

Letters to the Editor

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THE IMPACT OF INSECTICIDES AND HERBICIDES ON THE BIODIVERSITY AND PRODUCTIVITY OF AQUATIC COMMUNITIES

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To the Editor:

Relyea (2005a), asserts that current application rates for the glyphosate formulation Roundup are highly lethal to many amphibian species. We strongly disagree with this conclusion since it is based on inappropriate generalization and extrapolative inference from a single test that is arguably irrelevant to most real-world scenarios. We contend that the application rates and resultant aqueous exposure concentrations used in this experiment are quite atypical of those associated with dominant uses of glyphosate formulations in agriculture, forestry, and industrial sectors.

Two specific statements in the Relyea paper serve as focal points for concern:

1) This study represents one of the most extensive experimental investigations of pesticide effects on aquatic communities and offers a comprehensive perspective on the impacts of pesticides when non-target organisms are examined under ecologically relevant conditions.

2) Collectively, the available data indicate that, contrary to conventional wisdom, current application rates of Roundup can be lethal to many species of amphibians.

Both statements exemplify a tendency for over-generalization and excessive extrapolation. We submit that no single experiment represents an extensive investigation of pesticide effects on aquatic organisms. Neither could it offer a comprehensive perspective on such a broad topic area. Bartell et al. (1992), notes that ecological risk estimation is complex and requires, as a minimum, full characterization of concentration–response relationships and determination of the range of real-world exposure concentrations relevant to major use patterns. Resultant risk estimates are based on the probability that significant deleterious effects may be generated under real-world exposure regimes. A comprehensive perspective would require review and critical analysis of hundreds of previously published papers.

None of these fundamental requirements are met in this publication.

We certainly agree with the author on the general value of mesocosm studies in assessing potential effects of pesticides in aquatic systems. However, as discussed previously (e.g., Touart and Slimak 1989, Thompson 2004), mesocosm testing should be considered as one element in a multi-tiered research program that generates data, which when considered in whole, may be sufficient for effective estimation of ecological risk. The industry and complexity involved in conducting a single mesocosm study does not confer broad spectrum inference potential. Moreover, results must be considered in relation to relevant pre-existing data and with due consideration for the “weight of scientific evidence” principle. These criteria are also not met by the Relyea (2005a) publication. This is demonstrated by the fact that a number of directly relevant papers (e.g., Berrill et al. 1997, Lajmanovich et al. 2003, Chen et al. 2004, Edginton et al. 2004, Howe et al. 2004, Thompson 2004, Thompson et al. 2004, Wojtaszek et al. 2004) comprising a significant proportion of the preexisting body of knowledge on potential effects of glyphosate herbicides to amphibians were neither cited nor discussed in the paper. This omission is particularly disquieting given the author’s explicit statement (Relyea 2005a:625) that “Prior tests of glyphosate on amphibians have been rare,” the fact that several of these papers draw opposing conclusions, and the author’s assertion as to what the “collective data” on this topic indicate.

In our view, neither the specific results of this experiment nor the collective data support the conclusion that Roundup is likely to be lethal to many species of amphibians under environmentally realistic use scenarios. This viewpoint is based on the general lack of relevance of experimental results to typical use patterns and application rates, and available data on measured concentrations in lentic systems associated with the predominant use of glyphosate formulations in agriculture, forestry or industrial sectors. In addition, several methodological issues and design flaws result in substantial uncertainty in extrapolation of test results to effects that might be expected in natural aquatic systems.

To facilitate cross-comparisons and avoid confusion, all application rates and aqueous concentration values in this letter have been converted to units of glyphosate acid equivalents (a.e.). In the case of formulations containing the isopropylamine (IPA) salt, as for the test material in the Relyea study, the appropriate conversion factor is based on the molecular weight ratio of glyphosate acid to isopropylamine salt (356/480 =

TABLE 1. Comparative maximum application rates for various North American uses of glyphosate herbicide formulations containing the POEA surfactant.

Use scenario	Typical formulated product	Maximum application rate (product L/ha)†	Application rates (kg a.e./ha)‡	Ratio§
Relyea (2005a)	Roundup Weed and Grass Killer	64	12.8¶	1
Forestry				
Site preparation	Vision, Roundup Original	12	4.27	3
Conifer release	Vision, Roundup Original	6	2.14	6
Agriculture				
Annual weeds	Roundup Original	3.5	1.25	10
Perennial weeds	Roundup Original	12	4.27	3
Typical use USA	Roundup Original	4.5	1.6	8

† Maximum label rates were taken from product specific labels.

‡ The abbreviation "a.e." indicates acid equivalents.

§ Ratio calculated as Relyea (2005a) study rate divided by the maximum application rate allowed for a particular use as specified on the product label (e.g., the site preparation maximum rate for forestry is one-third of Relyea's rate).

¶ The unspecified product tested by Relyea was subsequently identified as Roundup Weed and Grass Killer Concentrate which contains 25% glyphosate isopropylamine salt (IPA) by mass. Given that 15.3 mL of product was applied to a surface area of 2.41 m² and the formulation density is 1.09 g/mL, the actual application rate was 1.73 g glyphosate IPA salt/m² equating to 17.3 kg glyphosate IPA salt/ha or 12.8 kg of glyphosate acid equivalents (a.e.)/ha.

|| Average use rates for major agricultural crops to which glyphosate is commonly applied in the USA.

0.74). Although incompletely described in the Relyea paper, the test material has been subsequently identified as Roundup Weed and Grass Killer Concentrate. This particular formulation contains the polyethoxylated tallowamine (POEA) surfactant. Both the IPA salt and POEA surfactant are components of several other glyphosate formulations marketed under a wide variety of registered trade names. Exact specification of the formulation tested is critical since a variety of glyphosate salt types, surfactant compositions, and other formulants occur within a family of herbicides with glyphosate as the active ingredient and not all formulations involve the POEA surfactant. Formulations containing the POEA surfactant are of particular interest in aquatic ecotoxicology since these are known to exhibit relatively greater toxicity to many aquatic organisms including amphibians as compared to either glyphosate alone or other glyphosate-based formulations (Perkins et al. 2000, Howe et al. 2004).

