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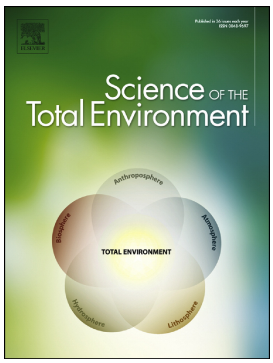

Letter to the editors regarding Battisti et al (2021): is glyphosate toxic to bees? A meta-analytical review

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Letter to the editors regarding Battisti et al (2021): Is glyphosate toxic to bees? A meta-analytical review.

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In Battisti and colleagues recent article (Battisti et al., 2021) they conduct a meta-analysis to answer the question 'Is glyphosate toxic to bees?', and find the answer to be yes. Here I argue that their study is insufficient to support their conclusion on four grounds: (i) the inclusion of studies in their meta-analysis that tested glyphosate-based formulations rather than just the active ingredient glyphosate, (ii) their data extraction, (iii) whether all appropriate literature was captured, and (iv) the categorisation of the data.

'Glyphosate kills bees' or 'glyphosate-based formulations kill bees', and data extraction.

Glyphosate is applied in agriculture and amenity settings not as a pure active ingredient, but instead in formulations that contain a range of co-formulants. Co-formulants are ingredients added to a product that are designed to improve its efficacy.

The dataset in Battisti et al. (2021) list a total of 18 publications with 34 separate experiments, 21 which use glyphosate, 10 which use formulations and 4 where the substance is not listed, Motta et al., (2020) tests both glyphosate and a formulation and is discussed later. Ruiz-Toledo and Sánchez-Guillén (2014) does not report the source of their glyphosate.

The analysis in Battisti et al., (2021) made no distinction between experiments with just the active ingredient glyphosate and those which tested a formulation that contains both glyphosate and a range of co-formulants. Had this been included in the analysis it would have allowed any effects observed to be attributed to the specific chemicals which cause them.

The distribution of the studies which use a formulation relative to effect size and support is most worrying, with Figure 1 demonstrating that substantial support is drawn from studies that instead use a formulation. The role of co-formulants in bee toxicity is poorly understood, but there is good evidence that contact exposure to surfactants, common co-formulants in glyphosate-based formulations (Mesnage, Benbrook, and Antoniou, 2019) can cause mortality in bees (Goodwin and McBrydie, 2000, Straw, Carpentier and Brown 2021).

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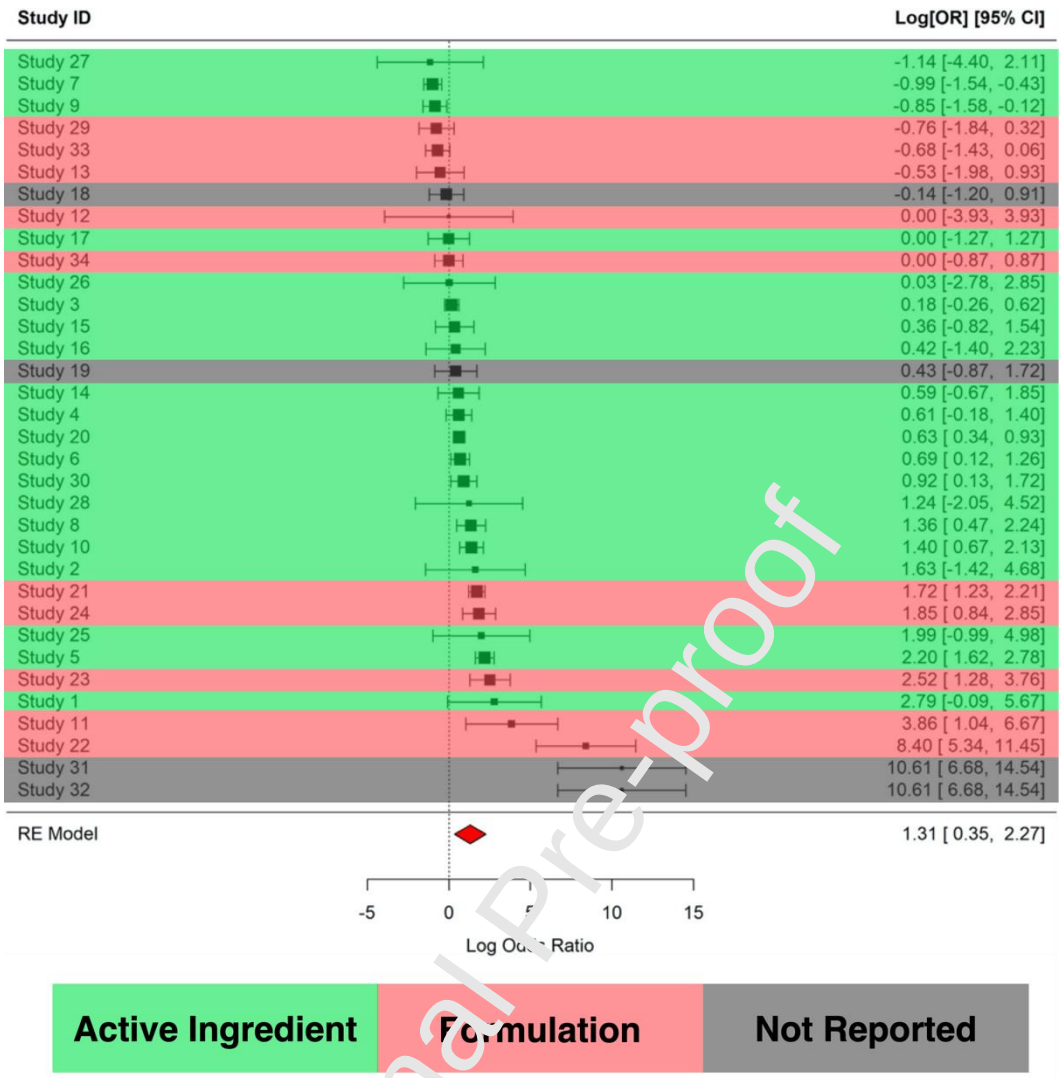

