Use of *In Vitro* to *In Vivo* Extrapolation (IVIVE) for Estimating Preliminary Margin of Exposure of Flavor Compounds via Oral Intake

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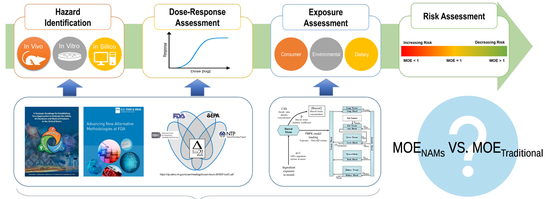
Society of Toxicology 61st Annual Meeting and ToxExpo
March 27-31, 2022
Abstract ID: 3889/P566

Introduction

- Traditional risk assessments usually utilize levels of toxicological concern derived from *in vivo* testing (e.g., JECFA ADI, Cramer Class) or acceptable regulatory exposure limits (e.g., FEMA PADI).
- New approach methodologies (NAMs), such as *in vitro* and *in silico* methods, may offer novel approaches for chemical risk assessment without additional *in vivo* testing.
- In this study, we used publicly available in vitro bioactivity data (ToxCast [1,2]/ Tox21 [3]) and generic pharmacokinetic models (httk packages for R) to conduct a preliminary risk assessment of selected flavor ingredients used in oral tobacco products.
- The resulting margin of exposure (MOE_{NAMs}) estimates were compared to the traditional MOE ($MOE_{traditional}$) as a preliminary evaluation.



NAMs (New Approach Methodologies): *in vitro* and *in silico*

Summary

- The study demonstrated the feasibility of using *in vitro* bioactivity data and PBPK models for preliminary risk assessment of oral ingredients. The median MOEs of selected 25 flavor ingredients between the NAM-based and traditional method differed by up to 2 orders of magnitude but were overall within the comparable range.
- Limitations and gaps were identified for future considerations.
  - *In vitro* assays and their biological responses: In this study, we limited the selection of *in vitro* assays to those publicly available (e.g., Tox21/ToxCast). Also, the median of EADs based on plasma C_{max} was mostly between 1 and 100 mg/kg BW/day, possibly due to the assay type and the dose range tested in the cHTS database. Therefore, if the selected assays do not represent the mechanisms related to the adverse outcome, experimental assays may be necessary.
  - Available exposure limit and uncertainty factors: The results showed a wide variation of MOE_{NAMs} among 25 example ingredients, which mainly reflected the impact of different possible average exposure level estimated for oral ingredients (i.e., PADI). Uncertainty factors (UFs) are usually considered in traditional risk assessment. However, in the NAM-based approach the appropriateness of applying UFs has not been defined.

References

1. Dix, D.J., Houck, K.A., Martin, M.T., Richard, A.M., Setzer, R.W., Kavlock, R.J.(2007) The ToxCast program for prioritizing toxicity testing of environmental chemicals. *Toxicol Sci.* 95(1):5-12. doi: 10.1093/toxsci/kfl103
2. Judson, R.S., Houck, K.A., Kavlock, R.J., Knudsen, T.B., Martin, M.T., Mortensen, H.M., Reif, D.M., Rotroff, D.M., Shah, I., Richard, A.M., Dix, D.J. (2010) *In vitro* screening of environmental chemicals for targeted testing prioritization: the ToxCast project. *Environ Health Perspect*. 118(4):485-92. doi: 10.1289/ehp.0901392.
3. Tice, R.R., Austin, C.P., Kavlock, R.J., & Bucher, J.R. (2013). Improving the human hazard characterization of chemicals: a Tox21 update. *Environ Health Perspect.* 121(7), 756–765. https://doi.org/10.1289/ehp.1205784
4. ECHA. (2017) Read-across assessment framework (RAAF). Doi: 10.2823/619212
5. Abedini, J., Cook, B., Bell, S., Chang, X., Choksi, N., Daniel, A.B., Hines, D., Karmaus, A.L., Mansouri, K., McAfee, E., Phillips, J., Rooney, J., Sprankle, C., Allen, D., Casey, W., Kleinstreuer, N. (2021) Application of new approach methodologies: ICE tools to support chemical evaluations. *Computational Toxicology*. 20, 100184. https://doi.org/10.1016/j.comtox.2021.100184.
6. Pearce, R.G., Setzer, R.W., Strope, C.L., Sipes, N.S., Wambaugh, J.F. (2017) httk: R Package for High-Throughput Toxicokinetics. *J Stat Softw*. 79 (4): 1-26. doi: 10.18637/jss.v079.i04.
7. Mansouri, K., Grulke, C.M., Judson, R.S., Williams, A.J. (2018) OPERA models for predicting physicochemical properties and environmental fate endpoints. *J Cheminformatics*. 10:10; doi:10.1186/s13321-018-0263-1.
8. Bell S., Abedini, J., Ceger, P., Chang, X., Cook, B., Karmaus, A.L., et al. (2020) An integrated chemical environment with tools for chemical safety testing. *Toxicol In Vitro.* 67:104916; doi:10.1016/j.tiv.2020.104916.
9. EPA's Center for Computational Toxicology and Exposure. 2020. ToxCast invitrodb data download page: https://epa.figshare.com/articles/dataset/ToxCast_Database_invitroDB_/6062623
10. Filer, D., Kothiya, P., Setzer, R.W., Judson, R.S., Martin, M.T. (2017) tcpl: the ToxCast pipeline for high-throughput screening data. *Bioinformatics*. 33(4): 618-20.

