Background:
A 2005 publication (Relyea 2005) reports the results of a 2-week study, conducted in cattle tanks containing approximately 1000 L of water, to determine effects of an unspecified Roundup® brand herbicide formulation (as well as 2, 4-D, carbaryl, and malathion) on an aquatic community containing algae and twenty-five species of aquatic animals, including six species of amphibians. The paper indicates that a Roundup brand herbicide formulation was tested at a surface application rate of 6.4 mL/m², which is equivalent to 64 L/ha or 27.4 quarts/acre of the formulation used. The author reports nearly complete mortality for tadpoles of the following amphibian species: leopard frog (Rana pipiens), gray tree frog (Hyla versicolor), and the wood frog (Rana sylvatica); no significant effects were observed for the American toad (Bufo americanus), the spring peeper (Pseudacris crucifer) or the spotted salamander (Ambystoma maculatum).

Response:
This study does not test a natural wetland system and also does not represent realistic use conditions for Roundup brand herbicides, or any Monsanto glyphosate-containing formulation for the following reasons:

- There are no Roundup brand formulations approved in the US or Canada for application over water. In fact, all current Roundup brand herbicide product labels specifically prohibit application over water.
- Glyphosate-containing products designed for direct applications to water, such as AquaMaster™ herbicide, are specifically formulated for this use to enhance their safety to aquatic animals, including the kinds of organisms studied in this report.
- Compared to typical agricultural applications, the application rate used in this study is unrealistically high, more than 7 times the typical use rates. Furthermore, most agricultural applications are made using ground application equipment, and are designed to avoid application to standing water of any depth.
- Typical application rates for industrial, turf and ornamental applications for roadside vegetation management are also significantly below the application rate of the Relyea study.
- For lawn and garden uses as well as some industrial and ornamental uses, application to water can easily be avoided as specified on the label since hand-held sprayers are used for these applications.
- Finally, for forestry applications, previous studies conducted using realistic application rates and methods in natural wetlands with glyphosate-containing products labeled for forestry use.

Note that the author does not clearly identify in the Ecological Applications article which commercial “Roundup®” product was tested. Subsequent information provided by the author on his website indicated that “Roundup Weed and Grass Killer” was the product tested.

Although glyphosate is not the most toxic component of Roundup brand formulations for aquatic organisms, formulations are often compared on the basis of the amount of glyphosate present in these formulations. For a product containing 25% glyphosate isopropylamine salt, an application rate of 64 L/ha is equivalent to a rate of 12.8 kg glyphosate acid equivalent (a.e.)/ha, or 11.4 lb glyphosate a.e./acre. In the 1000-L tank system described in the paper, the concentration, expressed as glyphosate a.e., obtained from the application of 15.3 mL of the Roundup product would be 3.09 mg glyphosate a.e./L, equivalent to 10.2 mg/L of a Roundup brand formulation containing 41% glyphosate isopropylamine salt.
have shown no adverse effects on aquatic animals, including some of the same organisms studied in this report.

Furthermore, contrary to Dr. Relyea’s statements, the results of this work should not have been surprising based on the toxicity data already publicly available for the original Roundup herbicide formulation, the surfactant POEA and glyphosate.

**Specific Points:**

1. The “over water” application method used in this study is not a realistic environmental exposure.

   The direct application of Roundup brand herbicides over water is specifically prohibited by the U.S. and Canadian product labels. The results obtained in this study, therefore, are not representative of results that would be obtained from the terrestrial application of Roundup brand herbicides.

   The vast majority of Roundup brand herbicide applications are made using ground application equipment, not aircraft. Ground application equipment operators avoid standing water of any depth to prevent the equipment from getting stuck in the mud.

2. Even if direct application to water was permitted or occurred unintentionally, the application rate used in this study (11.4 lb glyphosate a.e./acre) is over 7 times greater than typical application rates for agricultural uses (≤1.5 lb glyphosate a.e. per acre) and over 3 times greater than the maximum single application rate for agricultural uses (3.75 lb glyphosate a.e. per acre). For industrial, turf and ornamental applications, although higher rates are allowed for specialized uses such as brush control, typical broadcast application rates of glyphosate-containing formulations for roadside vegetation management are in the range of 0.38 to 1.5 lb glyphosate a.e. per acre. The application rate used in the Relyea study is 7 to 30 times higher than these typical application rates used for roadside vegetation management.

3. The application rate used in the Relyea study was calculated from a product designed for use in lawns and gardens, where the application equipment is much different than the equipment used for agricultural or forestry applications. The application rate specified for these small-scale applications is significantly higher than the rates used for large-scale applications to account for differences in the application equipment. Since small-scale applications are made with hand-held sprayers, application to water can easily be avoided as specified on the label.

4. The results of the Relyea paper are inconsistent with actual field studies conducted at realistic application rates with a glyphosate formulation (Vision® herbicide) labeled for forestry applications.

