In Vitro to in Vivo Extrapolation (IVIVE) for Evaluating Exposure and Health Impacts of Whole Product E-liquid: Case Study

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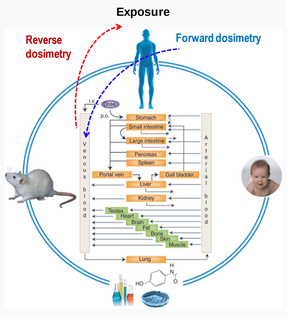
Abstract

E-Liquid formulations are typically comprised of nicotine, carrier chemicals (propylene glycol [PG] and glycerol [VG]), and flavor mixtures. While most flavor ingredients used in e-cigarettes (EC) are 'generally recognized as safe' (GRAS) for oral consumption, there is limited available information to evaluate their inhalation toxicity. In addition, recent publications that use in vitro assays report some market e-cigarettes (EC) may have adverse toxicity potential. Previously, in vitro to in vivo extrapolation (IVIVE) was performed to translate the in vitro cytotoxicity responses of EC aerosols to human equivalent administered doses (EADs), utilizing reported EC aerosol concentrations of nicotine and flavors (Chang et al 2021), and found that the human exposures needed to match the in vitro bioactivity exceeds the typical human usage. However, composition data on some major ingredients such as carriers were not available and therefore not included in this analysis.
Here we follow up previous IVIVE work with the whole product mixture, including carriers and organic acids, to estimate EADs representative of the whole product to support EC risk assessment of e-liquid consumption. Multi-compartment pharmacokinetic (PK) models with different exposure scenarios (2 h and 24 h dosing intervals) were used to evaluate effects of modeling approaches on EAD estimation. MTT cytotoxicity data for e-cigarette aerosol (Omaiye et al., 2019) were used to predict corresponding human exposure considering a mass balance for the whole product. Using an additive modeling approach, the IVIVE analysis of whole product ingredients in EC aerosols showed up to five-fold higher EAD estimates compared to previous results performed without carriers and organic acids. This is likely because carriers comprise a large volume of the mixture and PG and VG are subjected to extensive intrinsic clearance, reducing their in vivo availability in systemic circulation. While the estimated EADs greatly exceeded typical usage, future studies may evaluate different toxicity endpoints. This case study demonstrates that the pharmacokinetics of whole product ingredients including carriers should be considered when extrapolating in vitro assay data to relevant human exposure to e-cigarettes.

Introduction

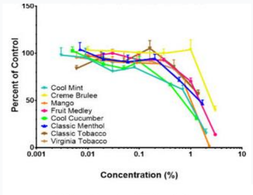
In Vitro To In Vivo Extrapolation (IVIVE)

A modeling approach to use the in vitro bioactivity concentration to estimate human-relevant exposures



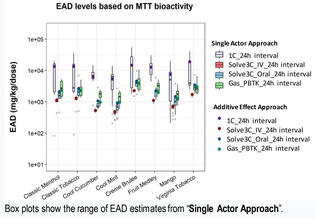
Research Question:
Would the full formulation (including PG, VG and benzoic acid) make a difference in the IVIVE outcomes (EAD) compared to those reported in Chang et al., 2021 which only evaluated flavors and nicotine?

Ref> Chang et al. (2021): IVIVE for Exposure and Health Impacts of EC Flavor Mixtures



Literature e-vapor aerosol in vitro (MTT) assays obtained from Omaiye et al. (2019).

Chang et al performed IVIVE using open-source PBPK modeling and two dosing regimens (see below)



EAD (Equivalent Administered Dose) as reported in Chang et al (2021)

Box plots show the range of EAD estimates from "**Single Actor Approach**".

The solid circles with varied colors represent EAD estimates from "**Additive Effect approach**".

1C_24h interval: 1-compartment model with 24-hour dosing interval; **Solve3C_IV (or Oral)_24h**: 3-compartment model with intravenous injection (or oral gavage dosing) at 24-hour dosing interval; **Gas_PBTK_24h**: a gas PBTK model with 24-hour dosing interval. The 1C model estimates Css (steady state plasma levels) whereas the 3C and Gas_PBTK models estimate Cmax (maximal plasma levels) which is a more conservative estimate. See details in Chang et al., 2021.

