Evaluation of Inhalation Toxicity IVIVE and Potential Health Impacts of Tobacco-Free Products and Mixtures

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Conflict of Interest Statement

The author declares no conflict of interest.

Outlines

- Background
  - Exposure and biologically effective dose
  - Forward and reverse dosimetry using physiologically based pharmacokinetic (PBPK) modeling
  - *In vitro* to *in vivo* extrapolation (IVIVE)
- Case study: Exposure and health impact of flavors and their mixtures in electronic nicotine delivery systems (ENDS)
  - Research question
  - Method: data, models, and approaches for IVIVE of mixtures
  - Result and discussion
- Summary
- Reference

Exposure and Biologically Effective Dose

Understanding and quantitating the biologically relevant dose is one of the keys in toxicological risk assessment.

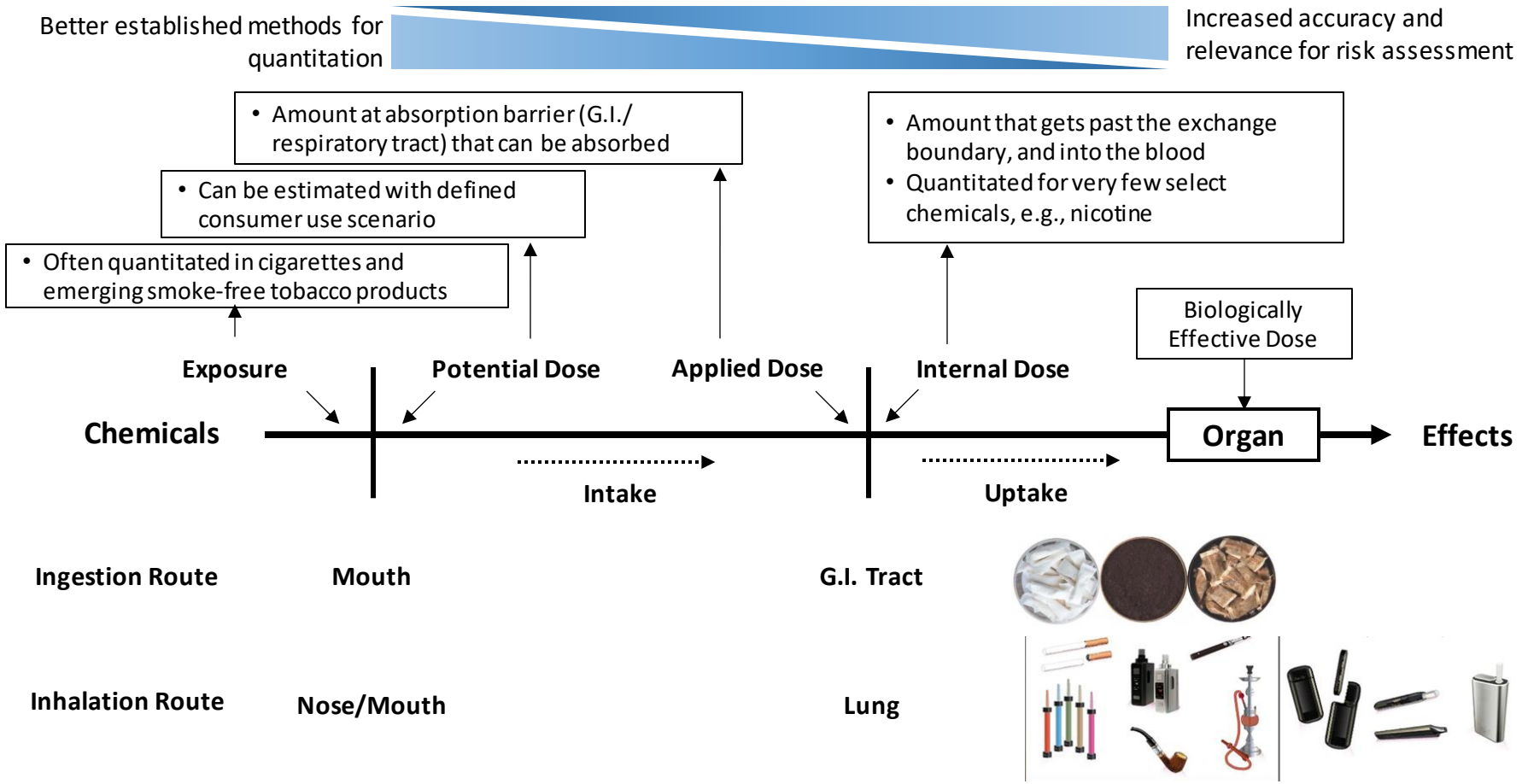
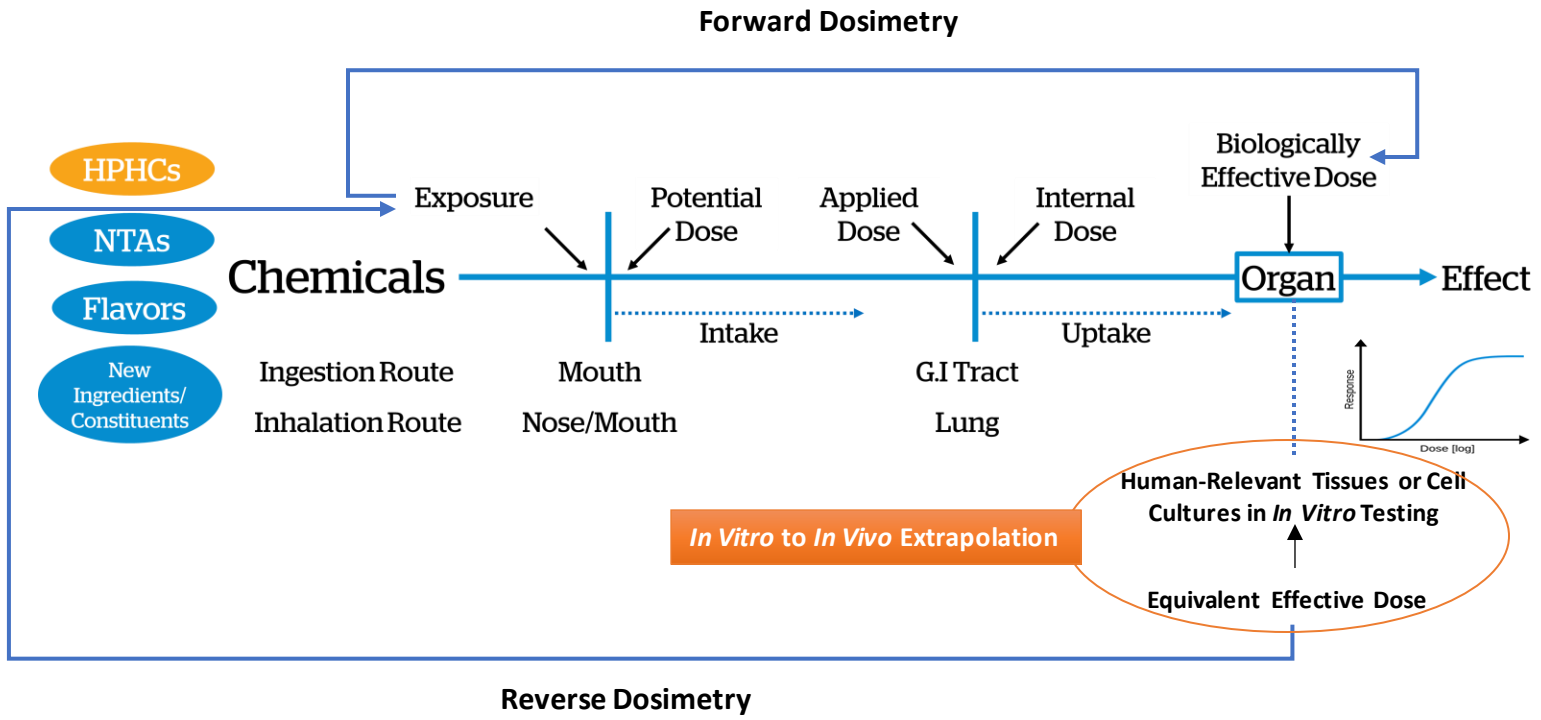
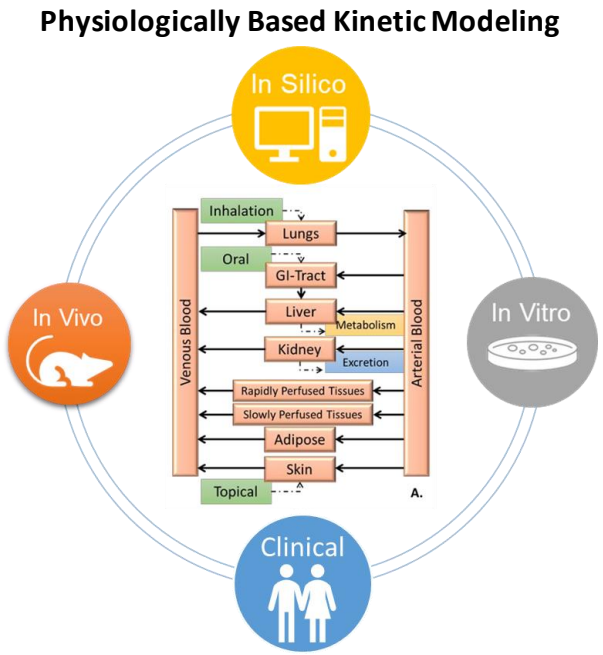


Diagram adapted from: https://www.epa.gov/expobox/exposure-assessment-tools-routes-ingestion

Forward and Reverse Dosimetry

- **Forward dosimetry** is an estimation of internal exposure from measurements of external exposure in studies characterizing chemical toxicities
- **Reverse dosimetry** is to estimate an external exposure to a chemical from internal body measurements or the equivalent of a given substance from biomonitoring studies