Several arguments suggest that the second concluding statement made by Relyea is erroneous and these are presented briefly here.

The application rate used in this study is atypical of those commonly employed in the major agriculture, forestry, and industrial use sectors.—Although the actual rate employed by Relyea may have been consistent with the maximum rate stated on the label, it assumes an illegal or unlikely scenario in which homeowners or other applicators would apply the product directly and uniformly to water surfaces constituting amphibian breeding habitat. Moreover, the application rate employed by Relyea (12.8 kg a.e./ha) is ~3–10 times greater than legally binding maximum label rates for the most common formulations of glyphosate used on non-cropland, forestry, or agriculture (Table 1). While application rates vary with site-specific conditions,

maximal rates are relatively uncommon as evidenced by the average of 0.85 kg a.e./ha for glyphosate use in a wide variety of agricultural field crops (USDA 2004) and 1.92 kg a.e./ha for conifer release in Canadian forestry (Thompson et al. 2004). These typical application rates are 15 times and seven times, respectively, lower than the rate employed in the Relyea study. Thus, the rate employed in the Relyea experiment must be considered to be atypical and essentially irrelevant in terms of extrapolative prediction of potential toxic effects on amphibians associated with dominant use of glyphosate products in agriculture, forestry, or industrial use sectors.

Based on the available data, the single exposure concentration tested in this experiment is substantially greater than aqueous concentrations observed in natural surface waters.—Although the nominal aqueous exposure concentration was slightly miscalculated by the author, and not analytically verified in the experimental units (cattle tanks), the true nominal exposure concentration in the Relyea experiment may be assumed to be 4.17 mg glyphosate/L as the isopropylamine salt, or its equivalent of 3.1 mg glyphosate acid per liter. (Based on the data provided in the paper, we would calculate the actual exposure concentration as 6.4 mL product/m² × 2.41 m²/1000-L tank = 15.3 mL product/1000 L. Given 15.3 mL of formulation applied per tank, a formulation density of 1.09 g/mL and a fractional IPA salt content of 0.25 yields 15.3 × 1.09 × 0.25 = 4.17 g IPA in 1000 L = 4.17 mg IPA/L or 3.1 mg glyphosate acid equivalents (a.e.)/L.) Such a nominal concentration greatly exceeds concentrations typically observed in surface waters representative of amphibian breeding and foraging habitats associated with agricultural, forestry, and rights of way use scenarios (Table 2). For example, available surface water monitoring data associated with agriculture, orchard, or rights of way uses in Canada, the

TABLE 2. Summary of aqueous glyphosate concentrations in surface water monitoring studies.

Use scenario and location	Site type	Application rate (kg a.e./ha)	Concentration in water (mg a.e./L)	Reference§
Experimental Study				
Pennsylvania	cattle tank	12.8	3.1	1
Roadside				
Oregon	runoff, ditch, and stream	1.07	<0.005	2
Hard surface				
Netherlands	runoff from two sites	NA	<0.004†	3
Agricultural				
Ontario	multiple low-flow agricultural drainages (soybeans, corn)	NA	<0.01	4
Netherlands	various	NA	<0.001	5
Norway	seven cereal cropland drainages, four years	NA	<0.00008†	6
Netherlands	21 orchard sites, five years	NA	<0.023†	7
Forest wetlands				
Ontario	16 buffered 11 adjacent 24 aerial over spray	1.92	<0.01† <0.39 <0.55†	8
Forest ponds				
Quebec	three ponds, over-sprayed from ground	1.5	0.47‡	9
Manitoba	three ponds, over-sprayed from air	2.1	0.06	10
Manitoba	20 sites adjacent to blocks	1.44	<0.155	11

Note: "NA" indicates that data are not available.

† Upper 99th percentile confidence limit for all reported concentrations, with non-detectable values conservatively assumed as equivalent to analytical limits of detection.

‡ Average concentration measured at 12 h post-treatment.

§ 1, Relyea (2005a); 2, Wood (2001); 3, Puijker and Janssen (1999) as cited in Horth et al. (2004); 4, J. Struger and D. G. Thompson (*unpublished data*); 5, G. Soppe, *personal communication*, cited in World Health Organization (1994); 6, Bechmann et al. (1999) as cited in Horth et al. (2004); 7, ZHEW (2002) cited in Horth et al. (2004); 8, Thompson et al. (2004); 9, Legris and Couture (1989); 10, Goldsborough and Brown (1993); 11, Beck (1987).

United States, and Europe, show maximal levels <0.023 mg a.e./L or 122-fold less than the concentration tested by Relyea. Not surprisingly, the highest surface water concentrations observed in extensive monitoring studies were associated with shallow wetlands receiving deposition from direct over spray. For example, Thompson et al. (2004) reported an upper 99th percentile confidence limit for glyphosate concentrations in over-sprayed forest wetlands of 0.55 mg a.e./L which is lower than the test concentration used in the Relyea (2005a) study by at least fivefold. Interestingly, in a follow-up study (Relyea 2005c) test concentrations of either 0.074 or 0.74 mg a.e./L more closely resembling the typical range of reported surface water concentrations (Table 2), generated no significant mortality in five different amphibian species tested even when exposed for a period of 16 days with exposure concentrations renewed every four days.

We emphasize here that the low concentrations of glyphosate observed in natural surface waters are not surprising given that glyphosate formulations containing the POEA surfactant are not intended for direct application to surface waters. In addition, the physico-chemical and environmental fate characteristics of glyphosate, as well as POEA, indicate that these compounds will be strongly sorbed to organic matrices including plants, soils, and sediments and rapidly

degraded (Feng et al. 1990, Wang et al. 2005) thus limiting the potential for significant indirect inputs to surface waters through surface runoff or subsurface movement. Finally, where inadvertent over spray or drift occurs, the greatest proportion of depositing spray clouds are intercepted by target vegetation or by riparian and emergent plants which typically border wetland systems, thereby limiting resultant aqueous concentration levels in these systems (Thompson et al 2004).

