Reviewer Report

Title: Map and Model - moving from observation to prediction in toxicogenomics

Version: Revision 1 **Date:** 3/24/2019

Reviewer name: Katharine Horzmann, DVM, Ph.D., MPH

Reviewer Comments to Author:

This is the second review of the submitted manuscript, "Map and Model - moving from observation to prediction in toxicogenomics". Overall, the manuscript is still well written and describes a very interesting model. The changes the authors have made to the manuscript are generally favorable and help provide additional information to clarify reviewer concerns or provide additional information. However, some reviewer comments could be further addressed.

The points below refer to the numbered sections in the Author's Response to Review's Comments.

Response 2.2: Previously, I requested a more explicit explanation of the limitations of the described model with respect to variation in experimental design factors of the zebrafish transcriptomic datasets. I disagree with the authors' statement about how using fold change data normalized to respective experimental controls would prevent factors such as time chemical exposure was initiated and incubation temperature from influencing the analysis. The dynamic nature of gene expression during development makes these factors critical, as genes are normally up- and downregulated during development and chemical exposure during different periods can cause different gene expression profiles. Likewise, difference in incubation temperatures would change the 'length' of those developmental windows and would influence microarray results. Comparing normalized fold change data would not adequately compare for these differences, as the controls would be different.

The authors cited in their clarification the SchÃ¼ttler et al. (2017) manuscript. This article does a good job about discussing the limitations of comparing methods in zebrafish embryo transcriptomic studies. I think the current manuscript would benefit from a larger discussion of the previous article or otherwise a greater discussion of the problems associated with comparing across differently designed studies. The comment, "Additionally, co-expression of genes is not necessarily consistent across different perturbations [68]. This would not be captured in the dynamic toxicogenomic fingerprints as shown here." needs to be expanded and more explicit.

Response 2.3: I can agree with the assumption that a slower development would not influence the toxicogenomic responses within a single study. I do think it limits comparisons of multiple studies at different incubation temperatures because the response to a chemical over time would not proceed at the same rate, due to the difference in rate of development. This can be addressed along with the previous comment, but I do think this is an important limitation of the study that needs to be mentioned in greater detail.

Response 3.4: The authors added to the manuscript the statement, "For example, toxnodes showing a biphasic response on the concentration scale would not be accurately captured." I think this is an important point and think some examples would be beneficial. For example, endocrine disrupting compounds typically have non-monotonic dose responses and a statement to this effect could be

added.
I really think it is important to highlight limitations, not to diminish the accomplishments of the research, but to add to its practicality and self-awareness.

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