Reference

Bell, S.M., Phillips, J., Sedykh, A., Tandon, A., Sprankle, C., Morefield, S.Q., Shapiro, A., Allen, D., Shah, R., Maull, E.A., Casey, W.M., Kleinstreuer, N.C., 2017. An Integrated Chemical Environment to Support 21st-Century Toxicology. Environmental Health Perspectives 125, 054501.

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.

Linakis, M.W., Sayre, R.R., Pearce, R.G., Sfeir, M.A., Sipes, N.S., Pangburn, H.A., Gearhart, J.M., Wambaugh, J.F., 2020. Development and evaluation of a high throughput inhalation model for organic chemicals. J Expo Sci Environ Epidemiol 30, 866–877.

Omaiye, E.E., McWhirter, K.J., Luo, W., Pankow, J.F., Talbot, P., 2019. High-Nicotine Electronic Cigarette Products: Toxicity of JUUL Fluids and Aerosols Correlates Strongly with Nicotine and Some Flavor Chemical Concentrations. Chem. Res. Toxicol. 32, 1058–1069.

Pearce, R.G., Setzer, R.W., Strope, C.L., Sipes, N.S., Wambaugh, J.F., 2017. httk: R Package for High-Throughput Toxicokinetics. Journal of Statistical Software in press.

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, 756–765.

Method

In vitro data used in the IVIVE analysis

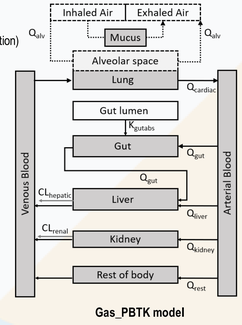
- In vitro cytotoxicity data on EC aerosols from a commercial EC (JUUL, 8 different flavors; Omaiye et al. 2019):
  - Half-maximal inhibitory concentrations (IC50s) from cytotoxicity assays (e.g., MTT) of EC aerosols
  - Mass fraction of individual flavor compounds in the EC aerosols (estimated based on reported analytical figure)
  - Mass fraction of PG, VG, and benzoic acid (BA) in the EC aerosols (estimated based on 5% nicotine nominal fraction)
- In vitro mechanistic data of individual flavor compounds from Tox21 database (Tice et al. 2013):
  - Half-maximal activity concentrations of the most sensitive (lowest AC50s) Tox21/ToxCast assays, as available (18 flavors, nicotine, PG, and BA)
  - In vitro data obtained from the Integrated Chemical Environment (ICE) (Bell et al. 2017)

PK model inputs for individual chemicals

- Fraction of chemical unbound to protein, hepatic clearance ($CL_{Hepatic}$), renal clearance (CL_{Renal}), Uptake rate of chemical from the gut (K_{gutabs}), tissue: plasma partition coefficients (LogP), (not shown in the figure), etc.
- Parameters for this model 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 for reverse dosimetry

- Solve3C: three-compartment (3C) PK model (Pearce et al. 2017) – IV bolus modeling
- Gas_PBTK model (Linakis et al. 2020) – Mimicking e-vapor aerosol modeling

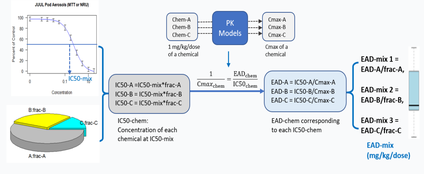


Gas_PBTK model

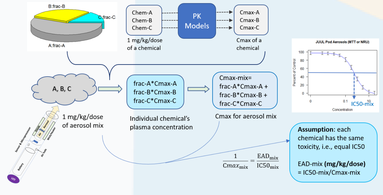
Outcomes:

Human equivalent administered dose (EAD): the amount of chemical or mixture given per dose (mg/kg/dose) that would result in a plasma concentration equal to the in vitro bioactivity concentration selected (e.g. IC50 or AC50).

- 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.

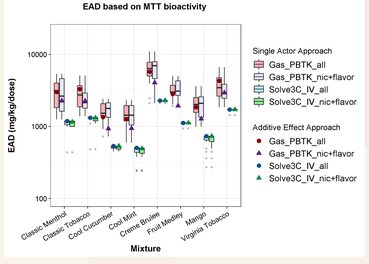
- EAD-Mix calculation with and without additional ingredients (PG, VG and BA)
  - Single Actor: $EAD - Mix = \frac{IC50 \times frac - chemical}{C_{max} - chemical}$ for each chemical
  - Additive Effect: $EAD - Mix = \frac{IC50 \times \sum Frac - chemical}{\sum (C_{max} - chemical \times frac - chemical)}$
    - WITH: $\sum frac - chemical$ = 1 as all chemicals are considered. All chemicals in the EC aerosol contribute to the toxicity and the EAD.
    - WITHOUT: the fraction of PG, VG and BA is set to zero as their contribution to the toxicity and the EAD are not counted.