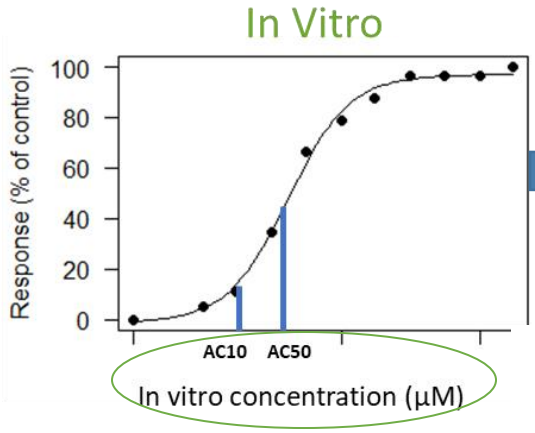
Schematic representation of PBK model adapted from OECD (2021). [Guidance document on the characterization, validation and reporting of Physiologically Based Kinetic (PBK) models for regulatory purposes (oecd.org)]

In Vitro to *In Vivo* Extrapolation (IVIVE)

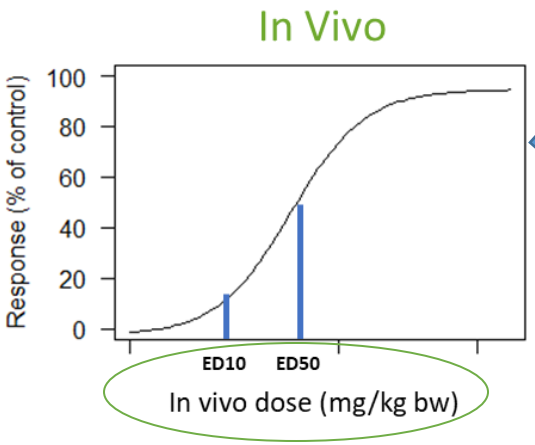
In Vitro to *In Vivo* Extrapolation (IVIVE) is a computational approach that calculates an equivalent *in vivo* administered dose based on the *in vitro* response concentration

In vitro activity concentration (ACx)

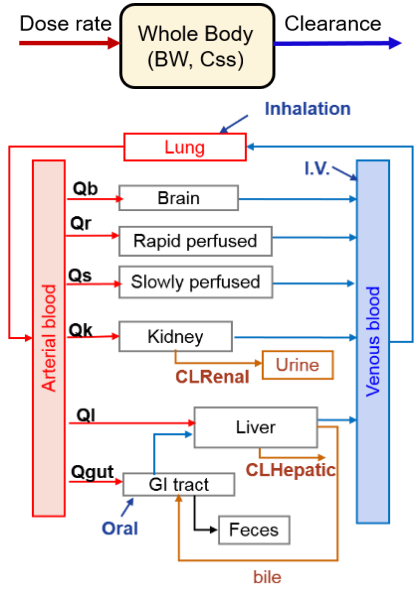
Predict equivalent effect dose (EDx)



Pharmacokinetic (PK) models



ACx from the *In Vitro* Assay ≈ *In Vivo* Tissue Level



Schematic representation of a PBK model for gas

Adapted from Bell (2021) Training Material for Continuing Education Courses

Research Question

How much exposure in human could result in the cytotoxic concentrations *in vivo* approximating the *in vitro*?

Chemical Research in Toxicology

Article

Cite This: *Chem. Res. Toxicol.* 2019, 32, 1058–1069

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High-Nicotine Electronic Cigarette Products: Toxicity of JUUL Fluids and Aerosols Correlates Strongly with Nicotine and Some Flavor Chemical Concentrations

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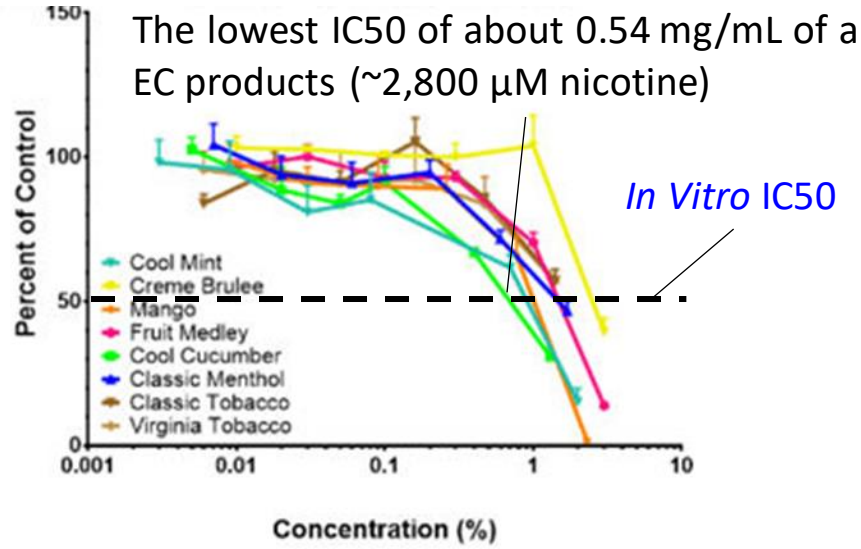
"the concentrations of nicotine and some flavor chemicals. . . are high enough to be cytotoxic in acute in vitro *assays, ..."*

The lowest IC50 of about 0.54 mg/mL of all EC products (~2,800 µM nicotine)

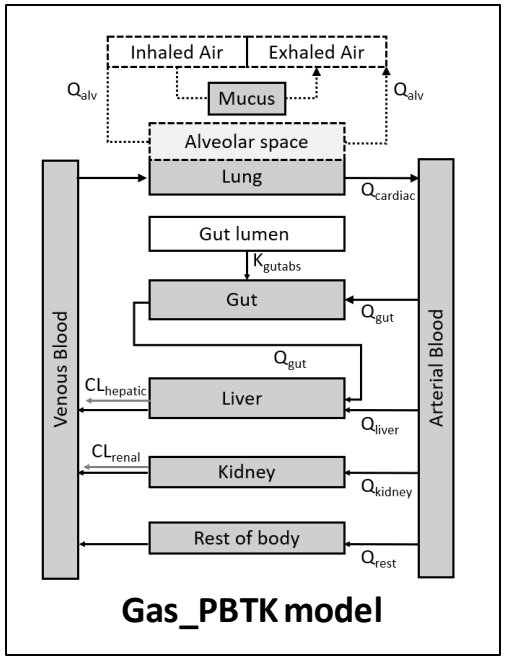


Concentration-Response Curves of a Cell Viability Testing (MTT) for BEAS-2B cells

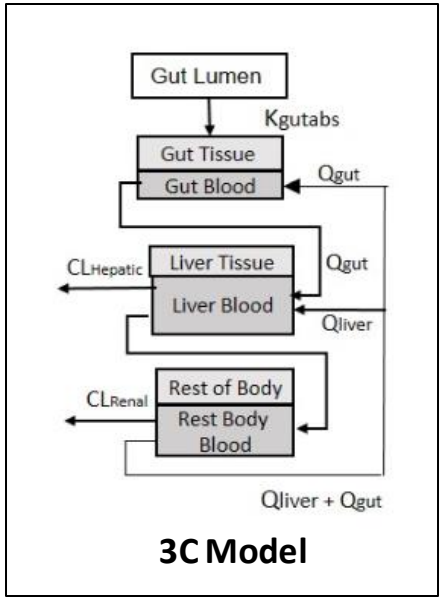
Omaiye et al., (2019) *Chem Res Toxicol* 32:1058–1069.

Method—Data and Model Input

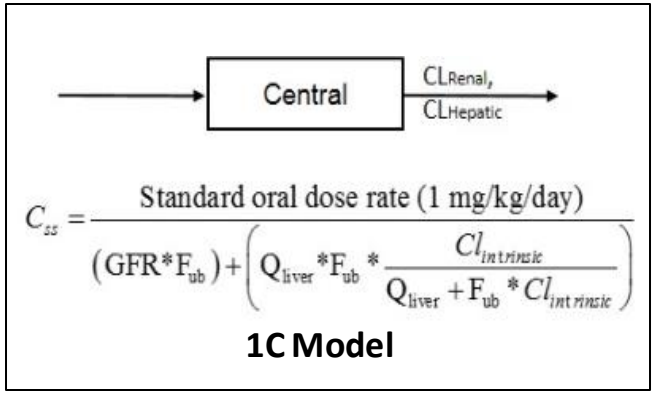
- ***In Vitro* Cytotoxicity data on E-cig aerosols from a commercial product (Omaiye et al., 2019), only reported flavor compounds are considered for modeling**
  - Half-maximal inhibitory concentrations (IC50s) from cytotoxicity assays (e.g., MTT) of EC aerosols
  - Mass fraction of individual flavor compounds in the EC aerosols (estimated based on analytical data)
- **PK Model inputs for individual flavor chemicals**
  - Fraction of chemical unbound to protein, hepatic clearance, and renal clearance
  - For 3-compartment model: uptake rate of chemical from the gut, tissue: 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**
  - One-compartment steady state model (1C) (Wetmore et al., 2012)
  - Three-compartment PBPK model (3C) (Pearce et al., 2017)
  - Gas_PBTK model (Linakis et al., 2020)—clinical relevance



Gas_PBTK model



3C Model



$$C_{ss} = \frac{\text{Standard oral dose rate (1 mg/kg/day)}}{(GFR * F_{ub}) + \left(Q_{liver} * F_{ub} * \frac{Cl_{intrinsic}}{Q_{liver} + F_{ub} * Cl_{intrinsic}}\right)}$$

1C Model

Chang et al., (2021) *Toxicol In Vitro*. 72: 105090
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

