Exploring IVIVE for Exposure and Health Impacts of E-Cigarette Flavor Mixtures

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Abstract

In vitro to in vivo extrapolation (IVIVE) refers to the usage of in vitro experimental data to predict corresponding in vivo exposures. This approach can be used to estimate clinical exposure scenarios (e.g., equivalent administered dose (EAD)) that may pose an adverse health risk based in vitro responses, potentially bypassing the need for animal testing. Interpretation of IVIVE results and confidence in modeling predictions are affected by model type and kinetic assumptions for the test article, and choice of in vitro assay(s).

Exposure scenarios can be complicated, particularly for mixtures. Here we use e-cigarette (EC) aerosol, a complex mixture including carriers, flavors, and nicotine, as a case study to explore IVIVE modeling of mixtures. We utilized literature in vitro cytotoxicity data on EC flavor mixtures and publicly available mechanistic (Tox21) in vitro data for individual flavors to predict exposure scenarios that could lead to adverse toxicities. Several pharmacokinetic (PK) models were explored including a simple steady state model and a 3-compartment model with repeat dosing. Our results suggest that in vitro assay selection has a greater impact than modeling approach and treatment of mixtures. For example, >1,000 pods (>700 mL EC liquid)/day were estimated for human exposures for certain flavors using cytotoxicity data on the mixtures. In contrast, mechanistic (Tox21) data on individual chemicals yield a lower but wider exposure range (3 to 100,000 pods) for some flavors. These proof-of-concept results highlight challenges and complexities in IVIVE for mixtures.

Data and Pharmacokinetic (PK) Model Inputs

Test Items: E-cigarette (EC) aerosols of flavor mixtures

IVIVE is explored based on: 1) in vitro assay data of EC aerosols' toxicity of interest and 2) a parameterized PK model describing the movement of flavor(s) in the body.

In vitro data used in the IVIVE analysis:

- In vitro cytotoxicity data on EC aerosols from a commercial EC (JUUL, 8 different flavors; Omaiye et al. 2019) :
  - Half-maximal inhibitory concentrations (IC50s) from cytotoxicity assays (e.g., MTT) of EC aerosols
  - Mass fraction of individual flavor compounds in the EC aerosols (estimated based on analytical data)
- In vitro mechanistic data on individual flavor compounds from Tox21 database:
  - Half-maximal activity concentrations of the most sensitive (lowest AC50s) Tox21 assays (Tice et al. 2013)
  - Data obtained from the Integrated Chemical Environment (ICE) (Bell et al. 2017)

PK Model inputs for individual flavor chemicals:

- Fraction of chemical unbound to protein, hepatic clearance, and renal clearance
- Additional inputs for 3-compartment model: uptake rate of chemical from the gut, tissue-to-plasma partition coefficients

All above parameters were obtained via US NTP's ICE using OPERA model predictions (Mansouri et al. 2018) or Httk R package (Pearce et al. 2017)

PK Models used:

- One-compartment steady state model **(Figure 1A)** (Wetmore et al. 2012)
- Three-compartment PBPK model **(Figure 1B)** (Pearce et al. 2017)

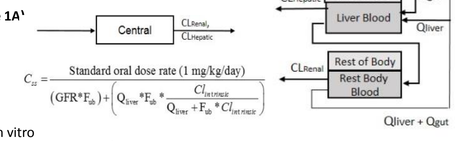


Figure 1A

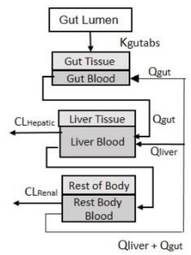


Figure 1B

Outcomes:

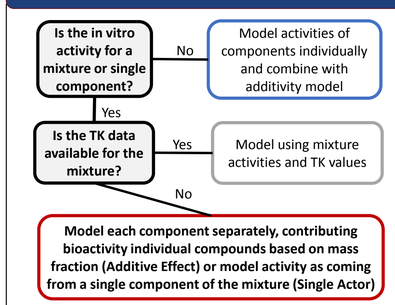
Human equivalent administered dose (EAD) resulting in a plasma concentration equal to the in vitro bioactivity concentration.

$$EAD = ACC(or\ AC50) \times \frac{1}{C_{ss}} (mg/kg/day)$$

Number of Pods:

$$\text{Number of pod} = (\text{EAD} * 70(\text{kg})) / (0.7\ (\tfrac{\text{ml}}{\text{pod}}) * \text{total flavor concentraion}\ (\tfrac{\text{mg}}{\text{ml}})$$

Framework for IVIVE of Mixtures

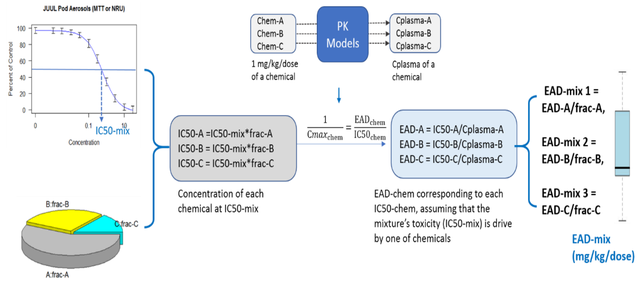


In vitro assays are attractive systems for testing mixtures like extracts and formulations, but it becomes difficult to tease out the bioactivity of each component. The decision tree (left) highlights the key modeling questions we addressed in this work.

