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Veterinary Medicine & Biomedical Sciences

Concentration-response modeling of in vitro bioactivity data from complex mixtures of priority Superfund compounds

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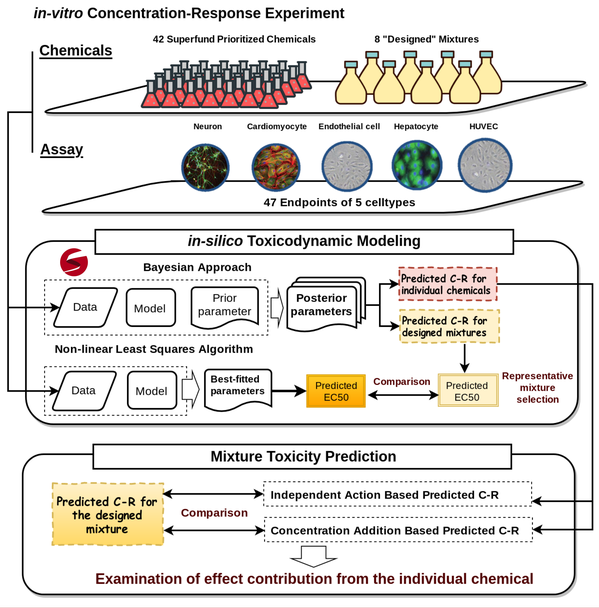
INTRODUCTION

- Environmental chemicals at Superfund sites are composed of diverse compounds that include heavy metals, pesticides, industrial chemicals, polycyclic aromatic hydrocarbons, and plasticizers.
- Traditionally, most exposure-effect studies focus on the adverse effects of a single chemical or a mixture with few compounds. The approach might not reflect the "real-world" exposure scenario that contains dozen of pollutants and can cause additive or synergistic effects of human health.
- The high throughput screening-based toxicity testing with *in-silico* approach provides the opportunity to more fully examine the biological responses from complex mixtures.

WORKFLOW



METHODS

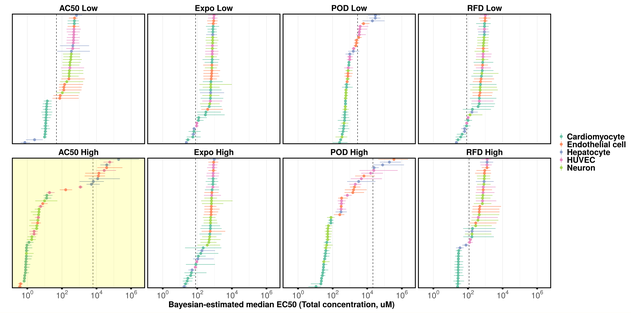
- At first, the sigmoidal dose-response model that derived from Fréchet distribution function (also known as Weibull distribution) was used to quantify concentration-response profiles from individual chemicals and mixture [1].
- The traditional Marquardt–Levenberg non-linear least-squares (NLS) algorithm [2], as well as Bayesian approaches [3], were used. Two algorithms were compared to determine the better method to describe the concentration-response properties [4].
- Making the predictions as to mixture toxicity under the assumptions of independent action (IA) and concentration addition (CA), and compared the predictions with mixture concentration-response data [5].
- Finally, we investigated the effect of contributions from the individual chemicals in the designed mixture.

The study objects include:

- **To examine the robustness of the probabilistic approach in quantifying the bioassay concentration-response profile.**
- **To address the property of toxicity from the designed mixture that was composed of dozens of chemicals.**
- **To identify the contribution of bioactivity responses from individual chemicals and conduct the prioritization.**