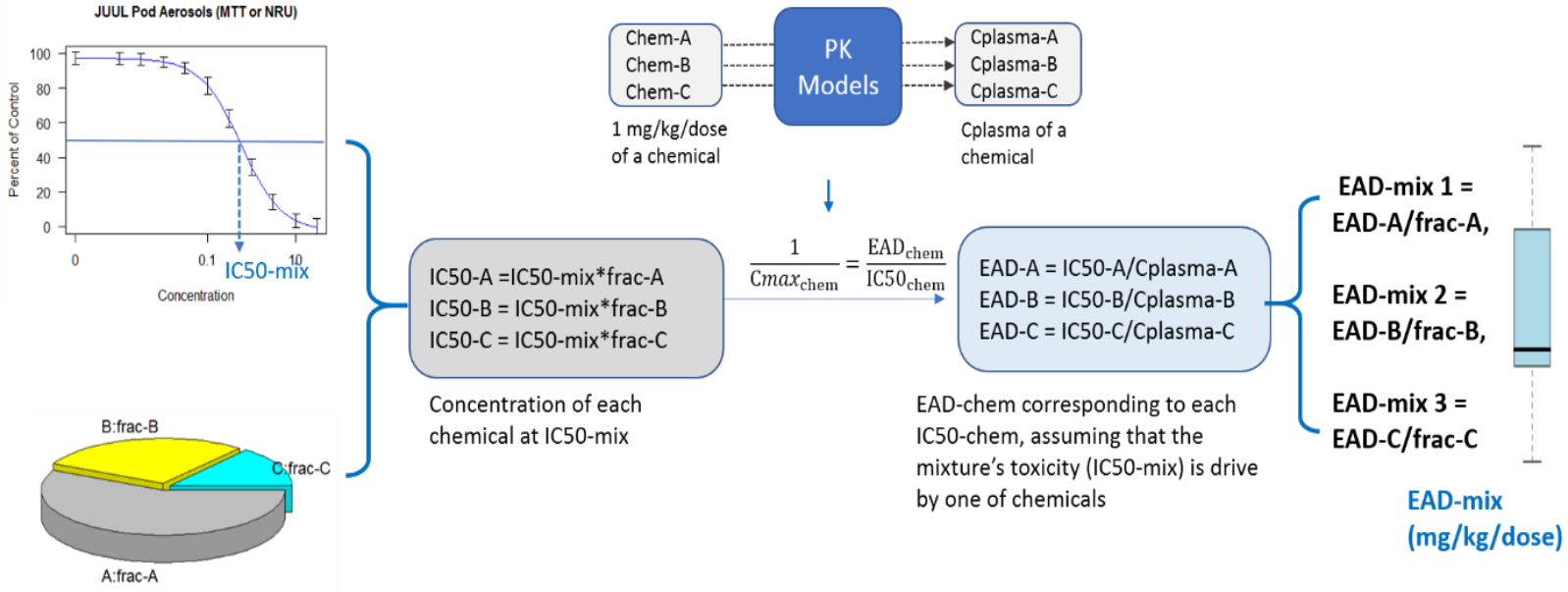
Method—Outcome

- **Human equivalent administered dose (EAD)**
  - An estimated dose resulting in a plasma concentration equal to the *in vitro* bioactivity concentration
  - $EAD = ACC(or\ AC50) \times \frac{1}{C_{ss}} (\frac{\frac{mg}{kg}}{day})$
- **Number of pods: as specific to the E-cig product**
  - Assuming an average human body weight of 70 kg, 0.7 ml of e–liquid per pod
  - $Number\ of\ pod = (EAD * 70(kg)\ /\ (0.7\ \left(\frac{ml}{pod}\right) * total\ flavor\ concentraion\ (\frac{mg}{ml})$

Chang et al., (2021) *Toxicol In Vitro*. 72: 105090

Method-Single Actor Approach for Mixtures

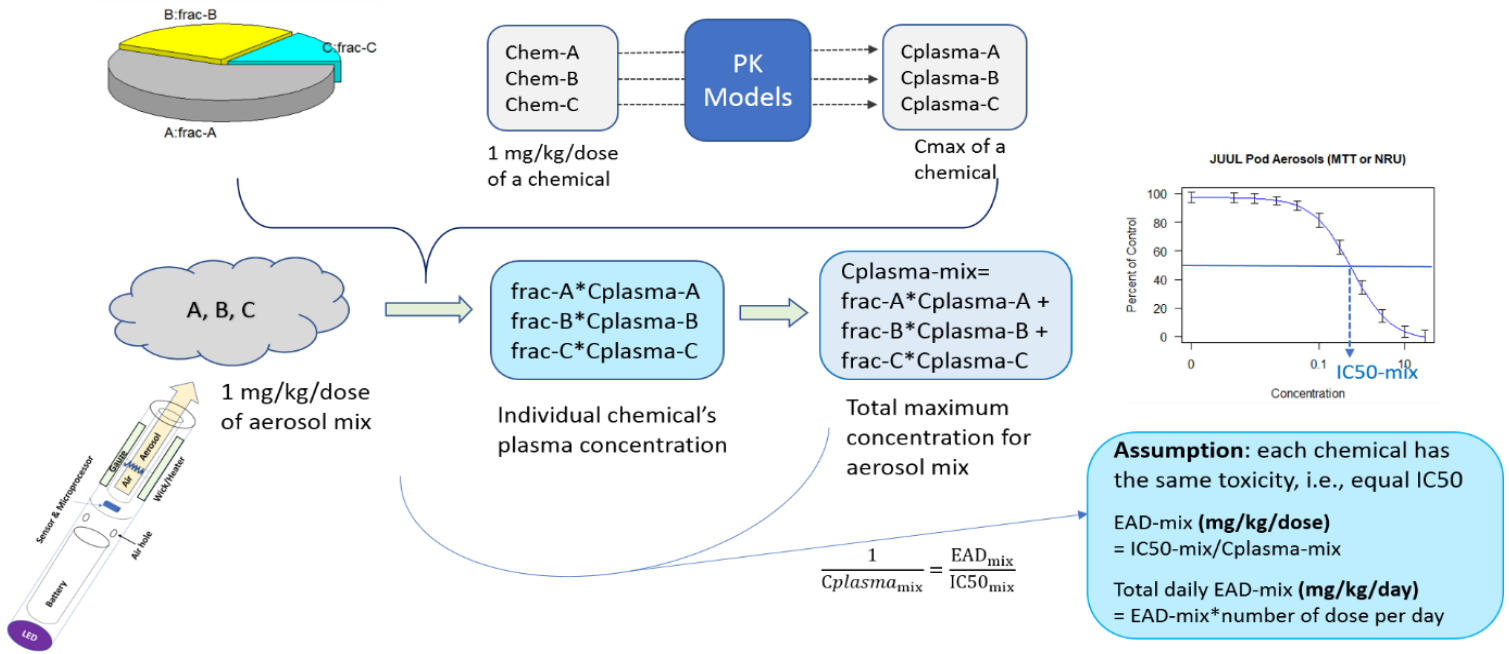
- Single actor approach (based on the measured mixture bioactivity)
  - This approach treated the *in vitro* activity of EC aerosol mixture as though the activity is caused by a single chemical in the mixture. This estimated a range of EAD-mix estimates, as an EAD was calculated for each chemical in the aerosol independently.



Chang et al., (2021) *Toxicol In Vitro*. https://doi.org/10.1016/j.tiv.2021.105090

Method-Additive Effect Approach for Mixtures

- Additive effect approach (based on the measured mixture bioactivity)
  - This approach assumed all the active 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.



Chang et al., (2021) *Toxicol In Vitro*. https://doi.org/10.1016/j.tiv.2021.105090

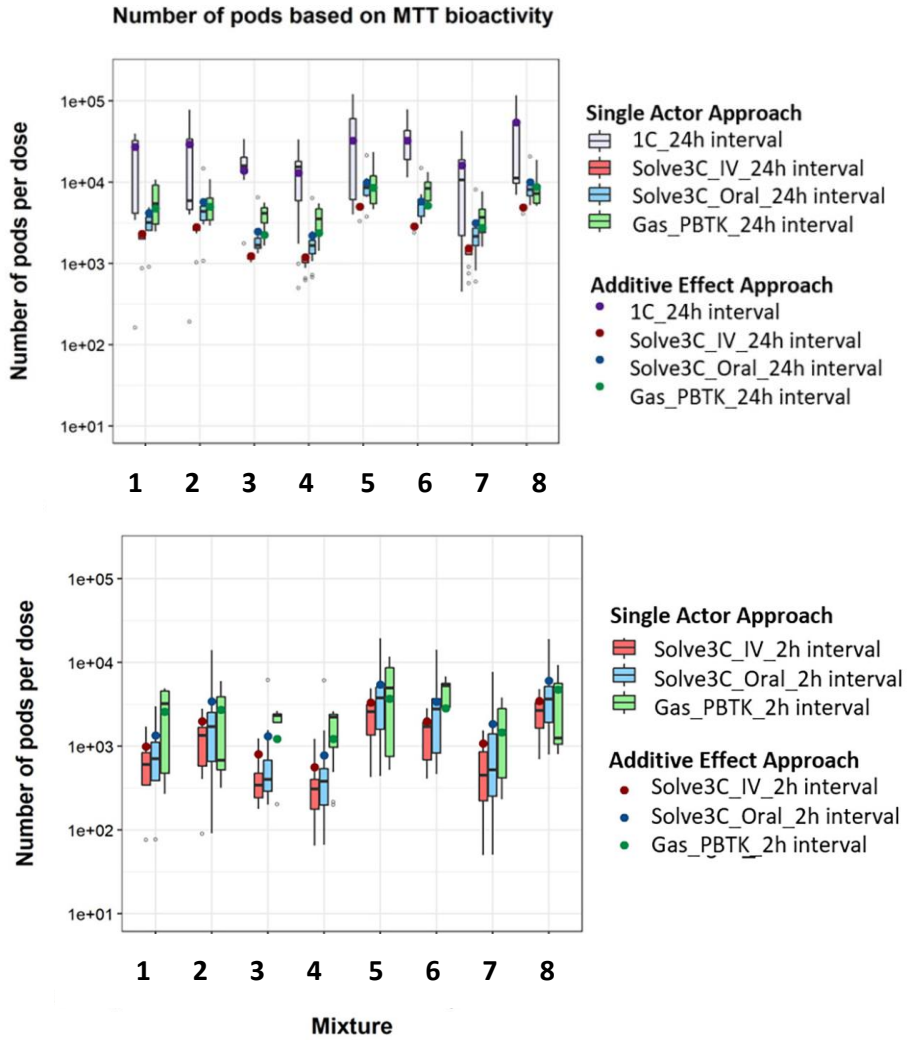
Method-Equations for EAD Calculation

- General Equation
  - $EAD_{mix} = \frac{AC50_{total} * \sum_{i=1}^{m} frac_i}{\sum_{i=1}^{m} (Cmax_i * frac_i)}$
  - m, the number of active ingredients; n, the total number of ingredients in the mixture; m ≤ n
- For the whole product, i.e., all ingredients are considered as active ingredients, m = n,
  - $\sum_{i=1}^{m} frac_i = 1$, therefore, $EAD_{mix} = EAD_{total} = \frac{AC50_{total}}{\sum_{i=1}^{n} (Cmax_i * frac_i)}$
- When a single ingredient in the mixture drives the toxicity,
  - $EAD_{mix} = EAD_i = \frac{AC_i / frac_i}{Cmax_i} = \frac{AC50_{Total}}{Cmax_i}$

Chang et al., (2021) *Toxicol In Vitro* 72: 105090
Zhang et al., (2022) *Front. Toxicol*. 3:787756

EAD Estimation Based on MTT IC50

- **Single dose per day (24-hr interval)**
  - For all eight flavored products, 1,000–100,000 pods need to be consumed to result in a plasma concentration equivalent to *in vitro* cytotoxic level
  - 1C steady-state model resulted in consistently higher projected pod number
  - Other modes, despite of the route of administration (i.v., oral, and gas inhalation), results in a pod number between 1,000 and 10,000
- **12 doses per day (2-hr interval, exaggerated)**
  - Under exaggerated user scenario, the estimated pod number is about one order of magnitude lower, still around several hundreds to thousands
- **The estimated daily pod consumption is unrealistically high, indicating a potentially high margin of acute toxicity of the E-cig products**
- **Impact of the modeling approaches**
  - The wide range of the values generated by single actor approach, suggesting some individual flavors could preferentially drive the cytotoxicity



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.

