

Reviewer Recommendation and Comments for Manuscript Number CBTO548
Cytotoxicity of herbicide Roundup and its active ingredient, glyphosate in rats

Original Submission
Charles Healy (Reviewer 2)

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Recommendation: Reject

Overall Manuscript Rating (1-100): 5

Reviewer Blind Comments to Author:

1. Introduction

The references provided by the authors do not provide a sound foundation for their working hypothesis.

For example the use of a single reference, Dallegrave et al. 2007, describing the authors findings from an experiment with Wistar rats with a glyphosate-based formulation as the basis for claiming that pesticides persist in the environment and pose health hazards to humans and animals is completely inaccurate and inappropriate.

No where does the author reference materials from governmental regulatory agencies whose specific jobs are to register and regulate pesticides to ensure the safe use of these products for humans and the environment.

Regulatory agencies world wide such as the United States Environmental Protection Agency (1993; 2006) and the European Commission (2002) as well as scientific organizations such as the World Health Organization (2004), whose responsibilities are to evaluate the use of pesticides and determine their safety for human health and the environment have concluded after reviewed extensive data sets that glyphosate has low toxicity to humans and wildlife, is not a carcinogen, not a mutagen, not a teratogen, is not neurotoxic and is not a reproductive toxicant and when used according to label directions do not pose unreasonable risks to human health and the environment.

It is clear the author does not understand what glyphosate is or the composition of glyphosate-based Roundup branded formulations. Glyphosate is the active ingredient in commercially available herbicide formulations around the world. Glyphosate is NOT an organophosphate, as is reflected by the high mM IC50 AChE inhibition values reported in the study. Organophosphate pesticide AChE inhibition IC50 values are typically in the range of μM -nM. Monsanto's leading glyphosate-based products are sold under the brand name "Roundup". Glyphosate-based formulations typically contain glyphosate (in a various salt forms), water and a surfactant - a soapy like substance. The proportion of glyphosate to surfactant in the herbicide formulation does not vary according to the country they are marketed but rather are developed based on the type of glyphosate salt that is used, the type of surfactant system used, the weed spectrum, the climatic conditions etc. Monsanto continues to develop new surfactant systems. These are proprietary and contribute to the tremendous efficacy seen in weed management with Roundup branded products. The formulations today are not as simple as the original "Roundup" formulation that contained only POEA or polyethoxylated tallow amine - the term used by the herbicide industry but include complex mixtures. Today's surfactant systems are blends not just the single surfactant, POEA. The use of Relyea, 2005 to describe what a Roundup formulation is unfortunate because he too did not understand the complexity of Roundup branded products today and used on off-label and illegal application of the herbicide in his experiments.

No where in the Williams et al., 2000 reference can one find the information that it is used to "control weeds in emerged grasses, broad-leaf weeds, pastures and cultures such as rice, corn and soy". Likewise no where in the Peluso et al, 1998 reference can you find the details of aquatic weed control "in fish ponds, lakes, canals and slow running water".

The author cites a World Health Organization (WHO) 2003 reference. There is no such document. When one looks at the reference list one finds:

World Health Organisation (WHO) (1994) United Nations Environment Programme, the International Labour

Organisation. Glyphosate. Environ. Health Crit.#159. Geneva, Switzerland.
World Health Organisation (WHO) (2003) Glyphosate, Environmental Health Criteria, 159:1-177
These are the same references - see references to "159" and in some form "Environmental Health Criteria" .
Here is the correct reference:
WHO. (1994) Glyphosate. Environmental Health Criteria No. 159. World Health Organization, Geneva.
<http://www.inchem.org/documents/ehc/ehc/ehc159.htm>

In addition there is a more recent review by the WHO in 2004:
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 September 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/agpp/agpp/Pesticid/JMPR/DOWNLOAD/2004_rep/report2004jmpr.pdf

The author accurately reflects the conclusions of the WHO's repeated reviews and Williams et al review of the extensive database on glyphosate, that it is not considered to be a public health concern in normal usage. The author then offers as a contrast to these extensive reviews, examples of "more accurate screening studies" on the toxicity of "Roundup" in fish and other taxonomic groups. The first example, a fish study by Jiraungkoorskul et al, 2003. Fish in aquaria were exposed to high concentrations of a Roundup branded formulation for 1, 2 or 3 months. It has been known since 1979 that Roundup branded products can be toxic to aquatic organisms such as fish. See 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. These formulations contain surfactants, soapy-like substances. That is why Roundup branded products are not allowed to be used in aquatic vegetation management. Other products like AquaMaster - that contain no surfactant are used.

