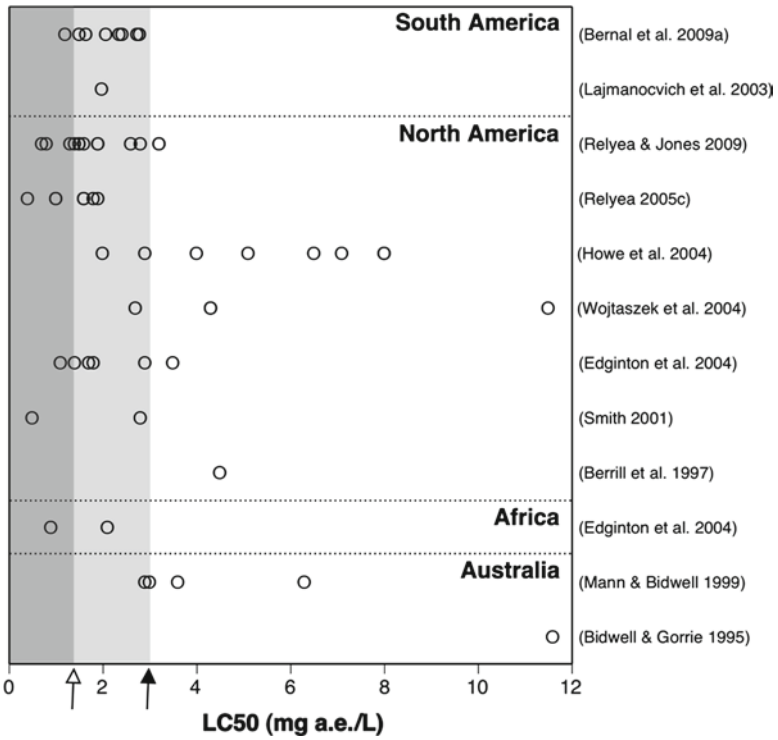

Fig. 9.2 All larval amphibian LC50 studies of glyphosate-based herbicides including the POEA surfactant or an unknown surfactant with similar toxicity as POEA. LC50 values were estimated from data in two cases (Berrill et al. 1997; Smith 2001) and all data were converted to the common units of mg a.e./L. Each *circle* represents an individual test of an amphibian species under a given set of conditions (pH, predator cues, etc.). The *closed arrow* indicates a worst-case scenario concentration of 3 mg a.e./L and the *open arrow* indicates the Canadian government's worst-case scenario in forestry applications of 1.43 mg a.e./L

surfactant, other leading surfactants that were effective at helping glyphosate kill plants were also effective at killing amphibians (Mann and Bidwell 2000, 2001). Unfortunately, the same properties that allow the surfactant to penetrate leaf cuticles also make it particularly good at rupturing the gill cells of fish and larval amphibians, leading to suffocation.

This was bad news for companies selling glyphosate-based herbicides. Media coverage at the time in Australia highlighted the threat that applications of Roundup around water posed to amphibians. In the Sydney Morning Herald, Leigh Dayton (1995) reported, "Mr. Nicholas Tydens, regulatory and environmental affairs manager for Monsanto Australia Ltd, said yesterday that the scientists were wrong. 'We have investigated every claim about Monsanto and Roundup affecting aquatic organisms. All of the evidence to date shows there is no adverse effect,' he said." Despite the conclusions of the scientists and the agreement from the Australian government that Roundup posed a risk to amphibians, the Monsanto representative

continued to proclaim that Roundup posed no risk. After they had reported their findings, Joe Bidwell had lunch with a Monsanto representative who was quite upset with the amphibian results and warned that Joe would never land a job in the chemical industry. As an academic scientist with no desire to work in the chemical industry, this warning amused Joe greatly (J. Bidwell, personal communication).

Interest in Testing Amphibians Slowly Builds

Following the Australian studies, there were three additional studies on glyphosate and amphibians in three countries that received considerably less media attention. One reason may be that two of the studies did not calculate LC50 values, which makes it difficult to compare these studies to past results and limits their utility for assessing general patterns of toxicity and risk.

As part of a large screening of several amphibians against a wide variety of pesticides in Canada, Berrill et al. (1997) conducted tests on green frog tadpoles (*Rana clamitans*) exposed to Roundup. After 4 days of exposure, they found very little death with 3 mg a.e./L but nearly 100% death with 6 mg a.e./L. While the LC50 value was not estimated, the data suggest that the $LC50_{4-d}$ was ~4.5 mg a.e./L (Fig. 9.2).

Smith (2001) tested a formulation sold as Kleeraway® on larval western chorus frogs (*Pseudacris triseriata*) and plains leopard frogs (*Rana blairi*) in the USA. Based on his reported ratios of formulation to water and the nominal concentration of glyphosate in the bottle, one can estimate Smith's concentrations in units of mg a.e./L. For the chorus frogs, he found no deaths in the controls, 45% death in his lowest concentration (~0.5 mg a.e./L), and 100% death in all higher concentrations (>5 mg a.e./L). This would suggest that chorus frogs had an $LC50_{1-d}$ of ~0.5 mg a.e./L. For the leopard frogs, he found no deaths in the control or in the lowest concentration (~0.5 mg a.e./L), but 100% death in all higher concentrations (>5 mg a.e./L). This would suggest that plains leopard frogs had an $LC50_{1-d}$ of ~2.8 mg a.e./L (Fig. 9.2).

Finally, Lajmanovich et al. (2003) conducted a study in Argentina on tadpoles of *Scinax nasicus* and found an $LC50_{4-d}$ value of 1.98 mg a.e./L (Fig. 9.2). Together, these three studies produced LC50 values that were well within the range of values that would later be published on a wide variety of amphibian species.

The First Roundup Studies by the Relyea Lab

At about the time that the latter two glyphosate studies were being published, my lab was expanding its own research in toxicology. Our initial discovery that adding a bit of ecological reality – the smell of predators in the water – could make pesticides more lethal to tadpoles, turned out to be a common outcome in a variety of tadpole species (Relyea and Mills 2001; Relyea 2003). Clearly, there was a need for more pesticide studies that incorporated ecological reality.

Laboratory Experiments

With funding from the US National Science Foundation in 2001, we began exploring how amphibians reacted to simultaneous exposure to several pesticides. Whereas most studies were examining one pesticide at a time, the reality in nature was that animals were being exposed to suites of chemicals. Using commercial formulations of four different pesticides under laboratory conditions (carbaryl, malathion, diazinon, and Roundup), we measured survival and growth in five species of tadpoles when exposed to the four pesticides separately and in pairwise combinations (Relyea 2004b). The impacts of the paired pesticides were largely additive in these experiments, but the individual effects of Roundup were quite interesting. Based on the earlier Australian research, we did not expect any substantial mortality at the concentrations that we used (0.75 and 1.5 mg a.e./L). However, at the higher concentration we found 48% mortality in American toads (*Bufo americanus*), 60% mortality in green frogs, and 30% mortality in bullfrogs (*Rana catesbeiana*).

The following year we decided to examine the synergy between predator cues and pesticides using a wider range of pesticides, including Roundup. Because these experiments were conducted using a range of pesticide concentrations, they also allowed us to estimate the LC50 values for the pesticides during the 16-day experiments. For Roundup, we found that $LC50_{16-d}$ values ranged from 0.4 to 1.9 mg a.e./L (Relyea 2005c). This was consistent with the mortality we observed in the previous paired-pesticide experiment. This was important because it meant that Roundup could now be classified by the EPA as moderately to highly toxic to amphibians. Because Roundup had been on the market for nearly three decades, I had assumed that this high toxicity must be well documented. I was wrong. Only a handful of geographically scattered studies existed in the world (as described above).

Outdoor Mesocosm Experiments

As a community ecologist, finding high rates of mortality with Roundup under laboratory conditions was interesting, but testing the effect under more natural conditions was the more relevant question. Given that testing pesticides in natural wetlands has a number of logistical and ethical issues (i.e., few people are interested in intentionally contaminating their wetlands), often the best compromise is to use simulated wetlands. In aquatic ecology experiments, a standard technique is to fill large plastic water tanks with hundreds of liters of water and add many components of a natural wetland including leaf litter, algae, and zooplankton. In essence, these wetland "mesocosms" are intended to simulate (although not exactly mimic) real wetlands.

