Chemical Research in Toxicology

LETTERS TO THE EDITOR

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

To the Editor: Regarding the recent article by Paganelli et al. (*Chem. Res. Toxicol.* (2010), *23*, 1586−1595) Glyphosate-Based Herbicides Produce Teratogenic Effects on Vertebrates by Impairing Retinoic Acid Signaling, we write to (a) confirm the high degree of confidence in the substantial toxicological database for glyphosate; (b) discuss the unsubstantiated basis provided by the authors as rationale for this published research; and (c) provide context for the dosing levels evaluated by the authors with respect to human health risk assessment.

(a) Multiple high quality toxicological studies and expert review panels consistently agree glyphosate is not a teratogen or reproductive toxicant: The GLP studies that Paganelli et al. infer as untrustworthy "industry-funded studies" have been exhaustively reviewed by multiple government scientific regulators, often comprising academic expert scientists and all of which have strongly supported the conclusions put forth in those studies. Glyphosate does not cause adverse reproductive effects in adult animals or birth defects in offspring of these adults exposed to glyphosate, even at very high doses. These conclusions are based on multiple studies in laboratory animals that have been conducted to examine the potential for multigenerational and teratogenic effects. These studies have been repeated by different companies at different laboratories across the globe over the last 30 or more years, with consistent results demonstrating that glyphosate does not pose the concerns raised by the authors. Regulatory authorities and independent experts who have documented this position include WHO/FAO,[1] U.S. EPA,[2] the European Commission,[3] and Williams et al.[4]

(b) Flawed premise: The authors provide no valid basis, other than an opinion, of an increase in the rate of birth defects in Argentina. The referenced epidemiology paper[5] implied by the authors as justification for implicating glyphosate as a chemical of concern does not mention glyphosate or even distinguish between herbicide, insecticide, molluscicide, rodenticide, or fungicide potential exposures to pregnant women. This small epidemiological study, conducted in Paraguay, investigated associations between proximity or assumed exposure to pesticide use/storage and congenital malformations in neonates. The association between "living near treated fields" (distance and pesticide types unspecified) and congenital malformations was weak, with an odds ratio about six times lower than the reported association between pesticide storage in the home and congenital malformations. There is nothing unusual about the wide variety of birth defects reported in the Paraguay study and it provides no support for the authors' allegation that they "strikingly resemble the wide spectrum phenotypes resulting from a dysfunctional RA or Shh signaling pathway".

The authors cite a number of papers[6−9] suggesting that glyphosate or glyphosate based formulations are a cause for concern regarding endocrine disruption or human reproduction and development. These studies were all based on unvalidated in vitro test systems. Such methods, and some of the specifically referenced literature, have been reviewed by regulatory authorities around the world[3,4,10−13] and other expert panels[14] and were consistently deemed inappropriate and irrelevant for human health risk assessment purposes.

(c) Irrelevant routes of exposure and inappropriately high doses: The research described by Paganelli et al. exposed two-cell frog embryos via direct injections of 360 pg and 500 pg glyphosate acid per cell, bypassing the developing amphibian protective gel coat. Assuming a cell diameter of 1 mm to determine spherical volume, the cellular doses are approximately 690 to 950 μg/L within each treated cell. Frog embryos were also bathed in glyphosate formulation at 1/5000 to 1/3000 dilutions of the glyphosate formulated product (approximately 70000 μg/L to 120000 μg/L glyphosate, respectively). These doses are 9−15 times greater than the acute LC_{50} value of 7900 μg/L for frog embryos of the same species.[15] Fertilized chicken eggs were also exposed via an unrealistic scenario, by opening a window in the shell and directly dosing 20 μL of 1/3500 and 1/4500 dilutions of the glyphosate formulated product (2.0 and 1.6 μg/chicken embryo). Using a similar chick embryo assay, Kobayashi et al.,[16] found the commonly consumed substance caffeine, to cause malformations in chick embryos.

A recent pharmacokinetic study in rats,[17] found that a 400 mg/kg oral dose of glyphosate resulted in blood C_{max} concentration of 4.6 μg/mL. Assuming linear pharmacokinetic behavior in rats for glyphosate, the dose necessary to produce a blood concentration of 72 μg/mL (as in the "low dose" of 72000 μg/L in the frog embryo culture experiments) in rats would be over 6200 mg/kg body weight (72 μg/mL/4.6 μg/mL × 400 mg/kg body weight = 6261 mg/kg body weight). Thus, the in vitro concentration used by the authors was equivalent to a glyphosate oral dose to rats of 6261 mg/kg body weight. This dose is over an order of magnitude greater than the already high doses of glyphosate shown not to cause developmental or reproductive effects in rats and rabbits (NOAELs), which are used for risk assessment purposes by some regulatory authorities to establish safe human allowable daily intakes (ADIs).

On the basis of the findings from their report, the authors express their concern for "families living a few meters from where the herbicides are regularly sprayed". This exposure scenario of concern is similar to that directly evaluated in the Farm Family Study[18] in which spouses were biomonitored for glyphosate exposure during a period of intense spraying of the herbicide only a few yards from their homes. Yet, even with that exposure proximity, the maximum systemic dose to spouses in the Farm Family Exposure Study was 0.04 μg/kg body weight, with more than 95% of the spouse exposures below the limit of detection. The margin of exposure of this human biomonitored−measured dose relative to the rat equivalent dose used in the frog embryo bathing experiments exceeded 150,000,000 (rat equivalent dose of 6,261 mg/kg equals 72 ug/mL in frog embryos;

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MOE = 6,261,000 ug/kg/[0.04 ug/kg human dose] = 156,525,000). The rat equivalent dose is the appropriate comparator to develop the Margin of Exposure calculation in that mammalian toxicology studies are the primary data sets to assess human exposure risks, and indicates that the frog embryo in vitro doses used in this study were exceedingly unrealistic relative to potential human exposures resulting from the field use of glyphosate.