The nominal test concentration employed in the Relyea study, is far in excess of typical concentrations observed in monitoring studies of amphibian habitats associated with agriculture, forestry, or industrial sites. It is also above LC50 values for nine different amphibian species as reported in five prior studies (Mann and Bidwell 1999, Lajmonovich 2003, Edginton et al. 2004, Howe et al. 2004, Wojtaszek et al. 2004). As such, we find no scientific basis to support the author's contention that typical environmental exposures would generate widespread mortality in native amphibian larvae.

Control adjusted mortality levels observed are predictable given the high exposure concentration and do not constitute an unusual or dramatic new finding.—Adjusting for the high levels of mortality which occurred in the controls, percentage mortality in amphibian larvae that might be attributable to a direct chemical effect of the

glyphosate formulation treatment would approximate 37%, 65%, 50%, 12%, and 28% for the *H. versicolor*, *P. crucifer*, *R. sylvatica*, *B. americanus*, and *R. pipiens*, respectively. These levels of chemically induced mortality are consistent with interpolative predictions based on known concentration–response relations for similar larval amphibian species exposed under laboratory (Edginton et al. 2004) or field conditions (Wojtaszek et al. 2004). As such, the resultant “dramatic” effect is neither surprising nor shocking but simply a reflection of the high exposure concentration employed.

In our previous publications (Edginton et al. 2004), we document concentration–response relationships for larvae of several frog species (*Xenopus laevis*, *Rana clamitans*, *Bufo americanus*, and *Rana pipiens*) exposed to the glyphosate formulation Vision, which also contains the POEA surfactant. Estimated 96-h LC50 values ranging from 0.88 to 3.5 mg a.e./L were similar to those published for other amphibian species under similar exposure conditions (Mann and Bidwell 1999, Howe et al. 2004) as well as for much longer durations (Relyea 2005b). In the latter paper, the author reported similar LC50 values ranging from 0.98 to 1.82 mg a.e./L for six different native amphibian species exposed to a formulated glyphosate via static renewal for 16 d or 384 h. In fact, the upper 99th percentile confidence limit for glyphosate concentrations observed in lentic surface waters (0.55 mg a.e./L) reported by Thompson et al. 2004, is below 96 hr LC10 values of 0.83, 0.85, and 0.89 mg a.e./L as determined for *R. pipiens*, *R. clamitans*, and *Xenopus laevis*, respectively, exposed under laboratory conditions with deleterious interactive influence of high pH (Edginton et al. 2004). This relation provides further support for our contention that typical environmental exposures represent a low probability of risk for acute mortality in amphibian larvae. The fact that parallel field studies (Wojtaszek et al. 2004), demonstrated consistently higher 96 h LC10 estimates for larvae of both *R. pipiens* (7.31 and 3.26 mg a.e./L) and *R. clamitans* (1.78 and 1.20 mg a.e./L) in two different wetlands, illustrates the general mitigative effect of chemical dissipation and degradation which reduce the exposure magnitude and duration for labile chemicals in natural systems.

The experimental design and artificial nature of the experimental units seriously limit the validity of extrapolative inferences for natural aquatic systems.—As part of a tiered program of empirical study, mesocosm testing provides substantial value in terms of overall ecological risk assessment (e.g., Graney et al 1994, Thompson 2004). However, that value depends largely on the ability to simulate natural conditions, thereby reducing the uncertainty associated with extrapolation of laboratory results to real-world scenarios. The experimental design used in this study does not allow for demonstration of concentration-dependence for suspected chemical-induced toxicity, estimation of no observable

effect levels, or interpolative prediction of effects that might occur under other exposure levels of interest, all of which are critical to predicting potential ecological risk. Failure to include sediments and macrophytes is a critical oversight since both glyphosate and the POEA surfactant are known to be removed from aqueous phase through binding to organic substrates in aquatic environments with concomitant reduction in toxicity to aquatic organisms (Zaranyika and Nyandoro 1993, Wang et al. 2005). In addition, excluding sediments and macrophytes from the test system design is very likely to enhance the susceptibility of amphibian larvae to predation mortality and sublethal predation stress. Finally, lack of aquatic plants may have limited dissolved oxygen concentrations in the system, potentially adding an additional stressor to organisms in the test system. The requirement to delete two of six replicate tanks assigned to untreated controls owing to development of an “unusual red periphyton community” suggests that the test systems were not developing uniformly prior to treatment and raises concerns with respect to possible influence of other undetected stress factors influencing results.

In summary, given the atypical application rates, unrealistic high aqueous exposure concentrations, and design limitations of this experiment, we do not believe that the resultant data support Relyea’s conclusion that Roundup, at current rates of application (i.e., as typically employed in major use sectors), is likely to be lethal to many species of amphibians. Moreover, such a conclusion is certainly not supported by evidence from directly pertinent studies conducted either by ourselves (Edginton et al. 2004, Thompson et al. 2004, Wojtaszek et al. 2004), other researchers (Chen et al. 2004, Howe et al. 2004) nor by several general scientific and regulatory reviews (Giesy et al. 2000, Solomon and Thompson 2003, U.S. Environmental Protection Agency 1993, World Health Organization 1994) all of which consistently conclude that glyphosate-based end-use products, used in accordance with label recommendations, do not pose unacceptable risk to non-target organisms in the environment.

Finally, in both this paper and subsequent journal articles (Relyea 2004, Relyea 2005a, b, c) as well as in derivative mass media communications, the author repetitively links experimental results to the global amphibian decline phenomenon with the implication, intended or otherwise, of possible causality. We do not believe that this implication is scientifically defensible based on either Relyea’s work per se, or with respect to the available scientific evidence in general.

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Response:

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Assessing how hundreds of pesticides affect thousands of nontarget organisms in nature is certainly a daunting challenge. When it comes to glyphosate-based products (including Roundup), Thompson et al. (2006) have taken exception to the conclusions of Relyea (2005a). To support this position, they discuss application rates, environmental concentrations, possible methodological flaws, and prior risk assessments. Here, I provide evidence to demonstrate that application rates are wider ranging than the authors suggest, that environmental concentrations cited by Thompson et al. (2006) are a highly biased subset of existing data, that the suspected flaws reflect a lack of knowledge of aquatic ecology, and that previous risk assessments are largely irrelevant to assessing Roundup's risk to tadpoles. Finally, I assess the current weight of the evidence as to whether Roundup (in its many formulations containing the surfactant POEA) is likely to be lethal to amphibians under environmentally relevant concentrations.

