Comparison of ToxCast HTS and high-throughput toxicokinetic data with in vivo animal data to evaluate the utility in food chemical safety assessment

Alexandra E. Turley[1], Janet Zang[1], Katie Paul Friedman[2], Richard S. Judson[2], Suzanne C. Fitzpatrick[1]

[1]Center for Food Safety and Applied Nutrition, U.S. Food and Drug Administration [2]National Center for Computational Toxicology, U.S. Environmental Protection Agency

Abstract

New toxicological approaches and technologies are currently being developed and evaluated for use in chemical safety assessment. These technologies encompass a variety of methods, including in vitro high-throughput screening (HTS) used in the ToxCast/Tox21 programs. The relationship between data from these new approach methodologies (NAMS) and traditional in vivo animal toxicology data, as well as the utility of these assays in risk assessment situations, is still under evaluation. HTS assay data has been used to prioritize chemicals for further analysis, however, the use of these assays in food chemical safety risk assessment has not been determined. Many compounds used directly or indirectly in foods have been run in these HTS assays, and the goal of this study is to evaluate the utility of the ToxCast/Tox21 HTS data in food safety assessment. To do this, concentrations demonstrating bioactivity in the ToxCast assays for a group of food-use compounds were identified and converted into oral administered equivalent doses (AEDs) via in-vitro to in-vivo extrapolation (IVIVE) using the US EPA HTTK package with in vitro- or in silico- based toxicokinetic parameters. These AEDs were then compared to low-observed effect levels or low-observed adverse effect levels reported in in vivo animal studies, initially using values from the ToxValDB. The initial comparison showed great variability between the ToxCast and in vivo datasets. From the initial list of 216 compounds, a subset of 18 compounds were identified for further investigation to refine both the in vitro and in vivo estimates of activity based on the presence of published pharmacokinetic data. For these 18 compounds, the ToxCast AEDs were generally roughly equivalent to or lower than the doses demonstrating effects in vivo, though the magnitude of the difference between the two estimates varied greatly, spanning 6 orders of magnitude with a median difference of roughly fifty-fold. 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

The authors would like to thank Patra Volarath for her helpful discussion on this project.

References

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

Materials and Methods

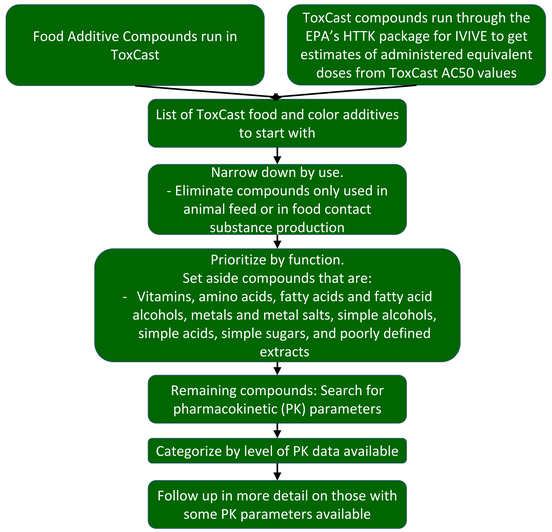

Table 1. Criteria for Pharmacokinetic Data Classification

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

Results

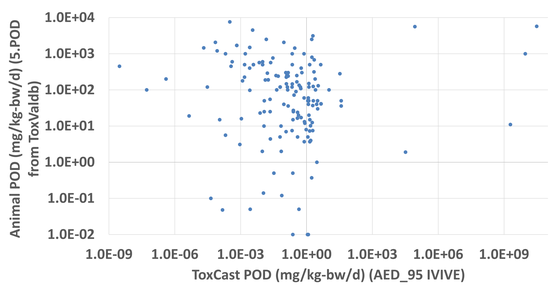

Figure 1. Comparison of ToxCast AEDs with in vivo animal data values for all initially identified compounds. The active ToxCast assays for each compound were filtered based on curve-fitting caution flag and uncertainty information, and the AC50 values remaining were classified into percentiles for each 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.

Results and Discussion

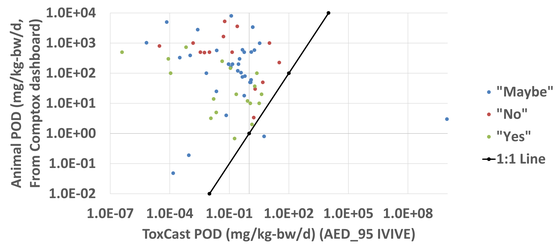

Figure 2. Comparison of ToxCast AEDs with in vivo animal data values for prioritized compounds. The 5th percentile filtered AC50 values from ToxCast for each compound (as determined in Figure 1) were converted to administered equivalent doses using the HTTK package, and plotted against the lowest low effect level in animals from the CompTox Dashboard in. 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. Initial values and chemical properties for the 18 compounds selected for further analyses. * indicates a predicted value as opposed to an experimentally determined value.

