



Update on Insights from Reverse Dosimetry for *in vitro* Electronic Nicotine Delivery System (ENDS) and Heated Tobacco Product Testing (HTP)

IIVS Workshop 11

November 12, 2025

Gaithersburg, Maryland



**Michael J. Oldham¹ ; Kei Yoshino²; Liam Simms³; Yuki Kanemaru⁴;
Utkarsh Doshi⁵; Roman Wieczorek³; Ito Shigeaki²; Hiromi Ohara²; Ali A.
Rostami⁵, Ali Salehi⁵, Amy K. Madl⁶; Michael Hollings⁷; Srikar Dudi³;
Jingjie Zhang⁵; Arkadiusz K. Kuczaj⁸; Brian Keyser⁹; Robert Leverette⁹;
Stanely Gilliland III¹⁰; Amit Gupta¹¹; Aditya R. Kolli⁸; Ramez Labib¹⁰;
Francesco Lucci⁸; Kostas Papikinos³; Todor Antonijevico; Marjory
Moreau^{12, 13}**

**1. Juul Labs, Inc.; 2. Japan Tobacco Inc.; 3. Imperial Brands; 4. JTI SA; 5. Altria Client Services
LLC; 6. Valeo Sciences LLC; 7. LabCorp; 8. Phillip Morris Products S.A.; 9. RAI Services
Company; 10. Sapphire Sciences; 11. Battelle; 12. ScitoVation, LLC.; 13. Currently at ESQ Labs**



Outline

- **Background**
- **Process**
- **Calculations for Nicotine**
- **Considerations for *In Vitro* Studies**
- **Comparison with Published Literature**

- Extrapolation from *in vivo* data to doses for *in vitro* studies must include;
 - Considerations for target organ anatomical parameters
 - Dynamics of ENDS aerosol composition
 - Dynamics of ENDS aerosol exposure
 - *In vivo* metabolism of the compound(s) of interest



Dynamics of ENDS aerosol considerations

- **Target Organ Anatomical Parameters**

- The lung contains 58 different types of cells originating from all three germ layers (Travaglini et al., 2020; Knudsen & Ochs 2018)
- The surface area of the lung has been estimated to range from 70m² to 140m² (Thurlbeck, 1967; Knudsen & Ochs 2018)
- Alveoli (Type 1 and Type II cells) are covered in surfactant = surface film and an aqueous hypophase:
 - 90% lipids and 10% proteins
 - Biophysical and immunomodulatory functions
 - Hydrophobic and hydrophilic properties (Knudsen & Ochs 2018;)

- Tracheobronchial airway epithelium region is covered by mucus:

- Trachea – 10 - 30µm
- Bronchi – 2 - 5µm
- Neutral pH
- Osmolarity is isotonic to plasma
- Non-Newtonian viscoelastic material
- Mucus is continually cleared and swallowed

(Patton, 1996; Sanders et al., 2009; Wine, 1999; Leal et al., 2017)



Dynamics of ENDS Aerosol & Exposure

- **Rapid evaporation of propylene glycol that decreased with the addition of nicotine or several flavors** (Luo et al., 2021)
- **Nicotine and propylene glycol evaporation and water absorption started immediately after ENDS particle formation** (David et al., 2021)
- **Nicotine and propylene glycol evaporation was complete in approximately 20–30 s when exposed to 65% relative humidity and 20° C air** (David et al., 2021)
- **In an adult oral-pharyngeal hollow model using aerosols of pure glycerin, propylene glycol, or mixtures of both, inclusion of wet wall surfaces substantially increased aerosol deposition** (Castro et al., 2024)