A BIT OF BACKGROUND ON ROUNDUP

Roundup (sold under a wide variety of names including Vision; Monsanto Corporation, St. Louis, Missouri, USA) is the most widely applied herbicide in the world. The most commonly used formulations in North America contain an active ingredient (glyphosate) that impedes photosynthesis plus a surfactant (polyethoxylated tallowamine; POEA) that is required for glyphosate to permeate the waxy cuticle of plant leaves. This is important because POEA is the lethal component to fish and amphibians (Giesy et al. 2000). There are

glyphosate formulations that contain glyphosate and no surfactant (e.g., Rodeo, Eagre), but the user must add a surfactant or glyphosate will be ineffective. Concentrations of Roundup can be expressed either in units of active ingredient (mg a.i./L) or acid equivalents (mg a.e./L). For consistency with Thompson et al. (2006), I report all data below as acid equivalents (1 mg a.i./L = 0.75 mg a.e./L).

THE EXPERIMENT OF RELYEA

In Relyea (2005a), I examined how wetland communities were impacted when exposed to no pesticide, carbaryl, malathion, Roundup Weed and Grass Killer Concentrate, or 2-4, D. The experiment was conducted in outdoor mesocosms that contained leaf litter, algae, nine species of zooplankton, three species of snails, five species of tadpoles, and several species of predatory insects. The purpose in conducting this experiment was not to definitively examine the impact of Roundup on tadpoles, but to examine the direct and indirect effects of different pesticides on a community. Quite unexpectedly, I found that Roundup caused a 70% decrease in tadpole diversity, an 86% decrease in tadpole biomass, and 98–100% mortality in four of the five tadpole species. At the time of the study (2002), little was known about the impact of Roundup on tadpoles other than laboratory studies on four species of Australian tadpoles (Bidwell and Gorrie 1995, Mann and Bidwell 1999), two species of North American tadpoles (Smith 2001), and one species of African embryos (Perkins et al. 2000). This may seem surprising given that Roundup had been used for nearly 30 years, but pesticide registration does not require amphibian testing.

Relyea (2005a) was one of the most extensive experimental investigations of pesticide effects on aquatic communities and offered a comprehensive perspective on the impacts of pesticides on nontarget organisms. Thompson et al. (2006) interpret this to mean that I claim to have conducted the most extensive and comprehensive study of Roundup on amphibians. They are incorrect. They present this conclusion by focusing on just one of the pesticides used (i.e., Roundup) and only one of the taxonomic groups (i.e., tadpoles), despite the context conveyed in the article. The statement actually refers to the fact that the study examined how four different pesticides affected a community of 25 species of animals. Our use of multiple pesticides and a large number of species was what made the study extensive and comprehensive.

Thompson et al. (2006) also conclude that it was “disquieting” that I did not cite a series of papers that they published (Chen et al. 2004, Edginton et al. 2004, Thompson et al. 2004, Wojtaszek et al. 2004). However, my paper was submitted seven months prior to the publication of their work. What they found to be disquieting is simply the result of a long time to

publication. Subsequent publications on Roundup (Relyea 2005*b, c*, Relyea et al. 2005) cited their work.

ROUNDUP IN WATER: LEGALITY VS. REALITY

Thompson et al. (2006) attempt to discredit the Relyea (2005*a*) study as representing an “illegal application.” This is surprising given their previous recognition that such applications happen in nature, given that these applications are not illegal for small wetlands, and given their own history of applying Roundup to water. It is true that Roundup is registered for terrestrial use and it is illegal to apply a pesticide in a way that is inconsistent with its registered use. Herein lies the ecological problem. Despite the regulations, numerous scientists (including Thompson and colleagues) have noted that Roundup is found in aquatic habitats (Newton et al. 1984, Feng et al. 1990, Chen et al. 2004, Edginton et al. 2004, Thompson et al. 2004, 2006, Wojtaszek et al. 2004). For example, consider Thompson et al.’s (2004:843) motivation for their study of Roundup and amphibians, “One important, but often overlooked, environmental risk pertains to potential deleterious effects in small wetlands that are ubiquitous in many forest landscapes. Unlike larger fish-bearing aquatic systems (lakes, ponds, rivers, and streams), there are no requirements to protect small wetlands with no-spray buffer zones. Additionally, small wetlands are difficult to avoid during aerial application of herbicides.” Unfortunately, a great many amphibians spend their larval lives in these small, seemingly unimportant habitats. Thompson et al. (2004:843) go on to say, “Thus, small wetlands occurring within the target site are likely to be directly over-sprayed, resulting in relatively higher potential exposures and effects for constituent biota as compared with those in adjacent or buffered wetlands. Many of these small wetlands constitute prime breeding and foraging habitat for frog species.” Thus, in 2004, Thompson and colleagues recognized that small wetlands are directly over-sprayed with Roundup in forests and felt that it was of prime importance to examine the impacts of these direct over sprays on amphibians. Such applications are not designed as illegal applications, but the reality is that direct applications to water happen inadvertently. Despite the fact that applications over water should not occur, Thompson and colleagues previously shared the view of Relyea (2005*a*) that we need to examine the impacts of this unintended reality. Indeed, they have conducted several experiments in the laboratory, in mesocosms, and in over-sprayed ponds to address this very issue (Edginton et al. 2004, Thompson et al. 2004, Wojtaszek et al. 2004).