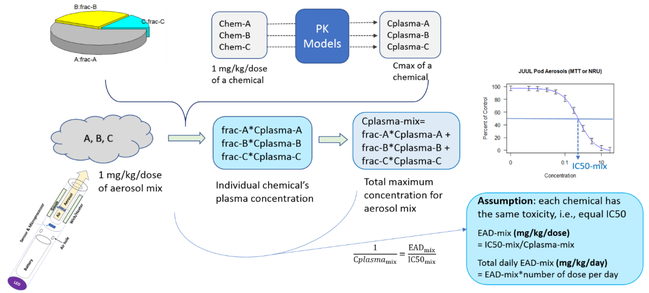
- Most published mixture studies consider individual chemical activities and mathematically explore combinations using IVIVE (blue box).
- If TK data is available for the mixture, then it may be possible to model the mixture directly (grey), provided the assays are appropriate.
- The work here is to address the case of no TK data on the mixture (red).

Approaches for Calculating EAD of Mixtures (EAD-Mix)

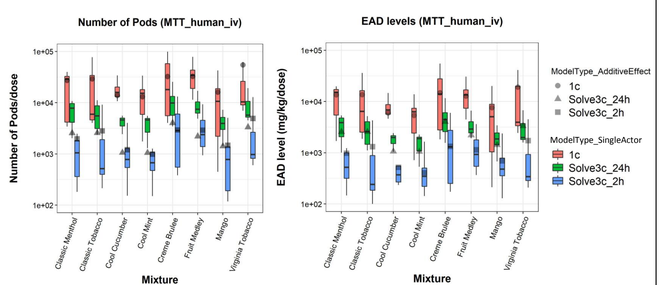
Single Actor: This approach treated the in vitro activity of EC aerosol mixture as though the activity is caused by a single chemical in the mixture. This estimated a range of EAD-mix estimates, as an EAD was calculated for each chemical in the aerosol independently.



Additive Effect: This approach assumed all the chemicals contribute proportionally to the in vitro activity of EC aerosol mixture according to their mass fraction in the mixture. This created a single estimate of the EAD-mix due to the integration of the activities.



IVIVE Using MTT Cytotoxicity Assay Data



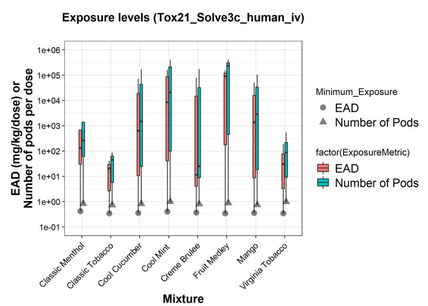
Number of pods (left) and the EAD (right) per dose based on cytotoxicity assays using different PK models as indicated. Box plots show the range of values based on the "Single Actor Approach," in which one flavor chemical of the e-fluid is responsible for the toxicity. The black symbols (circle, triangle, and square) represents the "Additive effect approach," in which all flavor chemicals of e-fluid mixtures assume to equally contribute to the toxicity.
1C: 1-compt model; Solve3C_24h: 3-compt model with 24-hour dosing interval; Solve3C_2h: 3-compt model with 2-hour dosing interval. The 1C model estimates Css (steady state plasma levels) whereas the 3C models estimate Cmax (maximal plasma levels) which is a more conservative estimate.

