Reviewer’s report

Title: A Comprehensive Analysis of the Animal Carcinogenicity Data for Glyphosate from Chronic Exposure Rodent Carcinogenicity Studies

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Reviewer: Michael Nicolas Antoniou

Reviewer's report: This is a very timely, expertly constructed and well-executed study. The author has uniquely collated and collectively re-evaluated the data from studies investigating the oncogenic potential of glyphosate in laboratory rodents (mice, rats). The author has rightly applied very strict eligibility criteria as to which publications should be included and as a result 13 studies were considered. The data from these was subjected to established and appropriate statistical analytical methods. Another major strength of this study is that all 13 investigations selected for consideration employed glyphosate alone and not as a commercial herbicide formulation. This avoids toxicity arising from surfactants and other substances in the adjuvant mixture present in the commercial formulations and any carcinogenic (and other toxic outcomes) observed can unequivocally be attributed to glyphosate. Thus, the author has convincingly arrived at the conclusion that this most widely used herbicidal active ingredient can indeed act as a carcinogen, which led to various types of cancers in both mice and rats.

However, the following points should be addressed before publication.

1. In the abstract the author refers to "glyphosate-resistant genetically modified plants". Strictly speaking these genetically modified crop plants are "tolerant" rather than "resistant" to glyphosate as they do not degrade this compound but tolerate its presence. Thus, this statement here (and elsewhere) needs to be corrected.

2. In the first paragraph of the Background it is stated that glyphosate-tolerant crops were introduced in 1995. This is not the case. The first such crops (eg soybeans) were not grown commercially until 1996. This needs to be corrected.

3. On page 5, line 21 the glyphosate concentration of the commercial formulation Roundup Original is given as 36g/L. However, Roundup Original is marketed as containing 41% glyphosate as the isopropylamine salt. Thus, the concentration of glyphosate per litre is 360g not 36g. This needs to be corrected.

4. On page 6, line 22 it is stated that Seralini GE et al conducted a 24-month "carcinogenicity" study (listed as reference 17). This is incorrect. The study by Seralini and colleagues was a chronic toxicity study conducted in accordance with OECD guidelines for such an investigation and not a carcinogenicity study, but which unexpectedly found an increased mammary tumour incidence. In addition, the strain of rats used was not Wistar as stated but Sprague-Dawley. These errors need to be corrected.
5. The first several paragraphs of the Discussion section attempt to try and provide some mechanistic insight into the oncogenic effects of glyphosate observed in the studies considered in this submission. However, what is presented here is largely irrelevant and leads to confusion rather than providing information about possible glyphosate carcinogenic mechanisms. First, and most important, virtually all the studies referred to here were conducted using commercial herbicide formulations rather than glyphosate alone. Thus, the ill-effects reported cannot unequivocally be attributed to glyphosate since the adjuvants present in the formulations used are highly toxic. Overall, the objective of this study was to provide evidence that glyphosate can act as a carcinogen. There is no need for it to provide the mechanisms of how glyphosate is carcinogenic, which is a huge question in its own right and well beyond the scope of what is presented here. I therefore recommend that the first 11 paragraphs of the Discussion be deleted. After a brief recount of the objectives of this study, the Discussion very logically should continue with the 12th paragraph beginning with "The evaluation of any one animal cancer study involves a large number of statistical tests that could lead to false positives". From here all that is discussed is of direct relevance of what is presented and very insightful.

6. The Discussion paragraph beginning "Both agencies note that a lack of preneoplastic or related lesions led to the exclusion of some tumors", ends with the statement "In addition, there was considerable scientific evidence in the peer reviewed literature supporting these findings". This last statement needs to be referenced.

7. The Discussion paragraph beginning "The mechanisms though which glyphosate causes these tumors in laboratory animals are as controversial as the cancer findings themselves", ends with the statement "In addition, the cancers may be due to glyphosate altering hormonal balance in the adrenal, pituitary and thyroid glands". This last statement needs to be referenced.

8. The Discussion paragraph beginning "The mechanisms though which glyphosate causes these tumors in laboratory animals are as controversial as the cancer findings themselves", it is stated that the IARC Working Group found evidence that glyphosate induces oxidative stress leading to genotoxicity and thus contributing to oncogenesis. Since the IARC report there have been a number of studies further demonstrating the genotoxic potential of glyphosate (eg López González EC et al. (2017) Ecotoxicol Environ Saf. 136: 84-89; Santovito A et al. (2018) Environ Sci Pollut Res Int. 25(34): 34693-34700), which the author may consider making reference to in support of their arguments.

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