Chang et al., (2021) *Toxicol In Vitro*. 72: 105090

EAD Estimation Based on MTT IC50—Caveat

- Impact of using the cytotoxicity assay on EAD estimates
  - The standard cytotoxicity assay such as MTT and NRU is colorimetric assays without mechanistic insight and is commonly used for hazard screening
    - It may miss detecting underlying bioactivity and possibly underestimate the risk associated with *in vivo* exposure
    - It may fail to detect delayed toxicity as efficiently as assays measuring growth/division
- The current approaches assume ingredients have the same cytotoxicity potential
  - How about considering the specificity of single ingredients?
  - If individual chemical data is used, how to model the additive effect?
- Only flavors are considered as active ingredients. However, nicotine, benzoic acid (BA) and humectants (propylene glycol [PG] and glycerol [VG]) comprise a large volume of the E-liquid.
- Limitation of the current PK models
  - For E-cig aerosols, a multi-phase inhalation PBPK model may work better than a gas model
  - The current PK model does not consider metabolic saturation

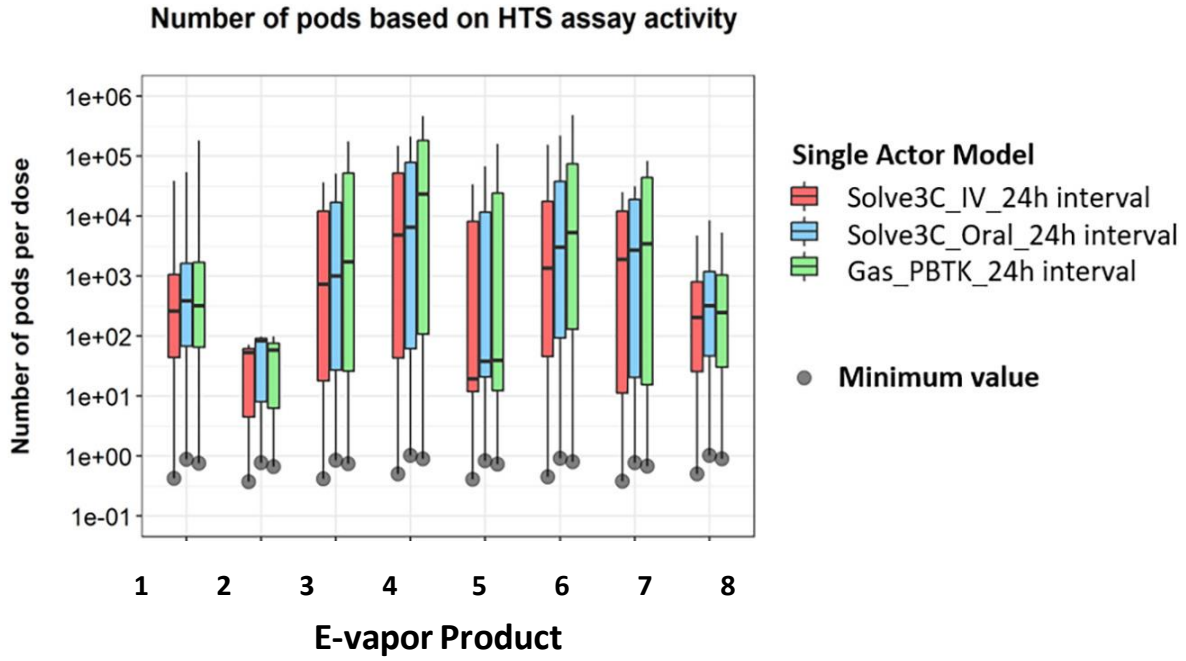
EAD Estimation Based on cHTS of Flavors—Data and Model

- **Curated high-throughput screening (cHTS) data on individual flavor ingredients**
  - *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 and nicotine)
  - *In vitro* data obtained from the Integrated Chemical Environment (ICE) (Bell et al., 2017)
  - The lowest AC50 (i.e., most sensitive endpoint) for IVIVE
- **Single actor approach**
- **PK Models used for EAD Estimation**
  - 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

Chang et al., (2021) *Toxicol In Vitro*. 72: 105090
Zhang et al., (2022) *Front. Toxicol*. 3:787756
Tice et al., (2013) *Environ Health Perspect* 121, 756–765
Bell et al., (2017) *Environmental Health Perspectives* 125, 054501
Peace et al., (2017*) J Stat Softw*. 79(4):1
Linakis et al., (2020) *J Expo Sci Environ Epidemiol* 30, 866–877

EAD Estimation Based on cHTS of Flavors

Flavor Chemical	Flavors[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



- Lower and wider range for majority of flavors (excluding nicotine) than obtained using the MTT data
- Reflecting the potential difference between early sub-toxic perturbation and cytotoxic outcomes
- The most sensitive scenario might be off target

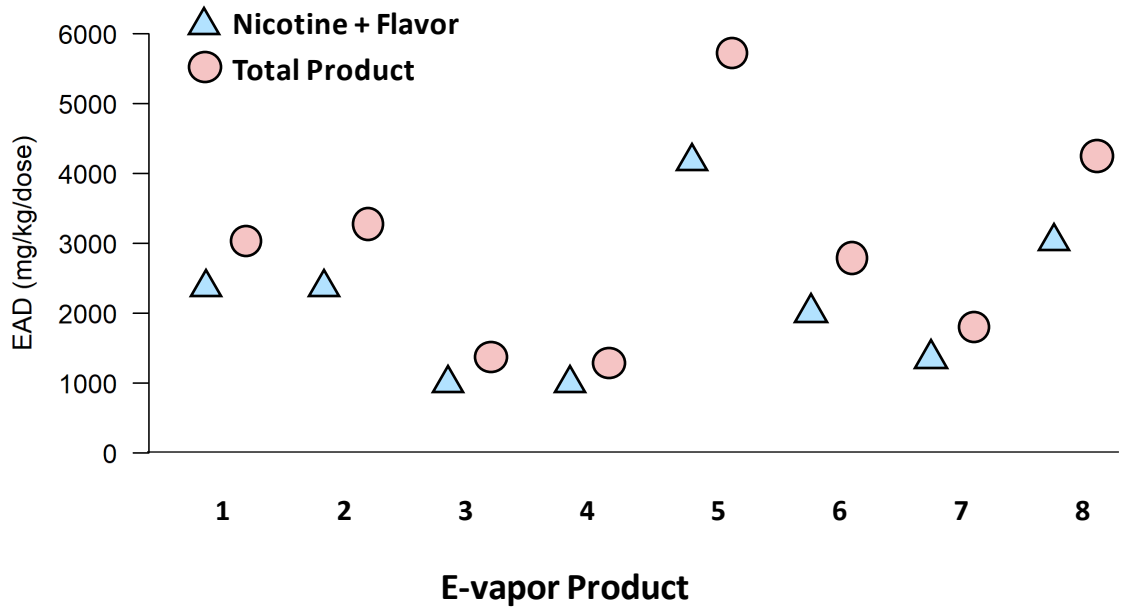
[1] The number of the flavor chemical was detected in 8 of JUUL pods: Cool Mint, Cool Cucumber, Mango, Classic Menthol, Virginia Tobacco, Classic Tobacco, Fruit Medley, and Crème Brulee

[2] Nicotine is not a flavor but expected to contribute the bioactivity of E-liquid: Nicotine is included for comparison with values from the aerosol mixture activity

Chang et al., (2021) *Toxicol In Vitro*. 72: 105090

EAD Estimation of Total Product Based on MTT

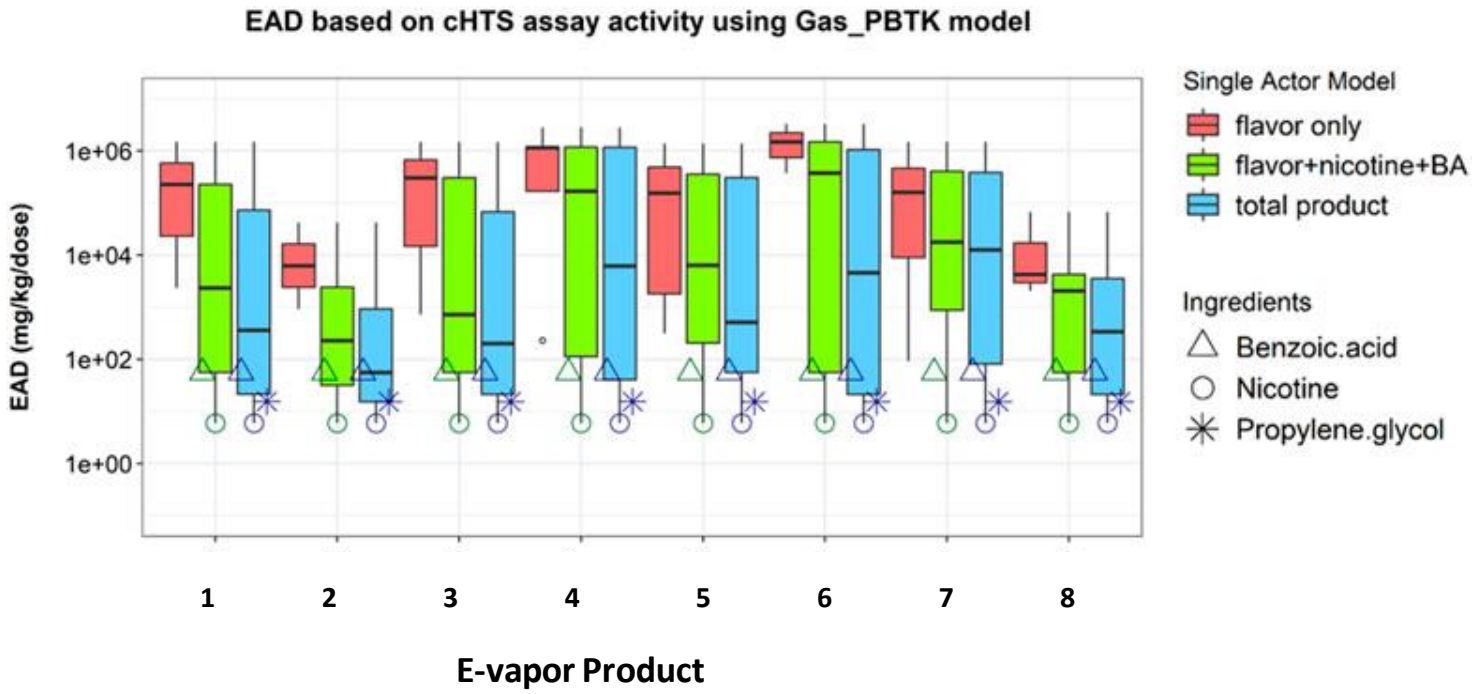
- **Research Question**
  - Would the full formulation (including PG, VG and benzoic acid) make a difference in the IVIVE outcomes (EAD) compared to those reported in Chang et al., which only evaluated flavors and nicotine?



