Prediction of potential passive exposure from commercial electronic nicotine delivery systems using exhaled breath analysis and computational fluid dynamic techniques

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Abstract

Use of computational fluid dynamic (CFD) modeling to predict temporal and spatial constituent exposure for non-electronic nicotine delivery systems (ENDS) users (passive exposure) provides a more efficient methodology compared to conducting actual exposure studies. We conducted a clinical study measuring exhaled breath concentrations of glycerin, propylene glycol, nicotine, benzoic acid, formaldehyde, acetaldehyde, acrolein, menthol and carbon monoxide from use of eight different commercial ENDS devices and a non-menthol and menthol cigarette. Because baseline adjusted levels of other constituents were not consistently above the limit of detection, the mean minimum and maximum per puff exhaled breath concentrations (\(N=20\)/product) of glycerin (158.7–260.9 \(\mu\)g), propylene glycol (0.941–3.58 \(\mu\)g), nicotine (0.10–1.06 \(\mu\)g), and menthol (0.432–0.605 \(\mu\)g) from use of the ENDS products were used as input parameters to predict temporal and spatial concentrations in an environmental chamber, office, restaurant, and car using different ENDS use scenarios. Among these indoor locations and ENDS use scenarios, the car with closed windows resulted in the greatest concentrations while opening the car windows produced the lowest concentrations. The CFD predicted average maximum glycerin and propylene glycol concentration ranged from 0.25 to 1068 \(\mu\)g m\(^{-3}\) and 1.5 pg m\(^{-3}\) to 13.56 \(\mu\)g m\(^{-3}\), respectively. For nicotine and menthol the CFD predicted maximum concentration ranged from 0.16 pg m\(^{-3}\) to 4.02 \(\mu\)g m\(^{-3}\) and 0.068 pg m\(^{-3}\) to 2.43 \(\mu\)g m\(^{-3}\), respectively. There was better agreement for CFD-predicted nicotine concentrations than glycerin and propylene glycol with published reports highlighting important experimental and computational variables. Maximum measured nicotine levels from environmental tobacco smoke in offices, restaurants, and cars exceeded our maximum average CFD predictions by 7–97 times. For all the measured exhaled breath constituents and CFD predicted constituents, except for propylene glycol and glycerin, concentrations were less from use of ENDS products compared to combustible cigarettes. NCT number: NCT04143256

1. Introduction

One of the concerns with the recent increase in electronic nicotine delivery systems (ENDS; also called e-cigarettes) use is the potential exposure of non-users to constituents in the exhaled breath of ENDS users (Bam et al. 2014, US FDA 2019). Originally, researchers utilized puffing machine generated ENDS aerosols that omitted gas phase and particle deposition within the human respiratory tract to generate exposure estimates of exhaled constituents from ENDS use (McAuley et al. 2012, Schripp et al. 2013, Czogala et al. 2014, Geiss et al. 2015). More recently, clinical trials have been conducted in rooms and offices (Shober et al. 2014, O’Connell et al. 2015, Maloney et al. 2016, Protano et al. 2017, 2018), well
defined exposure chambers (Liu et al 2017, Lampos et al 2019, van Drooge et al 2019, Oldham et al 2021), or have been measured opportunistically in locations where ENDS use has occurred (Ballbe et al 2014, Fernández et al 2015, Zwack et al 2017, Nguyen et al 2019). The uniqueness of each room, office, chamber, or location has limited extrapolation of each study’s results, however. These studies have demonstrated that exposure of non-ENDS users is possible, however the vast majority have also demonstrated that this exposure is significantly less than exposure to environmental cigarette smoke (Flouris et al 2012, Long 2014, Fernández et al 2015, Marco and Grimalt 2015, Hess et al 2016, Liu et al 2017, Papaefstathiou et al 2020).

The use of exhaled breath data to estimate the potential passive exposure to exhaled constituents from use of ENDS is an emerging field of research (Long 2014, St Helen et al 2016, Samburova et al 2018, Papaefstathiou et al 2020, Edmiston et al 2021). After performing a study measuring room air constituent concentrations in an environmental chamber where ENDS use occurred, Liu et al (2017) suggested use of a verified modeling approach would be a more efficient method to predict potential exposure of non-ENDS users to exhaled ENDS constituents. Subsequently, Rostami et al (2018) used computational fluid dynamic (CFD) techniques to predict potential spatial and temporal concentrations of some ENDS constituents that agreed with experimental data.

