

REPLY TO REEVES AND DUNN:

# Risk for autism in offspring after maternal glyphosate exposure

Kenji Hashimoto<sup>a,1</sup> and Bruce D. Hammock<sup>b,c</sup>

Autism spectrum disorder (ASD) is a developmental disorder that affects communication and behavior. Environmental factors, including exposures to synthetic chemicals (i.e., the herbicide glyphosate) during pregnancy, might increase the risk for ASD (1). A population-based case-control study in California showed that the risk of ASD was associated with the use of glyphosate (odds ratio = 1.16) as well as other pesticides (odds ratio = 1.10 to 1.13) (2). We report that maternal exposure to the formulated glyphosate (0.098%) causes ASD-like behaviors in juvenile murine offspring (3). Furthermore, exposure to formulated glyphosate during pregnancy caused differential expression of microRNAs and antioxidant-related genes in the brain of rodent offspring (4, 5).

Reeves and Dunn (6) point out that ASD-like behaviors after maternal exposure to formulated glyphosate might stem from other ingredients in the formulated herbicides such as Roundup Maxload (48% [wt/vol] glyphosate potassium salt, 52% other ingredients including water and surfactant). Furthermore, they point out that the body weight of pregnant mothers exposed to formulated glyphosate was lower than that of the control group, indicating that the mothers exhibit signs of malnutrition (6). They also point out that a quaternary amine surfactant may affect the outcome in offspring after maternal exposure to formulated glyphosate. Collectively, they address the possibility that the ingredients in Roundup Maxload may contribute to ASD-like behaviors in offspring after maternal exposure to formulated glyphosate.

In contrast, we recently reported that maternal exposure to pure glyphosate (0.098%, Sigma-Aldrich

Co., Ltd) caused ASD-like behaviors in male juvenile offspring (7). Body weight of pregnant mothers exposed to pure glyphosate was not different from the control group, indicating that pure glyphosate did not affect the body weight of pregnant mothers (7). Thus, it is possible that the ingredients included in the Roundup Maxload can decrease the body weight of mothers during exposure to formulated glyphosate (3). In addition, a recent study using pure glyphosate showed that maternal exposure to glyphosate during pregnancy caused neurobehavioral alterations (i.e., development of reflexes, motor activity, and cognitive function) (8). Taken together, it is likely that maternal exposure to a high dose of glyphosate without ingredients could cause behavioral abnormalities such as ASD-like behaviors in offspring. Importantly, we know that the rodent data do not necessarily translate to humans. Further research is needed to identify the molecular mechanisms of maternal glyphosate exposure in ASD etiology.

The urinary levels of glyphosate in some occupationally exposed subjects varied from 0.26  $\mu\text{g/L}$  to 73.5  $\mu\text{g/L}$ , and the urinary levels in environmentally exposed subjects ranged from 0.16  $\mu\text{g/L}$  to 7.6  $\mu\text{g/L}$  (9). A prospective birth cohort study showed that >90% of pregnant women had detectable levels of glyphosate (0.1  $\mu\text{g/L}$ ) in the urine, and that these levels were correlated significantly with shortened pregnancy lengths (10). Finally, a further cohort study on measurement of blood (or urine) levels of glyphosate and its major metabolite aminomethylphosphonic acid in pregnant mothers who have their offspring with or without ASD is of great interest.

- 1 L. A. Sealey *et al.*, Environmental factors in the development of autism spectrum disorders. *Environ. Int.* **88**, 288–298 (2016).
- 2 O. S. von Ehrenstein *et al.*, Prenatal and infant exposure to ambient pesticides and autism spectrum disorder in children: Population based case-control study. *BMJ* **364**, 1962 (2019).
- 3 Y. Pu *et al.*, Maternal glyphosate exposure causes autism-like behaviors in offspring through increased expression of soluble epoxide hydrolase. *Proc. Natl. Acad. Sci. U.S.A.* **117**, 11753–11759 (2020).

<sup>a</sup>Division of Clinical Neuroscience, Chiba University Center for Forensic Mental Health, Chiba 260-8670, Japan; <sup>b</sup>Department of Entomology and Nematology, University of California, Davis, CA 95616; and <sup>c</sup>UC Davis Comprehensive Cancer Center, University of California, Davis, CA 95616

Author contributions: K.H. and B.D.H. wrote the paper.

The authors declare no competing interest.

Published under the [PNAS license](#).

<sup>1</sup>To whom correspondence may be addressed. Email: hashimoto@faculty.chiba-u.jp.

Published December 22, 2020.

- 4 H. Ji, L. Xu, Z. Wang, X. Fan, L. Wu, Differential microRNA expression in the prefrontal cortex of mouse offspring induced by glyphosate exposure during pregnancy and lactation. *Exp. Ther. Med.* **15**, 2457–2467 (2018).
- 5 J. S. de Souza *et al.*, Maternal glyphosate-based herbicide exposure alters antioxidant-related genes in the brain and serum metabolites of male rat offspring. *Neurotoxicology* **74**, 121–131 (2019).
- 6 W. Reeves, S. E. Dunn, Additional observations regarding glyphosate-based herbicides and developmental toxicity. *Proc. Natl. Acad. Sci. U.S.A.*, 10.1073/pnas.2015865118 (2020).
- 7 Y. Pu *et al.*, Autism-like behaviors in male juvenile offspring after maternal glyphosate exposure. *Clin. Psychopharmacol. Neurosci.*, in press.
- 8 R. Coullery, A. M. Pacchioni, S. B. Rosso, Exposure to glyphosate during pregnancy induces neurobehavioral alterations and downregulation of Wnt5a-CaMKII pathway. *Reprod. Toxicol.* **96**, 390–398 (2020).
- 9 C. Gillezeau *et al.*, The evidence of human exposure to glyphosate: A review. *Environ. Health* **18**, 2 (2019).
- 10 S. Parvez *et al.*, Glyphosate exposure in pregnancy and shortened gestational length: A prospective Indiana birth cohort study. *Environ. Health* **17**, 23 (2018).