   In a study conducted in a natural wetland to investigate potential effects of a direct over-water aerial application (Thompson et al., 2004), no treatment-related effect on mortality of the leopard frog, *Rana pipiens*, or the green frog, *Rana clamitans*, was observed at an average application rate of 1.92 kg glyphosate a.e./ha. This rate is very close to or exceeds the maximum application rate for aerial applications that would be made on a recurring basis. The mean measured glyphosate concentration in oversprayed wetlands was 0.33 mg

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c The Monsanto products containing glyphosate for the industrial, turf, and ornamental markets are Roundup Pro® for terrestrial weed control and AquaMaster™ for aquatic weed control.

d The Vision herbicide formulation has been determined to have comparable aquatic toxicity to the Roundup brand herbicide used in the Relyea study when compared on the basis of glyphosate acid equivalents (which is related to surfactant content), based on tests conducted on three aquatic animals frequently used in toxicity testing.
glyphosate a.e./L, again well below the 3.09 mg glyphosate a.e./L° concentration used in the Relyea study. Even the 99% confidence limit concentration calculated in the Thompson study (0.55 mg a.e./L) was well below the rate tested in the Relyea study.

It is not unusual for studies conducted in artificial systems to demonstrate greater toxicity than studies conducted under actual environmental conditions. It is appropriate, therefore, to consider the Thompson et al. study conducted in a natural wetland with realistic application rates as more representative of effects that could be observed in the environment, rather than a study conducted in small confined cattle tanks at elevated application rates.

5. Data regarding the toxicity of Roundup brand herbicide formulations to aquatic animals from standard laboratory tests was first published in 1979 (Folmar et al., 1979). The toxicity was attributed primarily to the surfactant present in the formulation. In addition, Giesy et al. (2000) clearly discussed the influence of surfactants added to glyphosate formulations and potential toxicity to nontarget aquatic organisms. Because of the Folmar data, the U.S. Environmental Protection Agency has specified that product labels must contain wording to prohibit application directly to water (U.S. EPA 1993).

Surfactants are added to herbicide formulations to help the drops of spray solution (primarily water) containing the active ingredient adhere to the waxy surface of plant leaves and help cross the waxy barrier of the plant leaf.

Surfactants similar to those found in herbicide formulations are also found in laundry detergents, liquid cleansers, shampoos and shower products. Their chemistry makes it possible for them to remove soiling from many different materials, including skin and hair, so that oil and grime can be rinsed away. Some surfactants are harsh to the skin while others are very mild, depending on their type. Based on this wide variety of available surfactants, not all cleansers are the same.

Aquatic animals, like tadpoles and fish, are sensitive to surface-acting substances like surfactants. Like the example above, not all surfactants used in weed management products are the same. That is why Roundup brand formulations are not registered for direct water applications. Other glyphosate-containing products are specifically formulated for this use, such as AquaMaster™ herbicide, to enhance their safety to aquatic animals.

6. A risk assessment considering toxicity data for amphibians and other aquatic organisms and relevant exposure concentrations concluded that terrestrial use of glyphosate formulations is predicted to pose minimal acute and chronic risk to amphibians, including tadpoles (Giesy et al., 2000). For applications to water, although the theoretical safety margins are narrow if a Roundup brand formulation containing POEA is applied directly to extremely shallow bodies of water (less than or equal to 0.15 m), the risk to amphibians is minimal because interception by target vegetation, degradation, and dissipation of the product components can explain the lack of adverse effects under field conditions.

A recently published study (Wang et al., 2005) reports that the POEA surfactant dissipates rapidly in two water-sediment systems with half-lives of 13 and 18 hours.

Studies investigating the relationship between acute aquatic toxicity and biodegradation with alkylamine ethoxylates, which are structurally similar to POEA, have demonstrated that loss of toxicity correlates with biodegradation and dissipation from the water column (van Ginkel et al., 1993).

° Note that the 3.09 mg glyphosate a.e./L value for the Relyea work is a correction from the 3.8 mg/L value cited in the paper, which is actually a value given as glyphosate isopropylamine salt, not glyphosate acid. The 3.8 mg/L value did not take into account that the density of the formulation is greater than 1 g/mL. Also, unlike the concentration value from the work of Thompson et al. (2004), the value in the Relyea paper is not a measured value; it is calculated based on the amount of “Roundup” stated to have been added to the water.
7. It is misleading for the author to suggest that “Roundup” and “glyphosate” are synonymous. Glyphosate, in the form of a salt, is the active ingredient in numerous formulations marketed under the Roundup brand name\(^\text{f}\), as well as under other brand names. Most, but not all, of these formulations contain a surfactant. Since the Relyea paper acknowledges that the toxicity to aquatic animals observed after “Roundup” treatment is likely due to the surfactant and not to glyphosate, it is inappropriate to attribute the effects observed in the Relyea paper to glyphosate.

References:

Folmar LC, Sanders HO, Julin AM. (1979) Toxicity of the herbicide glyphosate and several of its formulations to fish and aquatic invertebrates. *Archives of Environmental Contamination and Toxicology* 8: 269-278.


\(^f\) Some Roundup brand formulations also contain other active ingredients in addition to glyphosate.