Applying a Tiered Risk-based Approach to Prioritizing Thousands of Chemicals for Further Evaluation: A Comparison of Current High Throughput Computational Approaches

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INTRODUCTION

There is a need to prioritize thousands of environmental chemicals for further testing with the help of current computational technologies. Here we present a case study on applying a risk-based approach to chemical prioritization based on an initial triage by ranking the ratios between ExpoCast[1] high-throughput exposure estimates and Thresholds of Toxicological Concern (TTCs)[2]. To demonstrate the applicability of TTCs for the initial triage, 358 ToxCast[3] chemicals were processed as follows: 1) oral equivalent doses (OEDs)[4] were calculated based on ToxCast bioactivity measurements and available metabolism data for estimating in vivo clearance, 2) TTC values were determined using the Cramer[1] classification system, 3) OEDs and TTCs were then compared with available ExpoCast exposure estimates to determine their respective activity:exposure ratios (AERs). TTCs were lower than OEDs for 349 (97%) of the evaluated ToxCast compounds, implying that TTCs can serve as a conservative estimate of hazard in the absence of chemical-specific data. The TTC approach was then applied to a curated dataset of ~45,000 chemicals. In order to ground-truth the results, we curated an ensemble of compounds with established points of departure (e.g., no observed adverse effect levels (NOAELs)). TTC values were lower than NOAELs for all 128 compounds that overlap with the chemical database and have published NOAEL values based on a daily oral exposure. This study demonstrated the utility of exploiting computational approaches as part of a tiered risk-based approach to prioritize thousands of chemicals. ***This abstract does not necessarily reflect U.S. EPA policy.***

OBJECTIVES

1. Investigate whether TTC-based chemical prioritization approach is relevant for environmental chemicals of interest.
2. Compare TTC-based chemical prioritization to ToxCast OED-based chemical prioritization.
3. Ground-truth prioritization results by comparing available regulatory points of

METHODS

- OEDs: The httk R package[5] was used to generate 10th percentile OEDs based on a distribution of AC50 values for each active ToxCast[1] compound that also had toxicokinetic data[4] needed for doing *in vitro* to *in vivo* extrapolation (HT-IVIVE).
- TTCs: The ToxTree (v2.6.13)[6] application was used in batch mode to compute Cramer[3] TTC classifications
- ExpoCast Exposures: A 2nd generation of the systematic empirical evaluation of models (SEEM2)[1] predictor was used to estimate 95th exposure estimates.
- Margin of Exposures (MOEs): MoEs were calculated by dividing the OEDs in mg/kg/d by the SEEM2 exposure estimates in mg/kg/d.
- Ground-truthing: U. S. EPA IRIS[7] no-observed adverse effect levels (NOAELs) were compared against ToxCast OEDs.

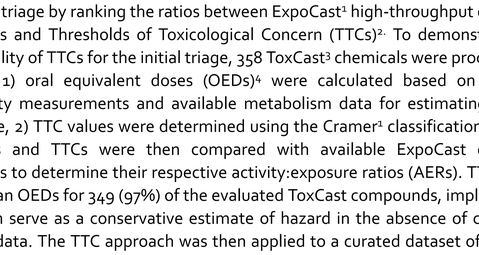

Figure 1. Concept map for exposure case study scope and design.

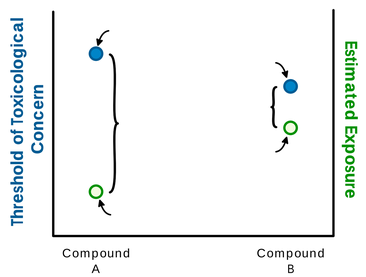

Figure 2. Depiction of the margin of exposure concept, which is the ratio of the Threshold of Toxicological Concern and the estimated exposure. Compound B is prioritized greater than Compound A due to its smaller relative margin of exposure.

RESULTS AND DISCUSSION

As the Cramer TTC method was based primarily on the toxicity of food additives[8] rather than environmental contaminants, here we demonstrate whether existing TTCs are useful for environmental chemicals that lack data for informing risk-based prioritization decisions. TTC's may be useful as a high-throughput risk-prioritization approach if it is more protective than existing lower throughput approaches such as those that require reverse toxicokinetic data or *in vivo* experimentation.

OEDs and Exposures vs. TTC Class for 360 Chemicals that HT-IVIVE data

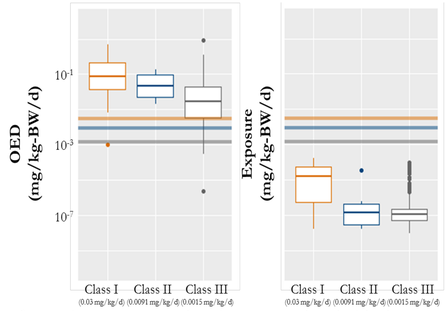

Figure 3. Left panel: comparison of TTC category thresholds (horizontal lines) with Oral Equivalent Doses for 360 ToxCast chemicals[1] that have the necessary metabolism data to support HT-IVIVE2 using the NCCT 'httk' R package (boxes and whiskers). Right panel: comparison of TTC category thresholds (lines) with estimated exposures using USEPA SEEM2 for the same compounds (boxes and whiskers). Chemicals are grouped by TTC class I (orange), II (blue), and III (grey). In every case the TTC category threshold provides a conservative estimate of a dose below the OEDs calculated for that category, and the ratio between the TTC and the median OED for each category is similar.