The combination of exhaled breath data and CFD modeling techniques can provide predictions of individual constituent concentrations in any indoor environment. This is a more efficient methodology than conducting actual exposure studies in each indoor environment. The exhaled breath of ENDS users is the source of the constituents measured in all the various environmental studies used to estimate potential exposure of non-users to exhaled ENDS aerosol. We designed a study to measure exhaled breath from users of four ENDS product flavors, with each flavor containing both 5.0% and 3.0% nicotine (by weight). The minimum and maximum per puff values for the major exhaled constituents (nicotine, propylene glycol, glycerin, and menthol for those Juul products that had menthol) were then used as input into the CFD model of Rostami et al (2018) to estimate potential exposure over time in an environmental chamber, idealized office, idealized restaurant, and car.

2. Materials and methods

2.1. Clinical study

All pertinent study documents were reviewed by the IntegReview Institutional Review Board prior to study initiation at the three clinical sites. This clinical study was conducted at Frontage Clinical Services, Inc. (Secaucus, NJ) and the Rose Research Center (Raleigh and Charlotte, NC). The clinical trial was conducted in accordance with the ethical principles that have their origin in the Declaration of Helsinki as stated in 21 Code of Federal Regulations (CFR) §312.120(c)(4); consistent with good clinical practice and all applicable regulatory requirements. The study was listed on clinicaltrials.gov website with the NCT number: NCT04143256.

Daily ENDS users and cigarette smokers were recruited to participate. Subjects were screened for participation up to 28 d before Day 1 (table 1) based upon inclusion and exclusion criteria (supplemental tables 1 and 2 (available online at stacks.iop.org/JBR/15/046006/mmedia)). All subjects were randomized between the three clinical sites. ENDS users who met all eligibility criteria were further randomized to Groups I–IV; however, combustible cigarette users who met all eligibility requirements were assigned to Groups V or VI based on their cigarette flavor smoking preference. Non-menthol combustible cigarette users were assigned to Group V and menthol combustible cigarette users were assigned to Group VI. All subjects agreed to abstain from use of alcohol, any nicotine products, consumption of any products containing mint or menthol flavors (e.g. chewing gum, mouth wash, toothpaste, etc) for 12 h before the start of testing.

A total of 120 subjects (20 for each group) were planned for this study. Of 264 subjects that were screened, 149 healthy males and females between the ages of 21 and 65 years were enrolled. Of these 149 enrolled subjects, 136 were assigned to six groups (Groups I through VI). The remaining 13 enrolled subjects were not assigned to any group, but were included in the safety population. Groups I–IV were to use both concentrations (e.g. 5.0% or 3.0%, in randomized order) of one ENDS flavor, and Groups V and VI were assigned to cigarette use (non-menthol and menthol, respectively). Each study group consisted of approximately 20 subjects and no study group included greater than 60% of either sex. Details of the study design are shown in table 1.

Groups I–IV completed two periods of baseline and test sample collections with a 3 h break in between each period. Each period consisted of two baseline samples (the first for analysis of nicotine, propylene glycol and glycerin and the second for analysis of acetalddehyde, acrolein, benzoic acid, formaldehyde, and menthol), followed by product use, and then two test samples. Exhaled carbon monoxide (CO) was measured before the first baseline sample, after the second baseline sample, before the first test sample and after the second test sample. Pods used during the test sessions were weighed before and after use. Groups V and VI completed one period of baseline and test sample collections, consisting of two baseline sample collections and two test sample collections.

A controlled puff sequence (product puffing time and subsequent inhalation time) was used to reduce
| Screening day | Study day | Follow-up |
|---------------|-----------|-----------|
| Day −28 to −1 | Day 1     | 24 h (the day following check-out) |

**Table 1. Clinical study design and products used in the study.**

| Juul ENDS groups | Number of subjects | Number of subjects in each sequence | Period 1 | Period 2 |
|------------------|--------------------|------------------------------------|----------|----------|
| I                | 20                 | 10                                 | A        | B        |
| II               | 20                 | 10                                 | B        | A        |
| III              | 20                 | 10                                 | C        | D        |
| IV               | 20                 | 10                                 | D        | C        |

**Cigarette groups**

| Number of subjects | Period 1 | Period 2 |
|--------------------|----------|----------|
| V                  | 20       | I        | N/A      |
| VI                 | 20       | J        | N/A      |

*Subjects were assigned to a Group based on product use history (ENDS, non-menthol smoker, menthol smoker). ENDS users were assigned to one Juul ENDS Product flavor and used 5.0% and 3% nicotine strengths in sequence according to the randomization.

*Subjects in Groups V and VI used one product, thus were discharged from study site after completion of Period 1.