The second reference regarding other taxonomic groups, is a laboratory study by Tsui and Chu 2003. The bacterium, microalgae, protozoa and crustaceans in glass flasks were exposed to various test substances ... this study also tells us nothing new. Franz et al,1997. Glyphosate--a unique global herbicide. ACS Monograph 189. American Chemical Society, Washington , DC. and Giesy JP, Dobson S, and Solomon KR. (2000) Ecotoxicological Risk Assessment for Roundup® Herbicide. Reviews of Environmental Contamination and Toxicology 167: 35-120. discuss in detail the effects of glyphosate, glyphosate-based formulations and the surfactants on numerous taxa. Again, when used in real world situations and according to label directions Roundup branded products do not pose an unreasonable hazard to human health or the environment

What does the author mean by "Roundup branded products are more acutely toxic than glyphosate alone? What route of exposure? Do they mean orally?dermally? Via inhalation? Let's use orally as an example. The oral LD50s of Roundup branded products and glyphosate are both greater than 5000 mg/kg - see Williams et al 2000.

The references the author used in this section Adams et al 1997 and Martinez and Brown 1991 are completely inappropriate. The author neglects to tell you the route of administration in these studies in rats was by intratracheal injection. Pulmonary exposure is not characteristic for glyphosate or glyphosate-based formulations. It is non-volatile and is not used in aerosol form so droplets are unlikely to be small enough to be respirable. The authors investigations were based on a concern for direct contact with the lung, resulting from aspiration of vomit after intentional ingestion of large amounts of the herbicide as might occur during a suicide attempt. The investigators in these studies via intratracheal administration placed large amounts of test substances directly into the lungs of the rats. Any liquid directly placed into the lungs would cause a violent reaction in the lungs. It should not be a surprise to anyone that a surfactant that is used to penetrate the waxy coat of plant leaves would be damaging to delicate lung tissue following intratracheal injection. These tests with intratracheal injections as well the intraperitoneal injection route of exposure used in this study do not reflect real world exposure to these products.

Marc et. al., 2002 and 2004 reported findings from in vitro studies where sea urchin embryos were directly exposed to Roundup branded formulations containing a surfactant or glyphosate. The results of these studies are secondary due to the effect of surfactants on cell membranes of cells in a Petri dish and are not validated to predict effects in whole animals. There is no evidence of synergy. First, the authors only tested glyphosate and the formulations, but they never tested formulations without glyphosate. Second, surfactants found in personal care products have been found to produce similar findings under the same experimental conditions:

Amouroux et. al., 1999. Mechanisms of Cytotoxicity by Cosmetic Ingredients in Sea Urchin Eggs Arch. Environ. Contam. Toxicol. 36, 28-37

The Peixoto 2005 study was not in whole animals but used isolated rat liver mitochondria. As noted above surfactants including those in personal care products, substances that people intentionally put on their bodies everyday, have effects on unprotected cells in Petri dishes. It should therefore not be a surprise to anyone that glyphosate had no effect on isolated mitochondria in a Petri dish while the formulated product with a surfactant did.

The Acquavella et al., 2004 reference has nothing to do with the information in this paragraph. The Acquavella paper reports on the results of a glyphosate biomonitoring study in farm families in Minnesota and South Carolina.

2. Materials and Methods

2.1 Chemicals

It is unclear that the authors understand what they are testing and what are the dose levels they used.

The report is inconsistent in the description of the test articles. The author uses Roundup, Roundup formulation, Roundup and its fundamental substance glyphosate, and glyphosate-Roundup. There are no Roundup formulations on the market anywhere in the world that contain 52% w/v POEA. Surfactants are typically 15% or less of the formulation.

Is not clear that the authors understand that glyphosate is the active ingredient and that it is formulated as a salt. There a number of salts - potassium salts, isopropylamine salts, ammonium salts, trimesium salts etc. The active ingredient is not the iso-propylamine salt of n-phosphonmethyl glycine. Also note the author does not present the chemical names correctly...isopropylamine is one word and it should be n-(phosphonomethyl)glycine.

2.2 Animals and treatment

Where were the animals purchased from? What is the specific strain of albino rat? What was the justification of using only males? Were the rats grouped or individually housed? What was the source/supplier of the food and water?

Why was an intraperitoneal injection route of administration? This is an irrelevant route of exposure for products like Roundup herbicides. What type of saline was used? Was the saline control also given by i.p. injection? Was saline used as the vehicle in the treated animals?

Roundup herbicide labels often state the concentration in two ways: a) lbs per gal of formulated glyphosate and b) lbs per gal of acid equivalent of glyphosate. For example, if a Roundup branded formulation contains 4 lbs per gal of the isopropylamine salt of glyphosate but only 3 lbs per gal acid equivalent of glyphosate. The first value includes the weight of the salt formulated with glyphosate, whereas the second only measures how much glyphosate is present. What then does this mean?:

"Doses of Roundup (1 mM) concentrations are expressed in terms of the final glyphosate concentration presented glyphosate (N-phosphonomethylglycine) contains 480 g/L of the active ingredient glyphosate in the form of its isopropylamine salt, equivalent to about 360 g/l of the acid, glyphosate."

