Evaluation of the utility of ToxCast HTS and high-throughput toxicokinetic data for food chemical safety risk assessment via comparison with *in vivo* animal data.

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Abstract

New approach methodologies (NAMs) are currently being developed and evaluated for use in chemical safety risk assessment, including chemicals used in food. NAMs include in vitro high-throughput screening (HTS) assays, such as the ToxCast and Tox21 assays. The ToxCast/Tox21 assays have been run for thousands of compounds, including hundreds of compounds used in food. However, the relationship of these NAM data with traditional in vivo animal data, and the utility of NAMs for risk assessment, remain under evaluation. The goal of the present study is to evaluate the utility of ToxCast/Tox21 HTS data in food safety risk assessment. To do this, bioactive concentrations of a subset of food-use compounds in ToxCast were converted to oral equivalent doses (OEDs) via in-vitro to in-vivo extrapolation (IVIVE) using either in vitro or in silico-based toxicokinetic parameters for a subset of food-use compounds. These OEDs were then compared to doses demonstrated to cause effects in in vivo animal tests (using data compiled by EPA and FDA). Initial comparisons demonstrated great variability in the correlation between ToxCast and in vivo data, so steps are being taken to further refine the toxicokinetic information, chemical groups, and in vivo endpoints in an effort to identify additional information and conditions necessary to utilize HTS data for preliminary food safety assessment. This work does not reflect the official policy of the US EPA or the US FDA.

Introduction

- The development and implementation of NAMs in food and chemical risk assessment is an ongoing goal in toxicology.
- High-throughput screening data have been generated for a large number of compounds through the ToxCast/Tox21 project, including several food-use chemicals.
- Use of these HTS data in food chemical safety risk assessment remains under evaluation.
- Ongoing work is being done to relate concentrations in HTS assays to doses given orally in animal studies by *in vitro* to *in vivo* extrapolation (IVIVE).
- Work done by Friedman *et. al*. (2019) determined administered equivalent doses (AEDs) for 448 ToxCast compounds using the high-throughput toxicokinetics (HTTK) package for the IVIVE, and did a screening level comparison to *in vivo* animal data[1].
- The present study builds on these data, with the goal of evaluating the utility of ToxCast/Tox21 HTS data in food safety risk assessment.

Acknowledgements

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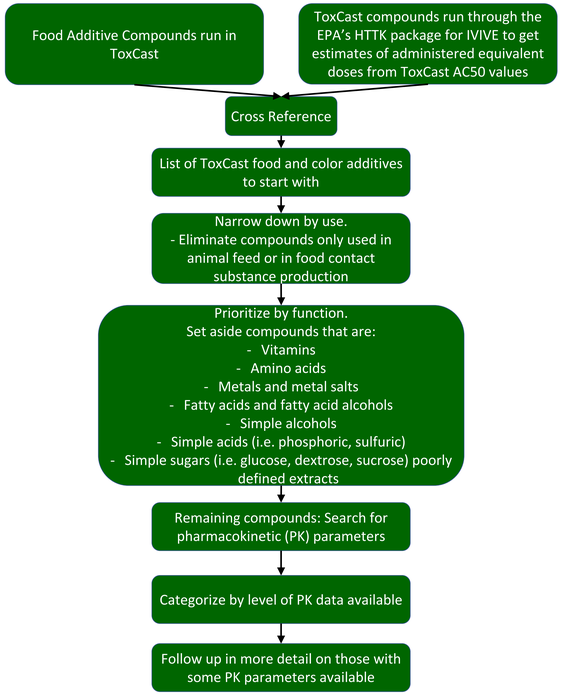
References

1. Friedman, K.P. Et. Al. Toxicol Sci. 2019 Sep 18

Materials and Methods



Results

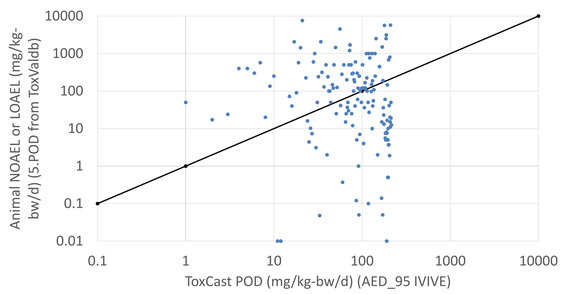


Figure 1. Comparison of ToxCast AEDs with in vivo animal data values for all initially identified compounds.
The active ToxCast assays for each compound were filtered based on curve-fitting caution flag and uncertainty information, and the AC50 values remaining were classified into percentiles for each compound. The 5th percentile AC50 value for each compound was converted to an administered equivalent dose (AED) using the HTTK package, and plotted against the lowest dose reported in *in vivo* animals studies in the ToxVal database. The black line delineates the 1:1 identity line.

