

Response to Comment on “Mutagenic Azo Dyes, Rather than Flame Retardants, are the Predominant Brominated Compounds in House Dust”

We appreciate the opportunity to respond to the comments by P. Lee Ferguson and Heather M. Stapleton regarding our article, and we also hope to further clarify the findings of our work.

The major concern of Ferguson and Stapleton¹ about our article² was that $[M-H]^-$ was presumed as the only ion. As stated in our original method study,³ highlighted in Figure 5 of that article,³ we have clearly shown that multiple possible neutral molecular formulas might exist for a detected ion. In the present study,² we also stated that other ions might be formed. For example, $[M-Br+O]^-$ ions of PBDEs were detected and shown in the main body of the manuscript and also in the supporting data. In fact, in addition to the $[M]^-$ ions mentioned by Ferguson and Stapleton, other ions such as $[M+formic\ acid-H]^-$ can also exist. As stated in our previous studies,^{3,4} we could use several strategies such as positive mode ionization, derivatization, or ESI to distinguish among these ions, though for most chemicals, these strategies might not be feasible due to insufficient sensitivity. Thus, these different ions are technically difficult to distinguish, and validation by use of authentic standards as was done by Ferguson and Stapleton is likely the only/best way to confirm ions. Considering this, we did not distinguish specific types of ions in our manuscript nor did we provide neutral formulas in either Table 1 or the supporting data. Instead, formulas of detected ions were provided, which is beneficial to other groups that might use different types of ions to match in their personal database, as was done by Ferguson and Stapleton.

Ferguson and Stapleton also mentioned that the formula of the first ranked azo dye was incorrectly annotated. As stated in this and previous studies,^{2,3} we have not yet applied a statistical false discovery rate (FDR)-controlled strategy to report our results, and only top formula with the least mass error or largest computational scores were shown for each detected compound. We acknowledge that $C_{21}H_{21}BrN_6O_6$ might be the most likely formula for the first and seventh ranked chemicals. In fact, $C_{21}H_{21}BrN_6O_6$, which was correctly assigned as the seventh ranked chemical (potential isomer) was also predicted as a top 3 formula for the first ranked chemical, though only the formula with the greatest score, $C_{23}H_{23}BrN_3O_7$, was reported. The uncertainty (or FDR) associated with predictions is due possibility of multiple formulas existing for a given m/z value, within the mass tolerance. This uncertainty is particularly great for azo dyes which have large molecular masses and great numbers of oxygen and nitrogen atoms, which creates a larger search space for predictions of formulas. To reduce uncertainty, we also checked MS² spectra, but this method does not guarantee exclusive prediction of formulas. For example, the two formulas $C_{21}H_{21}BrN_6O_6$ ($m/z = 532.0706$) and $C_{23}H_{23}BrN_3O_7$ ($m/z = 532.0719$), cannot be distinguished, even by use of their MS² spectra.

Ferguson and Stapleton also proposed several strategies to distinguish two formulas in the absence of authentic standards. First, they claimed that the LEWIS rule may be useful to exclude unreasonable formulas. But this would be difficult since adducts of these ions could not be distinguished. In fact, for the proposed formula $C_{23}H_{23}BrN_3O_7$, there are many matches in the Chemspider database if the putative compound analogue of $C_{23}H_{23}BrN_3O_7$, is queried. Second, Ferguson and Stapleton proposed that additional chemical informatics software (Sirius 3.3) might be useful, but these also have some imitations. For example, the Sirius software is not suited to batch analysis of DIPIC-Frag results, which would be very time-consuming if thousands of chemicals were searched individually. The use of fragmentation information is a major difference of Sirius compared to our computation algorithm (although we have checked MS² data manually). This strategy does not work well in many cases when there is insufficient MS² spectral information, as was the case for the two predicted chemical formulas, $C_{23}H_{23}BrN_3O_7$ and $C_{21}H_{21}BrN_6O_6$, mentioned above. Third, Ferguson and Stapleton claimed that the Orbitrap Fusion has greater mass accuracy and mass error was 0.33 ppm, but as shown in Figure 1B in the comments, the actual mass error of m/z 532.07236 was calculated to be 3.3 ppm. In our experience, following multiple injections and analysis with the DIA operating mode, <3 ppm is a more reasonable expectation of mass error for Q-Exactive.