Dynamics of ENDS Exposure

- Based upon measuring puffing behavior over 24 hours in 24 participants, there was an average of 156.2 ± 10.3 puffs, clustered in 10.2 ± 7.9 puffs per puffing session resulting in 15.3 ± 8.0 sessions (Kosmider et al., 2018)
- 14 established smokers* averaged 5.3 sessions/day (3.4 - 7.2 = 95% CI) while 6 non-established smokers* averaged 3.5 sessions/day (2.1 - 5.0 = 95% CI) using their own products over a 1-week period (Lee et al 2018)
- 20 subjects using their own products over a week averaged 6 ± 1 SE sessions/day with a range of 1 - 31 sessions/day (Robinson et al., 2016)

* Established smokers were defined by the authors as those individuals who had smoked at least 100 tobacco cigarettes in their entire life

- **The lungs have both Phase I (CYP, CYP reductases, Flavin-dependent monooxygenases, alcohol dehydrogenases, NAD(P)H:quinone oxidoreductases, Hydrolases) and Phase II enzymes (Glutathione S-transferases, UDP-Glucuronosyltransferases, Sulfotransferases, N-Acetyltransferases) and are inducible** (Aitio, 1976; Courcot et al., 2012; Kua et al., 2012; Oesch et al., 2019; Enlo-Scott et al., 2021)
- **The extent of xenobiotic metabolism varies regionally within the lungs. Club cells, which are a predominant cell type involved in lung metabolism, are concentrated within the bronchial region, where metabolism generally contributes more significantly to xenobiotic clearance compared to the peripheral region** (Oesch et al., 2019; Enlo-Scott et al., 2021)
- **Pulmonary CYP1A1/A2 expression is inducible** (Zhang et al., 2006; Jamaludin et al. 2013)
- **Cell lines don't have the same metabolic enzymes as the human lung cells** (Courcot et al., 2012)

Reverse Dosimetry – Process

- Define amount or range of inhaled/absorbed constituent (e.g., nicotine)
- Use Dosimetry modeling software (e.g., MPPD – Asgharian et al., 2024; Mori et al., 2024; etc.) to predict in what part of the respiratory tract (i.e., extra-thoracic, tracheobronchial, alveolar) target constituent deposits (both in particulate and vapor phase*; e.g., nicotine)
- Divide the deposited amount of target constituent by the surface area of the specified respiratory tract region

*As defined in MPPD, vapor phase contains volatile aerosol components (gas-phase) that deposit along the respiratory tract simultaneously with the particulate phase.



Reverse Dosimetry - Nicotine

- **Nicotine Pharmacokinetic studies (October 2024 cutoff date):**

- 66 unique pK studies (N = 2052) using controlled and/or ad-libitum use of ENDS products conducted worldwide

- 39 unique Studies (N = 1323) collected nicotine pK data with ad libitum ENDS product use over varying lengths of time

- 25 pK studies (N = 1038) provided Device Mass Loss data – 19 (N = 894) of these conducted with ad libitum ENDS product use over varying lengths of time

- 15 pK studies (N = 519) using controlled and/or ad-libitum use of HTP products conducted worldwide