In conclusion, the model systems employed by Paganelli et al., in which materials are tested at unrealistically high doses, may offer interesting results that help screen for early tier toxicological effects and perhaps offer some utility in elucidating hypothesized toxicological mechanisms. However, the results from this research cannot be used in isolation to reach the conclusions expressed in the publication. Instead, the type of data in this research paper must be interpreted relative to all other available data on the specific materials under study and with balanced consideration for higher tier apical studies. When all data including the extensive in vivo toxicological database are evaluated together in this manner, the weight of evidence supports the corroborated conclusion of regulatory experts across the globe that glyphosate is not a developmental or reproductive toxicant.

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REFERENCES

(1) WHO/FAO (2004) Pesticides Residues in Food, 2004, Report of the Joint Meeting of the FAO Panel of Experts on Pesticide Residues in Food and the Environment and the WHO Core Assessment Group on Pesticide Residues (JMPR), Rome, Italy, 20−29 Sep 2004. FAO Plant Production and Protection Paper 178. World Health Organization and Food and Agriculture Organization of the United Nations, Rome, Italy, http://www.fao.org/ag/agp/agpp/Pesticid/JMPR/DOWNLOAD/2004_rep/report2004jmpr.pdf

(2) U.S. EPA (1993) Reregistration Eligibility Decision (RED) for Glyphosate, U.S. Environmental Protection Agency, Office of Pesticide Programs, EPA 738-R-93-014, http://www.epa.gov/oppsrrd1/REDs/old_reds/glyphosate.pdf

(3) European Commission (2002) Report for the Active Substance Glyphosate, Directive 6511/VI/99, January 21, http://ec.europa.eu/food/fs/sfp/ph_ps/pro/eva/existing/list1_glyphosate_en.pdf

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

(5) Benitez Leite, S., Macchi, M. A., and Acosta, M. (2009) Malformaciones Congenitas asociadas a agrotoxicos. *Arch. Pediatr. Drug 80*, 237–247.

(6) Richard, S., Moslemi, S., Sipahutar, H., Benachour, N., and Seralini, G. E. (2005) Differential effects of glyphosate and roundup on human placental cells and aromatase. *Environ. Health Perspect. 113*, 716–720.

(7) Marc, J., Mulner-Lorillon, O., Boulben, S., Hureau, D., Durand, G., and Belle, R. (2002) Pesticide Roundup provokes cell division dysfunction at the level of CDK1/cyclin B activation. *Chem. Res. Toxicol. 15*, 326–331.

(8) Benachour, N., and Seralini, G. E. (2009) Glyphosate formulations induce apoptosis and necrosis in human umbilical, embryonic, and placental cells. *Chem. Res. Toxicol. 22*, 97–105.

(9) Gasnier, C., Dumont, C., Benachour, N., Clair, E., Chagnon, M. C., and Seralini, G. E. (2009) Glyphosate-based herbicides are toxic and endocrine disruptors in human cell lines. *Toxicology 262*, 184–191.

(10) Afssa (2009) Avis de l'Agence française de sécurité sanitaire des aliments relatif au glyphosate et aux préparations phytopharmaceutiques à base de cette substance active, Afssa − saisine no 2008-SA-0034 − Glyphosate. le 26 mars 2009, http://www.afssa.fr/Documents/DIVE2008sa0034.pdf

(11) Australian Pesticides and Veterinary Medicine Authority, APVMA (2010), Glyphosate is being reviewed in the United States and Canada. Is it still safe to use? 31 Aug, 2010, http://www.apvma.gov.au/news_media/community/2010-13_glyphosate_au.php

(12) Health Canada, Information note: request for a special review of glyphosate herbicides containing polyethoxylated tallowamine, http://www.hc-sc.gc.ca/cps-spc/pubs/pest/_fact-fiche/glyphosate/index-eng.php

(13) U.S. EPA (2009) Public comments regarding the Health Effects Division's (HED's) human health assessment scoping document in support of registration reiview of 3-Jun-2009. HED's response to public comments. EPA-HQ-OPP-2009-0361-0041, http://www.regulations.gov/search/Regs/home.html#docketDetail?R=EPA-HQ-OPP-2009-0361

(14) ECETOC Technical Report No. 106, Guidance on Identifying Endocrine Disrupting Effects, Brussels, June 2009.

(15) 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* (4), 815–822.

(16) Kobayashi, T., Nishida, A., Kurokawa, A., and Fumio, A. (1995) Cardiovascular malformations induced by caffeine amd Phenobarbital in chick embryos. *AATEX 3*, 17–27.

(17) Anadóna, A., Martínez-Larranaga, M. R., Martínez, M. A., Castellano, V. J., Martínez, M., Martin, M. T., Nozal, M. J, and Bernal, J. L. (2009) Toxicokinetics of glyphosate and its metabolite amino-methyl phosphonic acid in rats. *Toxicol. Lett. 190*, 91–95.

(18) Acquavella, J. F., Alexander, B. H., Mandel, J. S., Gustin, C., Baker, B., Chapman, P., and Bleeke, M. (2004) Glyphosate biomonitoring for farmer-applicators and their families: results from the Farm Family Exposure Study. *Environ. Health Perspect. 112*, 321–326.