- **Nicotine + Flavor**
  - EADs Ranging from 1,000–4,000 mg/kg BW
  - Several thousands of pods per person per day
- **Total Product (Nic + Flavor + PG + Gly + BA)**
  - Slight increase in EADs likely due to the fast clearance of the three compounds (PG/Glycerol/Benzoic Acid)

Zhang et al., (2022) *Front. Toxicol*. 3:787756

EAD Estimation of Total Product Based on cHTS

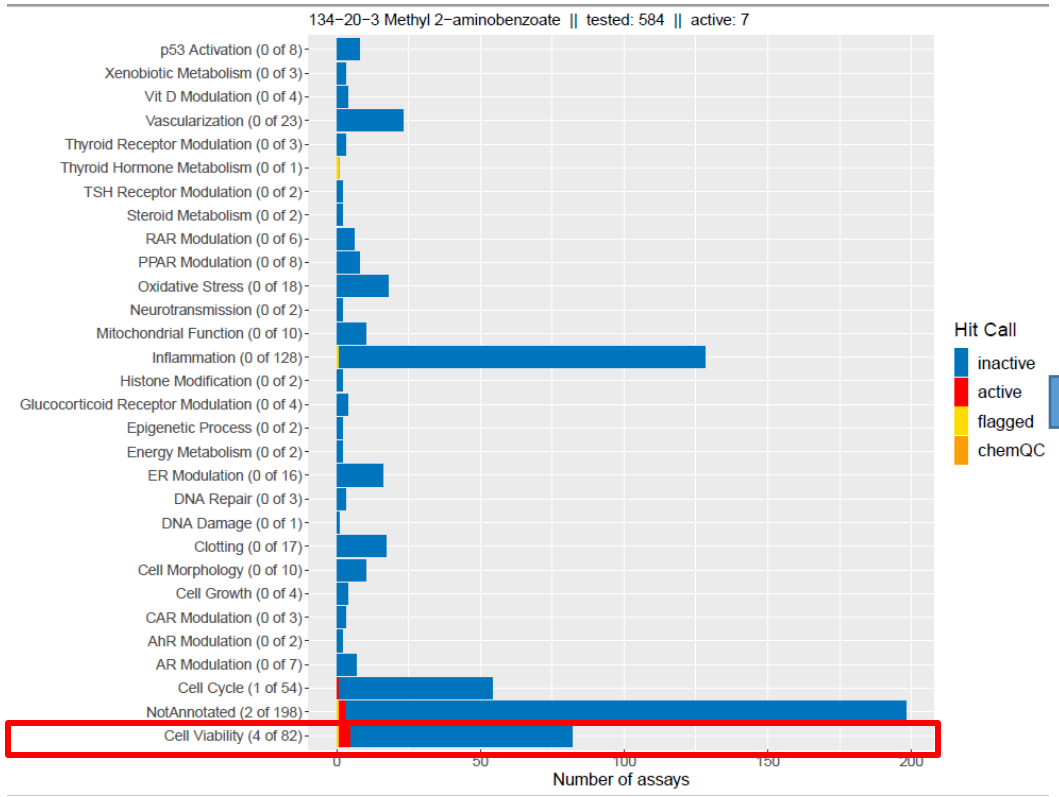


- EADs estimated using flavors only are overall consistently higher than those using full formulations, suggesting the minimal EAD is likely driven by other bioactive ingredients
- When the whole product is evaluated, the minimal EAD is obtained using the lowest AC50 of nicotine assays across all flavor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

Zhang et al., (2022) *Front. Toxicol*. 3:787756

EAD Estimation of Total Product Based on cHTS—Ingredient Breakdown

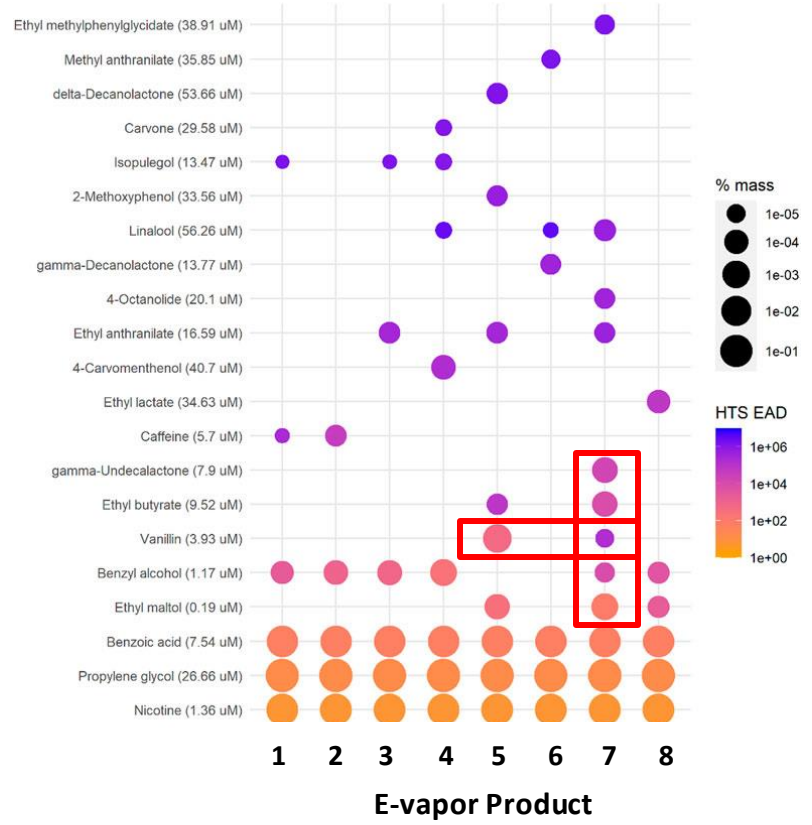
- An example of curated high-throughput screening (HTS) database
  - ToxCast/Tox21 programs
  - Covering multiple mechanistic targets



Lowest AC50 for the most conservative estimation

How could this be relevant to cytotoxicity for comparison?

- A single ingredient in the mixture drives the toxicity
- $EAD_{mix-i} = \frac{EAD_i}{frac_i} = \frac{AC_i/Cmax_i}{frac_i}$



Zhang et al., (2022) *Front. Toxicol.* 3:787756

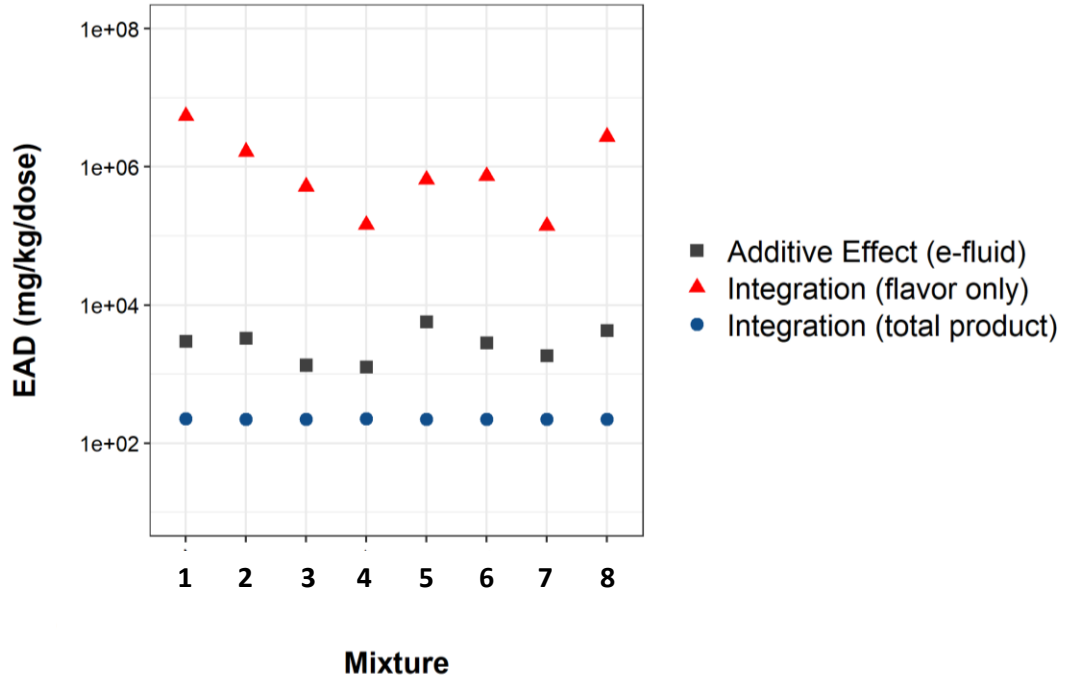
Equivalent Administered Dose (EAD) Calculation for Mixtures