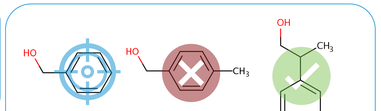
Other Resources

1. Chang X., Abedini J., Bell S., Lee K.M. (2021) Exploring *in vitro* to in vivo extrapolation for exposure and health impacts of e-cigarette flavor mixtures. Toxicol In Vitro. 72:105090. doi: 10.1016/j.tiv.2021.105090.
2. Hines, D.E., Zhang, J., Chang, X., Bell, S.M., & Lee, K.M. (2021). Application of Open-Source PBPK Models in Rat-to-Human Pharmacokinetic Extrapolation of Oral Nicotine Exposure. Computational Toxicology, 20, 100182. https://www.x-mol.com/paperRedirect/1431398773661876224
3. Lee and Bell. (2021) Advancing New Alternative Methods (NAMs) for Tobacco Harm Reduction: Introduction. CORESTA, Virtual. https://sciences.altria.com/-/media/Project/Altria/Sciences/presentations/2021/11_CORESTA-2021_NAM00_Monica-Lee_Final.pdf
4. Zhang, J, Change, X, Holland, T.L., Hines, D.E., Karmaus, A.L., Bell, S., Lee, K.M. (2022) Evaluation of Inhalation Exposures and Potential Health Impacts of Ingredient Mixtures Using in vitro to in vivo Extrapolation. Frontiers in Toxicology. https://doi.org/10.3389/ftox.2021.787756

Methods: Benzyl Alcohol as an Example

1. Chemical of Interest
2. Read Across
3. Available *In Vitro* Data (cHTS)
4. Evaluate PK Using httk [6] Package
5. Reverse Dosimetry for IVIVE
6. MOE Assessment

Example Chemicals
- Flavor compounds generally recognized as safe (GRAS) for oral consumption in food
- Compounds with traditional exposure/risk assessment available

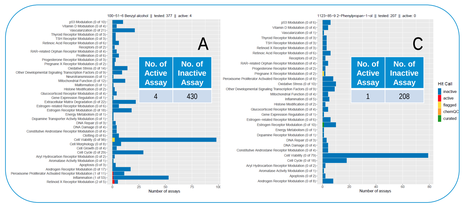


Chemical of Interest: Benzyl alcohol (A) | Match 1: 4-Methylbenzyl alcohol (B) | Match 2: 2-Phenylpropan-1-ol (C)

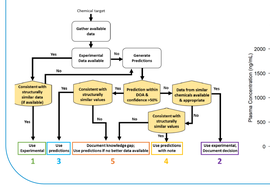
Chemical Similarity Match and Read-Across [4]
- Combine CDK, PaDEL and MACCS fingerprints to determine the structural similarity with Tanimoto score of 0.7 – 1.0
- Confirm identified similar structures with visual analysis
- Consider *in vitro* data intensity: at least 1 active assay found in the Tox21 / ToxCast for read-across

	B	C
Tanimoto Score	0.938	0.882
No. of Active Assay	0	2

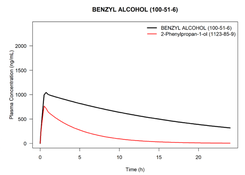
In Vitro Data Curation
- ICE curated data (cHTS) [5,8]
  - Retrieve HTS data from ICE v3.4
  - ICE [9,10] curated data includes some automated curve-based filtering and integration of publicly available chemical QC info
- Manual curation
  - Active curves were evaluated and omitted due to noise data, borderline active, or poor curve fit



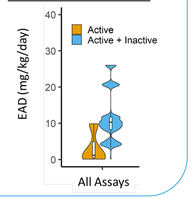
PBPK Parameters: EPA Chemical Dashboard and *in silico* QSA/PR Model [7]



Plasma PK Profile: One Oral Single Dose Per Day

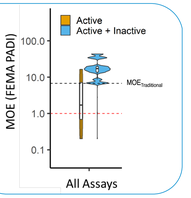


Estimated Administered Dose: Chemical A and C Combined



$$MOE_{traditional} = \frac{Toxicological\ Threshold}{PADI}$$

$$MOE_{NAMs} = \frac{EAD \times BW}{PADI}$$

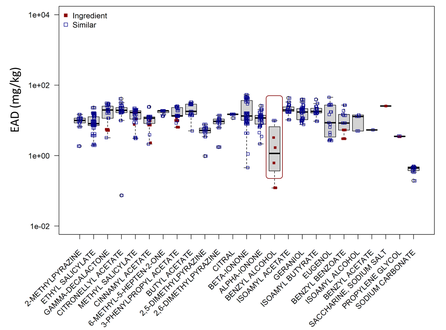


PBPK: Physiologically based pharmacokinetics; httk: high-throughput toxicokinetics; cHTS: curated high-throughput screening; QSA/PR: *in silico* quantitative structure activity/property relationship modeling; IVIVE: *In vitro* to in vivo extrapolation; MOE: Margin of Exposure; BW: Body Weight, assuming 70 kg for MOE_{NAMs}; EAD: Estimated Administered Dose, mg/kg BW/day; PADI: the possible average daily intake, mg/day