RATES OF APPLICATION AND AQUATIC CONCENTRATIONS: PAINTING A BIASED PICTURE

Thompson et al. (2006) state that the application rates and resulting aquatic concentrations of Relyea (2005*a*)

were atypical and do not represent real-world conditions. When it comes to application rates, Relyea (2005*a*:620) clearly states, “I applied each chemical at the manufacturer’s recommended maximum application rate.” Hence, for the Roundup treatment, I applied the herbicide at the rate listed on the back of the bottle of Roundup Weed and Grass Killer Concentrate (“6 ounces per 300 ft²”). Roundup is used at a wide variety of rates for a range of weed control goals. Whereas Thompson et al. (2006) provide a partial list of application rates (see their Table 1), their list only includes selective agricultural and forestry applications. The Roundup formulation used in Relyea (2005*a*) simply has a higher recommended application rate. Because application rates are variable (depending upon the weeds that need to be controlled), the more relevant point to consider is how Relyea’s (2005*a*) concentration of Roundup (3 mg a.e./L) compares to concentrations in natural wetlands.

When we consider the concentrations of Roundup in wetlands, we need to consider the expected worst-case concentrations and observed concentrations. As noted by Giesy et al. (2000), the first step in risk assessment is to conduct a tier I study in which one examines the impact of a pesticide under worst-case concentrations. If the worst-case scenario identifies substantial mortality, one then should examine lower concentrations. One presumes that Thompson and colleagues understand this approach since Solomon is a co-author of both Giesy et al. (2000) and Thompson et al. (2006). For Roundup, a number of worst-case concentrations have been estimated, ranging from the Canadian government’s estimate of 1.4 mg a.e./L to estimates of 2.7 mg a.e./L (Solomon and Thompson 2003), 2.8 mg a.e./L (Giesy et al. 2000), 2.9 mg a.e./L (Perkins et al. 2000), and 7.6 mg a.e./L (Mann and Bidwell 1999). The concentration used by Relyea (2005*a*) was 3 mg a.e./L, making the study a straightforward assessment of Roundup’s worst-case scenario.

There are few relevant field data on the concentration of Roundup in aquatic habitats of tadpoles. Thompson et al. (2006) provide a list of observed concentrations (see their Table 2) and conclude that Roundup only occurs in very low concentrations. However, a careful examination of these data suggests otherwise. First, Thompson et al. (2006) cite a number of studies from the non-peer-reviewed “gray literature” which fail to identify what was sampled, when it was sampled, or how it was sampled. For example, one study represents personal communication from G. Soppe to a chemical safety committee as cited by the World Health Organization without any relevant sampling protocols. Similarly, the studies of Bechmann et al. (1999), ZHEW (2002), and Puijker and Janssen (1999) are studies in the Netherlands and Norway that are cited in a European government report (all as cited in Horth 1994). Based

on the citation of Thompson et al. (2006), they were unable to obtain the original publications, preventing them from identifying critical variables including application rates and sampling protocols.

In contrast to the aforementioned studies, Wood's (2001) study is widely available and offers an excellent example of how Thompson et al. (2006) report only a subset of data to provide a biased perspective. In this study, the researchers sprayed roadside weeds with Roundup and then measured glyphosate in the roadside ditches. In the first experiment, they sprayed the roadsides, simulated a rain event the next day, immediately sampled the ditch water, and found 0.3–0.7 mg a.e./L. In the second experiment, the researchers sprayed the roadsides, waited 49 d until the first large natural rain event occurred, and then took their first water sample. As one might expect, glyphosate was not detected 49 d after the application (detection limit = 0.005 mg a.e./L). Curiously, Thompson et al. (2006) report the data from the second experiment (<0.005 mg a.e./L), but omitted the data from the first experiment, which had concentrations that were 60–140 times higher. Reporting only a subset of data clearly offers a biased perspective.

Second, many of the studies listed in Table 2 of Thompson et al. (2006) includes habitats that are largely irrelevant to tadpoles. This is a particularly strange decision given their statement that their opinions were based on “available data on measured concentrations in *lentic* systems.” For example, Puijker and Janssen (1999) measured Roundup concentrations in water wells, a habitat that lacks amphibians. Other included data are from streams (i.e., *lotic* systems), which are typically lower in pesticide concentration due to the flushing action of streams. For instance, J. Struger and D. G. Thompson (*unpublished data*) measured 19 stream sites with concentrations of <0.01 mg a.e./L. Curiously, Thompson et al. (2006) chose not to include the stream data of Feng et al. (1990), which detected 16-fold higher stream concentrations (0.16 mg a.e./L). It seems unlikely that these data were simply overlooked given that Thompson was a coauthor of Feng et al. (1990). They also left out the studies by Newton et al. (1984) who detected 27-fold higher stream concentrations (0.27 mg a.e./L) and Leveille et al. (1993) who found streams with 0.08–3.08 mg a.e./L. Despite all of these omissions, the problem with including stream data is that the vast majority of North American tadpoles do not live in streams. Including stream data to evaluate the risk of Roundup to tadpoles suggests either that one is unaware of tadpole biology or that there is a desire to present a highly biased perspective.

Given these issues, we should come to terms with the unfortunate fact that there are just very few data on how much Roundup appears in tadpole habitats (i.e., lakes, ponds, and wetlands). As noted by Goldsborough and

Brown (1993:1139), “As compared to the movement of glyphosate in lotic waters, little is known about the dissipation of glyphosate in lentic waters where, due to longer water residence time, herbicide residues may persist in sufficient concentration to induce toxicity.” Indeed, Thompson et al. (2006) provide data from only four studies from forest ponds and wetlands in Canada (Beck 1985, Legris and Couture 1989, Goldsborough and Brown 1993, Thompson et al. 2004). There are few data on Roundup concentrations from agricultural or residential areas and few data from outside of Canada.

If we examine these four studies, we can continue to gain insight into how Thompson et al. (2006) select data to make their case. For example, Thompson et al. (2004) aerially applied Roundup to coniferous forests in which there were wetlands that were directly over-sprayed by the airplane, adjacent to the path of the airplane, or buffered by a line of trees from the path of the airplane. While Thompson et al. (2006) reported the upper 99th percentile of 0.55 mg a.e./L for over-sprayed wetlands, they failed to disclose that these data were highly variable, with one of the ponds having up to 1.95 mg a.e./L (Thompson et al. 2004:846). Thus, their observed worst-case scenario for Roundup in wetlands was higher than the worst-case scenario estimated by the Canadian government (1.43 mg a.e./L) and the application rate was one-half of what can be sprayed on forests for site treatment. From the study of Beck (1985), Thompson et al. (2006) report the data collected 2 d after the application to water (<0.155 mg a.e./L), but did not report the initial concentration detected by Beck which was seven times higher (1.1 mg a.e./L). From the study of Legris and Couture (1989), Thompson et al. (2006) report low concentrations of glyphosate (0.47 mg a.e./L) in three over-sprayed forest ponds from measurements 12 h after the application, but they fail to report the sixfold higher initial measurements (2.8 mg a.e./L). If we had sampled our mesocosms after 12 h, we would have certainly obtained a lower concentration as well. The above studies support the expectations for worst-case scenarios and some are very close to the initial concentration of 3 mg a.e./L used by Relyea (2005a). Thus, these may not be worst-case scenarios after all, but may be frequent scenarios.