The m/z value for 533.0724 in Table 1 was reported in error, though the values were correctly reported in the supporting data. The fourth ranked chemical was correctly removed in the supporting data. The seventh ranked chemical might be an isomer of the first rank chemical, as they have very similar retention times (shown in supporting data). An erratum has been published to correct erroneous information in Table 1.⁵ There were four azo dyes successfully validated by Ferguson and Stapleton (7th, 2nd, 3rd, and 10th ranked chemicals were validated as Disperse Blue 373, Disperse Orange 61, Disperse Violet 93, and Disperse Blue 183:1). Using standards, we have validated 19 additional BFRs. The FDR of our computational algorithm to predict compound formulas was less than 5% (1/24).

Our group is developing a strategy independent of databases, for untargeted identification of environmental chemicals. The current version of DIPIC-Frag is a deterministic model without statistical FDR control, while we are working on the development of FDR-controlled strategy to more reasonably report our results. We have provided unique IDs for each identified compound (m/z , retention time, and potential formulas), which allow others to monitor these compounds in their own groups. The comments from Ferguson and Stapleton are part of a valuable follow-up to validate our

Published: March 10, 2017



chemical list. Because authentic standards of azo dyes were not commercially available to our group, Ferguson and Stapleton have provided important information regarding validation of the predicted azo dyes. Based on our screening results, and the validation of Ferguson and Stapleton, we have shown that brominated azo dyes are an important class of environmental chemicals, which need further research regarding their environmental occurrence, behaviors, and potential hazard and risk.

Hui Peng^{*,†}

David M. V. Saunders[†]

Paul D. Jones^{†,‡}

John P. Giesy^{*,†,§,||,⊥}

[†]Toxicology Centre, University of Saskatchewan, 44 Campus Drive, Saskatoon, SK Canada

[‡]School of Environment and Sustainability, University of Saskatchewan, 117 Science Place, Saskatoon, SK Canada

[§]State Key Laboratory of Pollution Control and Resource Reuse, School of the Environment, Nanjing University, Nanjing, People's Republic of China

^{||}Zoology Department, Center for Integrative Toxicology, Michigan State University, 1129 Farm Lane Road, East Lansing, Michigan United States

[⊥]School of Biological Sciences, University of Hong Kong, Hong Kong Special Administrative Region, Peoples republic of China

AUTHOR INFORMATION

Corresponding Authors

*Toxicology Centre, University of Saskatchewan, Saskatoon, Saskatchewan S7N5B3, Canada. Telephone (direct): 306-966-2096. Telephone (assistant): 306-966-4680. Fax: 306-966-4796. E-mail: jgiesy@aol.com.

*E-mail: huisci@gmail.com.

ORCID

Hui Peng: 0000-0002-2777-0588

Notes

The authors declare no competing financial interest.

REFERENCES

- (1) Ferguson, P. L.; Stapleton, H. M. Comment on "Mutagenic azo dyes, rather than flame retardants, are the predominant brominated compounds in house dust". *Environ. Sci. Technol.* **2017**, DOI: [10.1021/acs.est.7b00372](https://doi.org/10.1021/acs.est.7b00372).
- (2) Peng, H.; Saunders, D. M. V.; Sun, J. X.; Jones, P. D.; Wong, C. K. C.; Liu, H. L.; Giesy, J. P. Mutagenic azo dyes, rather than flame retardants, are the predominant brominated compounds in house dust. *Environ. Sci. Technol.* **2016**, *50* (23), 12669–12677.
- (3) Peng, H.; Chen, C. L.; Saunders, D. M. V.; Sun, J. X.; Tang, S.; Codling, G.; Hecker, M.; Wiseman, S.; Jones, P. D.; Li, A.; Rockne, K. J.; Giesy, J. P. Untargeted identification of organo-bromine compounds in lake sediments by ultrahigh-resolution mass spectrometry with the data-independent precursor isolation and characteristic fragment method. *Anal. Chem.* **2015**, *87* (20), 10237–10246.
- (4) Peng, H.; Saunders, D. M. V.; Sun, J. X.; Garry, C.; Wiseman, S.; Jones, P. D.; Giesy, J. P. Detection, identification, and quantification of hydroxylated bis(2-ethylhexyl)-tetrabromophthalate isomers in house dust. *Environ. Sci. Technol.* **2015**, *49*, 2999–3006.
- (5) Peng, H.; Saunders, D.; Sun, J. X.; Jones, P. D.; Wong, C. K. C.; Liu, H. L.; Giesy, J. P. Correction to mutagenic azo dyes, rather than flame retardants, are the predominant brominated compounds in house dust. *Environ. Sci. Technol.* **2017**, DOI: [10.1021/acs.est.7b00919](https://doi.org/10.1021/acs.est.7b00919).