- Outcome-oriented ingredient integration
  - This approach assumes that chemicals affecting the same targeted outcome contribute in an additive manner and the Cmax from each chemical occurs at the same time (i.e., Tmax is the same)
  - A weight factor is considered to obtain relative It also assumes that the potency of ingredients is inversely proportional to AC50
    - Weight factor $W = \frac{AC50_c}{AC50_i}$
  - $EAD_{total\_select\_outcome} = \frac{AC50_c}{\sum_{i=1}^{n}\left(\frac{AC50_c}{AC50_i} * Cmax_i * frac_i\right)}, c \in [1, n]$
    - Ingredient c is any ingredient that contributed to a targeted outcome

Chang et al., (2021) *Toxicol In Vitro*. https://doi.org/10.1016/j.tiv.2021.105090

Estimated EAD Based on cHTS Cytotoxicity

- The same toxicity outcome: Cytotoxicity
- Different weight factor: To obtain relative Cmax assuming the potency of ingredients is inversely proportional to AC50
- An order of magnitude difference from the EAD predictions based on mixture cytotoxicity data
- Cytotoxicity data were not available for some ingredients in the HTS database



Integration Based on ingredient cHTS cell viability assays

Additive Effect Based on mixture MTT assays

Summary

	IVIVE approach	*In Vitro* Data	Equations
General equation assuming all ingredients have the same toxicity potential	Additive effect approach	Mixture data	$EAD_{mix} = \frac{AC50_{total} * \sum_{i=1}^{m} frac_i}{\sum_{i=1}^{m}(Cmax_i * frac_i)}$
For the total product where $\sum_{i=1}^{m} frac_i = 1$	Additive effect approach	Mixture data	$EAD_{mix} = EAD_{total} = \frac{AC50_{total}}{\sum_{i=1}^{n}(Cmax_i * frac_i)}$
When a single ingredient drives the toxicity	Single actor approach	Mixture data, or individual ingredient data	$EAD_{mix} = EAD_i = \frac{AC_i/frac_i}{Cmax_i} = \frac{AC50_{Total}}{Cmax_i}$
When a targeted outcome or endpoint is the focus	Outcome-oriented ingredient integration	Individual ingredient data	$EAD_{total\_select\_outcome} = \frac{AC50_c}{\sum_{i=1}^{n}(\frac{AC50_c}{AC50_i} * Cmax_i * frac_i)}, c \in [1, n]$

Discussion

- **What is the research question?**
- How much do I know about the mixture?
  - What's the composition?
  - Are the biochemical and biophysical data available for each ingredient?
- What *in vitro* data are available?
  - Is the mixture tested as one test article? Is the assay tested for hazard identification or dose-response? How relevant is it to *in vivo* exposure?
  - Are the bioactivity data available for individual ingredient?
- **Is it feasible to use an IVIVE approach to answer the question?**

- Is my PK model sufficient? Or do I need a better model?
  - Route of administration and structure of the model
  - Assumptions and limitations
  - Input parameter: from experiments or from *in silico* prediction
  - Model verification with experimental data
- IVIVE approaches (assumptions) for mixtures
  - Is the bioactivity driven by a group of ingredients or likely a single ingredient?
  - Is additive effect applied to the mixture? Or do other interactions need to be considered?
- **Does the current model answer the research question?**
  - If not, which part needs to be improved? Additional *in vitro* data? A better PK model with metabolism refinement? Or additional considerations of chemical interactions in the mixture?

Acknowledgement

Please contact me if you have any questions.

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Case Example I

HPHC Forward Dosimetry

- Problem Formulation:
  - For FDA's 93 listed HPHCs[1] in the tobacco products, what would be the estimated internal exposure with various exposure scenarios?
    - Cigarettes vs. Reduced Risk Product Candidate (e.g., Heated Tobacco Product)

- NAM-based Dosimetry:
  - To apply Forward dosimetry modeling of selected cigarette smoke constituents (HPHCs) in humans to quantify target tissue doses using open-source PBK modeling

[1] https://www.fda.gov/tobacco-products/rules-regulations-and-guidance/harmful-and-potentially-harmful-constituents-tobacco-products-and-tobacco-smoke-established-list

Key Steps of Verification and Utilization of a PBK Model for HPHC Forward Dosimetry

Select PBPK Models: US EPA's high-throughput toxicokinetics package (HTTK) [1]

- Open source
- Generic model
- Supported by database

Evaluate the suitability of the model for 93 HPHCs

- The current HTTK models are not suitable for metals and small molecules.
- Nine metals and two small molecules (ammonium and hydrazine) are excluded.
- Compounds amenable = 82

Obtain parameters for PBPK models

- Source: experimental data and QSAR (quantitative structure activity relationship) prediction
- Parameters
  - Intrinsic clearance (CLint)
  - Fraction unbound in the plasma (fu)
  - Lipophilicity (pKa)
  - Acidity (pH)
  - Henry's Law constant (HL)

Verify PBPK models with experimental data (Literature)

- Nicotine
- NNK
- Formaldehyde

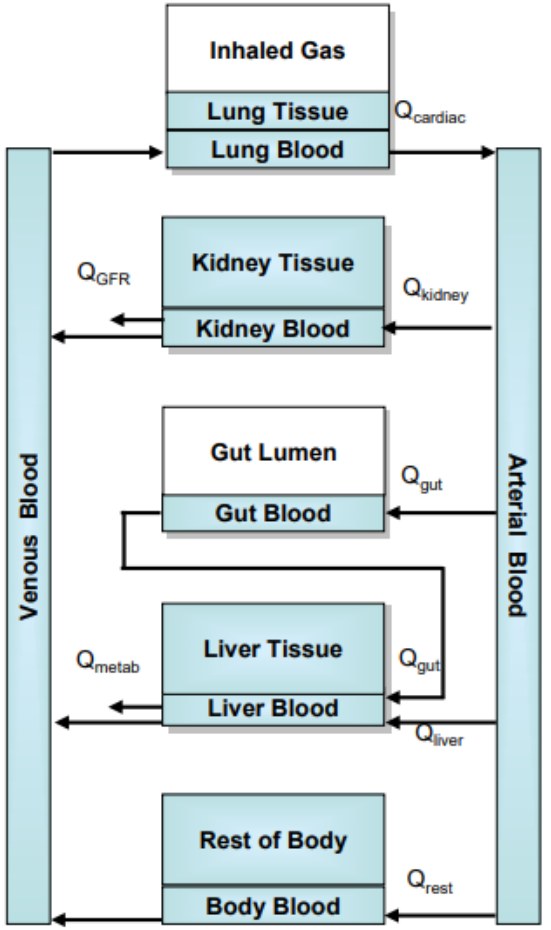
Estimate PK profiles and tissue levels

- Cmax for repeat-dose via inhalation: 3R4F vs. a heated tobacco product
- PK profile for a single dose: inhalation vs. oral ingestion (Annex)

[1] EPA: Environmental Protection Agency

HTTK Package: Open-Source PBPK Models

- EPA's High-throughput Toxicokinetics (HTTK) Package [1, 2]
  - A generic PBK Model
  - Constant partitioning is applied
  - Lung, kidney, gut and liver are modeled explicitly, others (e.g., fat, brain, bones) are lumped into "the Rest of Body" compartment
  - The only ways chemicals "leave" the body are either through metabolism in the liver or excretion by the kidney
- Input Parameters
  - Source: experimental data and QSAR (quantitative structure activity relationship) prediction
    - Intrinsic clearance (CLint)
    - Fraction unbound in the plasma (fu)
    - Lipophilicity (pKa)
    - Acidity (pH)
    - Henry's Law constant (HL)



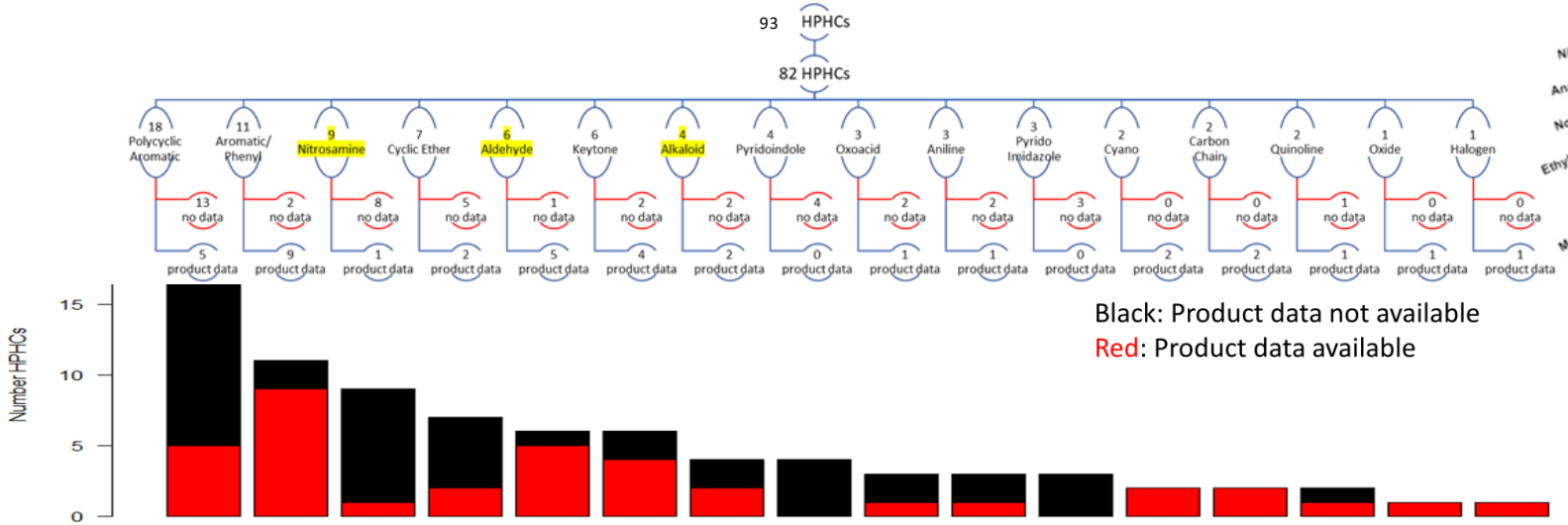
PBPK model diagram is adapted from: https://www.epa.gov/chemical-research/httk-epas-tool-high-throughput-toxicokinetics