At the same time we were conducting the Roundup lab experiments, we initiated our first mesocosm experiment to test the effects of different pesticides under more

seminatural conditions. In 2002, we decided that it might be insightful to assemble diverse communities designed to mimic simple natural wetland communities. To that end, we established mesocosms consisting of algae, nine species of zooplankton, three species of snails, five species of tadpoles, and eight species of predators. Once these communities were set up, we could apply different insecticides and herbicides to each tank. The idea behind the experiment was to ask two basic questions. First, would insecticides have "top-down" effects on the community (Polis and Strong 1996) by knocking out many of the insect predators and allowing their prey to flourish? Second, would herbicides would have "bottom-up" effects on the community by removing some fraction of the algae which forms the base of the food web thus decreasing food availability for grazers and ultimately decrease food availability for predators as well? To ask these questions, we used five treatments: a control, one of two popular insecticides (carbaryl and malathion) and one of two popular herbicides (Roundup Original and 2,4-D). We applied each pesticide at the rate recommended on the back of the bottles assuming that each was directly over sprayed on a wetland. For Roundup, this translated to a nominal (i.e., expected) concentration of 3 mg a.e./L (Relyea 2005a).

This was how I came to stare into that tank one summer day and see dead tadpoles littered across the bottom. The day after applying the pesticides we found very high tadpole mortality in the tanks treated with Roundup. Based on the Australian work, we expected some death, but nothing so widespread. In the end, the study produced a number of very interesting insights about the direct and indirect effects of pesticides on aquatic communities; however, the effect of Roundup on tadpoles was the most striking. Compared to the controls, mesocosms receiving Roundup experienced a 70% decline in amphibian species richness and an 86% decline in tadpole biomass (Fig. 9.3). For example, there was 100% mortality in both leopard frogs (*R. pipiens*) and gray tree frogs (*Hyla versicolor*) and 98% mortality in wood frogs (*R. sylvatica;* Relyea 2005a). These effects were similar to the effects we were observing in the lab that same summer (Relyea 2005c). It was immediately clear that we needed to do more research on the toxicity of Roundup. Thus, we made plans to examine the effects of Roundup under a variety of ecological conditions.

Our Roundup Results Hit the Fan

On April 1, 2005, the mesocosm study was published in the journal *Ecological Applications*. It was on this day that my career took an unanticipated turn.

In preparing for the paper's publication, it occurred to me that the impact of Roundup on tadpoles was so devastating that I needed to inform more than my fellow scientists. I needed to inform the public about the lethal consequences of applying glyphosate formulations containing POEA around wetlands that contain tadpoles. After giving this a good deal of consideration, I contacted the University's press office and inquired about how a scientist might conduct a press release.

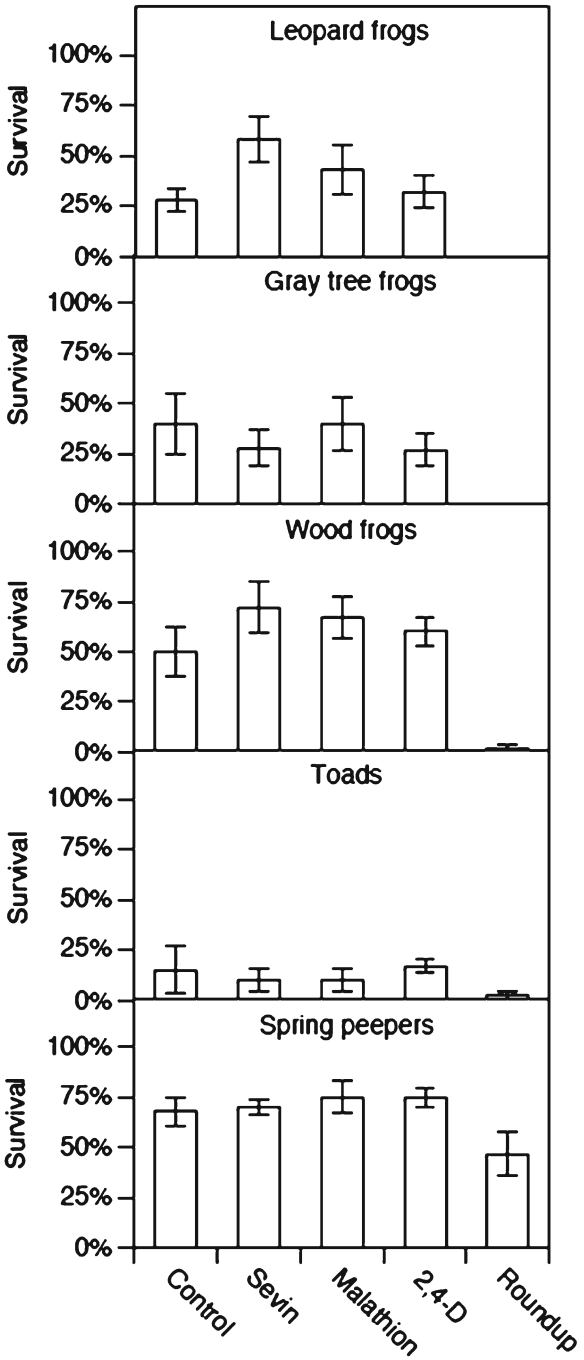

Fig. 9.3 The impact of a worst-case application of Roundup on pond mesocosms containing five species of North American tadpoles (Relyea 2005a)

As it is for most scientists, this was a shaky leap into a completely foreign world and I had no idea where such attention to my work might take me and my research group. The press release was issued on the same day the paper was published and it highlighted the lethal effects of Roundup on tadpoles that we observed.[3]

It would be an understatement to say that I was unprepared for what happened next. In April 2005, I still remember sitting in our small cabin at the University's field station when the journal article and press release were issued. I was bombarded by hundreds of e-mails per hour, many of which were very positive and supportive. Others cautioned me about what I was getting myself into. A few individuals were quite unhappy with our results.

Most of the e-mails that questioned our work were very professional, stating valid points from a variety of perspectives including from individuals who applied pesticides for a living. Some voiced genuine concerns about the intricacies of the potential for Roundup to impact tadpoles. For example, one individual said, "Roundup breaks down very rapidly once applied and is a very safe chemical. It literally does its job and degrades." This latter statement is certainly true. Roundup does break down relatively rapidly compared to other longer lasting environmental contaminants. The half-life for both the active ingredient (glyphosate) and the surfactant (POEA) ranges from 7 to 70 days in water, depending on environmental conditions (Giesy et al. 2000). However, the problem for tadpoles is that they die on the first day or two of exposure.

A few e-mails were personal attacks. In reading such e-mails, I noticed several patterns. First, some of the most outspoken critics read the press release and voiced strong criticisms without even reading the original journal article. Second, many people knew that pesticides went through extensive testing for registration, but had no idea that amphibians had not been tested in North America for the first 25 years of Roundup's existence. Third, most people knew that Roundup formulations were designed for terrestrial applications, so applying these formulations to water is an illegal act. Therefore, in their view, asking what would happen if Roundup got into water would be pointless. During a period of e-mail exchanges, a Fish and Wildlife Service biologist defended our work by noting "People are missing the point of this research. Adding the chemical of concern directly to water is a standard first step in research on chemicals and their impacts to aquatic life. Now research is needed on what concentrations may be problematic, does the chemical get transported to water bodies, and what are the methods of transport." He was correctly arguing that we need to first know the effects of Roundup *if* it gets into water, then we need to assess the probability of Roundup actually getting in the water. Together, these two facts help assess risk. Finally, some people saw our research as a serious threat to their jobs that involved promoting the use of glyphosate-based herbicides.

[3] http://mac10.umc.pitt.edu/m/FMPro?-db=ma.fp5&-format=d.html&-lay=a&-sortfield=date&-sortorder=descend&keywords=relyea&-max=50&-recid=36262&-find= (last accessed June 2010).

Monsanto's Response

Shortly after the article's publication and the press release, Monsanto put out its own press release.[4] They had three main criticisms. First, the application rate that we used was higher than used in agricultural settings. This is true, when compared to only *agricultural* uses. The application rate we used was based on home and garden use, which came straight from the bottle of Roundup Original. Regardless of the application rate used, what really matters, of course, is the concentration that ended up in the water. Our nominal concentration was 3 mg a.e./L and this represented a worst-case scenario for a direct over spray of a wetland. Past researchers had estimated the worst-case scenario for an over spray to be between 2.7 and 7.6 mg a.e./L, depending on assumptions about the depth of the water and whether vegetation intercepts any of the pesticide prior to landing on the water (Mann and Bidwell 1999; Giesy et al. 2000; Solomon and Thompson 2003). Observed worst-case scenarios range from 1.7 to 5.2 mg a.e./L (Edwards et al. 1980; Giesy et al. 2000; Thompson et al. 2004). The value in asking whether the worst-case scenario harms an organism is that if it does not, future testing at lower concentrations is not necessary. If it does cause harm, we need to consider lower concentrations of the pesticide to determine the lowest concentration which poses no harm.