Figure 1. Replication of the forest plot from Battisti et al., (2021) (their figure 2). Colour coded by treatment type Green: Active Ingredient (glyphosate), Red: Formulation (glyphosate-based formulation), Grey: Not Reported (publication contained insufficient information to discern chemical used).

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This concern goes hand in hand with the interpretation of Motta et al., (2020)(study 21), where Battisti and colleagues find heavy support for contact mortality effects of glyphosate. This is however a misinterpretation of Motta et al., (2020) which contains multiple contact and oral toxicity experiments. One group of experiments uses five different dilutions of the formulation Roundup® ProMAX, and a separate experiment testing a similar concentration of pure glyphosate. The experiments found severe mortality with most doses of the formulation and no mortality with the glyphosate treatment. Motta and colleagues even note "*In initial trials, bees sprayed with 1.0% glyphosate in water did not die more than bees sprayed with only water, even 24 h after exposure, suggesting that other components of the formulation are responsible for the increased mortality of these topically exposed bees*".

Motta et al., (2020) also appears to have had its data extracted incorrectly. In the supplementary materials for Battisti et al., (2021), it notes that the dose used was 1.2 mL of 0.1% glyphosate. This is incorrect, as there is no treatment with 0.1% glyphosate in Motta et al., (2020). The entry for 0.1% glyphosate could be referring to 0.1% Roundup® ProMAX, which they list as 'toxic'. This is also incorrect as the 0.1% Roundup® ProMAX contact treatment did not cause significant mortality. Under Battisti et al.'s (2021) analysis scheme Motta et al., (2020) should have been separated into several entries, one for each concentration of formulation used, as well as an additional entry for the pure glyphosate treatment. There are also several additional experiments in Motta et al., (2020) including oral exposure to Roundup® ProMAX which appear to have been completely ignored.

Another paper where Battisti et al., (2021) find support for contact mortality effects of glyphosate is Abraham et al., (2018), which also used a glyphosate-based herbicide, Sunphosate 360 SL. In the light of Motta et al., (2020), Goodwin and McBrydie (2000) and Straw, Carpentier and Brown (2021) it seems highly unlikely that this result is driven by glyphosate and not surfactant co-formulants.

If we exclude publications that tested formulations or did not report the test substance, we can still attempt to answer the question 'does glyphosate cause toxicity (specifically just mortality) in bees?'. There are six experiments using just glyphosate that find significant toxicity (Studies 5, 6, 8, 10, 20 and 30). Collectively, these represent just three publications

(Vázquez et al., 2018, Almasri et al., 2020 and Herbert et al., 2014). So, support for the conclusion that glyphosate causes mortality in bees rests on three publications, all of which use *Apis mellifera*. Below I detail those three studies and critically assess how much they support mortality effects of glyphosate.

Vázquez et al., (2018) is a publication whose results heavily weigh the analysis towards the finding of a mortality effect (Studies 5 through 10, see Figure 1). However, the data extraction here appears to have been mishandled. Vázquez et al., (2018) tested the effects of chronic oral exposure to glyphosate on *A. mellifera* larvae, with the experiment repeated in 2014 and 2015. Each year three concentrations of glyphosate, 1.25, 2.5 and 5 mg/L, was fed to three colonies. Vázquez and colleagues performed individual statistical testing for each year-colony-concentration combination, because of strong colony effects. This is not seen in the extracted data which reports three 5 mg/L treatments and three 2.5 mg/L treatments. It is not obvious how Battisti and colleagues have converted 18 possible comparisons against a control into just six. It is also not obvious why the concentration of 1.25 mg/L, which never caused significant mortality, was not included in the analysis.

Almasri et al., (2020) fed honey bee workers 0.1, 1 or 10 µg/L for 20 days and saw just 10% corrected mortality in the most lethal dose (10 µg/L), a relatively small effect size for 20 days of chronic exposure. Almasri et al., (2020) also had three treatments, but only one entry in the data extraction, again highlighting fears of incorrect data extraction.