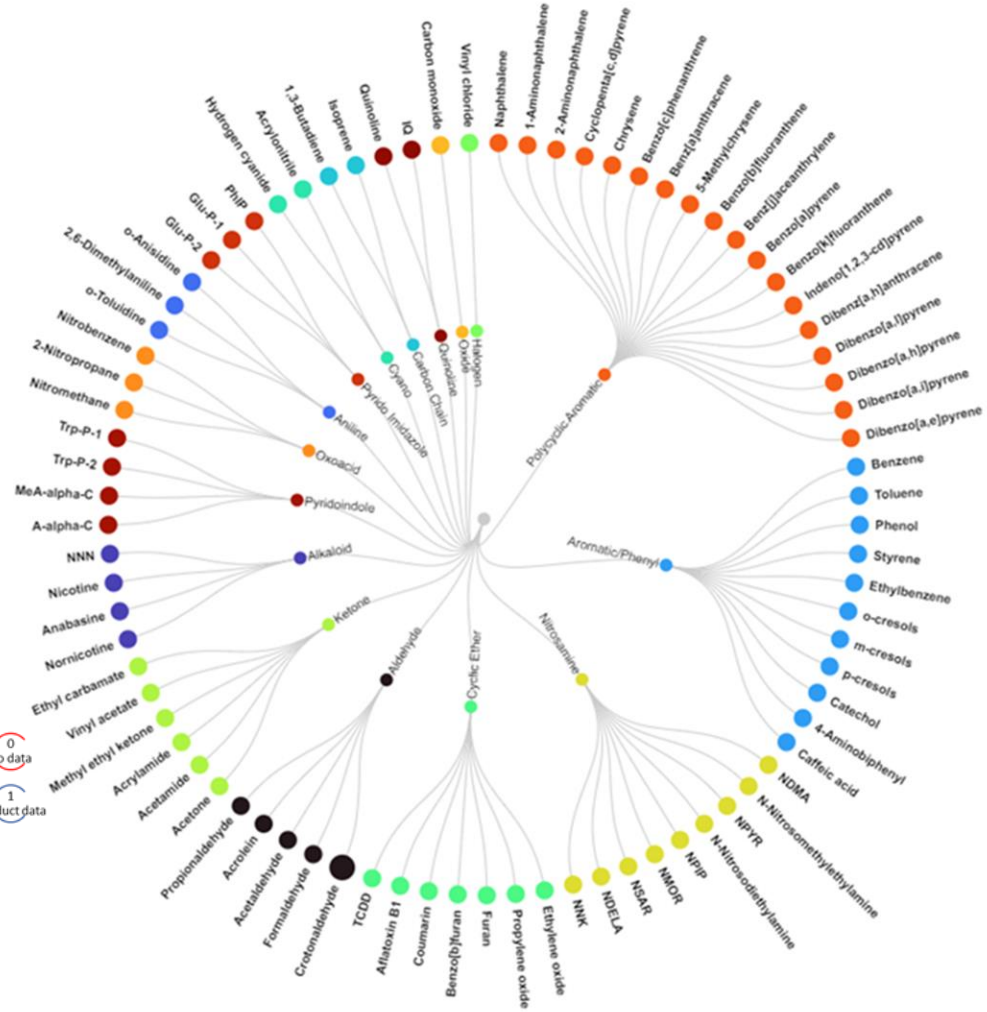
[1] https://pubmed.ncbi.nlm.nih.gov/30220889

[2] https://ice.ntp.niehs.nih.gov

Evaluation of the Model's Suitability for FDA's 93 Listed HPHCs

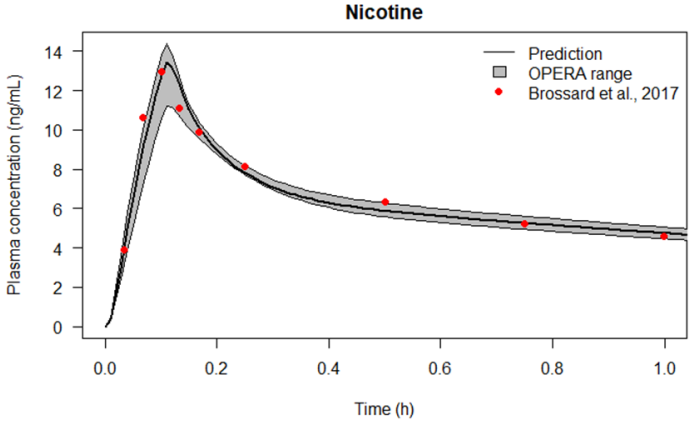
- Availability of the input parameters
  - 9 metals and 2 small molecules (ammonium and hydrazine) are excluded
  - Compounds amenable: 82
- Structural grouping of the 82 HPHCs
  - 16 groups based on similar functional groups and molecular descriptors and fingerprints
  - 3 chemicals from three groups were selected for model verification
- Availability of the product analytical data: 37 constituents for 3R4F and a heated tobacco product (HTP)





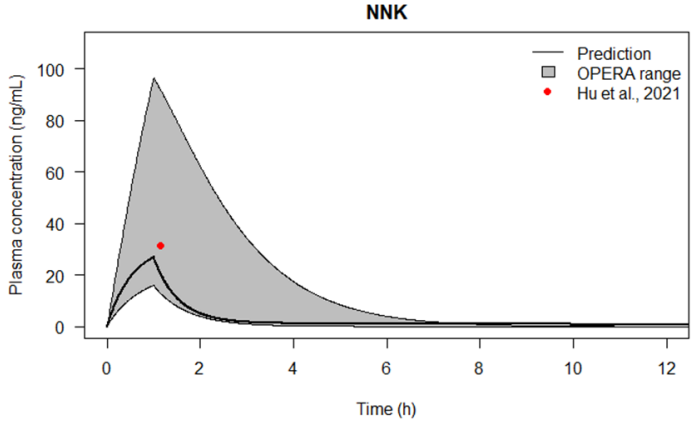
Verification of the PBK Model with Experimental Measurements

- Three HPHCs of varying characteristics and PK data availability are tested.
- Model predictions generally matched in vivo data well with the data points falling within the prediction range.
- Uncertainty of the prediction is associated with the uncertainty of the parameter prediction with a QSAR tool (OPERA[4]).



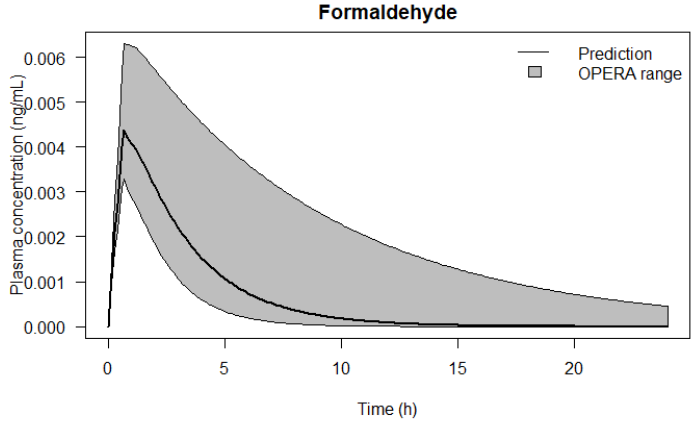
Nicotine (Alkaloid)

- Brossard et al. (2017) [1]
- Clinical study
- Exposure: one stick of THS 2.2 reduced risk
- The PK profile is compared between the measured and the predicted



NNK (Nitrosamine)

- Hu et al. (2021) [2]
- Non-clinical study in rats
- Exposure: 1-hour exposure to 1.7 mg/L NNK in rat
- Cmax and Tmax are compared between the measured and the predicted



Formaldehyde (Aldehyde)

- Heck et al. (1985) [3]
- Clinical study
- Exposure: 40-min inhalation exposure to 1.9 ppm formaldehyde
- No significant accumulation in the blood

[1] Brossard et al. 2017. "Nicotine Pharmacokinetic Profiles of the Tobacco Heating System 2.2, Cigarettes and Nicotine Gum in Japanese Smokers." Regulatory Toxicology and Pharmacology 89: 193–199.
[2] Hu et al. 2021. "Toxicokinetic and Genotoxicity Study of NNK in Male Sprague Dawley Rats Following Nose-Only Inhalation Exposure, Intraperitoneal Injection, and Oral Gavage." Toxicological Sciences 182 (1): 10–28.
[3] Heck et al. 1985. "Formaldehyde (CH_2O) Concentrations in the Blood of Humans and Fischer-344 Rats Exposed to CH_2O under Controlled Conditions." American Industrial Hygiene Association Journal 46 (1): 1–3.
[4] https://ntp.niehs.nih.gov/whatwestudy/niceatm/comptox/ct-opera/opera.html

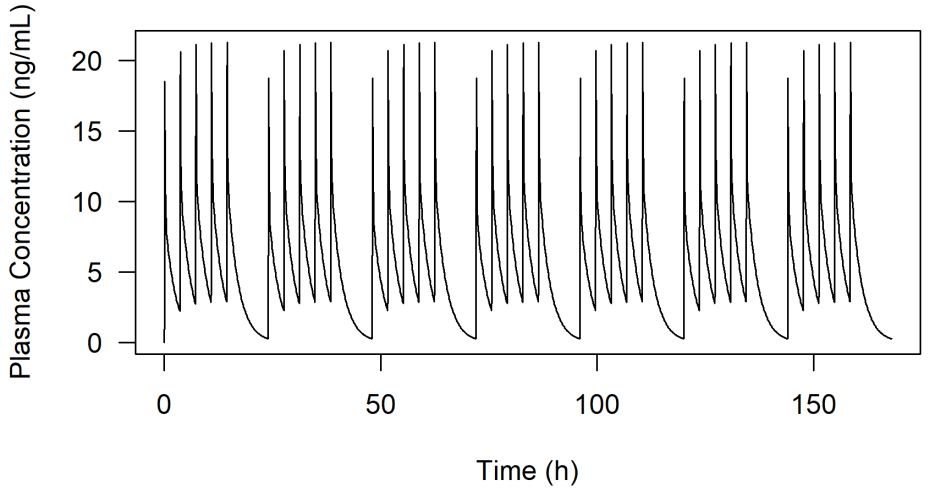
Plasma Level of Select Constituents after Repeat Dose