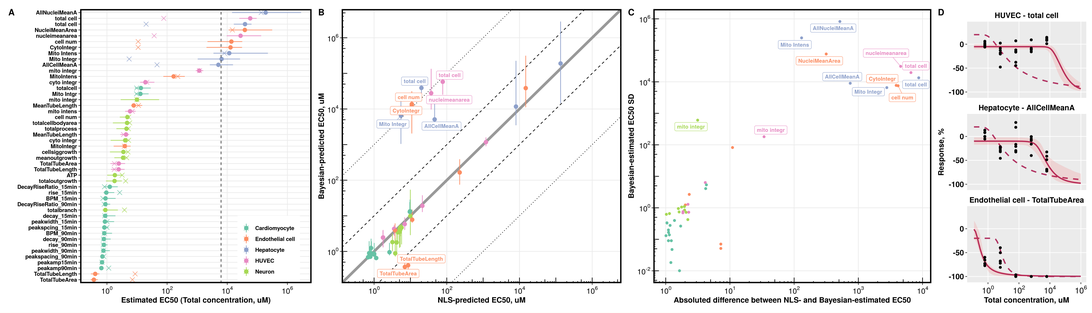
RESULTS

Bayesian curve fitting-estimated EC50 for designed mixtures



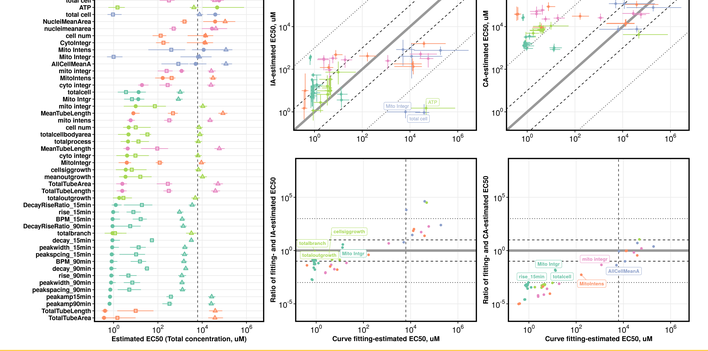
The Bayesian-estimated EC50 with the 95% confidence interval of designed mixtures. The dashed line is the designed maximum concentration for each mixture. The different phenotypes in the specific cell type are displayed by the same color. The mixture designed by the maximum AC50 (yellow highlight) was selected to conduct the following analysis due to the lower estimation of EC50 (high toxicity) compare with other mixtures.

Comparison of Bayesian and NLS approach

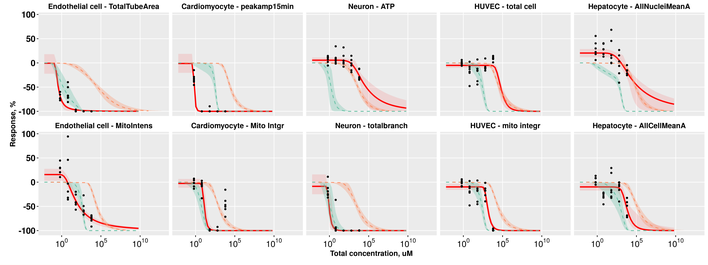


(**A**) Comparative analysis of estimated EC50 from Bayesian and non-linear least-squares approach. The solid circle is the estimated median EC50 (with 95% confidence interval). The cross represents the nonlinear least-squares-estimated EC50. (**B**) The association between Bayesian and NLS-estimated EC50. The dashed lines represent the ratio of curve-fitted and predicted median EC50 equal to 10 and 100, respectively. (**C**) The constructed relationship between the estimated standard deviation of EC50 from the Bayesian approach and the calculated difference with NLS-estimated EC50. The example of the concentration-repose profiles from the phenotypes that had higher differences in estimated EC50.