Results and Discussion

Table 1. Criteria for Pharmacokinetic Data Classification

Determination	Criteria
No	Lack of data OR unsuitable for in vitro comparison (compound completely transformed before absorption in the GI tract)
Maybe	Some PK data, with potential issues
Yes	Some PK data available to use

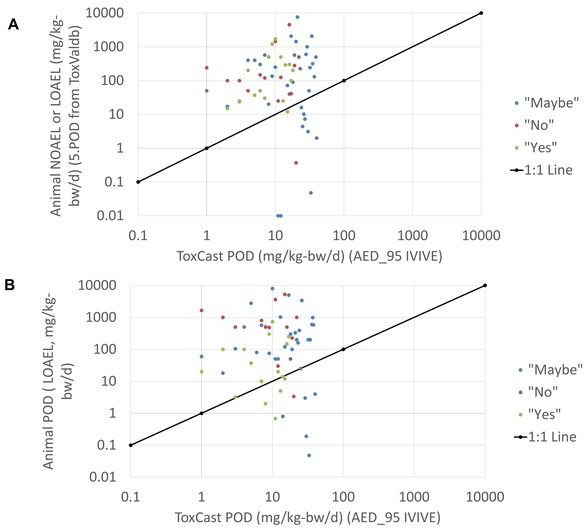


Figure 2. Comparison of ToxCast AEDs with in vivo animal data values for prioritized compounds.
The 5th percentile filtered AC50 values from ToxCast for each compound (as determined in Figure 1) were converted to administered equivalent doses using the HTTK package, and plotted against the lowest value reported for *in vivo* animals studies in the ToxVal database(A), or against the lowest low effect level in animals from the CompTox Dashboard in (B). Black line delineates the 1:1 identity line. Compounds are divided into those with no PK or are unsuitable for comparison ("no"), those that have potentially some PK data ('maybe"), and those with some level of PK data available for use ("yes")

Table 2. ToxCast data for 18 compounds selected for more detailed analyses

Compound	CASRN	ToxCast Assays run	ToxCast assays active	ToxCast 5th % AC50 (µM)
Styrene	100-42-5	211	1	100.0000
Cyclohexylamine	108-91-8	639	6	0.0016
Butylated hydroxytoluene	128-37-0	401	61	7.8826
Sodium saccharin	128-44-9	211	1	69.8740
Estragole	140-67-0	427	5	21.2958
Butylated hydroxyanisole	25013-16-5	211	22	17.0508
Etidronic acid	2809-21-4	211	5	65.9696
Sodium benzoate	532-32-1	670	1	100.0000
Glycerol	56-81-5	669	17	0.0028
1,2-Propylene glycol	57-55-6	640	12	0.0334
Caffeine	58-08-2	676	53	1.4245
Sodium nitrate	7631-99-4	210	0	100.0000
Sodium nitrite	7632-00-0	638	4	2.5073
Potassium nitrate	7757-79-1	427	7	6.5230
Saccharin	81-07-2	428	4	1.22E-05
Propylparaben	94-13-3	719	99	7.4093
Eugenol	97-53-0	696	28	0.1320
Methylparaben	99-76-3	690	23	0.1215

Table 3. Initial values and pharmacokinetic data available for the 18 compounds selected for further analyses

Compound	CASRN	Use	Initial ToxCast AED (mg/kg-bw/d)	Initial in vivo animal effect level (mg/kg-bw/d)	PK refinement
Styrene	100-42-5	Polymer production	4.2277	0	PBPK model
Cyclohexylamine	108-91-8	Boiler water additive	0.0001	15	Reported PK parameters in the literature
Butylated hydroxytoluene	128-37-0	Preservative	0.0118	25	Reported PK parameters in the literature
Sodium saccharin	128-44-9	Sweetner	2.4606	200	Reported PK parameters in the literature
Estragole	140-67-0	Flavor	1.9242	37	PBPK model, some human data
Butylated hydroxyanisole	25013-16-5	Preservative	1.1452	50	Reported PK parameters in the literature
Etidronic acid	2809-21-4	Boiler water additive, sanitizer	3.2042	30	Some reported PK parameters in the literature
Sodium benzoate	532-32-1	Preservative	1.3959	500	Some reported human PK parameters
Glycerol	56-81-5	Multiple	8.47E-05	1200	Reported PK parameters in the literature
1,2-Propylene glycol	57-55-6	Multiple (incl. antioxidant, flavor, stabilizer, solvent, humectant)	6.64E-04	1700	Reported PK parameters in the literature
Caffeine	58-08-2	Additive	0.1856	0	Reported PK parameters in the literature
Sodium nitrate	7631-99-4	Preservative	0.2244	500	PBPK model (based on nitrate ion)
Sodium nitrite	7632-00-0	Preservative	0.0220	25	PBPK model (based on nitrite ion)
Potassium nitrate	7757-79-1	Preservative	0.0164	290	PBPK model (based on nitrate ion)
Saccharin	81-07-2	Sweetner	3.96E-07	200	Reported PK parameters in the literature
Propylparaben	94-13-3	Preservative, antimicrobial, flavor	0.8345	12	Reported PK parameters in the literature
Eugenol	97-53-0	Flavor	0.1140	300	Reported PK parameters in the literature
Methylparaben	99-76-3	Antimicrobial, flavor	0.0437	100	Reported PK parameters in the literature

Discussion

- On a first pass through the compounds, the ToxCast AED is often lower than the *in vivo* point of departure from animal studies, but not for all compounds.
- Many compounds run in the ToxCast assays are difficult to directly compare to *in vivo* animal data, for a variety of reasons, including things such metabolism or reactivity of the parent compound, compound volatility, and type of compound such that the compound is a vitamin, amino acid, or other component of normal metabolism in the body, among others.
- Results from these 18 prioritized chemicals can be used to help interpret the results of other chemicals in ToxCast.

Future Directions

- Use PK parameters identified in the literature to refine the IVIVE AEDs (and compare).
- Curate *in vivo* animal data to compare to studies used to make regulatory decisions.

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