Monsanto also argued for the irrelevancy of any aquatic experiment. They said, "This study does not represent realistic use conditions for Roundup brand herbicides for applications to aquatic environments. In fact, there are no Roundup brand formulations approved in the US or Canada for application over water." Thus, the argument being made is that Roundup formulations containing the POEA surfactant are not approved for application over water; therefore, Roundup should not be in wetlands and any tests of Roundup's effects on aquatic organisms are irrelevant. This argument stands up only if the terrestrial use of Roundup never causes the herbicide to end up in water bodies. While there are few data on concentrations of glyphosate in natural water bodies, there are enough data to confirm that the herbicide, while not intentionally sprayed on water, is either unintentionally sprayed on water, leaches out of the soil, or washes off vegetation and into bodies of water (Thompson et al. 2004). Monsanto's press release goes on to cite a single study of Roundup that found no effect of Roundup on tadpoles as the one definitive study (Thompson et al. 2004; discussed below), even though they claimed that any test of Roundup on aquatic life is irrelevant and despite contradictory studies showing that the Roundup posed a risk to amphibians (Mann and Bidwell 1999).

In hindsight, it was probably Monsanto's press release that brought more attention to the issue of Roundup's toxicity to amphibians than anything I could have done. The strong negative reaction from Monsanto brought a great deal of media attention to the issue and I found myself in a strange position thrust before the press. Thankfully, the vast majority of the reporters were quite professional and objective,

[4] http://www.monsanto.com/monsanto/content/products/productivity/roundup/bkg_amphib_05a.pdf (last accessed June 2010).

although there was one interesting case in which a newspaper reporter from Nebraska wrote a criticism of the research for her local farm community. In the article, she interviewed an extension agent who had not read the article, and, when I contacted her, she admitted that she had neither read the article nor tried to contact me to get a balanced story. Fortunately, this type of interaction was a rare exception.

One of the most significant events happening during this time happened behind the scenes. In June 2005, 2 months after the release of the paper, I attended the World Congress of Herpetology in Stellenbosch, South Africa. Herpetologist Ron Heyer (from the Smithsonian Museum) informed me that when my article came out, he had been contacted by Monsanto to see if he would criticize the article. He read the article, found no reason to criticize it, and declined Monsanto's request. Seven months later, while attending the Illinois Crop Protection Conference, toxicologist Allan Felsot (from Washington State University) told the same story to the conference audience. He said that when our article first came out, Monsanto had called him and asked him to publicly criticize our work. As he told the audience, he read the article and did not agree with Monsanto's criticisms, and refused to speak out against the study. I do not know how many other scientists were contacted, but clearly Monsanto was trying to find scientists that would criticize our research and these scientists were not going along.

The Roundup Studies of 2004

What is particularly interesting about the strong opposition from Monsanto in 2005 is that this opposition was not voiced a year earlier when a series of papers were published by Canadian researchers saying very similar things. In 2004, after our article was already in press, these researchers published a series of four papers examining tadpole exposure to Vision® (Monsanto's Canadian version of Roundup which also contains POEA). In the first paper, Edginton et al. (2004) examined the toxicity of Vision to four species of tadpoles under multiple pH conditions (pond pH in nature typically ranges from 4 to 9). They found that all four species had similar sensitivities and that sensitivity increased with pH. Indeed, the LC50 values they produced (1.8–3.5 mg a.e./L at pH = 6; 0.9–1.7 mg a.e./L at pH = 7.5; Fig. 9.2) were very similar to those that we published the following year ($LC50_{16\text{–}d}$ = 0.4–1.9 mg a.e./L at pH = 8; Relyea 2005c). In the end, the authors state, "we concluded that, at EEC [environmentally expected concentration] levels, *there was an appreciable concern of adverse effects to larval amphibians in neutral to alkaline wetlands*. The finding that the mean pH of Northern Ontario wetlands is 7.0 further compounds this concern" (Edginton et al. 2004, p. 821).

In the second paper, Chen et al. (2004) examined how the herbicide interacted with different levels of pH (5.5 vs. 7.5) and different levels of food stress. After conducting lab experiments on a single zooplankton species and a single amphibian species, they found that higher pH caused significantly more mortality. Indeed, even their lowest herbicide concentration (0.75 mg a.e./L) cause 100% mortality under

conditions of high pH and low food. As a result, the authors concluded that "For both species, significant effects of the herbicide were measured *at concentrations lower than the calculated worst-case value* for the expected environmental concentration" (Chen et al. 2004, p. 823).

In the third paper, Wojtaszek et al. (2004) investigated the impact of Vision when green frog and leopard frog tadpoles were living inside enclosures that had been set up in natural wetlands. The enclosures had polyethylene sidewalls that were anchored to the bottom of the wetland. Two wetlands were chosen for the experiment, one with a lower pH (6.4) and one with a higher pH (7.0). They then added different amounts of Vision to each enclosure to determine the $LC50_{4-d}$ values for the two species under natural conditions. Overall, they found that the two tadpole species appeared to be less sensitive in these two wetlands compared to past lab studies, but there was substantially greater tadpole mortality in the pond with higher pH. The $LC50_{4-d}$ for green frogs was 4.3 mg a.e./L in the lower pH wetland, but 2.7 mg a.e./L in the higher pH wetland. Similarly, the $LC50_{4-d}$ for leopard frogs was 11.5 mg a.e./L in the lower pH wetland, but 4.3 mg a.e./L in the higher pH wetland (Fig. 9.2). Although these LC50 values were a bit higher than the companion lab studies of Chen et al. (2004) and Edginton et al. (2004), it was still clear that the herbicide would cause tadpoles to die at environmentally relevant concentrations, whether one considers worst-case concentrations of 3 mg a.e./L (as used in our studies) or even if one uses the more conservative estimates of 1.43 mg a.e./L used by the Canadian government for forest applications (Wojtaszek et al. 2004). Based on the LC50 estimates, 50% of the tadpoles would not die, but 10 or 20% of the tadpoles would die. Despite this expectation, the authors concluded, "The results of this in situ enclosure study provide no evidence to conclude that environmentally relevant concentrations of Vision cause significant mortality, abnormal avoidance, or reduced growth in native larval amphibians used in this study."(Wojtaszek et al. 2004, p. 841).

The final paper in this series set out to determine the concentrations of Vision that could actually occur in wetlands when a forest is sprayed from a helicopter to kill broadleaf trees and favor the more marketable conifer trees. A second goal was to determine the impact on tadpole mortality during these sprayings (Thompson et al. 2004). To achieve these goals, helicopters applied Vision either directly over wetlands, adjacent to wetlands, or with a buffer of vegetation separating the sprayed area and the wetlands. In terms of the average concentration found in each scenario, there were no surprises; buffered wetlands had the least herbicide (0.03 mg a.e./L), adjacent wetlands had moderate amounts of herbicide (0.18 mg a.e./L), and over sprayed wetlands had the most herbicide (0.33 mg a.e./L). It is also not surprising that the data were quite variable among wetlands due to differences in pond depth. For example, while the average concentration of an over sprayed wetland was 0.3 mg a.e./L, the values range from undetectable (<0.01 mg a.e./L) to quite high (2.0 mg a.e./L), exceeding the Canadian government's own worst-case scenario estimates (1.43 mg a.e./L). In assessing risk, however, one typically uses the *average* natural concentration and ignores the fact that some wetlands have much higher concentrations than the average. If we were to consider the wide range of observed

concentrations in Canadian wetlands, we would predict that some wetlands would experience no mortality while other wetlands would experience high rates of mortality. Curiously, in a subsequent paper published 4 years later, Thompson and colleagues (Struger et al. 2008) report glyphosate concentration data from a survey of streams and wetlands in Ontario. These samples, which were not taken immediately after the herbicide was sprayed, never exceeded 0.041 mg a.e./L. As a result, they concluded that glyphosate concentrations in wetlands are far below any concentrations that would cause harm to amphibians. They further concluded, "Aqueous environmental exposure concentrations for glyphosate residues as observed in this study were very similar to other surface water concentration values published in monitoring studies in the USA and in various European countries." (Struger et al. 2008, p. 380). This conclusion was made despite the fact that Thompson and colleagues reported detections of up to 2.0 mg a.e./L in wetlands, a concentration that is nearly 50 times higher (Thompson et al. 2004).

