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Peer Review

Review of: "Mixture Toxicity: Evidence from Experimental Studies on Concurrent Chemical Exposures"

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QEIOS review of

Mixture Toxicity: Evidence from Experimental Studies on Concurrent Chemical Exposures

General comments: There is too much of a mixture of different fields and chemical classes. Everything is mixed! Metals and organics, ecotoxicity and human health. The author is recognized as an expert in human health but not in ecotoxicity, and many of the concepts in the exceedingly short aquatic toxicity chapter do not cover major contributors to the field (see list of example references below). The author recommends a mechanistic approach, but many of the references are at least 20 years old, and the field has progressed significantly since 2005. Notably, the manuscript purports that mechanistic information is paramount to understanding mixtures, but none of the mechanistic schemes which are well known in the environment. Particularly, mode of action schemes such as Verhaar et al. (2000), Russom et al. (1997), and more recently new generation mechanistic alerts from Sapounidou (2019) and Bauer et al. (2018) are completely absent. These set the basis for a new approach to consideration of toxicological impacts for both human health and the environment for organic chemical toxicity.

Research work on metals (an area the author seems to be particularly familiar with) should be separated from work on organics. And it probably makes sense to have different frameworks when considering mixtures of metals from mixtures of organics. Mixtures of metal & organics would represent yet another level of complexity.

Methodological Approaches and Theoretical Frameworks

The study revealed that while most combined effects were close to dose-additive for hazard assessment purposes, particularly for congeneric chemicals sharing similar reactive mechanisms, the underlying

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mechanisms were considerably more complex than simple additivity models would suggest.

Addition of time-dependent dosing actually rectified the problem, so while more complex, it was explainable.

This publication would have been useful to include in methodological approaches:

European Food Safety Authority (EFSA), Dorne, J. L. C., Crépet, A., te Biesebeek, J. D., Machera, K., & Hogstrand, C. (2020). *Human risk assessment of multiple chemicals using component-based approaches: A horizontal perspective* (Vol. 17, No. 1, p. 1759E).

Experimental Animal Studies

Perhaps should be renamed Experiments on mammals, as the next section, Aquatic Toxicology Studies, also discusses animals.

Aquatic Toxicology Studies

This section merits a publication in itself rather than just a section. Once again, the mixing of metals and organics is inappropriate; for metals, the Biotic Ligand Model is well respected to consider metal bioavailability (see ref below for overview):

Paquin, P. R., Gorsuch, J. W., Apte, S., Batley, G. E., Bowles, K. C., Campbell, P. G., ... & Wu, K. B. (2002). The biotic ligand model: a historical overview. *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology*, *133*(1-2), 3-35.

For organics, there are two aspects: mechanistic toxicity and the additivity/independent action aspects. None of the authors in this area have been cited in this publication, and inclusion of the references below would have provided a much more balanced and informative article.

Refs for MoA / mechanistic approaches:

Verhaar, H. J., Solbé, J., Speksnijder, J., van Leeuwen, C. J., & Hermens, J. L. (2000). Classifying environmental pollutants: Part 3. External validation of the classification system. *Chemosphere*, *40*(8), 875-883.

Russom, C. L., Bradbury, S. P., Broderius, S. J., Hammermeister, D. E., & Drummond, R. A. (1997). Predicting modes of toxic action from chemical structure: Acute toxicity in the fathead minnow (Pimephales promelas). *Environmental toxicology and chemistry*, *16*(5), 948-967.

Sapounidou, M. (2019). *In Silico Classification of Industrial Chemicals Associated with Acute Aquatic Toxic Action Utilising Molecular Initiating Events*. Liverpool John Moores University (United Kingdom).

Bauer, F. J., Thomas, P. C., Fouchard, S. Y., & Neunlist, S. J. (2018). A new classification algorithm based on mechanisms of action. *Computational Toxicology*, *5*, 8-15.

Refs for environment (aquatic) mixture toxicity approaches not cited in this review:

ECETOC Exploring community-based environmental hazard assessment of mixtures using mode-of-action based approaches Technical Report No. 135 Brussels, May 2020 ISSN-2079-1526-135 (online)

Backhaus, T., & Faust, M. (2012). Predictive environmental risk assessment of chemical mixtures: a conceptual framework. *Environmental science & technology*, *46*(5), 2564-2573.

Rolf Altenburger, Thomas Backhaus, Wolfgang Boedeker, Michael Faust, Martin Scholze, L Horst Grimme, Predictability of the toxicity of multiple chemical mixtures to *Vibrio fischeri*: Mixtures composed of similarly acting chemicals, *Environmental Toxicology and Chemistry*, Volume 19, Issue 9, 1 September 2000, Pages 2341–2347, https://doi.org/10.1002/etc.5620190926

Brian, J. V., Harris, C. A., Scholze, M., Backhaus, T., Booy, P., Lamoree, M., ... & Sumpter, J. P. (2005). Accurate prediction of the response of freshwater fish to a mixture of estrogenic chemicals. *Environmental health perspectives*, *113*(6), 721-728.

Schmidt, S. N., Holmstrup, M., Smith, K. E., & Mayer, P. (2013). Passive dosing of polycyclic aromatic hydrocarbon (PAH) mixtures to terrestrial springtails: Linking mixture toxicity to chemical activities, equilibrium lipid concentrations, and toxic units. *Environmental science & technology*, *47*(13), 7020-7027.

Bicherel, P., & Thomas, P. C. (2021). Aquatic toxicity calculation of mixtures: a chemical activity approach incorporating a bioavailability reduction concept. *Environmental Science & Technology*, *55*(16), 11183-11191.

The aquatic toxicology section is so light that it might be better to remove it altogether.

Synergism is noted but without any attempt to explain these findings. Especially as there are recognised reasons for this relatively rare occurrence, and some examples could have been provided as case studies.

Statistical Methods and Data Analysis

Concerning PBTK modelling, the field is still rather young, and software currently still poorly predicts physico-chemical properties influencing ADME, so without in vitro information on absorption, Cmax, metabolism, etc., the models tend to predict rather poorly even for single substances. It seems hard to imagine that recent frameworks can provide accurate predictions for mixtures, and the Hertzberg et al. reference cited in the intro dates back to over 10 years ago, while the El-Masri reference is nearly 20 years old.

This is a more recent one:

Sylvain Bart, Stephen Short, Tjalling Jager, Emily J. Eagles, Alex Robinson, Claire Badder, Elma Lahive, David J. Spurgeon, Roman Ashauer, (2022). How to analyse and account for interactions in mixture toxicity with toxicokinetic-toxicodynamic models, Science of The Total Environment, Volume 843,157048,ISSN 0048-9697, https://doi.org/10.1016/j.scitotenv.2022.157048

Theoretical Considerations and Limitations

The Borgert et al. reference is also seriously out of date (see MoA approaches above, where the field has significantly changed since 2004 and since the work by Lambert & Lipscomb in 2007). Basically, all the citations in this chapter are out of date.

Neurotoxicity and Developmental Effects

This section is limited to three metals/organometals: lead, mercury/methyl mercury, and the interaction between mercury and aluminium.

There is plenty of other work performed on neurotoxicity that could have been included here.

Overall, this review is extremely limited, purports to cover many subjects but does not cover any of them thoroughly, and is largely dependent on outdated publications to support its arguments. To add advantageously to the debate, it is necessary to use the latest published work in the field.

Declarations

Potential competing interests: No potential competing interests to declare.