*Subjects were contacted via phone approximately 24 h after check-out. Site staff made two phone call attempts, separated by one day. A = Juul ENDS 5.0% (nicotine by weight) Virginia tobacco; B = Juul ENDS 3% (nicotine by weight) Virginia tobacco; C = Juul ENDS 5.0% (nicotine by weight) Mango; D = Juul ENDS 3% (nicotine by weight) Mango; E = Juul ENDS 5.0% (nicotine by weight) Mint; F = Juul ENDS 3% (nicotine by weight) Mint; G = Juul ENDS 5.0% (nicotine by weight) Menthol; H = Juul ENDS 3% (nicotine by weight) Menthol; I = US Cigarette, Tobacco flavor (Marlboro Gold King Size); J = US Cigarette, Menthol flavor (Newport King Size).
variability between subjects. All subjects were trained on the controlled puff sequence using video and instructions by clinic personnel. Subjects in Groups I–IV (ENDS groups) had the opportunity to try an ENDS product using the controlled puff sequence while subjects in the two cigarette groups had the opportunity to use their assigned cigarettes. All subjects were also trained on the exhaled breath collection.

2.2. Products tested
Table 1 indicates the products used in this study. The ENDS products contained 0.7 ml e-liquid, comprised of vegetable glycerin (glycerin; United States Pharmacopeia [USP] grade), propylene glycol (USP grade), nicotine (5.0% and 3.0% by weight; USP grade), benzoic acid (USP grade), and various flavorants. All ENDS products were provided by Juul Labs Inc and were stored in a locked, limited-access area in the study site and kept at controlled room temperature (defined as 20 °C–25°C [68–77°F]). All combustible cigarette products (Marlboro Gold King Size and Newport King Size) were purchased by the study site. Marlboro Gold King Size and Newport King Size cigarettes were selected because they are market leading non-menthol and menthol cigarette brands.

2.3. Exhaled breath sample (EBS)
EBS collection was performed consistent with previous method verification work (Oldham et al. 2017). In brief, EBS collection consisted of an RTube™ (Respiratory Research, Austin, TX) connected to dual, in series, filter holders, each containing a Respirgard II™ filter. Exhaled breath condensate from the RTube™ and filters were extracted for gas-chromatograph/mass spectrometer (GC/MS) analysis. The concentrations of nicotine, propylene glycol, glycerin, acetaldehyde, acrolein, formaldehyde, menthol and benzoic acid (including benzoate) were measured using two collections. The first collection (non-aldehyde) employed uncoated Respirgard II™ filters for nicotine, propylene glycol, glycercin, menthol and benzoic acid. The second collection (aldehyde) utilized 2,4-dinitrophenylhydrazine (DNPH) coated filters for carbonyl compounds (acetaldehyde, acrolein, and formaldehyde). Use of DNPH coated filters for collection of carbonyl compounds in exhaled breath was verified in previous work (Oldham et al. 2017) and is consistent with other analytical methods for collection of carbonyl compounds from air samples (ASTM D5197-16 2016; EPA Method TO-11A, 1999). Limit of detection (LOD) and limit of quantitation (LOQ) values for each analyte/collection (R-tube™ and filters) are provided in supplemental table 3. Exhaled CO measurement was performed using a CoVita Micro Basic Smokerizer portable CO meter (accuracy ≤ 2 ppm). The baseline-adjusted concentration (=post baseline value—baseline value of each EBS analyte) was used in the statistical analysis. Trained personnel ensured that each subject performed 10 inhalation/exhalations for each EBS collection.

2.4. Statistical procedures
Descriptive statistics including number of observations, mean, median, standard deviation, minimum, maximum, and 90% confidence interval (CI), for the baseline-adjusted measurements of each group were performed. As an exploratory analysis, the two sample t-test was used to test for the difference in each constituent level between any of the two cigarette groups with each of the ENDS study groups (5.0% and 3.0%) using a significance level of $p \leq 0.05$.

Primary analyses consisted of comparing between baseline-adjusted constituent concentrations (post-baseline concentration—baseline concentration) of EBS from the two ENDS Product nicotine concentrations (5.0% vs 3.0% by weight) for each flavor (Virginia tobacco, Mango, Mint, and Menthol). It was hypothesized that there would be no significant difference in the EBS concentration of nicotine between the 5.0% and 3.0% nicotine concentration products regardless of the flavor tested. Secondly, it was also expected that EBS constituent concentrations (except nicotine, propylene glycol, and glycerin) from use of ENDS products would be significantly lower than from use of the two cigarette products, Marlboro Gold King Size and Newport King Size.