To assess the impact of these three spray treatments on tadpoles, the researchers caged groups of five tadpoles (green frogs and leopard frogs) in each wetland for 2 days and then counted how many tadpoles survived. As noted earlier, the standard in toxicology studies is that not more than 10% of the control animals should die in short-term trials; more than 10% death in the controls suggests either that the animals are in poor condition or the environment is not hospitable enough for a reliable experiment. Their investigation found that the buffered wetlands, which contained almost no herbicide and thus could serve as control, experienced 15% death of leopard frogs and 26% death of green frogs. While it is unclear what was killing such a high fraction of tadpoles after only 2 days in a, tadpole mortality in the over sprayed wetlands, which experienced 14 and 36% death of leopard frogs and green frogs, respectively, was not significantly different from the buffered wetlands. Because the differences in mortality were not significantly different among the three application treatments ($p = 0.19$ for leopard frogs, $p = 0.13$ for green frogs), the authors concluded that Vision poses a low risk to amphibians. The EPA had a different interpretation of these data (Carey et al. 2008, p. 104):

> The results suggest that there was a large amount of variability that could have obscured detecting treatment effects especially given that these were naturally occurring wetlands that represented a range of environmental conditions.

In the end, Thompson et al. (2004) concluded, "Overall, results of this tiered research program confirm that amphibian larvae are particularly sensitive to Vision herbicide and that these effects may be exacerbated by high pH or concomitant exposure with other environmental stressors. Although results from laboratory studies were very useful in the comparative sense and in understanding mechanisms of interaction, they tended to overestimate effects as observed under natural exposure scenarios" (Thompson et al. 2004, p. 848). This final conclusion may be why Monsanto voiced no public opposition to these studies.

Counter to this result, one additional paper – produced by an independent research group (Howe et al. 2004) – examined the lethal and sublethal effects of Roundup Original (containing POEA) and several other glyphosate formulations to

four species of tadpoles under several exposure scenarios. Using a tadpole developmental stage similar to past studies (Gosner stage 25; Gosner 1960), the researchers found $LC50_{4-d}$ values that were similar to what others had found (2.0–5.1 mg a.e./L; Fig. 9.2). Even more interesting were the effects observed when tadpoles were reared under longer (i.e., chronic) exposures, from hatchling tadpole through metamorphosis. They found that even low concentrations (0.6 mg a.e./L) could cause 30% mortality. The authors concluded, "The present results indicate that formulations of the pesticide glyphosate that include the surfactant POEA at environmentally relevant concentrations found in ponds after field applications can be toxic to the tadpole stages of common North American amphibians." (Howe et al. 2004, p. 1933).

What is striking about these five papers is that three of them expressed clear concerns that glyphosate products containing the POEA surfactant posed a clear risk to amphibian survival, and the other two demonstrated substantial death rates, although the rates happened to be less than 50%. Yet, to my knowledge, not one of these studies was denounced by Monsanto.

Our Follow-Up Studies of 2005

A few months after our article and press release were issued in April 2005, we published three more articles reporting the results of four additional experiments. The first article was our work that documented interactions between the herbicide and predator cues (Relyea 2005c). Not only did we confirm that the herbicide could become more toxic when combined with the stress of predation, but we also estimated the LC50 values of Roundup for six species of North American tadpoles. We exposed six amphibian species to a wide range of glyphosate concentrations (0–15 mg a.e./L). To look for the synergy with predator cues, we also ran the experiments for a longer duration (16 days) than typical LC50 lab experiments (1–4 days). We estimated the $LC50_{16-d}$ values as 0.4–1.8 mg a.e./L (Fig. 9.2). These experiments predicted that we should observe 80–90% tadpole death at 3 mg a.e./L. This was consistent with the conclusions of the concurrent mesocosm study that a concentration of 3 mg a.e./L caused very high rates of tadpole mortality.

The second study tested the long-touted claim that, because glyphosate and POEA strongly bind to soil in agricultural fields, the presence of soil in aquatic environments should ameliorate the effects of Roundup on amphibians by removing the herbicide from the water. The extrapolation to wetlands sounded reasonable, but had not been tested. So we conducted an experiment to determine if the addition of soils made Roundup less toxic. Using 3 mg a.e./L again in outdoor mesocosms, we examined whether the survival of three tadpole species improved if we manipulated the soil type in experimental mesocosms to include either no soil, sand, or loam (the soil type in our region). Our results were clear: adding sand or loam did not improve tadpole survival; with a single application of Roundup, 98% of the animals died (Relyea 2005b; Fig. 9.4).

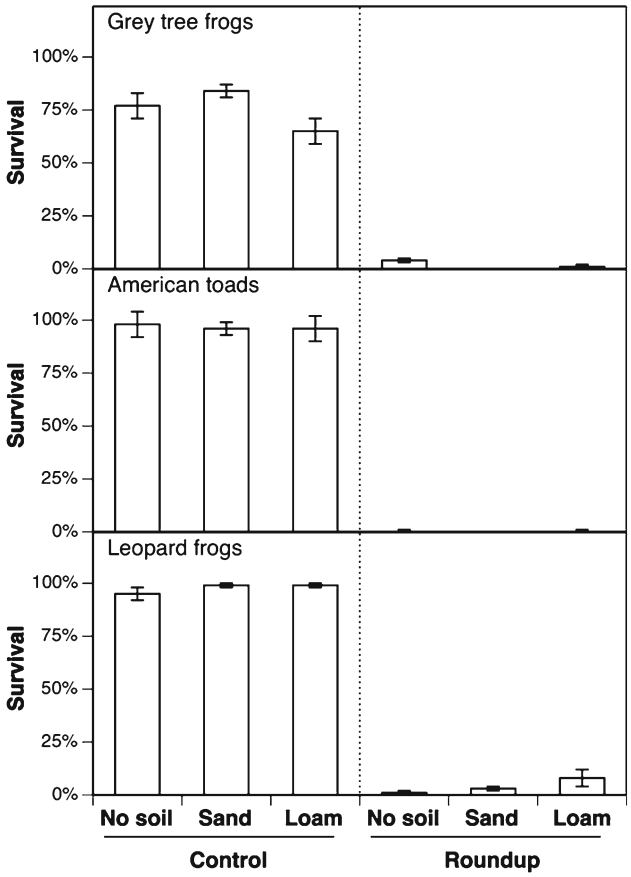

Fig. 9.4 The impact of a worst-case application of Roundup on pond mesocosms when combined with either no soil, a sand substrate, or a loam substrate (Relyea 2005b)

Later, we learned why soil plays less of a role than previously hypothesized. Although Roundup does strongly bind to soil, it is also highly soluble in water. The critical factor concerns what medium Roundup contacts first. If Roundup hits water first, as would happen during an over spray, the herbicide stays dissolved in the water and consequently remains toxic in the environment. While we currently do not know how much Roundup is bound by wetland soil or the rate at which this binding occurs in equilibrium with the water, it is clear that one cannot assume Roundup will be immediately removed by the sediments of a wetland and make the water safe for amphibians. We certainly need much more research on this issue.

The third study examined the direct and indirect effects of Roundup and malathion in communities with different species of lethal predators (Relyea et al. 2005). With regard to Roundup, the important aspect of this study is that it

examined a much lower concentration of Roundup (1 mg a.e./L) rather than the worst-case scenario concentration used previously (3 mg a.e./L). The resulting effect was not as lethal, but dramatic nonetheless. In the absence of predators, one-third as much Roundup caused no significant mortality in gray tree frogs, but caused 29% mortality in leopard frogs, and 71% mortality in American toads. In other words, even a much lower concentration caused substantial amphibian mortality.

In the fourth study, we examined the effects of Roundup on the terrestrial stage of amphibians (Relyea 2005c).[5] Almost nothing was known about the susceptibility of the terrestrial stage, except for a few Australian species that had been tested while living in water (Mann and Bidwell 1999). For our study, we collected newly metamorphosed gray tree frogs, wood frogs, and American toads. We then placed groups of ten frogs or toads into laboratory tubs containing wet paper towels and conducted an over spray at the manufacturer's recommended application rate. Across the three species, 79% of the animals died within 24 h.