Comparison of predicted mixture toxicity

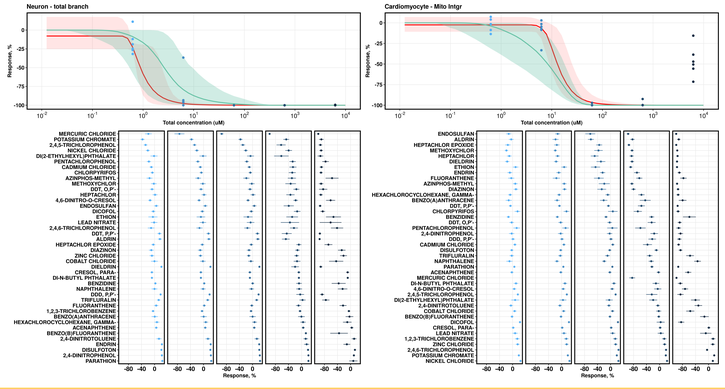


(**A**)The comparison of mixture toxicity properties. The square is the independent action (IA)-estimated median EC50 with 95% confidence interval. The triangle is the concentration addition (CA)-estimated median EC50 with 95% confidence interval. The comparison and calculated ratio of (**B**) IA and (**C**) CA-estimated EC50, which are used to determine the accuracy of prediction. The dashed lines represent the ratio of curve-fitted and predicted median EC50 equal to 10 and 1000W, respectively.

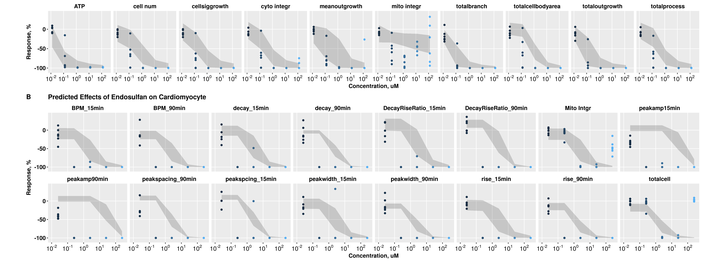


The example of concentration-response profiles based on the IA-predicted EC50 that had (top panel) different and (bottom panel) similar estimations. The red, green, and orange lines (shadows) are curve-fitting, IA-predicted, and CA-predicted median (with 95% confidence interval), respectively. The neuron cell and cardiomyocyte have similar concentration-response profile with curve-fitting results.

Effect contribution from the individual chemicals



(Top) The example of concentration-response profiles based IA-predicted EC50 that have relative lower fitting/predicted ratio of 2. The red and green lines/shadows are curve-fitting and IA-predicted median/95% confidence interval, respectively. (Bottom) The exposure based corresponding effects from individual chemical. The chemicals are ordered from top to bottom to represent the most to less toxicity. The mercury chloride and endosulfan are the most toxicity chemical for neuron cell and cardiomyocyte, respectively



The predicted concentration-response profiles (polygon) for the representative chemical, (**A**) mercury chloride and (**B**) endosulfan, which dominate the mixture toxicity for neuron cell and cardiomyocyte under the mode of independent action, respectively. The solid points are mixture bioassay response data with corresponding concentration from selected chemical to compare the effect from single chemical and mixture.

SUMMARY

- **"Whole mixture" testing can a much more realistic indication of the potential hazards. Such testing is possible with high throughput *in vitro* model systems.**
- **This study took advantage of the *in vitro* bioactivity data on both the individual chemicals and their "designed mixtures" to examine whether traditional paradigms of concentration addition or independent action are reasonable first approximations for the mixtures, in some cases mixture bioactivity is "greater than the sum of its parts."**
- **Compare with the traditional non-linear least square method, the Bayesian approach can provide informative and reliable predicted results.**
- **Compare with concentration addition in toxicity prediction, we find that the independent action assumption (using data on the individual chemicals) was the most reasonable approximation for the data on designed mixtures of diverse compounds; however, testing of the whole mixture may be needed to increase precision of hazard identification.**
- **Because the bioactivity of the designed mixtures were more similar to independent action assumption, it can be concluded that the effects of the mixtures were "likely" dominated by the effects of a few chemicals. The information on which chemicals were "drivers" of the effects of the whole mixture is important for ultimate risk assessment.**

ACKNOWLEDGEMENTS

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