Clinically Relevant Doses for Electronic Nicotine Delivery System and Heated Tobacco Product In Vitro Testing

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Michael J. Oldham[1]; Kei Yoshino[2]; Marianna Gaca[3]; Liam Simms[4]; Yuki Kanemaru[5]; Utkarsh Doshi[6]; Roman Wieczorek[4]; Ito Shigeaki[2]; Hiromi Ohara[2]; Ali A. Rostami[6]; Ali Salehi[6]; Amy K. Madl[7]; Michael Hollings[8]; Srikar Dudi[4]; Jingjie Zhang[6]; Arkadiusz K. Kuczaj[9]; Brian Keyser[10]; Robert Leverette[10]; Stanlezy Gilliland III[11]; Amit Gupta[12]; Aditya R. Kolli[9]; Ramez Labib[12]; Francesco Lucci[9]; Konstantinos Papikinos[4]; Todor Antonijevico[13]; Marjory Moreau[13,14]

1. Juul Labs, Inc.; 2. Japan Tobacco Inc.; 3. BAT (Investments) Ltd; 4. Imperial Brands; 5. JTI SA; 6. Altria Client Services LLC; 7. Valeo Sciences LLC; 8. Labcorp; 9. Phillip Morris Products S.A.; 10. RAI Services Company; 11. Sapphire Sciences; 12. Battelle; 13. ScitoVation, LLC.; 14. Currently at ESQLabs

Background

Extrapolating in vitro toxicology results to humans would be more meaningful if clinically relevant exposure doses were known. For both Electronic Nicotine Delivery Systems (ENDS) and Heated Tobacco Products (HTP) clinical studies reporting plasma nicotine values were used to calculate clinically relevant in vitro exposure doses for the extrathoracic (ET), tracheobronchial (TB) and alveolar (AL) regions of the human respiratory tract on a mass of nicotine/cm^2 – min basis.

Methods

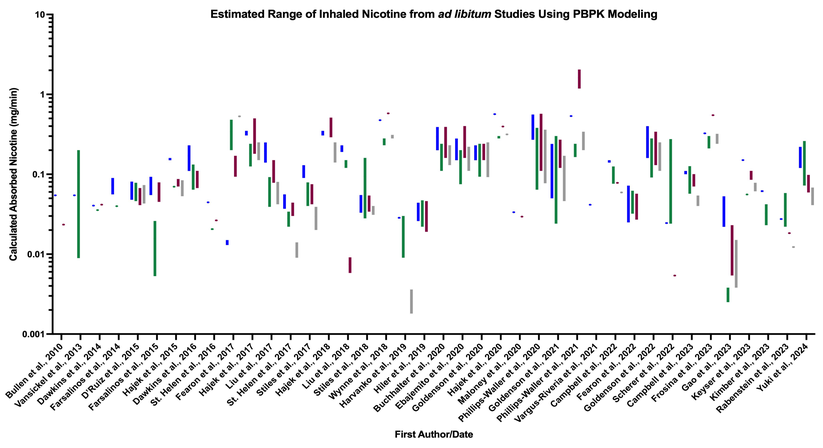
A recently published PubMed[1] search was duplicated and identified 95 papers as of November 2024, which were compared with previous summaries of pharmacokinetic studies of ENDS product use. Elimination of duplicates resulted in 65 and 15 unique clinical studies reporting nicotine plasma levels after ENDS and HTP use, respectively[2]. To calculate the total amount of absorbed nicotine from each clinical study reporting *ad libitum* ENDS use, 100% bioavailability was assumed and reverse dosimetry was performed using 3 recently published nicotine specific physiologically based pharmacokinetic (PBPK) models[3,4,5] and one commercial PBPK model (GastroPlus®). To calculate nicotine vapor and particle deposition within regions of the human respiratory tract, 3 published aerosol dosimetry models specifically designed for semi-volatile ENDS aerosols were used.[6,7,8] Finally, the surface of each respiratory tract region was used to determine the clinically relevant in vitro exposure doses on a nicotine mass/cm^2 – min basis.

Results

Forty of the 65 clinical trials conducted around the world reported plasma nicotine values from *ad libitum* ENDS product use by 1,323 participants. Participants used a variety of ENDS products with nicotine concentrations ranging from 0-59 mg/ml (0 – 5% nicotine) and product use from 4.5 minutes to 24 hours. ENDS products studied included first-generation disposables to the latest fourth-generation pod-based and refillable ENDS products.

The range of total absorbed nicotine predicted by the four PBPK models using reverse dosimetry from these 40 *ad libitum* use clinical trials was 0.0018 – 2.04 mg nicotine/min (**Figure 1**). Aerosol dosimetry model deposition efficiency predictions in the ET, TB and AL were different due to differences in mass median aerodynamic diameter, puff volume and duration of oral cavity hold used (**Table 1**). The clinically relevant in vitro exposure dose range for the ET region is 0.00009 – 5.0 µg nicotine/cm^2 – min., for the TB region is 0.008 – 102 ng nicotine/cm^2 – min, and for the AL regions is 0.0014 – 4.6 ng nicotine/cm^2 – min (**Table 2**).

Figure 1. Predicted total absorbed nicotine from clinical studies of *ad libitum* ENDS product use from 4 PBPK models.



