Chemical Research in Toxicology

LETTERS TO THE EDITOR

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Letter to the Editor Regarding the Article by Paganelli et al.

The article by Paganelli et al. (*Chem. Res. Toxicol.* (2010), *23*, 1586−1595) is seriously flawed in terms of the experimental designs, the lack of clear descriptions of the methods used, the interpretation of the results, and in the extrapolation of the responses observed in the eggs of frogs and chickens to humans. The more egregious errors in the article are discussed below, but the article was also lacking in details of the methods as well as rigorous use of statistical tests, such as regression analysis of the concentration responses.

In the first part of the study on embryos of *Xenopus laevis*, exposures to the formulated product (Roundup Classic; 480 g/glyphosate IPA salt/L) at 1/3000; 1/4000; and 1/5000 dilutions were equivalent to 71,158; 88,947; and 118,596 μg a.e./L, respectively. The concentrations are expressed as acid equivalents (a.e.) of glyphosate for comparison to the literature. The concentrations used in the exposures of embryos to the formulated product were large, 9-, 11-, and 15-fold greater than the median, lethal concentrations (LC50s) (7,900 μg a.e./L) for embryos of the same species reported from the literature.[1] Embryos showed a reduction in the development of the neural crest, but no concentration−response to the treatments was reported; data are only shown for one dilution (1/5000) in Figures 1 and 2 and, while there was a concentration−response in RA signaling shown in Figure 4, there was no statistically significant difference between the untreated controls and the embryos exposed to the 1/5000 dilution, in which developmental effects were reported. This calls the proposed mechanism of action into question.

Given the high concentrations used and the fact that the eggs were not protected by the naturally present jelly coat during the exposures (not specifically described in the methods but presumed from the Figures), these results are an artifact of the totally unrealistic concentrations used in this part of the study and not relevant to exposures experienced in the field. Monitoring data for lentic or slow-moving lotic systems that constitute natural amphibian habitats show very small exposures from the use of glyphosate-based herbicides in agriculture. Concentrations range from a maximum of 40.8 μg a.e./L[2,3] in amphibian habitats to 8.7 μg a.e./L for in streams.[4] Greater concentrations have been observed in conditions of use in forests, but these are still small. In intentional oversprays of shallow surface waters, concentrations of 162 μg a.e./L were reported[5] and 99th percentile concentrations of 90 μg a.e./L (with spray buffer), 390 μg a.e./L (adjacent to spray swath), and 550 μg a.e./L in directly oversprayed wetlands.[6] All of these values are much smaller than the lowest concentration used in the study by Paganelli et al. and underscore the lack of relevance of the exposures that may occur in the field and to risk assessment for amphibians. That exposures to concentration many times greater than lethal values caused adverse effects is not unexpected, but the extrapolation of this to effects in humans who are exposed to even smaller amounts[7] is irresponsible use of poor science.

That the effects of glyphosate reported in this study may have been mediated via increased "activity" of retinoic acid is of possible academic interest; however, the relevance of the proposed mechanism must be weighed against the use of unrealistically high concentrations, inappropriate routes of exposure, and the possible effects of pH (see below).

In the other parts of the study, exposures were via injection into the eggs of *X. laevis* and chickens. The authors reported that effects on neural development were observed, but that effect was most pronounced at the site of injection. Here, as in other sections of the article, the methods are unclear; in the Experimental Procedures section, it is stated that the eggs of chickens were injected with "20 μL of 1/3500 or 1/4500 dilutions of GBH", but, in the Discussion (p 1590), it is stated that the "Embryos were incubated with 1/3500 or 1/4500 dilutions of GBH...", suggesting emersion; which is correct?

The method of treatment was not realistic. Injection is not a relevant route of exposure for amphibians or birds in the field, and the data are not useful in risk assessment for amphibians, other wildlife, or humans. Although the estimated concentrations in the eggs were lower than that in the study on embryos (based on volume of the egg and diffusion throughout the egg after injection), there is no information on the movement of the injected material from the site of injection, and the actual exposure in the treated area is unknown. In fact, the pronounced effects only at the site of injection suggest that the responses were to highly localized concentrations. In addition, the effects observed after the injection of glyphosate acid into the eggs of frogs could be explained simply by pH. Nowhere in the article is there any mention of adjustment of the pH of the treatment solutions or even what the pH of these solutions was. The pK_as for glyphosate acid are 5.77 $\pm$ 0.03 and 2.18 $\pm$ 0.02,[8] and others have shown that buffering of the pH to the normal physiological range significantly reduces responses in *in vitro* assays with cells from the same species of frog.[9] It is not known what the pH of the dilutions of the formulated product were, but the pH of the commercial product is 4.9.[10]

The suggestion in this article that glyphosate causes teratogenic effects in animals is not consistent with the large number of studies that have been reviewed by regulatory agencies[11−13] and in the literature.[14] The NOEL and LOEL for teratogenicity in rabbits are 1,000,000 and 3,500,000 μg/kg/day, respectively.[11] The NOEL in a three-generation reproduction study in rats was >30,000 μg/kg/day.[11] In birds, no effects on reproduction were observed in mallard ducks or bobwhite quail after exposures to technical glyphosate up to 1,000,000 μg/kg diet.[11] Even when eggs of chickens were treated with a concentration of 5% formulated Roundup on days 0, 6, 12, and 18 days postlaying, there were no effects on hatching success.[15]

In their discussion, the authors suggest that their observation may explain some of the reported cases of malformations in children born of mothers exposed to herbicides during pregnancy. In support of this, they quote an epidemiological study where exposures to glyphosate were not characterized[16] or are based on anecdotal observations: "(Dr. Hugo Lucero, Universidad Nacional del Nordeste, Chaco; personal communication)".