Herbert et al., (2014) fed honey bee adults 2.5 or 5 mg/L glyphosate for 14 days and saw just 13.8% corrected mortality in the most lethal dose (2.5 mg/L). Again, this publication has multiple treatments, but only one entry in the data extraction. The 5 mg/L used in Herbert et al., (2014) was 500 times more concentrated than the 10 µg/L in Almasri et al., (2020), yet similar mortality is observed. This indicates that there may be additional factors explaining the mortality, perhaps even a *Serratia marcescens* infection which Motta, Raymann and Moran, (2018) demonstrated can synergise with glyphosate to cause mortality where glyphosate alone could not. Neither publication screened their bees for infection to rule out a *S. marcescens*, or other, infection, potentially providing an alternative explanation for the mortality observed.

Methodological concerns.

The field of herbicide research in bees is still very small, with Cullen et al., (2019) finding just 15 experimental publications on glyphosate. It seems that given the size of the field, it would have been possible to perform a broader search by not including leading terms like 'lethality' in the initial search. A better search could have used just "Glyphosate" AND "Bee", ideally with using wildcards terms to allow common names for bees like 'bumblebee' to be captured. This would have left a larger, and unbiased pool of literature to then be screened for mortality reporting. A lack of detail in the methods makes it not possible to repeat their search. For example, while the search terms are given the categories searched i.e. abstracts, titles or keywords are not.

Motta, Ryamann and Moran, (2018)(a publication cited by the authors) and Odemer et al., (2020) appear to fulfil the inclusion criteria, but were excluded. For Motta, Ryamann and Moran, (2018), it appears that because they report 'approximate' numbers of bees in their experiments (3 experiments with large sample sizes of 40-90 bees per treatment) they were excluded. Odemer et al., (2020) appears to have been excluded because it was published close to the deadline of October 2020 and potentially was not yet indexed by the databases used. Both publications were well powered and found no effect of glyphosate on mortality.

Not all research on pesticides and bees is academic, with regulators across the globe requiring ecotoxicology data prior to approving a substance. As such regulatory bodies have very rigorous data on the toxicity of glyphosate to bees. Crucially, these data were not considered in the meta-analysis. For the European Union risk assessment, there is public data on contact and oral exposure for *A. mellifera* to both glyphosate and glyphosate-based formulations which fits the inclusion criteria. For glyphosate, this is available publicly via an industry hosted website (Glyphosate Renewal Group, 2012). Regulatory testing of both glyphosate and glyphosate-based formulations consistently finds no mortality effects, so its exclusion has biased the results towards an effect.

Categorisation of the data.

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The authors categorisation of publications into “manufacturer recommended dose” and “ecologically relevant” is of questionable merit. The authors do not define these terms, so the reader is unable to determine the validity of their distinction. Their categorisation of some publications as “Ecologically relevant” is questionable given our current knowledge of bee exposure to glyphosate. Publications that used long term high exposure to glyphosate in nectar like Blot et al., (2019) which exposed honeybees to 210 mg/kg for 22 days are categorised as “ecologically relevant”. Yet this concentration is considerably above even the day one ~30 mg/kg peak nectar concentration recorded in semi-field residue trials (Thompson et al., 2014, Odemer et al., 2020).

Battisti et al., (2021) report data for four species of bees, but each species of bee other than *A. mellifera*, is included in just a single publication, so there is insufficient data to draw a robust, meta-analytical conclusion for these species.

Concluding thoughts

To conclude, Battisti et al., (2020) ask the question ‘Is glyphosate toxic to bees?’, and reports the answer to be yes. The data Battisti et al., (2020) cite does not appear to support this conclusion for several reasons. There is insufficient evidence to generalise to all bees from data principally collected on honeybees. They cite several publications which find glyphosate-based formulations cause mortality through contact exposure, but these results are almost certainly attributable to surfactant co-formulants and not glyphosate. The remaining publications which support their conclusion number just three, with each appearing to have had their data incorrectly extracted and/or alternative possible explanations for the mortality.

Aside from the interpretation and analysis of the results collated, there is a worrying trend of unwarranted exclusion of publications, treatments, or experiments which find no mortality effect. This adds to a range of methodological concerns like questionable data extraction, leading search terms and a failure to consider broader literature sources, which call into question the validity of making any assessment based on this dataset.

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Given that glyphosate is the worlds most used pesticide (Duke, 2018), linking it to mortality in bees requires a high bar of evidence. While it is still possible that glyphosate causes mortality in bees, there is very limited evidence to support this conclusion, and considerable evidence to refute it. There is, however, evidence that exposure to glyphosate or glyphosate formulations can cause mortality in bees through synergistic effects with parasites (Motta, Raymann and Moran, 2018, Motta et al., 2020), or exposure to co-formulants (Abraham et al., 2018, Motta et al., 2020). Instead of a narrow focus on mortality, future research could instead look to sublethal impacts, where there is evidence that glyphosate can have a range of effects (Reviewed in Farina et al., 2019).

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Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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