The AC50 Values of Tox21 In Vitro Assays for Individual Flavor Chemicals

Flavor Chemical	# Pods[1]	Assay Name	AC50 (μM)
Benzyl alcohol	6	ATG_RXRb_TRANS_up	1.17
4-Octanolide	1	ATG_PXRE_CIS_up	20.104
5-Heptyldihydro- 2(3H)-furanone	1	NHEERL_ZF_144hpf_TERATOSCORE_up	7.897
Ethyl butyrate	2	ATG_HNF6_CIS_up	0.0451
4-Hydroxy-3-methoxybenzaldehyde	2	NVS_ENZ_hMMP3	3.926
Methyl 2-aminobenzoate	1	ATG_Ahr_CIS_dn	61.756
2-Ethyl-3-hydroxy-4H-pyran-4-one	3	NVS_ENZ_oCOX1	0.187
Nicotine[2]	**All**	**NVS_LGIC_hNNR_NBungSens**	**1.362**
4-Methyl-1-(propan-2-yl)cyclohex-3-en-1-ol	1	ATG_PXRE_CIS_up	40.699
Caffeine	2	ATG_Sox_CIS_up	0.0901
6-Pentyltetrahydro-2H-pyran-2-one	1	ATG_PXRE_CIS_up	53.659
Ethyl methyl-phenylglycidate	1	OT_ER_ERaERb_1440	38.906
Linalool	3	ATG_PXRE_CIS_up	56.263
Ethyl anthranilate	3	ATG_RXRb_TRANS_dn	16.588
Isopulegol	3	ATG_ERE_CIS_up	13.475
2-Methoxyphenol	1	ATG_PXRE_CIS_up	84.215
2,5-Dimethylphenol	1	ATG_ERE_CIS_dn	0.009
alpha-Terpineol	1	TOX21_NFkB_BLA_agonist_viability	0.0901
dl-Carvone	1	ATG_PXRE_CIS_up	29.583

[1] The number of pods (out of 8 JUUL pods) that the flavor chemical was detected in Omaiye et al. (2019)
[2] Nicotine is not a flavor but expected to contribute the bioactivity of EC liquid: Nicotine is included for comparison with values from the aerosol mixture activity

IVIVE Using Tox21 Assay Data



A range of EAD-mix and corresponding number of pods were calculated using the 3-compartment PK model with a 24hr exposure interval. EAD calculations were based on the AC50 from Tox21 assay with the most sensitive (i.e. lowest AC50) response for each chemical where data were available. This resulted in a much lower but wider exposure range for the EADs compared to using the IC50 data on the EC aerosol mixtures.

Results and Conclusion

This case study demonstrates the feasibility of using IVIVE for risk assessment of EC liquid consumption; at the same time, it highlights challenges, opportunities and points of consideration in IVIVE for mixtures:

- EC liquid consumption needed to obtain a plasma concentration equivalent to the in vitro activity varied greatly based on in vitro assay selection, and to a smaller degree, the modeling of mixtures.
- Results using the 1 compartment (modeling steady state concentration) and 3 compartment (modeling maximal concentration) models had overlapping exposure range for same dosing interval.
- EADs calculated based on the mixture's in vitro MTT activity were substantially higher than those calculated using the more sensitive Tox21 assay for the individual chemicals.
- Using the Single Actor assumption, the range of estimated pods needed varies from 7- to 400-fold across the example 8 JUUL EC products.
- Values under Additive Effect were generally at the upper end of the interquartile range generated by Single Actor, suggesting the Additive Effect approach is less conservative and may underestimate the risk.
- Using most sensitive in vitro assays on single chemicals yield a lower but wider exposure range (3 to 100,000 pods) for flavors.
- Assay selection to put in vitro results into a biologically relevant context are important consideration when conducting an IVIVE.

Strengths and Limitations

Application of IVIVE in risk screening and prioritization compares the EAD to the expected exposure scenario. An EAD that is orders of magnitude above the expected exposure can support a low probability of adverse outcome due to exposure. Conversely, if the calculated EAD is close to the expected exposure then additional follow up would be needed to rule out possible risk. Models that can conservatively estimate the EAD would be desired.

The study presented here used a simplified modeling approach that required minimal inputs (1C), as well as a more complex modeling approach that required parameterization of chemical partitioning between tissue compartments (Solve3C). While the results were comparable between these models, EC aerosol exposure is primarily through inhalation. Modeling aerosol exposure is a complex problem as it considers the delivery and deposition of chemicals in the lung and along the respiratory tract. While an IV route is expected to be more conservative than the inhalation route, it bypasses the local interactions of chemicals in the respiratory tract.

To better address these questions, the following is to be considered:

- Replication of analysis of the mixture and the single chemical in vitro data using an inhalation model
- Identification or generation of in vitro data for both EC aerosol and the individual ingredient chemicals
- Compare modeling results using single chemical and EC aerosol mixtures for the same in vitro assay to assess the approach to modeling the mixture.

References

Bell SM et al. (2017) Environ Health Perspect 125:054501.
Ji EH et al. (2016) PLoS ONE 11(5).
Mansouri K et al. (2018) J Cheminformatics 10:10.
Omaiye et al. (2019) Chem Res Toxicol 32:1058–1069.
Pearce RG et al. (2017) J Stat Softw 79(4):1–26.
Tice RR et al. (2013 Environ Health Perspect 121:756–765.
Wetmore BA et al. (2012) Toxicol Sci 125:157–174. 0