Regarding this statement: "The dose was within of limits of NOAEL and equivalent to ¼ LD50 in rats as described elsewhere (WHO 1994)." What does that mean? Limits of NOAEL? Which NOAEL from which study from which species? Equivalent to ¼ LD50 in rats as described elsewhere? LD50 of what? Roundup? Glyphosate? An oral, dermal, intraperitoneal route? The intraperitoneal LD50 of glyphosate in rats has been reported to be 238 mg/kg in males (WHO 1994). The author gave in intraperitoneal injection of 134.95 mg/kg, over half the LD50 in male rats.

What really were the doses given these animals?

Why were the animals given 0.1 ml of saline and not 1 ml/kg as the treated animals?

What was the dosing regimen? What does "each 2 days, during 14 days" mean?

When and how were the animals sacrificed? Decapitation? Cervical dislocation? CO2 asphyxiation? Were the animals fasted or non-fasted before they were sacrificed?

2.3 Body and organ weights.

How old were these animals? When were the animals weighed, time of day? The author says the individual animal and body weights and organ weights were taken weekly. How can that be? See Table 1. How many animals are in the groups for week one? Week two? How can the organ weights be recorded weekly? Were groups of animals sacrificed at the end of week one; then the end of week two? What was the process of removal of the kidneys and livers? Were gross observations made?

After treatment, were there any inlife observations made post-dosing, daily, at sacrifice? If so when?

2.4 Biochemical Parameters

Were blood samples taken from right and left orbital sinuses? How many samples per animal were processed? Single, duplicates, triplicates? How much blood was taken? What kind of tubes were used to collect the blood?

2.5 Measurement of hepatic glutathione

Where whole livers homogenized or were specific lobes used?

2.6 Measurement of lipid peroxidation in liver

Where whole livers homogenized or were specific lobes used?

3. Results and 4. Discussion

The results of this study are more likely secondary effects mediated by local toxicity as a consequence of an intraperitoneal injection of high doses of glyphosate and a Roundup branded formulation (Heydens et al., 2008)

Glyphosate technical material is also known as glyphosate acid and has a pH of ~ 1.7. What was the pH of the injection solution? Roundup branded products do contain surfactants and can be very irritating to delicate tissues; such as the visceral and parietal peritoneum and to the organs themselves. Heydens et al., 2008 compared the results of intraperitoneal injections to oral dosing of a Roundup branded formulation, a Roundup branded formulation minus glyphosate and the vehicle control in mice. They authors found substantial changes in clinical chemistry values and histopathological lesions in livers and kidneys following intraperitoneal that were not observed when the animals were dosed orally. In addition, similar effects were observed following intraperitoneal injection of the Roundup branded formulation and the Roundup branded formulation minus glyphosate supporting the conclusion that the surfactant was involved in producing the observed effects.

The results of this study are more likely the consequence of the study design, an intraperitoneal injection of very high doses of irritating test materials. Given that oral ingestion is the more relevant route of exposure for the general population and that the estimation for dietary intake globally for humans to glyphosate is at extremely low levels (WHO/FAO, 2004) the results from this study have no real significance for human risk assessment.

William F. Heydens, Charles E. Healy, Kathy J. Hotz , Larry D. Kier, Mark A. Martens, Alan G. E. Wilson, and Donna R. Farmer. Genotoxic Potential of Glyphosate Formulations: Mode-of-Action Investigations. J. Agric. Food Chem. 2008, 56, 1517-1523

Reviewer Confidential Comments to Editor:

Review Sheet: General Judgement

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1. Is the paper acceptable for publication

(a) in its present form? NO

(b) with minor revisions? NO

Should the paper be reconsidered after

major revision? NO

Is it unacceptable for publication? YES

2. Please list any other general comments or specific suggestions in the separate blind comments to author's box.

Pesticide use and the potential for effects on human health and the environment are of great interest to many stakeholders however this paper does not provide any meaningful information to that discussion.

As presented this paper is fundamentally flawed and does not meet the rigor of a peer reviewed journal and should be rejected out right. The paper is full of suppositions with very little concrete information and what is provided is subject to question.

This study as reported in this manuscript due to the significant lack of detail could not be independently repeated by others and does not permit the reader to ascertain if real differences exist but are left to believe what the authors say.

There are a number of typographical errors, poorly structured sentences, and poor grammar some of which may be the result of errors of translation to English making the manuscript confusing and difficult to understand. One section contains verbatim text from another peer reviewed publication, Journal of Environmental Science and Health Part B 336 (1) Jan 2001, pp 29-42.

This study is unacceptable for publication as is, based on the flawed study design, the misrepresentation of published literature, the authors clear lack of understanding of the test material and the doses given as evidenced by this confusing statement: "Doses of Roundup (1 mM) concentrations are expressed in terms of the final glyphosate concentration presented glyphosate (N-phosphonomethylglycine) contains 480 g/L of the active ingredient glyphosate in the form of its isopropylamine salt, equivalent to about 360 g/l of the acid, glyphosate. The dose was within of limits of NOAEL and equivalent to ¼ LD50 in rats as described elsewhere (WHO 1994)." and that the results of the study are more likely a consequence of an intraperitoneal route of injection of high doses of irritation materials which is irrelevant for human risk assessment.

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