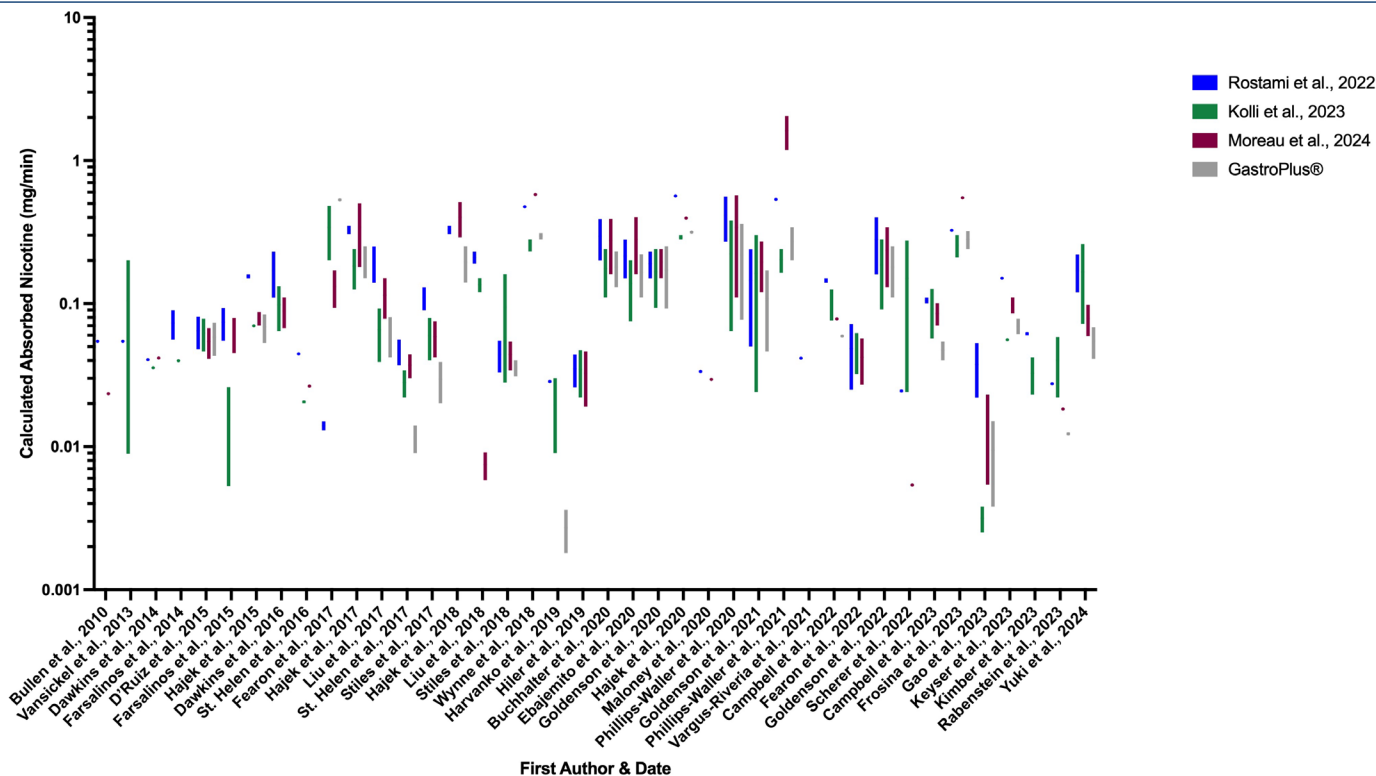
- 12 Studies (N = 448) collected nicotine pK data with ad libitum HTP product use over varying lengths of time



PBPK Models to Estimate Absorbed Nicotine

- Rostami et al. , 2022
- Kolli et al., 2023
- Moreau et al., 2024
- GastroPlus® (Simulation Plus)

PBPK Models Estimate of Absorbed Nicotine





Aerosol Dosimetry Models

Respiratory Tract Region	Asgharian et al., 2024 ^a				Mori et al., 2024 ^b		Lucci et al., 2025 ^c			
	Deposition Efficiency (Percent)			Surface Area (cm ²)	Total Deposition Efficiency (Percent)	Surface Area (cm ²)	Deposition Efficiency (Percent)			Surface Area (cm ²)
	Vapor	Particle	Total				Vapor	Particle	Total	
Extrathoracic	33.0	2.0	35.0	154.82	2.0	154.82	0	0.8	0.8	180.0
Tracheobronchial	2.5	2.5	5.0	4,717.0	19.7	4,717.0	0	1.0	1.0	2,620.0
Alveolar	30.0	22.0	52.0	709,254.0	62.0	709,254.0	7.0	76.3	83.3	438,000.0

a. 0.55µm MMAD, 50cm³ puff over 2 sec. with a 2 sec. hold in the oral cavity followed by 1000cm³ inhalation, taken from Figure 8A, surface area at mid inhalation.