Not only did Thompson et al. (2006) provide a biased reporting of the above four pond and wetland studies, they also chose not to report data on ponds and wetlands from at least four studies that found considerably higher glyphosate concentrations. First, they omitted the data of Newton et al. (1994) who conducted aerial over-sprays of forest ponds in Oregon, Georgia, and Michigan and found concentrations of 0.31, 0.35, and 1.24 mg a.e./L, respectively. Second, the authors did not report the data of Couture et al. (1995) who found initial glyphosate concentrations in ponds of 1.54 mg a.e./L. Third, they left out the data of Edwards et al.

(1980) who sprayed fields with high rates of Roundup to reestablish fescue grasslands found concentrations of up to 5.2 mg a.e./L in surface water runoff. Finally, Giesy et al. (2000) report that Monsanto supplied data to regulators indicating observed concentrations of 1.7 mg a.e./L. Because Solomon was a coauthor of Giesy et al. (2000) and is a coauthor of Thompson et al. (2006), Thompson et al. (2006) were aware of the Monsanto data. These four studies are widely cited by other investigators of glyphosate toxicity, making it unclear as to why Thompson et al. (2006) chose not to include these data in their review of glyphosate concentrations in nature.

So what can we conclude from the concentration data presented by Thompson et al. (2006)? First, the data selected by Thompson et al. (2006) includes several studies that have unknown sampling details and have not been subject to the quality control provided by peer-reviewed journals. Second, the data include well water and stream samples that are irrelevant to tadpoles. Third, and perhaps most disconcerting, when studies have taken multiple samples over time, Thompson et al. (2006) present the reader with the lowest concentrations following degradation. Fourth, they exclude well-known studies that have observed considerably higher concentrations. Collectively, the indisputable consequence of Thompson et al.'s (2006) failure to cite relevant data, even data coming out of their own research, is to bias the assessment and paint a picture of very low environmental concentrations. The plain fact is that ponds and wetlands that are over-sprayed with Roundup achieve considerably higher concentrations (1.1–5.2 mg a.e./L) than suggested by Thompson et al. (2006). These concentrations are quite similar to many estimated worst-case scenarios (1.4–2.9 mg a.e./L) and demonstrate that the 3 mg a.e./L used by Relyea (2005a) was a reasonable concentration to begin testing the effects of Roundup on tadpoles. Moreover, using only one-third as much Roundup (1 mg a.e./L) still causes up to 71% mortality (Relyea et al. 2005).

HOW MUCH DEATH WAS REALLY THE RESULT OF ROUNDUP?

Thompson et al. (2006) suggest that the high rates of observed mortality are predictable from past studies once mortality in the controls is taken into account. Once again, they are incorrect. They begin by expressing concern over the high rates of mortality that occurred in the control mesocosms (32% to 85% over a 12-d period, depending on species). This concern is especially ironic given Thompson et al.'s (2004) study in which their control treatments had 26% mortality after only 2 d with no explanation. Our no-pesticide controls (Relyea 2005a) had tadpole survival that was lower than would be expected in single-species lab LC50 studies (LC50 is the lethal concentrations needed to kill 50% of a test

population). However, Thompson et al. (2006) failed to grasp that our aquatic communities had tadpole predators. One cannot have high tadpole survival when mesocosms contain tadpole predators (e.g., Morin 1981).

There are three lines of evidence that the low survival of tadpoles in the control mesocosms was due to predation and not some unknown factor. First, when insecticides were present in the mesocosms (carbaryl or malathion), the biomass of insect predators was reduced by half and the survival of the tadpoles doubled. Second, in a subsequent mesocosm study using three species of tadpoles and the same concentration of Roundup but without predators (Relyea 2005c), the control tanks had excellent tadpole survival (75%, 97%, and 98%) whereas tanks treated with Roundup still exhibited very low survival (2%, 0%, and 4%). Third, in single-species laboratory studies, we have observed excellent survival in control treatments but the interpolated rate of mortality for 3 mg a.e./L of Roundup after 1 d (based on a range of concentrations) was 91–100% mortality in wood frogs, leopard frogs, gray tree frogs, and American toads (Relyea 2005b). Collectively, the evidence demonstrates that the low survival in the control treatments of Relyea (2005a) was due to predation and that when predators are not part of the experimental system we continue to observe very low survival with 3 mg a.e./L of Roundup. Once again, Thompson et al. (2006) failed to recognize what is well known among anuran ecologists: predators can cause high tadpole mortality (Morin 1981).

POSSIBLE METHODOLOGICAL FLAWS

Thompson and colleagues suggest that there are two methodological flaws associated with the Relyea (2005a) study. First, they focus on the fact that no single experiment stands alone as a definitive test of a pesticide's impact on organisms and that a tiered approach should be taken in which investigators examine the pesticide under a range of concentrations and experimental conditions. I could not agree more. As noted above, the Relyea (2005a) study was not designed to focus on Roundup and tadpoles, but rather to examine the effects of several different pesticides on aquatic communities. To better understand the effects of Roundup on tadpoles, I have conducted a number of additional studies to examine (1) how different concentrations of Roundup (alone and mixed with other pesticides) affect tadpole growth and survival across five species in the laboratory (Relyea 2004b), (2) how a range of Roundup concentrations affects the survival of six species of tadpoles with and without predatory stress in the laboratory (Relyea 2005b), (3) how Roundup interacts with the presence and absence of vertebrate and invertebrate predators to affect the growth and survival of three species of tadpoles in mesocosms

(Relyea et al. 2005), (4) how Roundup and different soils interact to affect the survival of three species of tadpoles in mesocosms (Relyea 2005c), and (5) how a range of Roundup concentrations affects the survival and growth of three species of tadpoles in mesocosms (R. Relyea, unpublished data). These studies clearly demonstrate that my lab is taking a tiered approach to understand how Roundup affects tadpoles under a range of concentrations and experimental conditions. Based on the citations of Thompson et al. (2006), it is also clear that they are aware of these studies.