Compound	CASRN	Initial ToxCast AED (mg/kg-bw/d)	Initial in vivo animal effect level (mg/kg-bw/d)	Molecular weight (g/mol)	LogP	Original HTTK method
Styrene	100-42-5	4.22769	20	104.152	2.9	in silico
Cyclohexylamine	108-91-8	0.00011	100	99.177	1.5	in silico
Butylated hydroxytoluene	128-37-0	0.01179	3.2	220.356	5.1	in silico
Sodium saccharin	128-44-9	2.46061	100	205.16	0.37*	in silico
Estragole	140-67-0	1.92424	37	148.205	3.14*	in silico
Butylated hydroxyanisole	25013-16-5	1.14517	10	180.25	3*	in silico
Etidronic acid	2809-21-4	3.20425	10	206.027	-2.05*	in silico
Sodium benzoate	532-32-1	1.39588	2	144.105	1*	in silico
Glycerol	56-81-5	0.00008	300	92.094	-1.76	in silico
1,2-Propylene glycol	57-55-6	0.00066	730	76.095	-0.092	in silico
Caffeine	58-08-2	0.18556	0.68	194.194	-0.07	in vitro
Sodium nitrate	7631-99-4	0.22442	20	84.994	-0.79*	in silico
Sodium nitrite	7632-00-0	0.02198	5	68.995	-2.37*	in silico
Potassium nitrate	7757-79-1	0.01635	14	101.102	-0.79*	in silico
Saccharin	81-07-2	3.95873E-07	500	183.18	0.91	in silico
Propylparaben	94-13-3	0.83448	12	180.203	3	in vitro
Eugenol	97-53-0	0.11403	147.9	164.204	2.27	in vitro
Methylparaben	99-76-3	0.04370	250	152.149	1.96	in vitro

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.

This work does not reflect the official policy of the US EPA or the US FDA.

Table 3. ToxCast data for 18 compounds selected for more detailed analyses

Compound	CASRN	ToxCast Assays run	ToxCast assays active, initial	ToxCast assays active after manual curation
Styrene	100-42-5	211	1	0
Cyclohexylamine	108-91-8	639	6	0
Butylated hydroxytoluene	128-37-0	401	61	12
Sodium saccharin	128-44-9	211	1	0
Estragole	140-67-0	427	5	3
Butylated hydroxyanisole	25013-16-5	211	22	6
Etidronic acid	2809-21-4	211	5	0
Sodium benzoate	532-32-1	670	1	0
Glycerol	56-81-5	669	17	0
1,2-Propylene glycol	57-55-6	640	12	4
Caffeine	58-08-2	676	53	38
Sodium nitrate	7631-99-4	210	0	0
Sodium nitrite	7632-00-0	638	4	1
Potassium nitrate	7757-79-1	427	7	3
Saccharin	81-07-2	428	4	3
Propylparaben	94-13-3	719	99	57
Eugenol	97-53-0	696	28	16
Methylparaben	99-76-3	690	23	8

Example: Butylated Hydroxytoluene

In vivo animal data:

- Effects reported in short term and subchronic assays are changes in liver weights and liver enzymes, and changes in thyroid weights
- 90 day rat LOAEL of 25 mg/kg-bw/d

Table 4. Details on the active ToxCast assays remaining after manual curation to for BHT

Assay Name	Intended Target	Gene name/Assay Target	AC50 Value (µM)
ATG_RXRb_TRANS_up	nuclear receptor	retinoid X receptor, beta	0.841
NCCT_TPO_AUR_dn	oxidoreductase	thyroid peroxidase	2.38
ATG_RXRa_TRANS_up	nuclear receptor	retinoid X receptor, alpha	14.1
TOX21_RXR_BLA_Agonist_ratio	DNA binding	retinoid X receptor, alpha	14.8
ATG_DR5_CIS_up	nuclear receptor	retinoic acid receptor, beta	17
ATG_PXR_TRANS_up	nuclear receptor	Pregnane X Receptor	20.6
TOX21_RT_HEK293_FLO_40hr_viability	cell cycle	Viability	24.5
NCCT_HEK293T_CellTiterGLO	cell cycle	Viability	25.7
TOX21_RXR_BLA_Agonist_ch2	background measurement	Background	30.1
OT_AR_ARSRC1_0960	nuclear receptor	androgen receptor	37.4
TOX21_MMP_ratio_down	cell morphology	Viability	43.9
TOX21_MMP_rhodamine	background measurement	Background	53.3

Discussion

- AECs derived from the ToxCast data are generally more sensitive than *in vivo* animal effect levels, with a large degree of variability in the margin between the two
- Generation of toxicokinetic data on compounds for more accurate IVIVE will likely help with this variability.
- Many compounds run in the ToxCast assays are difficult to directly compare to *in vivo* animal data, for a variety of reasons. These include: 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 other reasons.
- Effects noted in animals can be difficult to correlate to effects seen in *in vitro* assays.
- Results from the 18 prioritized chemicals can be used to help interpret the results of other chemicals in ToxCast.