Conclusion

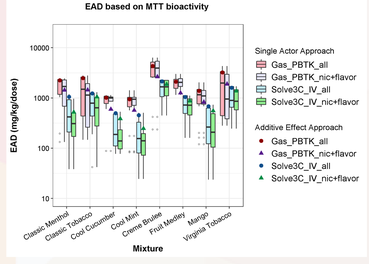
- For the whole formulation assessment, we included PG, VG and BA into previously developed IVIVE models to estimate the human EADs of the EC formulations and compared the results to the previous study (Chang et al. 2021).
- The comparison suggests that the added ingredients (PG, VG and BA) minimally impacted the EAD outcomes under the tested dosing regimens and the in vitro data used for the analysis.
- Among ingredients, nicotine was most bioactive based on the Tox21/ToxCast in vitro data, contributing lower EADs of full formulations compared to flavor-only EADs.

Result and Discussion

EAD estimate based on 24-h dose interval (single dose/day)



EAD estimate based on 2-h dose interval (12 doses/day)



EAD comparisons: Impact of additional ingredients (PG, VG, BA)

Single Actor

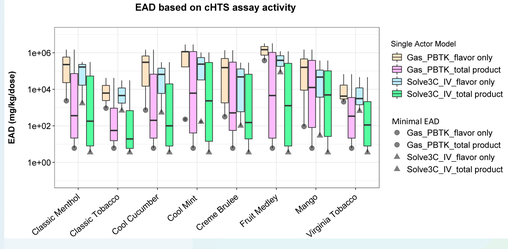
- PG, VG nor BA drive the lower limit of EAD in any PK models.
- Inclusion of PG, VG and BA does not change the upper limit of EAD for all EC formulations for 24-h interval, but raises the upper limit of EADs and in some EC formulations for 2-h interval (e.g., Classic Menthol with Solve3C_IV)
- The result suggests that PG, VG or BA are not driving toxicity concerns in terms of the in vitro data used for modeling.

Additive Effect

- Inclusion of PG, VG and BA result in higher EAD estimates compared to cases without them, which is likely due to the high concentration of PG and VG in the EC formulations while their in vivo bioavailability in the systemic circulation is poor (i.e., low C_{max}).
- BA has similar clearance and mass fraction as nicotine, but the fu of BA is much smaller than that of nicotine. Thus, the increased EAD after inclusion of PG, VG and BA is not likely driven by the addition of BA.

2-h vs. 24-h dose interval

- EADs (2-h) < EADs (24-h) suggests potential metabolic saturation or chemical accumulation with the exaggerated, repeated doses every 2 hours during a day.
- For the single actor approach, EADs (2-h) shows a wider range than EADs (24-h) suggesting a greater variation for multiple daily doses due to different metabolic saturation rates across individual chemicals.



IVIVE using the AC50 from most sensitive cHTS assay, 24-h interval

Flavors only vs. Whole Product

Based on cHTS assays (Single Actor Approach)

- EADs estimated using flavors only are overall consistently higher than those using full formulations, suggesting the minimal EAD is likely driven by other bioactive ingredients.
- When the whole product is evaluated, the minimal EAD is obtained using the lowest AC50 of nicotine assays across all flavor mixtures.

Strength and Weakness

Strengths

- The study, along with the previous study (Chang et al. 2021) demonstrates the feasibility of the IVIVE dosimetry of EC mixtures based exclusively on literature in vitro, Tox21 database, and open-source PK modeling tools.

Weaknesses

- This study used the estimated exposures using analytical results from Omaiye et al., 2019 (detected ingredients in EC and e-vapor aerosols) plus nominal levels for carrier and BA, which may not fully represent the actual EC formulations.
- The utilized inhalation PBPK model is based on gas, not aerosols. Additionally, for biological activities, there were limited ingredient-specific (Tox21) in vitro data.