The sample size estimation was based upon our hypothesis of no significant difference in EBS level of nicotine between the two nicotine levels tested, regardless of flavor (Groups I, II, III, and IV). A power calculation performed using two one-sided t-tests, assuming a 5% Type I error rate and 20% intra-subject variability, with 80% power, 20% equivalence margin, and 0% true difference, resulted in approximately 20 subjects being needed for each group.

A linear mixed model was implemented in SAS (ver. 9.4; Cary, NC) for the primary analysis. In the linear mixed model, the response variable was the baseline-adjusted EBS measurement; the model terms included product (3.0% or 5.0% nicotine), sequence and period as the fixed effects; with subject nested within sequence as the random effect. For each study group, the least squared mean difference between the 5.0% nicotine product (reference) and the 3.0% nicotine product and 90% CI were determined. Data outliers were identified according to the studentized residual in SAS (PROC MIXED or PROC GLM procedures). A value with a studentized residual larger than three (absolute value) was deemed an outlier and was removed in the exploratory sensitivity analysis. Values that were either below the limit of quantification or detection were used as the limit of quantification or detection divided by the squared root of 2 (EPA 1994, National Center for Health Statistics 2016).
2.5. CFD predictions
The Rostami et al (2018) CFD-based distributed computational model was used to predict selected constituent levels for four indoor spaces. Detailed CFD methods are contained in the supplemental methods. As noted by Rostami et al (2018), the CFD-based distributed computational model predictions were validated using the chamber data of Czogala et al (2014), which used an experimental machine puffing for aerosol generation.

The four indoor spaces included an environmental chamber (supplemental figure 1) used in a previous clinical study (Oldham et al 2021), an idealized office (figure 1), an idealized restaurant (figure 2), and a Toyota Corolla car (figure 3). The Rostami et al (2018) model is based on the physical and thermodynamic interactions between the air, gas, and particulate phases of the aerosol. All major physical and thermodynamic processes, including turbulence in air, gas-particle mass transfer, discrete particle-phase transport, constituent species transport, and aerosol emission rate are included. This enables prediction of temporal and spatial changes in the concentration of chemical constituents from a defined aerosol emission source as those constituents travel through an indoor space.

Three simplifying assumptions were used in this work. First, we assumed all constituents would completely evaporate instantaneously. In other words, the evaporation time scale is taken to be negligible as compared to the overall simulation timescale. Exhaled breath mixing with air in the indoor space and fresh air ventilation play important roles in rapid evaporation of constituents. The volume of air in the indoor space is far greater than that of the exhaled breath introduced, such that equilibrium between the particulate and gas phase will result in a minuscule particulate phase residual. This is true even for glycerin, which has the lowest vapor pressure in the exhaled breath mixture. Recent work reported the time scale for nicotine and propylene glycol evaporation from ENDS aerosol is approximately 20–80 s (David et al 2020). This assumption results in a slight over prediction of gas-phase constituent concentrations.

Our second assumption was that constituent loss to walls and surfaces were negligible. Particles would deposit on surfaces due to diffusion and...
sedimentation while gas phase constituents would be adsorbed by the surfaces of the indoor space. A clinical study by Liu et al. (2017) demonstrated that after ENDS products were used, the amounts of selected constituents in the surface samples taken were below the analytical method detection limits. This assumption also results in a slight over prediction of gas phase constituents as none are lost to surfaces. Our third assumption was isothermal conditions, meaning the exhaled breath and occupants did not affect the air temperature in the indoor space. As noted in our first assumption, the volume of air in the indoor space is far greater than that of the exhaled breath. Making the isothermal assumption also enabled omission of various heating and cooling scenarios within the indoor spaces. This assumption results in more homogenous predictions.

Using the three simplifying assumptions, gas transport in the indoor space is controlled by diffusion, convection, and turbulent mixing. The gas phase is treated as a continuous quantity, the concentration of which is calculated by solving species transport equations for each constituent through a Eulerian approach (Rostami et al. 2018). The necessary input data comprises two parts. One part provides detailed dimensions of the indoor space including a precise floor plan containing heating and ventilation inlets/outlets, fresh air ventilation rate, and internal re-circulation air flow rate (table 2). For a moving car, the ventilation rate depends on the speed, the circulation fan (on or off) and whether the windows are closed or open. Fruin et al. (2011) recommended an equation for a moving Toyota Corolla that was used in this work. The vehicle velocity used in this analysis was 30 mph. The second part of the input data are results from the clinical study on constituent concentrations in exhaled breath. Based upon the clinical results, three of the constituents comprising ≥90%–95% of ENDS formulations were chosen (Oldham et al. 2018): propylene glycol, glycerin, and nicotine. Menthol was added as it is a primary flavor category. The minimum and maximum per puff average amount for the four constituents (table 3), as well as the frequency and release location of the exhaled breath, and number of occupants in the indoor space (table 2) completed the necessary input data for constituent concentration predictions in the four indoor spaces.
3. Results