As we approached the publication date of these four studies, it became clear that we had a number of new and important messages to convey to the public. We produced a second press release[6] to highlight the discovery that soils had no ameliorating effect on Roundup's lethality and that terrestrial applications could cause very high rates of death in the terrestrial stage of frogs and toads. This prompted another round of press coverage including an article in the St. Louis Dispatch (Hand 2005), a newspaper that resides in the hometown of Monsanto's international headquarters. As part of that interview, I asked reporter Eric Hand to inquire why Roundup Biactive®, Monsanto's new formulation that was less toxic to fish and amphibians, was being used in Australia and Europe but was not available in North America. This safer formulation was brought to market in direct response to the Australian research that determined Roundup was moderately lethal to Australian amphibians. Their spokesperson, Donna Farmer, gave two answers. First, Roundup Biactive has a weaker surfactant, so although it was safer for amphibians, it was less effective at penetrating plant cuticles and therefore less effective at killing North American weeds. Second, she said that registering the formulation in North America would be "subject to a cumbersome EPA approval process." This is the same approval process that the company had gone through for countless other Roundup formulations, and the active ingredient (glyphosate) was already approved in North America.

It was at this time that the EPA Environmental Fate and Effects Division started making inquiries. The initial inquiries asked for reprints of my papers. I obliged. When reporters asked the EPA for comment, their representatives generally gave the same answer: they were aware of our studies and were examining them.

[5] See errata Ecol Appl 19:276.

[6] http://mac10.umc.pitt.edu/m/FMPro?-db=ma.fp5&-format=d.html&-lay=a&-sortfield=date&-sortorder=descend&keywords=relyea&-max=50&-recid=36355&-find= (last accessed June 2010).

A Letter to the Editor

Shortly after our first mesocosm study was published in April 2005, a group of scientists from Canada wrote a letter to the editor of *Ecological Applications*. The journal's editor contacted me and asked if I thought the letter criticizing our study should be published as long as I had an opportunity to publish a response. After reading the letter, I felt strongly that it should be published.

The letter was written by a group of scientists from the University of Guelph (Barbara Wojtaszek, Andrea Edginton, Gerald Stephenson, Keith Solomon, and Dean Thompson [now with the Canadian Forest Service]). This group included many of the authors who conducted the studies on Vision that were published in 2004. One individual, Keith Solomon, was not an author of the studies published in 2004, but had conducted multiple risk assessments of glyphosate and had been the graduate advisor for many of the other authors. The lead author of the letter, Dean Thompson, was recommending the widespread use of Vision across Canadian forests and was some facing local opposition to this plan. Our research results, if they stood the test of time, would make this recommendation more difficult to promote.

Their letter to the editor was sent to an anonymous reviewer and me as an 11-page manuscript. It appeared to both of us that the criticism was quite hurriedly written and we asked that the authors correct a number of fundamental errors. Given time to rethink their arguments, the revised letter expanded from 11 to 21 manuscript pages. The final version of the letter was published in October 2006 and made four major claims (Thompson et al. 2006): (1) our application rates were too high, (2) the concentrations we used were not relevant to those found in nature, (3) there were potential methodological errors, and (4) past risk assessments had already concluded that the herbicide to pose no risk to nontarget organisms. In my response (Relyea 2006) I systematically identified the flaws in each argument.

First, the authors provided a list of application rates that were all lower than the application rate used in my study. However, the authors failed to acknowledge that higher application rates existed. Regardless of differences in application rates for different uses, the real issue, of course, is the concentration that was in the water. As noted earlier, the aquatic concentration in our first mesocosm experiment was 3 mg a.e./L, which fell within the range of both estimated and observed worst-case scenarios in nature.

Thompson et al.'s (2006) second claim was that our concentration of glyphosate in the water was unusually high. To support this claim, they provided a list of aquatic concentrations that had been observed in nature. In my response, I pointed out that the authors tried to make the case that only low concentrations occur in nature by including a lot of data from habitats such as water wells and streams. These two water sources are known for having low concentrations of glyphosate. More importantly, these two water sources and are largely irrelevant for North American species of tadpoles – tadpoles do not live in underground wells and most species of tadpoles do not live in streams. They live in wetlands. I also pointed out that several well-known studies that documented high concentrations of glyphosate in nature were

omitted from their list. I made it clear that the authors were surely aware of these studies. They had co-authored several of them.

Their third claim was that our study contained a number of potential flaws that made the study unreliable. For example, they claimed that the absence of soil in our study detracted from its applicability to nature because soil would have taken the herbicide out of the water column. Of course, by this time we had already published our follow-up study demonstrating that adding soil did nothing to improve tadpole survival with Roundup (Relyea 2005b). They were also concerned that our mesocosms did not contain any aquatic plants (i.e., macrophytes) For example, they claimed that aquatic plants might add oxygen to the water, speculating that oxygen concentrations in the water were low and may have unnaturally stressed our animals. Repeated experiments have found very high oxygen levels in these tanks, with or without macrophytes. All of these arguments were weak attempts at finding flaws that were easy to refute. In response to these speculations, I came to the following conclusion, "The authors propose a number of methodological flaws that are not only without support, but, in many cases, demonstrate a lack of knowledge of aquatic ecology" (Relyea 2006, p. 2033).

The final claim took an approach that is a classic response to criticisms of a manufacturer's product. The authors argued that Roundup has been used to kill weeds for a very long time and multiple risk assessments have been conducted that have found "no unacceptable risk," so the product poses no risk to amphibians. This is a strong argument, until one investigates the details of the past risk assessments. Thompson et al. (2006) begin by citing reports from the EPA (1993) and WHO (1994). There was one small problem – neither of these reports included any amphibian data because no amphibian data existed in the world until Bidwell and Gorrie's (1995) work in Australia. They also cited the risk assessment by Giesy et al. (2000); this assessment included the Australian data and actually concluded that Roundup posed a potential risk to amphibians that needed further evaluation. Finally, they cited the risk assessment of Solomon and Thompson (2003); an assessment that included fish and invertebrates, but not amphibians. Collectively, these risk assessments actually tell us little about the risk of Roundup to larval and adult amphibian populations.

I concluded my response by quoting excerpts from the 2004 papers in which several of the authors agreed that Roundup/Vision posed a risk to amphibians in nature (as quoted above) and that the weight of the evidence from all studies continued to support this assessment. I further noted that a very likely reason some of the Canadian studies had produced lower (and more variable) death rates from the herbicide was due to the lower (and more variable) levels of pH in those studies. I suggested we should look more carefully at the role of pH to get a better idea of Roundup's impacts on amphibian mortality.

While writing my response, there was one issue that still bothered me. At the same time that my lab was conducting research on Roundup and amphibians, Tyrone Hayes (University of California, Berkeley) was in the midst of a debate over the effects of the herbicide atrazine on amphibians. Tyrone was finding that atrazine could cause male frogs to become feminized via endocrine disruption (Hayes 2004, see Chap. 10).

This debate involved the Syngenta pesticide company and EcoRisk, a consortium of pesticide companies that hires academic scientists to work as consultants. One of the people hired by EcoRisk was Keith Solomon, one of the authors of the letter to the editor. Several months after their letter to the editor was published, Keith Solomon admitted to a reporter that he had received funding from Monsanto (Lubick 2007). According to the University of Guelph,[7] Dean Thompson was also receiving funding from Monsanto. Accepting research money from a pesticide manufacturer is not a problem. Debating the safety of the company's product without full disclosure that the company is funding your research is a problem. It can affect the world's assessment of your independence and objectivity.

Roundup and the Colombian Drug War

While the Roundup-amphibians debate was ongoing, there was a war on drugs occurring in South America and these two issues came together quite unexpectedly. Beginning in the 1990s, a rebel group in Colombia known as FARC (Fuerzas Armadas Revolucionarias de Colombia) was obtaining a large fraction of its financial support from coca production. The coca plants were processed into cocaine and smuggled to other countries, including the USA. Since the United States considered FARC a terrorist group, US officials recognized that if they could curtail coca production in Colombia it would have the twin benefit of taking away the financial foundation of a terrorist group and reducing the amount of cocaine being smuggled into the USA.

Plan Colombia

In 1999, "Plan Colombia" was developed between the USA and Colombian governments that included funds for aerial fumigation of illegal coca fields. The herbicide of choice was a glyphosate formulation called Glyphos-Cosmo-Flux, which used the POEA surfactant. However, after a large number of complaints related to legal crops being sprayed as well as worries over unintentional impacts of glyphosate application to humans and wildlife, the Organization of American States in 2004 agreed to assemble an independent panel of outside experts to assess the potential risk that these aerial spraying might pose to amphibians and humans. The person selected to lead this independent panel was Keith Solomon, the same person who was funded by Monsanto.