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In fact, the case-control study in Paraguay[16] only enumerated self-reported association with pesticides ("agrotóxicos") in general, and it is unclear if glyphosate was even used in the region. The extrapolation of their findings in the eggs of frogs and chickens to humans are not supported by epidemiology studies in the literature,[14] in general, or specifically to neural development in humans.[17]

In summary, the study reported effects of glyphosate only at unrealistically high concentrations or via unrealistic routes of exposure. The data are inconsistent with the literature, are not suitable or relevant for risk assessment for humans or wildlife, and do not support the extrapolations to human health as stated in the conclusions.

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REFERENCES

(1) Edginton, A. N., Sheridan, P. M., Stephenson, G. R., Thompson, D. G., and Boermans, H. J. (2004)Comparative effects of pH and Vision herbicide on two life stages of four anuran amphibian species. *Environ. Toxicol. Chem. 23*, 815–822.

(2) Struger, J., Thompson, D., Staznik, B., Martin, P., McDaniel, T., and Marvin, C. (2008)Occurrence of glyphosate in surface waters of Southern Ontario. *Bull. Environ. Contam. Toxicol. 80*, 378–384.

(3) Byer, J. D., Struger, J., Klawunn, P., Todd, A., and Sverko, E. (2008)Low cost monitoring of glyphosate in surface waters using the ELISA method: An evaluation. *Environ. Sci. Technol. 42*, 6052–6057.

(4) Battaglin, W. A., Kolpin, D. W., Scribner, E. A., Kuivila, K. M., and Sandstrom, M. W. (2005)Glyphosate, other herbicides, and transformation products in midwestern streams, 2002. *J. Am. Water. Res. Assoc. 41*, 323–332.

(5) Feng, J. C., Thompson, D. G., and Reynolds, P. E. (1990)Fate of glyphosate in a Canadian forest watershed. 1. Aquatic residues and off-target deposit assessment. *J. Agric. Food. Chem. 38*, 1110–1118.

(6) Thompson, D. G., Wojtaszek, B. F., Staznik, B., Chartrand, D. T., and Stephenson, G. R. (2004)Chemical and biomonitoring to assess potential acute effects of Vision herbicide on native amphibian larvae in forest wetlands. *Environ. Toxicol. Chem. 23*, 843–849.

(7) Acquavella, J. F., Alexander, B. H., Mandel, J. S., Gustin, C., Baker, B., Chapman, P., and Bleeke, M. (2004)Glyphosate biomonitoring for farmers and their families: Results from the farm family exposure study. *Environ. Health Perspect. 112*, 321–326.

(8) BCPC (2003) *The e-Pesticide Manual*, 12th ed., British Crop Protection Council, Farnham, Surrey, UK.

(9) Hedberg, D., and Wallin, M. (2010)Effects of Roundup and glyphosate formulations on intracellular transport, microtubules and actin filaments in *Xenopus laevis* melanophores. *Toxicol. in Vitro 24*, 795–802.

(10) Monsanto (2002) *Material Safety Data Sheet Commercial Product*, p 8, Monsanto Canada, Winnipeg, Canada.

(11) USEPA (1993) *Reregistration Eligibility Decision (RED) Glyphosate*, p 291, United States Environmental Protection Agency, Washington, DC.

(12) World Health Organization (1994) *Glyphosate, International Program on Chemical Safety*, World Health Organization, Geneva, Switzerland.

(13) World Health Organization (2005) *Glyphosate and AMPA in Drinking-water. Background document for development of WHO Guidelines for Drinking-water Quality*, p 19, Wold Health Organization, Geneva, Switzerland.

(14) Williams, G. M., Kroes, R., and Munro, I. C. (2000)Safety evaluation and risk assessment of the herbicide Roundup® and its active ingredient, glyphosate, for humans. *Reg. Toxicol. Pharmacol. 31*, 117–165.

(15) Batt, B. D., Black, J. A., and Cowan, W. F. (1980)The effects of glyphosate herbicide on chicken egg hatchability. *Can. J. Zool. 58*, 1940–1942.

(16) Benítez-Leite, S., Macchi, M. L., and Acosta, M. (2007)Malformaciones congénitas asociadas a agrotóxicos. *Pediatr. (Asunción) 34*, 111–121.

(17) Rull, R. P., Ritz, B., and Shaw, G. M. (2006)Neural tube defects and maternal residential proximity to agricultural pesticide applications. *Am. J. Epid. 163*, 743–753.