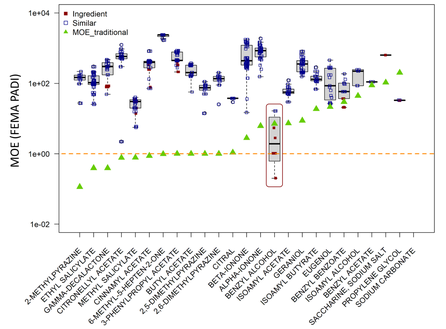
Benzyl Alcohol
- 5 out of in total 636 assays provide valid bioactivity data (e.g. AC50 from the Hill equation model) for IVIVE. For the rest of the 631 inactive assays which do not provide an AC50 value, the top dose tested were used for IVIVE.
- When active assays are used for the calculation, MOE_{NAM} < $MOE_{Traditional}$, which is less conservative. This may be due to the metabolism of benzyl alcohol to benzoic acid *in vivo*

Results

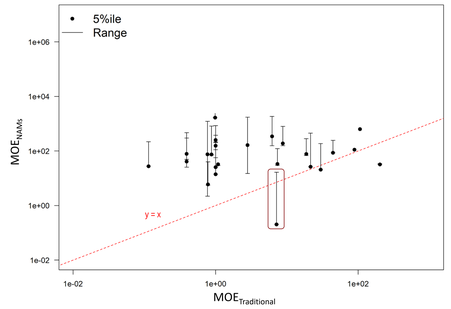
EAD Based on Plasma C_{max} Extrapolated from Active Assays
Plasma EAD (All Active AC50)



MOE$_{NAMs}$ Based on Plasma C_{max} Extrapolated from Active AC50



MOE Comparison: MOE$_{NAMs}$ VS. MOE$_{Traditional}$



Benzyl Alcohol was highlighted in a red box.

Toxicological Threshold (RfD) for Traditional MOE Assessment (MOE$_{Traditional}$)

Chemical Name	CASRN	FEMA PADI (mg/day)	RfD (mg/day)	References for RfD
2-METHYLPYRAZINE	109-08-0	4.73	0.54	Cramer Class
ETHYL SALICYLATE	118-61-6	5.36	5.36	FEMA PADI
GAMMA-DECALACTONE	706-14-9	4.59	1.80	Cramer Class
CITRONELLYL ACETATE	150-84-5	2.34	1.8	Cramer Class
METHYL SALICYLATE	119-36-8	38.41	30	JECFA ADI
CINNAMYL ACETATE	103-54-8	2.06	1.8	Cramer Class
6-METHYL-5-HEPTEN-2-ONE	110-93-0	0.55	0.54	Cramer Class
3-PHENYLPROPYL ACETATE	122-72-5	2.1	2.1	FEMA PADI
BUTYL ACETATE	123-86-4	6.16	6.16	FEMA PADI
2,5-DIMETHYLPYRAZINE	123-32-0	4.8	4.8	FEMA PADI
2,6-DIMETHYLPYRAZINE	108-50-9	4.8	4.8	FEMA PADI
CITRAL	5392-40-5	27.69	30	JECFA ADI
BETA-IONONE	14901-07-6	2.14	6	JECFA ADI
ALPHA-IONONE	127-41-3	0.97	6	JECFA ADI
BENZYL ALCOHOL	100-51-6	41.88	300	JECFA ADI
ISOAMYL ACETATE	123-92-2	24.49	180	JECFA ADI
GERANIOL	106-24-1	3.42	30	JECFA ADI
ISOAMYL BUTYRATE	106-27-4	9.64	180	JECFA ADI
EUGENOL	97-53-0	6.99	150	JECFA ADI
BENZYL BENZOATE	120-51-4	10.08	300	JECFA ADI
ISOAMYL ALCOHOL	123-51-3	4.06	180	JECFA ADI
BENZYL ACETATE	140-11-4	3.38	300	JECFA ADI
SACCHARINE, SODIUM SALT	128-44-9	2.83	300	JECFA ADI
PROPYLENE GLYCOL	57-55-6	7.44	1500	JECFA ADI
SODIUM CARBONATE	497-19-8	NA	NA	JECFA ADI not specified

CASRN: CAS Registry Number; RfD: reference dose; TTC: threshold of toxicological concern; FEMA: Flavor Extract Manufacturers Association; JECFA: the Joint FAO/WHO Expert Committee on Food Additives; ADI: acceptable daily intake based on 60 kg human BW; NA: Not Available