The US government was particularly concerned about potential impacts to wildlife in Colombia, especially after our research published in April 2005 showed that inadvertent over sprays of wetlands could be highly lethal to amphibians and many

[7] https://www.uoguelph.ca/research/summaries/2006/table1-oac.shtml. (last accessed June 2010).

of the coca fields had adjacent wetlands. This concern was exacerbated by the fact that Colombia has an incredibly high diversity of amphibians (746 species). Nearly a third of them (255 species) are currently classified as either vulnerable, near threatened, endangered, or critically endangered.[8] There also was a concern that the eradication program was having no effect on coca production. The Senate appropriations bill (US Senate 2005) stated the following:

> The Committee reaffirms its commitment to assist the efforts of Colombian President Uribe in destroying the threats of terrorism and narcotics in that country …. The Committee is increasingly concerned, however, that the aerial eradication program is falling far short of predictions and that coca cultivation is shifting to new locations. Since the start of Plan Colombia, over 525,000 hectares of coca crops have been sprayed, yet coca cultivation has decreased by only 7%. Last year alone, 136,555 ha were sprayed, but the total area under cultivation, estimated by the State Department at 114,000 ha, remained essentially unchanged from the previous year. There is no indication that the quantity of cocaine entering the United States has decreased …. The Committee directs the Secretary of State, in consultation with the EPA and appropriate Colombian authorities, to submit a report not later than 180 days after enactment of the Act, with the following information: the results of a GIS analysis of the proximity of small, shallow water bodies to coca and poppy fields and of tests to determine the toxicity of the spray mixture to Colombian amphibians; and, an assessment of potential impacts of the spray program on threatened species, including in Colombia's national parks.

In 2007, reporter Naomi Lubick contacted me about the risk assessment being conducted in Colombia. At the time, no formal assessment had yet been produced, although I had seen preliminary reports and I knew that Keith Solomon's research team in Colombia was finding similar levels of tadpole death using Glyphos-Cosmo-Flux as we had reported for Roundup. In her story that was published in *Environmental Science and Technology*, Naomi reported, "The team concludes that the glyphosate mixtures used in the program *are potentially harmful to tadpoles, particularly those living in shallow pools* [italics added]." (Lubick 2007, p. 3404). Moreover, she reported, "Solomon and his colleagues, however, predict moderate toxicity of glyphosate mixtures at levels of exposure *similar to some of Relyea's lowest concentrations* [italics added]" (Lubick 2007, p. 3405). At this point, I realized even our staunchest critic had finally arrived at the inescapable conclusion that glyphosate formulations containing POEA posed a significant risk to amphibians.

The Initial Risk Assessment for Colombia

In 2007, Solomon and colleagues produced a preliminary risk assessment for Plan Colombia (Solomon et al. 2007). This risk assessment did not contain any data on Colombian amphibian species, but took what was known about the sensitivity of amphibians in North America and Australia and applied it to the herbicide application

[8] Database search of AmphibiaWeb conducted on 11/02/09; http://www.amphibiaweb.org/.

Table. 9.1 The summary of potential environmental impacts associated with coca production as determined by Solomon et al. (2007; see their Table 14)

Impacts	Intensity score	Recovery time (years)	Impact score	Impact %
Clear cutting and burning	5	60	300	96.9
Planting the coca or poppy	1	4	4	1.3
Fertilizer inputs	1	0.5	0.5	0.2
Pesticide inputs (by farmers)	5	0.5	2.5	0.8
Cosmo-Flux spray (for eradication)	1	0.5	0.5	0.2
Processing and refining	2	1	2	0.6

Cosmo-Flux spray is the herbicide that contains the same active ingredient (glyphosate) and surfactant (POEA) as many commercial formulations of Roundup

rates that were being conducted in Colombia. Based on the application rates used to eradicate coca, the estimated concentration of glyphosate in a shallow wetland (15 cm deep) with no initial absorption to sediments was 2.47 mg a.e./L. Actual concentrations in sprayed fields and wetlands could not be obtained because coca fields are often protected by armed guards. Because published LC50 values for many tadpole species were considerably lower than 2.47 mg a.e./L, an inadvertent over spray of wetlands would kill more than 50% of all tadpoles living in shallow wetlands. In agreement with what Naomi Lubick had reported earlier, the research team concluded, "Moderate risks to some aquatic wildlife may exist in some locations where shallow and static water bodies are located in close proximity to coca fields and are accidentally oversprayed." (Solomon et al. 2007, p. 104). Our research completely agreed with this conclusion.

What Solomon et al. (2007) did next was an attempt to relativize the risk of Roundup on amphibians. They proposed subjective "intensity scores" (from 1 to 5) for how various steps in the coca production process would harm nontarget plants and animals (Table 9.1). They decided that the cutting and burning of the forest and the use of other pesticides by coca farmers should both receive an intensity score of 5 whereas the spraying of the herbicide should only receive an intensity score of 1. Next, they assigned values for how many years it would take the plants and animals to recover from each impact. They decided that amphibian habitats could recover from deforestation after 60 years and amphibian populations experiencing death from the herbicide could recover from the spraying of the herbicide in only 0.5 years. Given the high rates of mortality that can occur at environmentally expected concentrations of the spray program, however, it is not reasonable that an amphibian population could recover in 0.5 years (especially for amphibians that only breed once per year). Finally, they computed "impact scores" (calculated as the product of an intensity score and recovery time). Using these numbers, they came to the conclusion that clear cutting the forest for coca production was responsible for 96.9% of the effect on the environment whereas the herbicide spraying program was responsible for only 0.2% of the effect on the environment. As a result, they concluded, "When taken in the context of the environmental risks from other activities associated with the production of coca and poppy, in particular, the uncontrolled

and unplanned clearing of pristine lands in ecologically important areas for the purposes of planting the crop, the added risks associated with the spray program are small." (Solomon et al. 2007, p. 104). This conclusion, of course, is only correct if the subjective estimates are correct. Moreover, this conclusion does not address the question that the US Congress asked. The team was charged with the task of assessing the risk of the spray program to amphibians in coca fields *where deforestation has already occurred*. In other words, given that there amphibians living in and around coca fields, what is the impact of the spray program on them?

The Final Risk Assessment for Colombia

In August 2009, Solomon's research team published a series of articles in the *Journal of Toxicology and Environmental Health* in which they presented their research findings from the Plan Colombia experiments. This included studies of herbicide drift, impacts on human health, and impacts on amphibians. Because the eradication program flies airplanes over coca fields, often while being fired upon by the coca farmers (Lubick 2007), the researchers were unable to determine how much glyphosate lands on the fields, forests and wetlands. As in the initial assessment, they could only estimate the amounts based on application rates. They also never produced the valuable GIS analysis of wetland proximity to coca and poppy fields that the US Congress had requested.

The research team did conduct a number of experiments. In laboratory tests on eight species of Colombian tadpoles, they found that the $LC50_{4-d}$ for Glyphos-Cosmo-Flux ranged from 1.2 to 2.8 mg a.e./L (Bernal et al. 2009a; Fig. 9.2). These values are very much in line with values that we published on six species in North America ($LC50_{16-d}$ = 0.4–1.8 mg a.e./L; Relyea 2005c). Indeed, the team concluded, "Data suggest that sensitivity to Roundup-type formulations of glyphosate in these species is similar to that observed in other tropical and temperate species." (Bernal et al. 2009a, p. 961) and, in a companion paper, state, "There are some potential risks to amphibians from direct overspraying of shallow waters." (Marshall et al. 2009, p. 930). This seemed like a logical and reasonable conclusion from the data. The team, however, was not finished.

The next step they took was to examine the effect of the herbicide under "field conditions" to determine whether laboratory LC50 values were predictive of impacts under field conditions (Bernal et al. 2009b). They set up six outdoor mesocosms, each containing soil and 15 cm of water. To each mesocosm they added between 165 and 200 tadpoles (representing two species in each of two experiments), exposed each mesocosm to a different concentration of herbicide, and then determined survival 4 days later. In a fashion unlike most peer-reviewed scientific studies, their study included only one replicate of each treatment, thereby preventing any assessment of repeatability. Importantly, their mesocosm experiments had a lower pH (average pH = ~7) than their lab studies (pH = 8.2). Not surprising, the researchers found less tadpole death in the mesocosm experiments ($LC50_{4-d}$ = 9–11 mg a.e./L)

than in the lab experiments ($LC50_{4-d}$ = 1.2–2.8 mg a.e./L). As noted earlier, the herbicide is well known to be less lethal at lower pH (Chen et al. 2004, Wojtaszek et al. 2004). What was surprising is that the researchers attributed the lower death rates in the mesocosm experiments not to the lower pH, but to the presence of soil, despite the fact that studies had already shown that adding soil has no ameliorating effect on the lethality of Roundup (Relyea 2005b). Using their interpretation that soil was the underlying cause of the reduced mortality, the researchers concluded that the herbicide poses a low risk under "field conditions."

