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Letter to the Editor

Additional Safety Assessments Needed for Diamide Insecticides

We thank Almássy and coworkers for carefully reading our paper entitled *Comparison of Chlorantraniliprole and Flubendiamide Activity Toward Wild-Type and Malignant Hyperthermia-Susceptible Ryanodine Receptors and Heat Stress Intolerance* Pessah and Truong (2019). In their letter, highlighting several clear gaps in the science concerning the activity of insecticidal diamides towards mammalian ryanodine receptors (RyRs) to better understand the potential risks associated with their use, especially in individuals that express mutations and variants within the three genes that encode for the Ca^{2+} channel proteins RyR1, RyR2, and RyR3. Indeed, when we embarked on our experiments with chlorantraniliprole (CP), a broadly used anthranilic diamide, and flubendiamide (FD), a discontinued (in the United States) phthalic diamide, based on the prevailing literature, we expected a complete lack of activity of either diamide on wild-type (WT) mammalian RyR1. Unexpectedly, not only does CP modulate WT RyR1 from rabbit and mouse skeletal muscle, it is rather efficacious at triggering Ca^{2+} release from junctional sarcoplasmic reticulum membrane vesicle preparations, with CP showing significantly more activity in these assays. Importantly, ours was the first study that tested whether mutations residing either within the N-terminal (R163C) or C-terminal (T4826I) regions implicated for diamide binding to insect RyR enhanced sensitivity to these insecticides. As Almássy and coworkers remark, the genotype effects we report (sensitivity to both CP and FD in the range 0.01–100 μM was T4826I≫R163C∼WT) raise significant concerns regarding the unknown consequences of strong gene by environment interactions. To amplify the concerns indicated in our discussion, we strongly agree with the authors' concerns, especially when one considers the >400 RYR1 Riazi *et al.* (2018) and the growing number of RYR2 genetic variants reported, many of which are expressed and known to confer susceptibility to stress-triggered pathology, morbidity, and mortality, including catecholaminergic polymorphic ventricular tachycardia .

The seemingly paradoxical failure of our *in vivo* temperature stress study to show a clear influence of CP on time to trigger the fulminant malignant hyperthermia (MH) episode was not intended to be interpreted as proof that CP under all exposure paradigms should be predicted to have no health consequences in the genotypes studied, much less across all variants known to occur in RYR1 and RYR2. Our rationale, as described in the Methods section (Pessah and Truong, 2019), for choosing the acute high-dose oral gavage dosing protocol was simply that it closely followed the only published information available to us that also included pharmacokinetic data. Also, CP has very poor properties of solubility and, thus the high-dose oral dosing protocol was the most defensible option. However, we are keenly aware that lack of a measurable response using this extreme high-dose protocol should not and cannot be over-interpreted as an indicator of overall safety for populations expressing any RYR mutation, especially over more relevant long-term exposures. Hopefully, future study designs will address the data gaps, especially now that we know insecticidal diamides have a structure-activity relationship (SAR) towards RyRs and that mutations in RYR (at least RyR1 to date) can influence that SAR. Such information may be especially valuable as application and exposure rates increase in response to insect resistance to ryanoids.

SUPPLEMENTARY DATA

Supplementary data are available at *Toxicological Sciences* online.

Declaration of Conflicting Interests

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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REFERENCES

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Isaac N. Pessah[1] and Kim M. Truong
Department of Molecular Biosciences, School of Veterinary Medicine, University of California, Davis, California 95616-5270

[1]To whom correspondence should be addressed at Department of Molecular Biosciences, School of Veterinary Medicine, University of California, 1089 Veterinary Medicine Drive, Davis, CA 11 95616. Fax: (530)-752-4698. E-mail: inpessah@ucdavis.edu.

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