Second, Thompson et al. (2006) argue that our results are unreliable because the mesocosms did not include soil or macrophytes and that this fact has three potential consequences. The first potential consequence is that a lack of soil prevents adsorption of Roundup by the sediments which could reduce the concentration of Roundup in the water (Giesy et al. 2000). While soils were not included in the original experiment, we manipulated soil in a subsequent experiment as part of our tiered research program. We used identical mesocosms as before but added either no soil, sand, or loam and applied Roundup at 3 mg a.e./L. Adding soil made no difference; the three tadpoles species in the control tanks had 98%, 97%, and 75% survival whereas tadpoles in the Roundup tanks had 4%, 2%, and 0% survival (Relyea 2005b). Thus, while soil absorbs Roundup, it does not occur fast enough to prevent high rates of death. Thus, the original interpretation of Relyea (2005a) was not affected by a lack of soil.

The second potential consequence is that a lack of soil and macrophytes "is very likely to enhance the susceptibility of amphibian larvae to predation mortality and sublethal predation stress." (Thompson et al. 2006). The authors seem to have overlooked the fact that all tanks contained 300 g of leaf litter on the bottom, which permits the tadpoles a great amount of refuge in which to hide from predators (Relyea 2004a). Moreover, the authors appear to be unaware that leaf litter in the absence of macrophytes is actually a frequent characteristic of many temporary ponds. The argument being made by the authors is that the lack of structure caused higher predation rates, but this effect would be occurring in control tanks and Roundup tanks, meaning that if there were an enhanced predation effect, it would be enhanced across all treatments. Moreover, our subsequent study on tadpole communities without predators (Relyea 2005c) definitively demonstrated that the high mortality rates observed by Relyea (2005a) can be attributed entirely to Roundup and not to predation.

The third potential consequence is that the "lack of aquatic plants may have limited dissolved oxygen concentrations in the system, potentially adding an additional stressor to organisms in the test system." (Thompson et al. 2006). This is not only highly speculative, but also counter to a basic limnological

principle that shallow bodies of water that lack macrophytes are highly oxygenated due to both the close proximity to the air-water interface and oxygen production by periphyton and phytoplankton (Wetzel 2001). In summary, despite the fact that the original experiment (Relyea 2005a) did not contain soil or macrophytes, there is a great deal of data supporting the mechanism of direct toxicity and no data supporting the speculations of Thompson et al. (2006). When we used experiments that contained both soil and leaf litter, we observed the same high rates of tadpole mortality as in the original experiment.

ROUNDUP'S RISK ASSESSMENTS AND THE WEIGHT OF THE EVIDENCE

Thompson et al. (2006) try to support their position that glyphosate-based products does not pose a risk to amphibians by citing several previous risk assessments. The first two risk assessments (U.S. Environmental Protection Agency 1993, World Health Organization 1994) include no data on amphibians because there had not been a single study of Roundup's effect on tadpoles until Australian biologists began publishing their work in 1995 (Bidwell and Gorrie 1995, Mann and Bidwell 1999). This means that the U.S. Environmental Protection Agency (1993) and the World Health Organization (1994) assessments are irrelevant for assessing Roundup's risk to amphibians. Using the Australian data, Giesy et al. (2000) assessed glyphosate's risk and concluded that Roundup in a worst-case scenario produced a hazard quotient 2.82 to tadpoles, indicating that Roundup posed a potential risk that needed to be further evaluated. At this time, not a single study had yet been conducted on tadpoles from North America, South America, Europe, Asia, or Africa. Finally, Solomon and Thompson (2003) conducted a risk assessment that pooled all aquatic organisms (including the few available amphibian data). They concluded that Roundup posed minimal risk to aquatic organisms, but they did not address the specific risk that Roundup posed to amphibians. In short, of the four risk assessments, the first two contained no tadpole data and the second two contained minimal tadpole data. It is unlikely that many biologists would feel comfortable with such assessments of Roundup's risk to tadpoles.

The good news is that, during the past two years, we have added a considerable amount of data on the effects of Roundup on tadpoles. Laboratory LC50 studies have now been conducted on 11 species of tadpoles and these data have shown substantial species and population differences in susceptibility, ranging from slightly toxic ($LC_{50} > 10$ mg a.e./L) to highly toxic ($LC_{50} < 1.0$ mg a.e./L; Mann and Bidwell 1999, Edgington et al. 2004, Howe et al. 2004, Wojtaszek et al. 2004, Relyea 2005b). The LC50 estimates produced by my laboratory (1.0–1.9 mg a.e./L; Relyea 2005b) are within the range of

expected concentrations in nature and overlap with previous LC50 estimates for some of the species examined by Howe et al. (2004; 2.0 to > 8 mg a.e./L) and Edginton et al. (2004; 0.9–3.5 mg a.e./L) but are lower than many LC50 estimates observed in four species of Australian tadpoles (2.9–11.6 mg a.e./L; Bidwell and Gorrie 1995, Mann and Bidwell 1999) and two species of Canadian tadpoles (2.7–11.5 mg a.e./L; Wojtaszek et al. 2004). Variation in LC50 values likely reflects differences in species, populations, and experimental conditions. Clearly, the time has come to reassess Roundup's risk to amphibians.