As part of this same paper, the team investigated the lethal effects of the herbicide on postmetamorphic amphibians (i.e., metamorphosed juvenile and adult frogs). Using tubs containing moist soil and leaf litter, they sprayed the amphibians at a variety of application rates. At the typical application rate for spraying coca, they found that up to 30% of the animals died. Because fewer than 50% of the adults died at the typical application rate, they concluded that, "Data indicate that, under realistic, worst-case exposure conditions, the mixture of Glyphos and Cosmo-Flux … exerts a low toxicity to aquatic and terrestrial stages of anurans." (Bernal et al. 2009b, p. 966).

It is important to ask, however, whether a 30% loss of an adult population is unimportant to the persistence of the population. In amphibians, larvae typically experience high rates of death from natural causes (e.g., predation, competition, disease) whereas adults have much higher rates of natural survival. As a recently published life-history model of amphibian populations demonstrates, this means that the persistence of the population is much more dependent upon adult survival than larval survival (Taylor et al. 2006). This model found that a 20% decrease in annual adult survival would cause the population size of breeding females to decline by 45% while a 40% decrease in annual adult survival would cause the population size of breeding females to decline by 87%. Hence, 30% annual mortality of adults following terrestrial spraying of Roundup is expected to cause a substantial decline in the population. Moreover, given that the frequency of spraying the coca fields is every 6–12 months, this means that up to 30% of the adult population could be killed as frequently as every 6 months (Solomon et al. 2007).

Given the unavoidable conclusion now that glyphosate with POEA is moderately to highly toxic to amphibians in both the aquatic and terrestrial stages, the team again relativized their results. First, they examined the species ranges of numerous amphibians and found that the mortality caused by the herbicide would only impact a portion of many species' ranges. As a result, "… populations as a whole are at low risk." (Lynch and Arroyo 2009, p. 974). This means that the authors accepted that areas being sprayed will cause amphibian populations to decline locally, but argued that the remaining unsprayed areas would ensure the persistence of the species. For those species with only part of their range in Colombia, "the consequences of coca production may be more serious and may have placed several species of frogs at risk." (Lynch and Arroyo 2009, p. 974). In other words, if a species of frog had a small piece of its natural range in Colombia (and the rest of its range in neighboring countries), the eradication program might have a negative impact on the persistence of the species in Colombia.

As they did in their initial assessment in 2007 (Solomon et al. 2007), the team in 2009 again compared the impact of the herbicide against other factors that are known a priori to have much more devastating effects. For example, the team compared the toxicity of the herbicide against the toxicity of other pesticides and found that some insecticides are orders of magnitude more toxic (Brain and Solomon 2009). Relative to a very highly toxic insecticide such as endosulfan, Glyphos-Cosmo-Flux is not as toxic (Jones et al. 2009). This is analogous to saying that rat poison can be deadly to humans, but not as deadly as arsenic. While true, the comparison does not make rat poison any less toxic to humans and Brain and Solomon's (2009) comparison between the herbicide and endosulfan does not make the Glyphos-Cosmo-Flux any less toxic to amphibians. They went on to suggest that the deforestation caused by coca farmers causes habitat loss to amphibians and that this impact is also much larger than the impact of spraying the herbicide. Thus, while Roundup poses a risk, "the uncontrolled deforestation for the production of illicit crops such as coca will have a major effect on amphibians in Colombia through habitat alteration." (Brain and Solomon 2009, p. 944). While no real data are brought to bear on this question, it is likely true. Nevertheless, this conclusion had little to do with the assessment requested by the US Congress, which funded the researchers to determine the impact of the herbicide on amphibians living in and near cleared fields – not to assess the impact of the herbicide relative to other factors. Comparing the effect of the herbicide to complete deforestation of amphibian habitat allowed the team to conclude, "In summary, there are a number of human activities associated with the production of coca that present greater risks to amphibians than the glyphosate and Cosmo-Flux mixture used in the aerial eradication spraying." (Brain and Solomon 2009, p. 945). Although this is certainly correct, it only served to distract attention from the real issue at hand. All evidence from the Plan Colombia assessment pointed to the fact that the typical applications rate of the herbicide can kill larval, juvenile, and adult amphibians in large numbers.

An Independent Assessment of Plan Colombia

Nearly a decade has passed since the implementation of Plan Colombia using aerial fumigation with Glyphos-Cosmo-Flux. The original objective in 1999 was to reduce coca production by 50%. In 2008, the independent Congressional General Accounting Office (GAO) examined the program and found that from 2000 to 2006, the US government had spent over $6 billion to improve security and fight the drug war (US GAO 2008). By 2007, they were spraying 160,000 ha of coca fields annually. What was the result? The GAO found that between 2000 and 2007, coca production had *increased* by 15% (Fig. 9.5). Farmers quickly learned that they could replant new coca shrubs, prune their perennial shrubs after being sprayed, and move to new areas that can be cleared for new coca farms. Indeed, the aerial eradication program had pushed farmers out of their existing fields and motivated them to clear

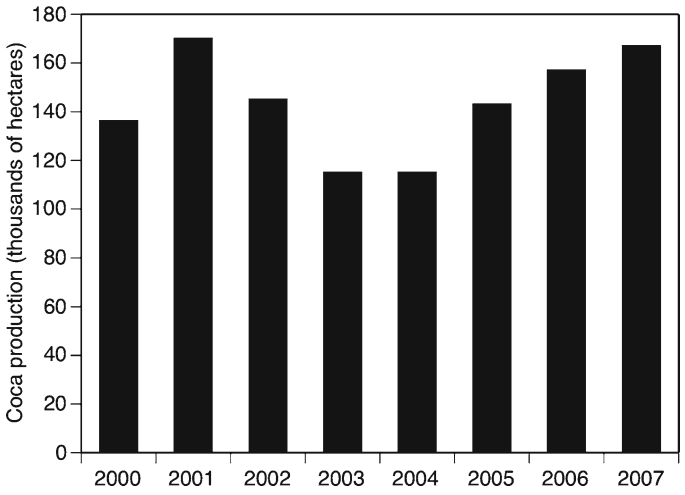

Fig. 9.5 Coca cultivation from the beginning of Plan Colombia (2000) until 2007 US GAO (2008)

new areas of rainforest habitat in promised no-spray zones in Colombian national parks and along the border with Ecuador. Thus, deforestation, one of the major threats to amphibian populations highlighted by Brain and Solomon (2009), is increasing *because* of the coca fumigation program. In summary, while providing no benefit of reduced coca production, the program ultimately contaminated large areas of Colombia and caused additional deforestation of amphibian habitats. Even the relativistic arguments of Solomon's group held no ground this time; directly or indirectly, the spraying of glyphosate was causing the loss of amphibian habitat in Columbia. While spraying Glyphos with Cosmo-Flux has been a complete failure in eradicating coca, it may be making excellent progress in eradicating Colombia's amphibians.

Ironically, this outcome was actually predicted by the US government. In 2000, the US Central Intelligence Agency was asked to assess the probability of being successful in the antidrug war in Colombia (US CIA 2000). In a document that was declassified in 2003, the CIA assessment stated, "A 50% decline in coca cultivation in the south over the next 5 years likely would encourage substantial new cultivation in other parts of Colombia. Farmers would probably be able to compensate for their losses by growing elsewhere in Colombia." Thus, at the start of Plan Colombia, it was estimated that the plan would fail to reduce coca production and would push farmers out to clear new areas for coca farms. Despite this prediction, the Plan still proceeded.

In 2009, the US State Department was asked about the lack of success in reducing coca production by aerially spraying. Naomi Lubick (2009) reported, "Despite this, a spokesman from the US State Department says that the USA will support Colombia's government as long as it chooses to continue the herbicide-spraying strategy to tackle its cocaine problem."

The Case of the California Red-Legged Frog

At the same time that Roundup's impact on amphibians was being evaluated in Colombia, there was an effort to also have its risk to amphibians assessed in California. In 2002, the Center for Biological Diversity sued the EPA, alleging that the EPA violated the Endangered Species Act by approving the registration of multiple pesticides to be used in California without considering the potential impacts of pesticides on the endangered California red-legged frog (*Rana aurora draytonii*). In 2005, the US District Court agreed, and an agreement was drawn up requiring the EPA to assess the potential impact of 66 pesticides on the red-legged frog and its habitat.[9] One of these pesticides was glyphosate.