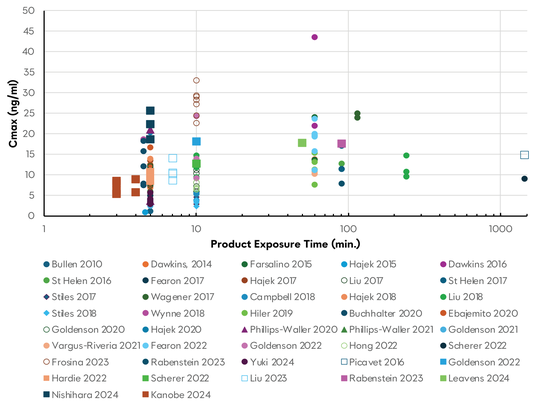


Figure 2. Reported nicotine Cmax values from *ad libitum* use of ENDS and heated tobacco products. Circles, diamonds and triangles represent ENDs product use. Cmax values with open circles = geometric mean values, filled circles = mean values, diamonds = geometric least square (LS) mean values and triangles = median values. Lines may not be visible due to overlap with other symbols. Squares and lines represent heated tobacco product use. Cmax values with open squares = geometric means, filled squares = mean values, and lines representing geometric LS mean values. Note that studies that prescribed *ad libitum* ENDS product use within 24 hours of their controlled ENDS product use or a required number of puffs within a certain time frame followed by an *ad libitum* ENDS product use session were excluded (e.g., Vansickel et al., 2013, D'Ruiz et al., 2015, Farsalinos et al., 2014, etc.).

Table 1. Aerosol dosimetry model predictions of vapor and particulate phase nicotine deposition in the adult human respiratory tract.

Respiratory Tract Region	Deposition Efficiency for Total Inhaled Nicotine (Percent)[a]	Calculated Surface Area cm^2	Total Deposition Efficiency for Absorbed Nicotine (Percent)[e]
	Asgharian et al., 2024[b]		
ET	35.0	154.82[c]	38
TB	5	4717.0[c]	5.43
AL	52	709,254[c]	56.57
	Mori et al., 2024		
ET	2.0	154.82[c]	2.3
TB	29.7	4717[c]	23.7
AL	62.0	709,254[c]	74.0
	Lucci et al., 2025		
ET	0.8	180[d]	0.9
TB	1.0	2,620[d]	1.2
AL	83.3	438,000[d]	97.9

a. Calculated from the dosimetry model and accounts for nicotine that may be exhaled. **b.** From Asgharian et al., Figure 8A. **c.** Calculated at mid-inhalation. **d.** Calculated at end of exhalation (i.e., functional residual capacity). **e.** Absorbed nicotine deposition efficiency adjusted based upon the ratio of respiratory tract region total inhaled nicotine deposition efficiencies to add up to 100%.

Table 2. Calculated inhaled nicotine exposure in human respiratory tract regions based upon ENDS product exposure using PBPK modeling.

Parameter	Units	Calculated Nicotine Amount		
		Asgharian et al., 2024	Mori et al., 2024	Lucci et al., 2025
Range of Total Absorbed Nicotine	mg/min.	0.0018 – 2.04		
Deposited in ET airways TB airways AL airways	 µg/min. µg/min. µg/min.	 0.68 – 775 0.1 – 111 1.01 - 1154	 0.041 – 46.9 0.43 – 483 1.3 - 1510	 0.016 – 18.4 0.022 – 24.5 1.8 – 1997
Cell Exposure Dose ET airways TB airways Al airways	 µg/cm^2 – min. ng/cm^2 – min. ng/cm^2 – min.	 0.004 – 5.0 0.02 – 23.5 0.0014 – 1.6	 0.0003 – 0.3 0.09 – 102 0.0019 – 2.1	 0.00009 – 0.1 0.008 – 9.3 0.004 – 4.6

Table 3. Applied doses in 16-HBE, BEAS-2B, and monocytes (U937) corresponding to the 10-1000 µM exposure concentrations used for seven flavor ingredients found in some ENDS products tested by Muthumalage et al., (2018).

Flavor Chemical			Exposure Duration (minutes)	Applied Doses[a] (µg/cm^2-min.)			
Name	CAS #	MW (g/mole)		10 µM	100 µM	500 µM	1000 µM
Diacetyl	431-03-8	86.06	1440	0.315	3.15	15.7	31.5
Cinnamaldehyde	104-55-2	132.16	1440	0.483	4.83	21.2	48.3
Acetoin	513-86-0	88.106	1440	0.322	3.22	16.1	32.2
Pentanedione	600-14-6	100.17	1440	0.366	3.66	18.3	36.6
O-vanillin	148-53-8	152.149	1440	0.556	5.56	27.8	55.6
Maltol	118-71-8	126.111	1440	0.461	4.61	23.1	46.1
Coumarin	91-64-5	146.145	1440	0.534	5.34	26.7	53.4

CAS = Chemical Abstract System; g= gram; MW= molecular weight; M = Mole
a. For 24-well plates each well has an area of approximately 1.9 cm^2 and typically 400 – 600 µl of apical media is used, however for a conservative comparison only 1 µl was used in our calculations (UNC School of Medicine seeding density guidelines) (https://www.med.unc.edu/marsicolunginstitute/wp-content/uploads/sites/547/2020/01/Cell-plating-guidelines-0107202Olf.pdf)

Discussion

Nine of the 15 clinical trials reporting *ad libitum* use of HTPs, reported plasma nicotine values (Cmax) that were similar to nicotine Cmax values from *ad libitum* use of ENDS products (**Figure 2**). Additionally, nicotine reverse dosimetry should allow extrapolation to clinically relevant doses of other ENDS and HTP constituents (i.e., flavors and harmful and potentially harmful chemicals) from characterized aerosols. These clinically relevant in vitro exposure dose ranges for nicotine are up to 545 (55.6/0.008) times lower than levels of ENDS flavors tested in some in vitro studies with tracheobronchial cells **(Table 3)**[9]. The magnitude of this difference is conservative since nicotine is typically used in ENDS products at a higher concentration than individual flavors.

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"Society of Toxicology/San Diego, California" – 260323

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Conflict of Interest: The authors declare no conflicts of interest

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