b. Average of 0.7 – 1.0µm MMAD, 1.8 GSD, 40°C aerosol, 55 cm³ puff over 0.5 sec. with a 0.5 sec. hold in the oral cavity, followed by 550cm³ inhalation, Surface Area at mid inhalation.

c. Average of 0.7 & 1.0µm MMAD, GSD 1.8, 55cm³ puff over 3 sec. with a 2 sec. hold in oral cavity followed by 1000cm³ inhalation, 1:1 ratio of nicotine to acid, surface area at end of exhalation (i.e. FRC).



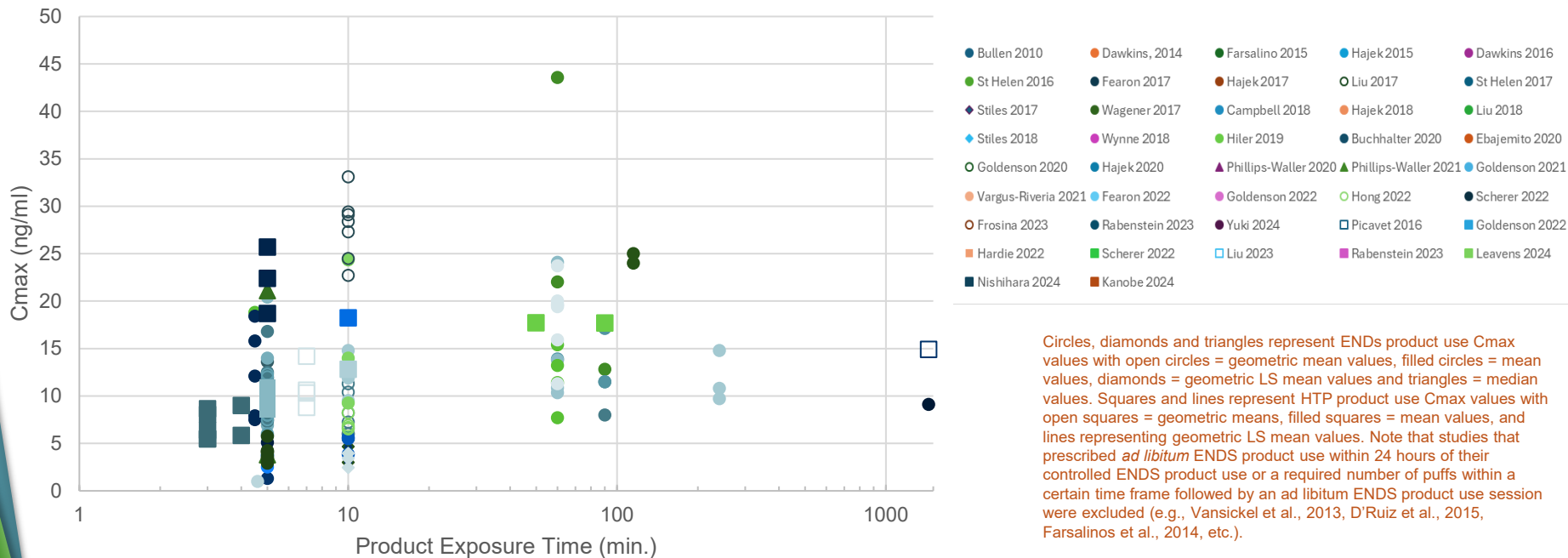
Reverse Dosimetry Calculations for Nicotine

- **PBPK estimates for absorbed nicotine are:**
 - **Minimum = 0.0018 mg nicotine/min.** (Harvanko et al., 2019)
 - **Maximum = 2.04 mg nicotine/min.** (Phillips-Waller et al., 2021)