In 2008, the EPA completed its assessment for glyphosate risks to the California red-legged frog (Carey et al. 2008). Because the California red-legged frog occupies both aquatic and terrestrial habitats, separate assessments were conducted for each habitat. Within each habitat, they assessed the potentially direct effects of the herbicide on survival, reproduction, and growth as well as the indirect negative effects that might occur if the herbicide application altered the frog's habitat.

The first risk assessment was conducted on the aquatic stage. The assessment includes lists of published studies on glyphosate toxicity on both fish and amphibians. When calculating the Risk Quotient, however, they used a study of bluegills (*Lepomis macrochirus*) that had an LC50 value of 3.17 mg a.e./L. They also calculated an expected concentration in the water based only on drift; they did not include inadvertent over spray that occurs during forestry applications. This produced an expected environmental concentration of 0.095 mg a.e./L. Using the bluegill LC50 value and the forestry expected environmental concentration value, they calculated a Risk Quotient of 0.095/3.17 = 0.03. Because the Risk Quotient value did not exceed the EPA's Level of Concern (i.e., 0.05), the EPA concluded there was no direct risk of Roundup applications to aquatic stages of amphibians. As I discuss below, more recent studies have discovered that at least two species of tadpoles have LC50 values that are much lower than bluegills (Relyea and Jones 2009), making the Risk Quotient now exceed 0.05.

Next, the risk assessment evaluated the impact on the terrestrial stage of the frog. As noted earlier, the sensitivity of terrestrial amphibians is assumed to be similar to the sensitivity of birds eating herbicide-contaminated food. It does not allow an assessment of potential impacts when the frog's skin is sprayed with the herbicide (we now know from the Colombian research that terrestrial applications of Roundup also can directly kill up to 30% of adult frogs; Bernal et al. 2009b). Using this approach, the EPA concluded that there were no concerns over direct effects of the herbicide on the terrestrial stage of the California red-legged frog.

Although the EPA did not identify sufficient evidence for direct negative effects, they did identify concerns regarding indirect negative effects on the frog's habitat

[9] http://www.biologicaldiversity.org/news/press_releases/rlf-10-19-2006.html (accessed June 2010).

and prey. In the end, they concluded using standard EPA categories, "Based on the best available information, the Agency makes a May Affect, and Likely to Adversely Affect determination for the CRLF [California red-legged frog] from the use of glyphosate." (Carey et al. 2008, p. 10). In plain language, the EPA concluded that the use of glyphosate in the habitats of the California red-legged frog would have a negative effect on the long-term persistence of the endangered frog's population. To my knowledge, this was the first time that the EPA had concluded that a glyphosate-based herbicide could negatively affect amphibians.

Reflecting on the Future of Amphibians and Roundup

As I reflect on the research that has been conducted on Roundup and amphibians, I am struck by the path that the research has taken and the attention it has received. In the mid 1990s, the Australian researchers determined that glyphosate-based herbicides containing the POEA surfactant were moderately toxic to amphibians. Monsanto declared that the researchers were wrong. My group has followed in their path confirming that the herbicide can be classified as highly toxic to amphibians in North America. Monsanto has declared that we also were wrong. Our latest work continues to confirm our initial studies. We recently conducted $LC50_{4-d}$ tests on 13 species of amphibians including – for the first time – tests on larval salamanders (Relyea and Jones 2009). We have also conducted additional mesocosm experiments that have demonstrated that the amount of mortality differs with tadpole age and that the herbicide becomes more lethal when combined with the stress of competition from other tadpoles (Jones et al. 2010, 2011). These studies have used a newer formulation that has been heavily marketed to farmers (Roundup Original Max®). A spokesperson for Monsanto, Scott Mortenson, called me in the summer of 2009 to insist that this formulation does not contain the POEA surfactant. He would not divulge what surfactant was added, claiming that it was a trade secret. In the end, the identity of the surfactant did not matter. The toxicity of Roundup Original Max was very much the same as the older formulations that contain POEA. Newer formulations, including Roundup PowerMAX® and WeatherMAX®, have received little testing on amphibians. However, a recent study found that chronic exposure to a relatively low concentration (0.572 mg a.e/L) of WeatherMAX caused 80% mortality in western chorus frog tadpoles (Williams and Semlitsch 2010).

In June 2009, the EPA announced it is seeking data on glyphosate toxicity as part of Monsanto's application to renew the herbicide's registration. The challenges will be familiar. The EPA has to assume that the herbicide is only used according to label directions. Hence, the inadvertent and unavoidable over sprays of herbicide on wetlands that are known to occur when spraying Colombian coca fields or Canadian forests will not likely factor into the risk assessment, although the impact of herbicide drift into water bodies will likely be considered. A large number of assumptions can be made about exposures in nature to obtain a variety of risk assessment outcomes (the depth of the water, how much herbicide is intercepted by overhanging

vegetation, how much herbicide is held by the soils, etc.). Unfortunately, there is a paucity of data on natural concentrations to evaluate the accuracy of these estimates. As Renier Mann (University of Technology in Sydney) recently said in an interview with Lubick (2009), "Everyone agrees on concentrations that cause toxicity. The argument is over whether the frogs are exposed to those concentrations." This is a critical area for future research.

For the aquatic stage of amphibians, we now have substantially more toxicity studies covering a broader range of species. The studies definitively show that glyphosate formulations containing POEA and other formulations that may or may not contain POEA (i.e. Roundup OriginalMAX, Roundup WeatherMAX) are highly toxic to tadpoles. Based on these most recent studies, the most sensitive tadpole species are American bullfrogs and the spring peepers (*P. crucifer*). Both have $LC50_{4-d}$ values of 0.8 mg a.e./L, which is considerably lower than the bluegill data that have been used in past amphibian risk assessments. If we were to follow the EPA risk assessment protocol (Jones et al. 2004) and divide this value by 20, the upper limit that would protect larval amphibians would be 0.04 mg a.e./L. In the case of the EPA's risk assessment of the California red-legged frog (Carey et al. 2008), the Risk Quotient would now be 0.095/0.8 = 0.12. This means that the Risk Quotient would exceed the Level of Concern value of 0.05 that has been set to protect amphibians. We also now have new data on the concentrations found in nature. For example, Battaglin et al. (2009) surveyed vernal pools in the Rock Creek National Park and found concentrations up to 0.328 mg a.e./L, which substantially exceeds the 0.065 mg/L freshwater standard for aquatic life exposed to glyphosate.

We still have few data on the terrestrial stages of amphibians. The only two published experiments (Relyea 2005b; Bernal et al. 2009b) have found that aerial oversprays at expected application rates can cause substantial death in adult frogs (30–86% death in 1–4 days). With these data now available, it is unclear whether the EPA will continue to use estimates of dietary ingestion in birds as surrogates to assess the effects of Roundup on terrestrial stages of amphibians. If this turns out to be the case, then any concerns about amphibian exposures to Roundup will likely follow the outcome of the California red-legged frog and emphasize the indirect effects rather than direct lethal effects.

It will be quite interesting to follow the re-registration process for glyphosate-based herbicides in the years to come. With Monsanto's share of the glyphosate sales market reaching $3.5 billon in 2009 (Monsanto 2009), there is been a lot of corporate profit hanging in the balance. Interestingly, however, increased foreign competition following the end of the glyphosate patent has forced large reductions in Monsanto's price point for its line of Roundup products (by approximately two-thirds) and Monsanto has announced that its long-term strategy is to reduce its manufacturing of chemicals and focus its efforts on its manufacturing of genetically modified seeds (Leonard 2010). Regardless of who makes glyphosate-based herbicides and seeds, there will be a strong push by the corporations to continue the use of glyphosate. Based on the history of such debates across many industries, the corporate strategy will likely be to proclaim that decades of past studies have determined there is no risk, to discredit any scientists who question past risk assessments with new

data, and to ultimately delay any decision that could impact profits (Michaels 2008). If I ran a corporation that made a great deal of money from a popular herbicide and associated genetically modified seeds, I suppose I might be tempted to do the same thing. As a scientist, however, my goal has always been to better understand how pesticides might be impacting our environment, to inform the public about the science, and to let the public and policy makers decide what, if anything, should be done next. What is clear based on published data is that unlike many species of Roundup-Ready crop plants, amphibians are not ready for Roundup.

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