There have also been several mesocosm and pond enclosure studies using Roundup that have reached a variety of conclusions. For example, while my research group has repeatedly found high rates of tadpole mortality in mesocosms using 1–3 mg a.e./L (Relyea 2005a, c, Relyea et al. 2005), Wojtaszek et al. (2004) found no significant mortality in their mesocosms using 1.4 mg a.e./L. Thompson et al. (2004) added tadpoles to cages placed in wetlands that were directly over-sprayed, adjacent, or buffered by a line of trees with Roundup at 0–1.9 mg a.e./L. After only 48 hours, *Rana clamitans* tadpoles experienced 36% mortality in over-sprayed ponds vs. 26% in buffered wetlands and 10% in adjacent wetlands. While the data were too variable to find significant differences ($P = 0.129$), there was no explanation for the high mortality rates after only 48 hours, making the results of Thompson et al. (2004) difficult to interpret. Such disparate results among research groups appear to form much of the basis of the disagreement regarding mortality rates put forth by Thompson et al. (2006).

As noted above, it is not at all surprising that different researchers have reached different conclusions when investigators work with different species, different populations, and different experimental venues. However, when one examines all of the studies together, a pattern emerges that explains much of the diversity in outcomes. Roundup is considerably more toxic under higher pH conditions (for unknown reasons; Chen et al. 2004, Edginton et al. 2004, Wojtaszek et al. 2004), and differences in pH among experiments are likely causing differences in experimental outcome. For example, the most lethal outcomes (under both laboratory and mesocosm conditions) have been found by experiments by my research group in which tadpoles were reared in well water with a pH = 8. In contrast, the experiments of Wojtaszek et al. (2004) were conducted at two different pond sites that differed in pH (6.4 vs. 7.0), with greater mortality at the higher pH site. If pH is the primary factor driving the different experimental outcomes, it would also explain the highly variable survival data of Thompson et al. (2004) across ponds that varied widely in pH (ranging from 4.8 to 9.0). In short, differences in pH may be a good biological reason that different

research groups reach different conclusions. Thus, rather than argue that one research group is right and another research group is wrong, we would be better served to come together and understand how variation in pH produces different mortality from Roundup.

THE RISK OF ROUNDUP TO AMPHIBIANS . . . IN THEIR OWN WORDS

Thompson et al. (2006) suggest that my experimental results are atypical and not in line with previous research. Specifically, they state, "In our view, neither the specific results of this experiment nor the collective data support the conclusion that Roundup is likely to be lethal to many species of amphibians under environmentally realistic use scenarios." Most researchers of Roundup do not share this opinion (including, amazingly enough, Thompson and colleagues in their writings from only two years ago). For example, Chen et al. (2004:828) studied zooplankton and tadpoles and concluded, "concentrations equal to and less than the EEC [expected environmental concentrations] were significantly toxic to both species. This suggests that both groups may be at risk of direct mortality at environmentally relevant concentrations." Edginton et al. (2004:821) state, "We concluded that, at EEC 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." Even Thompson et al. (2004:848) conclude that, "Overall, results of this tiered research program confirm that amphibian larvae are particularly sensitive to Vision [i.e., Roundup] herbicide and that these effects may be exacerbated by high pH or concomitant exposure with other environmental stressors." Howe et al. (2004:1933) state, "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." My experiments concur with the conclusion that Roundup with POEA can be highly lethal to tadpoles at environmentally relevant concentrations. In spite of their previous conclusions to the contrary, Thompson et al. (2006) now state that glyphosate-based products "do not pose unacceptable risk to nontarget organisms." What could have changed their opinion in only two years?

Finally, Thompson et al. (2006) claim that, "in both this paper and subsequent journal articles (Relyea 2004b, 2005a, b, c) as well as in derivative mass media communications, the author repetitively links experimental results to the global amphibian decline phenomenon with the implication, intended or otherwise, of possible causality." The authors cannot produce a single quote of me making such a connection because I have never made such a statement. As a longtime amphibian

biologist who has witnessed the global amphibian crisis over the past 15 years, I am well aware of the gravity of the situation and that we must refrain from drawing broad geographic conclusions from a small number of studies. While the media and others may have made connections between our research and global amphibian declines, I have always urged caution against any such extrapolation. We know that Roundup with POEA has the potential to kill a very high fraction of tadpoles. Whether Roundup applications to water cause population declines in nature is a much larger question that has yet to be addressed.

CONCLUSIONS AND FUTURE DIRECTIONS

In their attempt to discredit Relyea (2005a), Thompson et al. (2006) have made a number of missteps. The authors suggest that the application rate used was unusually high. In fact, the rate followed manufacturer recommendations and produced a concentration that was in line with estimated worst-case scenarios and many observed scenarios in ponds and wetlands. The authors offer a list of concentrations observed in nature that is largely composed of second-hand reports, irrelevant well water and stream data, and a subset of wetland data sampled at time points after the herbicide degraded. A complete list reveals that higher concentrations are often found in the environment. 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. The authors draw upon past risk assessments that contain little or no amphibian data and also contradict their own published conclusions on the impact of Roundup on tadpoles. When we examine the entire collection of relevant concentration data and experimental data (including those of Thompson and colleagues), it becomes clear that Roundup formulations containing POEA can be highly lethal to tadpoles at environmentally relevant concentrations.

It is also evident that we need much more data. There is a critical need to sample natural ponds and wetlands for Roundup at appropriate times across a variety of forest, agriculture, and residential settings. Further, there is a continued need to experimentally evaluate the impact of Roundup on amphibians under a range of experimental conditions. Prior to 2005, members of a single collaborative research group, often in collaboration with Monsanto, have been involved in all of the risk assessments of Roundup (World Health Organization 1994, Giesy et al. 2000, Solomon and Thompson 2003) and a majority of the experiments (Perkins et al. 2000, Chen et al. 2004, Edginton et al. 2004, Thompson et al. 2004, 2006, Wojtaszek et al. 2004) with a few notable exceptions (Bidwell and Gorrie 1995, Mann and Bidwell 1999, Smith 2001, Lajmanovich et al. 2003). There is a growing movement for other laboratories to

independently assess the impacts of Roundup (Howe et al. 2004, Relyea 2004b, 2005a, b, c, Relyea et al. 2005) and we need many more laboratories to conduct Roundup experiments to develop more accurate and relevant risk assessments. In doing so, we can replace unsubstantiated attacks with actual data and arrive at the truth.

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