Assumptions of Consumer Use Pattern

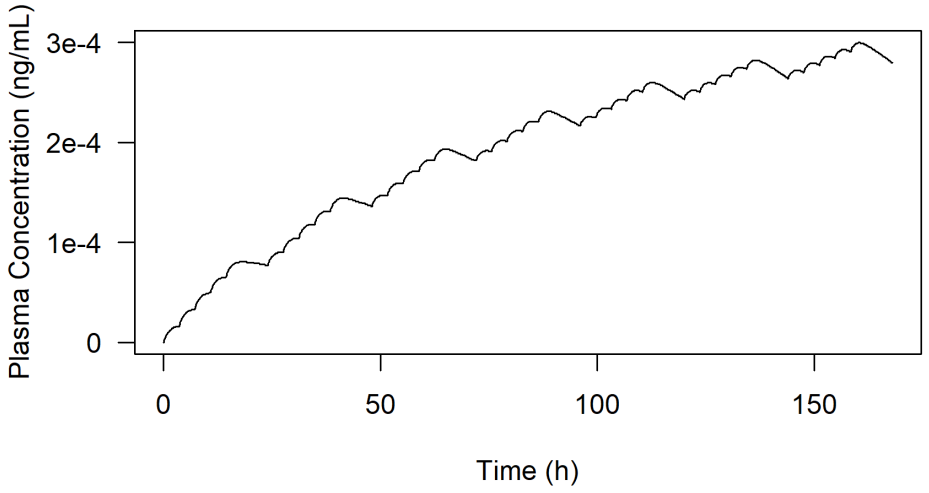
- Inhalation
- Seven consecutive days
- Use over 18 hours with six hours of abstinence
- Five sticks of 3R4F [1] (a reference cigarette) per day

Research Cigarette 3R4F [1]

Nicotine 3R4F



NNK 3R4F

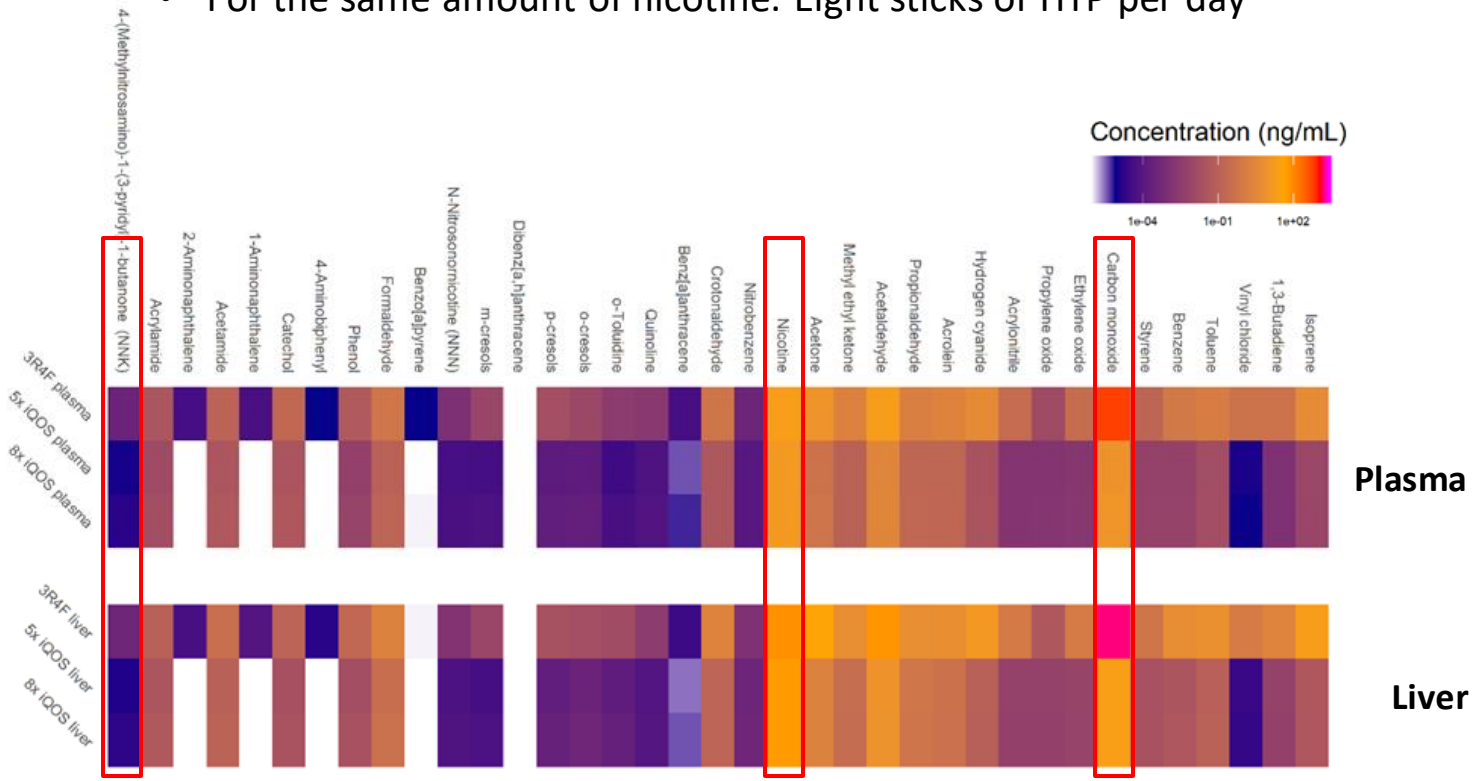


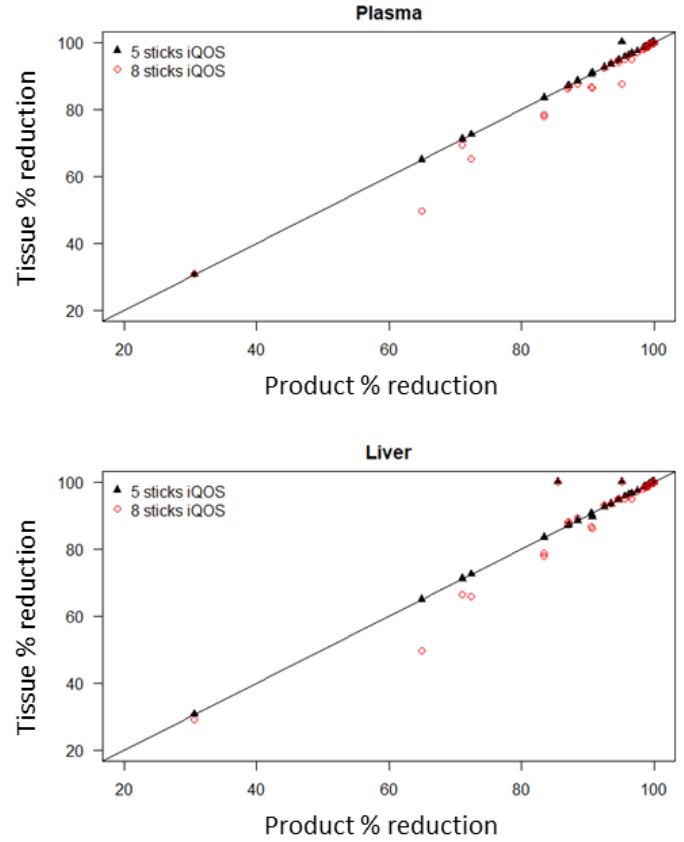
[1] https://www.coresta.org/university-kentucky-reference-cigarette

Estimated tissue level correlates with reduced exposure

Cigarettes vs. A Reduced Risk Product Candidate [1, 2, 3]

- For the same number of sticks: Five sticks of HTP per day
- For the same amount of nicotine: Eight sticks of HTP per day





[1] Uchiyama et al. 2018. Chemical Research in Toxicology 31 (7): 585–593.

[2] https://www.pmiscience.com/content/pmiscience/language-master/en/research/product-assessment-approach/platform-development/ths-mainstream-aerosol-compared-to-reference-cigarette-smoke.html.

[3] Helen et al. 2018. Tobacco Control 27 (Suppl 1): s30–s36.

Summary

Case Example I - HPHC Forward Dosimetry

- Using a publicly available, generic PBK model, the internal dose of selected HPHCs is estimated after various exposure regimen - via inhalation and from different products.
  - Proof-of-Concept: We verified the PBK model with three select compounds (nicotine, formaldehyde, and NNK) with experimental data from literature and showed consistent results.
  - We estimated the internal exposure of 82 out of the 93 FDA's listed HPHCs using a generic PBPK model. With HPHC level data in the products (37 out of 82), the target tissue level (Cmax) can also be estimated and compared for various products and use patterns.
  - The estimated target tissue levels can be used to design the exposure range for target tissue in vitro toxicity evaluation.

Strengths and Limitations

Case Example I - HPHC Forward Dosimetry

- Limitations
  - Modeling is a useful tool but the suitability is dependent on the chemical nature of the compounds, and availability of validation (experimental) data.
  - The generic model can be applied to a variety of compounds but may lack the specificity (e.g., lack of saturable metabolism).
  - Physiological parameters for compounds could be estimated using QSAR prediction tools if experimental data are not available. If not independently verified, the associated uncertainty needs to be taken into account.
- Strengths
  - The method provides an estimation of the *in vivo* dosimetry of compounds and is a critical initial step in the *in vitro* to *in vivo* extrapolation (IVIVE).
  - This is essential especially if *in vitro* data is used for toxicity evaluation for compounds with limited *in vivo* data.

Abbreviations

- **ENDS:** Electronic Nicotine Delivery Systems
- **EAD:** Equivalent Administered Dose
- **EPA:** Environmental Protection Agency
- **GI:** Gastrointestinal
- **HPHCs**: Hazard and Potentially Hazard Constituents
- **HTP:** Heated Tobacco Product
- **cHTS:** curated High-Throughput Screening
- **IVIVE:** In Vitro to In Vivo Extrapolation
- **NAMs:** New Approach Methodologies
- **NNK:** Nicotine-Derived Nitrosamine Ketone
- **NTA:** Non-Target Analysis
- **PBK:** Physiologically Based Kinetic
- **PG:** propylene glycol
- **BA:** Benzoic Acid
- **QSAR:** Quantitative Structure Activity Relationship