AER Plot for Top 50 Ranked ToxCast Chemicals with HT-IVIVE data

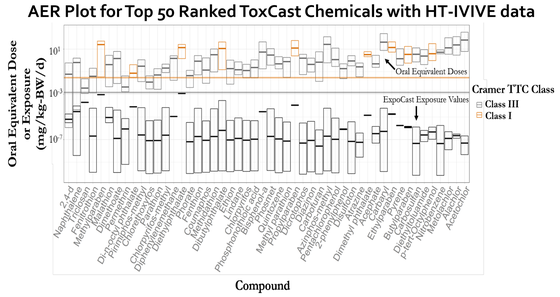

Figure 4. Ratio of exposure estimates (ExpoCast SEEM2) in black vs oral equivalence doses (OEDs) for 50 ToxCast compounds with HT-IVIVE data in grey and orange crossbars. Grey and orange lines refer to the Cramer TTC classifications: Class III (0.0015 mg/kg/d) and Class I (0.03 mg/kg/d), respectively. The upper end of the box corresponds to the OED for the 95th percentile AC50 while the lower end corresponds to the OED for the 5th percentile AC50. The exposure box plots represent the upper and lower bounds on the 95% confidence interval for the median, with a crossbar at the median. OEDs and their corresponding exposure estimates are ordered based on their ratio. Some compound names were truncated for display.

MoE's for Top 50 Ranked ToxCast Chemicals with HT-IVIVE by TTC Class

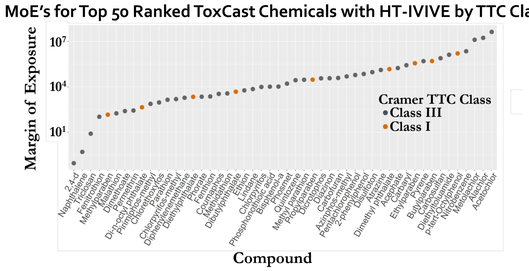

Figure 5. Margin of exposures (MoEs) plot for 50 ToxCast compounds with HT-IVIVE data in grey and orange dots (Cramer TTC Class III and Class I, respectively). MoEs were calculated by dividing the 10th percentile AC50-derived OEDs in mg/kg/d by the upper 95% bounds on the ExpoCast SEEM2 exposure estimates in mg/kg/d. Some compound names were truncated for display.

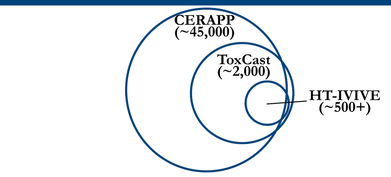

Figure 6. Cartoon representation of dataset overlap: while there is a growing database of ToxCast compounds with OEDs derived from HT-IVIVE, they are a small subset (~1%) of the 45,000 relevant environmental compounds in the CERAPP[9] database.

SEEM2 Exposure Distributions Across TTC Class for 45,000 Chemicals

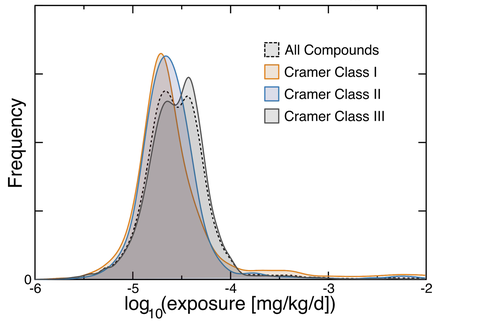

Figure 7. In order to determine spaces where the TTC-based margin of exposure approach appears to be most effective, the distribution of 95th percentile SEEM2 exposure estimates was plotted for 45,000 CERAPP compounds binned as Cramer TTC Class I (orange), Class II (blue), Class III (grey), and all compounds (dotted line).

Breakdown of TTC-Based Prioritization Approach for 45,000 Chemicals

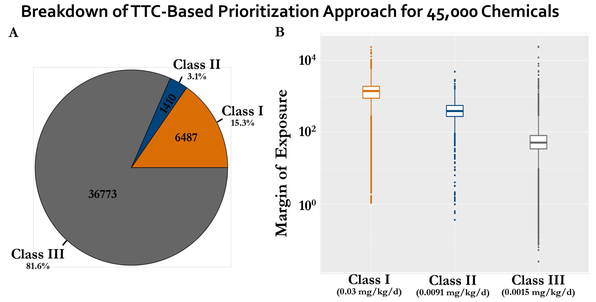

Figure 8. A) Breakdown of the 45,000 CERAPP compounds by their Cramer TTC Class and B) Distribution of Cramer TTC classes across their corresponding margins of exposure computed by dividing TTC values by the estimated SEEM2 exposure estimates for 45,000 CERAPP compounds