Parameter	Units	Dosimetry Model Calculations for Nicotine		
		Asgharian et al., 2014	Mori et al., 2024	Lucci et al., 2025
Deposited in:				
Extrathoracic Airways	µg/min.	0.63 – 714	0.036 – 40.8	0.018 – 16.3
Tracheobronchial Airways	µg/min.	0.09 – 102	0.35 – 402	0.022 – 20.4
Alveolar Airways	µg/min.	0.94 – 1061	1.12 - 1264	1.83 – 1,699
Cell Exposure Dose				
Extrathoracic Airways	µg/cm ² - min.	0.004 – 4.6	0.00023 – 0.263	0.001 – 0.09
Tracheobronchial Airways	ng/cm ² - min.	0.02 – 1.5	0.074 – 85.2	0.008 – 7.8
Alveolar Airways	ng/cm ² – min.	0.0013 – 1.5	0.0016 – 1.8	0.004 – 3.9

Reverse Dosimetry Calculations for Nicotine

Nicotine Cmax from All Ad Libitum Exposure pK Studies (Ends & HTP)





Reverse Dosimetry Considerations for *In Vitro* Studies

- **Since most *in vitro* studies use a single dose, how should clinically relevant doses be calculated?**
 - **Is Haber's rule (concentration x time = same response) reasonable for *in vitro* exposure duration used for ENDS?**
 - A one-day study found the number of sessions/day was 15.3 ± 8.0
(Kosmider et al., 2018)
 - A one-week study found mean number of sessions/day was 6 ± 1 SE
(Robinson et al., 2016)
 - Another one-week study found a sessions/day ranged from 2.1 – 7.2
(Lee et al 2018)
- **In a study of 20 chemicals (21 cases; 2 endpoints for one chemical) assessing Haber's rule for inhalation, all chemicals/cases failed Haber's rule, meaning effects were more related to concentration than time** (Belkebir et al., 2011)



Reverse Dosimetry Considerations for In Vitro Studies

- ENDS aerosol don't deposit on cells in the respiratory tract, the aerosol deposits on mucus or surfactant
- Mucus is continuously being created/digested
- Mucus in extrathoracic and tracheobronchial airways is moving with mucus swallowing occurring from both regions
- Recent work demonstrates acute increase in mucus velocity when ENDS are used. (Bennett et al., 2024)
- Typical cells lines used for *in vitro* studies have documented metabolic differences compared to the human lung cells (Courcot et al., 2012; Oesch et al., 2019), so how should potential toxicity measured in these *in vitro* studies be interpreted?



Comparison to Published *In Vitro* Data

Applied doses corresponding to the 10-1000 μM *in vitro* exposure concentrations used for seven flavor ingredients found in some ENDS products tested by Muthumalage et al., 2018 using U937 monocytic cells from human pleural tissue and monocyte-macrophage cell line Mono Mac 6, established from peripheral blood of a patient with monoblastic leukemia.

Flavor Name	CAS #	MW	Exposure Duration (minutes)	Applied Doses ¹ ($\mu\text{g}/\text{cm}^2\cdot\text{min.}$)			
				10 μM	100 μM	500 μM	1000 μM
Diacetyl	431-03-8	86.06	1440	0.315	3.15	31.5	315
Cinnamaldehyde	104-55-2	132.16	1440	0.483	4.83	48.3	483
Acetoin	513-86-0	88.106	1440	0.322	3.22	32.2	322
Pentanedione	600-14-6	100.17	1440	0.366	3.66	36.6	366
O-vanillin	148-53-8	152.149	1440	0.556	5.56	55.6	556
Maltol	118-71-8	126.111	1440	0.461	4.61	46.1	461
Coumarin	91-64-5	146.145	1440	0.534	5.34	53.4	534

1. For 24-well plates each well has an area of approximately 1.9 cm^2 (UNC School of Medicine seeding density guidelines (<https://www.med.unc.edu/marsicolunginstitute/wp-content/uploads/sites/547/2020/01/Cell-plating-guidelines-01072020lf.pdf>))

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