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LETTER TO THE EDITOR: TOXICITY OF ROUNDUP AND GLYPHOSATE

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To the Editor:

The authors of the bibliographic review "Developmental and Reproductive Outcomes in Humans and Animals After Glyphosate Exposure: A Critical Analysis" (Williams et al. 2012) intensively analyzed five of our articles concerning the cellular toxicity of Roundup, other glyphosate-containing products, and glyphosate (Marc et al. 2002; 2003; 2004a; 2004b; 2005). Although they admit that we have demonstrated Roundup toxicity on embryonic individual cells, they minimize our experimental evidence that glyphosate plays a role in the toxicity and discredit our findings.

Their article contains several errors about our experiments that we shall not detail. All scientific readers can refer to our original publications. We intend to focus on two main points, the first about the scientific context of our investigations curiously absent from the authors review and the second about the contested environmental significance of our findings.

We want to highlight that the context of our results is the field of cell cycle disorders and mechanisms at the origin of tumorization. The authors totally disregard this context and do not even state the DNA-damage checkpoint or G2/M cell cycle transition that are clearly at the center of our results and that situate glyphosate-based products as of human health concern. Using the same experimental model and same experimental procedures, we have further shown that chromium(III) (Le Bouffant et al. 2008) or methyl methanesulfonate (MMS) (Le Bouffant et al. 2007), both known carcinogens, lead to the same molecular phenotype than glyphosate containing formulations. This context is clearly stated in almost all our cited articles and has been reviewed by us (Bellé et al. 2007), including glyphosate-based products' effects, not quoted by the authors' bibliographic review. Involvement of the DNA-damage checkpoint at the origin of cancer is widely accepted by scientists (Jackson and Bartek 2009; Kastan and Bartek 2004; Nyberg et al. 2002). The concept that cancer originates from a few (if not one) stem cells that themselves leads by clonal selection to cancerous stem cells and further to cancer development is also well documented and accepted by a large community of scientists (Rahman et al. 2011; Ratajczak et al. 2006). We have worked on embryonic cells, and per se stem cells, and our results at the level of the DNA-damaged checkpoint therefore suggest that glyphosate-based products are of human health concern and warrant further investigation.

The authors consider our results as "not environmentally relevant" because of the concentrations used. The sentence was repeated five times in their article. This is a speculative assertion since (1) we observe effects at concentrations (8 m*M* affecting 100% of the individual cells at short time exposure) below the usage concentration (20 m*M*) of the herbicide. Therefore, regarding the considerable amount of glyphosate-based product sprayed worldwide, the concentration of Roundup in

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every single micro droplet is far above the threshold concentration that would activate the cell cycle checkpoint. (2) The effects we demonstrate were obtained by a short exposure time (minutes) of the cells to glyphosate-based products, and nothing excludes that prolonged exposure to lower doses may also have effects. Since glyphosate is commonly found present in drinking water in many countries, low doses with long exposure by ingestion are a fact. The consequences of this permanent long-term exposure remain to be further investigated but cannot just be ignored.

The authors do not take into account in their interpretation of our results the very poor cell membrane permeability of pure glyphosate (Riechers et al. 1994), although they do state that "commercial formulations include a surfactant system . . . allowing penetration of the active ingredient." Since our results were obtained for short exposure time at neutral pH, we ascribed the absence of cellular effect of pure glyphosate to this poor permeability. To our knowledge, pure glyphosate is not used as an herbicide in agriculture applications and we ignore whether, in such conditions, pure glyphosate is or not an herbicide.

Altogether, we consider that independent researches on the safety of glyphosate-based products need to be encouraged and that this article, minimizing our results and presenting them outside their scientific context, leads to underestimation of the human health risk.

Although we notice that Monsanto, the manufacturer of Roundup, financed their work, we would have expected strong scientific arguments against our results or alternative findings that would evidence the contrary. This is not the case, and, to our knowledge, our experiments, first published in 2002 and brought to Monsanto's knowledge as early as 1999, have not been demonstrated to be incorrect or biased.

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RESPONSE TO THE COMMENTS OF BELLE AND COLLEAGUES

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We thank Dr. Bellé and colleagues for their interest in our recent assessment of the potential developmental and reproductive effects of glyphosate. Bellé et al. believe that our article provided an incomplete critique of their molecular findings. They indicate that their experiments using sea urchin embryos are focused on understanding cell cycle disorders and mechanisms at the origin of tumorization. As the title of our article denotes, the emphasis of our review was on the potential developmental and reproductive effects that might be associated with glyphosate exposure. To that end, we included discussion of the Bellé group's studies because disruption of cell cycle progression is a possible mechanism whereby developmental toxicity could occur. Nothing in our publication, however, challenges the idea that altering the DNA-damage checkpoint or G2/M cell cycle transition could hasten tumorigenesis. Further, it was not our intent to suggest that chemicals that interfere with DNA checkpoints do not lead to cancer or that clonal selection is not a key step in cancer development.

Nevertheless, it must be emphasized that glyphosate has been shown to be nongenotoxic and noncarcinogenic in whole-animal studies. In studies conducted through the National Toxicology Program, glyphosate was not mutagenic in multiple strains of *Salmonella typhimurium* at concentrations of up to 10,000 μg/plate and did not induce micronuclei formation in mice at doses of up to 50 mg/kg (NTP 1992). Furthermore, the U.S. Environmental Protection Agency (EPA) has classified glyphosate as a class E compound (noncarcinogenic to humans) based on adequate studies in mice and rats (U.S. EPA 1997). International organizations and panels have come to similar conclusions about the absence of carcinogenic effects in mammals (European Commission 2002; WHO 1994; WHO/FAO 2004). Therefore, the relevance of their sea urchin studies with glyphosate-based formulations to understanding mechanisms involved with the origin of tumorization is unclear.

Another point of contention for Bellé et al. is our emphasis on environmentally relevant exposures. Although Roundup (a formulation containing glyphosate, surfactants, and other ingredients) is applied in the environment at a variety of concentrations, human cells are not bathed in the application solutions. Further, the concentration of the applied solution would be much higher than the glyphosate concentration found in surface or drinking water as a result of Roundup use. As such, the concentrations at which effects are seen in the sea urchin studies (8 m*M*, as calculated by the Bellé group) are not relevant to the concentrations that human embryos actually experience as a result of typical glyphosate exposures. Therefore, it is our opinion that the concentrations of Roundup used in the studies by Bellé et al. are not informative for assessing human developmental and reproductive risks because they have not been shown to be physiologically relevant.

It should be further noted that our focus was on the active ingredient, glyphosate, and not on the various glyphosate-based formulations because these differ considerably in their

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chemical makeup. To be inclusive, however, we provided information on formulations in our review when these were used. We note, however, that effects seen in studies as a result of exposure to herbicide formulations are not necessarily due to the active ingredient. In fact, we discussed studies (e.g., Levine et al. 2007) showing that the surfactants used in various herbicide formulations have significant effects in vitro that others have ascribed to active ingredients, such as glyphosate (which when used alone had no effect in the test system).

In conclusion, while we are appreciative of Bellé and colleagues' interest in our recent article, the focus of our article was on the potential human developmental and reproductive effects of glyphosate exposure. Although we included discussion of the Bellé group's studies, it was only to explore a potential mechanism that, if proven, could have implications for developmental toxicity. Our critical analysis, however, found that the results of these studies were not of relevance for understanding the potential human developmental and reproductive effects of glyphosate at expected environmental exposure concentrations.

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