MoE's for Top 50 Ranked Chemicals by TTC Class

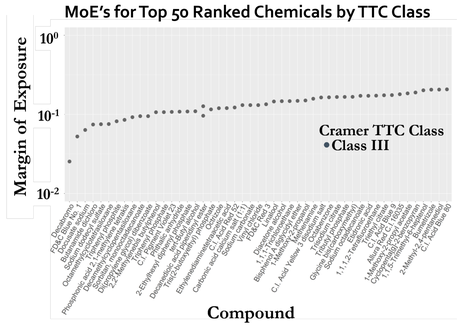

Figure 9. Margin of exposures (MoEs) for top 50 ranking CERAPP compounds in grey dots (Cramer TTC Class III). 24 of these are ToxCast compounds, 3 have data necessary for HT-IVIVE, and 0 are ToxCast active compounds that also have data for employing HT-IVIVE. MoEs were calculated by dividing the Class 3 TTC (0.0015 mg/kg/d) by the upper 95% bounds on the ExpoCast SEEM2 exposure estimates in mg/kg/d. Some compound names were truncated for display.

The breakdown of CERAPP compounds per Cramer class was roughly 82% (36773), 3% (1410), and 15% (6847), for Class III, Class II, and Class I, respectively (Figure 7a). Median margin of exposures for the three classes was 51.2, 392.1, and 1409.1 respectively (Figure 7b). Roughly 1000 chemicals have TTC-derived MoE of less than 10, while 164 have a margin of exposure of less than 1. These results are consistent with the a previous study[10] comparing Cramer classes across compounds with biomonitoring data from the National Health and Nutrition Examination Survey (NHANES)[11]; 66% of NHANES compounds are in Cramer Class III. Furthermore, this study also illustrated that MoEs of less than 1 are feasible (58 compounds). Overall, TTC's were more protective than 349 (97%) ToxCast OEDs. TTC's were more protective than all 128 CERAPP compounds that have IRIS NOAEL values based on an oral exposure. Out of these 128 chemicals, 57 of them also have ToxCast OEDs and TTC's were protective for 47 out of 57. Out of the 360 ToxCast OEDs that were evaluated, 58 of them had corresponding NOAEL values based on an oral exposure and 48 (83%) were more protective than NOAELs.

CONCLUSION & FUTURE WORK

We demonstrated that TTC values may be useful as a conservative proxy for toxicity for thousands of compounds for which neither *in vivo* nor *in vitro* data exists. Over 45,000 CERAPP compounds now have computed margins of exposure (MoE). In the future, this approach can be used to inform a read-across approach where we prioritize chemical spaces that may be amenable to read-across, based on an analogue selection procedure that considers a diverse set of descriptors. 45,000 environmentally relevant compounds that were binned into 17 chemical families (eg., dioxins, carbamates, phthalates), will be prioritized for read-across suitability. We will employ machine learning and supervised selection of chemical analogues, which may partially depend on their potentially active metabolites, for endpoint specific read-across. Also, we plan to incorporate the use of other exposure tools that feature comprehensive use scenario evaluations, which can be utilized to further demonstrate the use of a tiered approach for risk prioritization: 1) High-throughput Stochastic Human Exposure Dose Simulation model (SHEDS-HT), 2) Consumer Exposure Model (CEM), and 3) TRA ConsExpo.

REFERENCES

1. J. F. Wambaugh, et al., Environ. Sci. Technol., 2014, 48, 12760-12767.
2. G. M. Cramer, Ford R., Hall, R. L., Food. Cosmet. Toxicol., 1978, 16, 255-276.
3. R. Judson, et al., Basic Clin. Pharmacol. Toxicol., 2014, 115, 69-76.
4. B. A. Wetmore, et al., Toxicol. Sci., 2015, 148, 121-136.
5. J. F. Wambaugh, et al., CRAN, 2015
6. G. Patlewicz, et al., SAR QSAR Environ Res., 2008, 19(5-6):495-524.
7. U.S. EPA Integrated Risk Information System (IRIS), https://www.epa.gov/iris, Accessed June 25, 2017.
8. Kroes et al., Toxicol. Lett., 2002, 127(1-3):43-6
9. K. Mansouri, et al., J. Cheminform., 2017 Just Accepted, DOI: 10.1186/s13321-018-0263-1.
10. R. A. Becker, et al., Use of Threshold of Toxicological Concern (TTC) with High Throughput Exposure Predictions as a Risk-Based Screening Approach to Prioritize More Than Seven Thousand Chemicals, In preparation.
11. CDC (Centers for Disease Control and Prevention). Environmental and Related Chemicals Measured in Blood, Serum or Urine in NHANES., http://www.cdc.gov/nchs/data/nhanes/nhanes_03_04/environmentalhealth_03.pdf Accessed May 8, 2017.

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