3.1. Clinical trial—demography and product use

The mean age of subjects who enrolled in this study was 37.1 years, measured mean weight was 80.4 kg, and calculated mean body mass index (BMI) was 27.6 kg m$^{-1}$. No substantial statistical difference in weight, height, and BMI was measured (weight or height) or calculated (BMI) between Juul Product groups (Group I through IV) or cigarette groups (Groups V and VI). Mean number of cigarettes smoked per day was 14.9 and overall mean duration of cigarette usage was 19.4 years. A majority of the subjects that were enrolled in this study were White ($N = 116; 77.9\%$). Blacks or African Americans made up 18.1\% ($N = 27$) and other races (American Indian or Alaskan Natives, Asians, Native Hawaiian or other Pacific Islanders) represented less than 5.0\% of the overall enrollment. Non-Hispanic or Latino population made up 87.2\% ($N = 129$) and Hispanic or Latino comprised 11.4\% ($N = 17$) of the overall enrolled population. Complete demographic information can be found in supplemental table 4.

The observed number of inhalations/exhalations was 10 for each subject in all ENDS and cigarette groups, indicating good compliance with the controlled puffing sequence. For 5.0% ENDS products, mean product consumption ranged from 20.0 to 26.5 mg across groups for the non-aldehyde constituent collection (supplemental table 5) and 25.3–26.8 mg for the aldehyde collection (supplemental table 6). For 3.0% ENDS products, mean product consumption ranged from 21.8 to 28.7 mg across groups for the non-aldehyde constituent collection and 22.5–30.9 for the aldehyde collection (supplemental tables 5 and 6, respectively).

3.2. Exhaled breath constituent concentrations

There were no statistically significant differences (paired t-test) in the mean baseline concentrations between groups for any of the measured constituents (table 4). Comparison of the mean and median
values for baseline constituent concentrations confirm that the data was sufficiently normally distributed for use of the linear mixed model without data transformation.

The baseline adjusted concentration of all constituents measured above the limit of detection or quantification was highly variable, especially for the ENDS groups. There were statistically significant differences in the mean exhaled constituent concentrations between the ENDS groups and the non-menthol (Group V) and menthol cigarette (Group VI) groups (figure 4 and table 5). The baseline-adjusted concentration of exhaled acetaldehyde, acrolein, carbon monoxide, and formaldehyde was statistically significantly lower ($p < 0.05$) for all ENDS Products than in either of the two cigarette groups (Groups V and VI).

The baseline-adjusted concentration of glycerin was statistically significantly higher in all ENDS Products than in either of the two cigarette groups. The baseline-adjusted concentration of propylene glycol in exhaled breath between Groups I–IV and the non-menthol cigarette group (Group V) were statistically significantly higher except for the Virginia tobacco 5.0% and Menthol 5.0% ENDS products. Baseline-adjusted concentrations of propylene glycol were statistically significantly higher for the Mango and Mint flavors compared to the...
Table 2. Summary of ventilation rate and ENDS use conditions for CFD predicted constituent concentrations.

| Indoor space                      | Air exchanges per hour | Number of ENDS users | Puffing frequency (per min) | Use duration (min) | Exposure duration (min) |
|-----------------------------------|------------------------|----------------------|-----------------------------|--------------------|-------------------------|
| Environmental chamber—residential ventilation | 4.5<sup>a</sup>         | 10                   | 2                           | 5 min every half hour<sup>c</sup> | 240                     |
| Environmental chamber—office ventilation | 7.5<sup>b</sup>         | 10                   | 2                           | 5 min every half hour<sup>c</sup> | 240                     |
| Environmental chamber—hospitality ventilation | 15<sup>b</sup>          | 10                   | 2                           | 5 min every half hour<sup>c</sup> | 240                     |
| Office                            | 0.35                   | 1                    | 0.33                        | 30                 | 60                      |
| Office                            | 1                      | 1                    | 3.3                         | 3                  | 30                      |
| Office                            | 0.35                   | 1                    | 3.3                         | 3                  | 30                      |
| Restaurant                        | 2.02                   | 5                    | 1.2                         | 60                 | 120                     |
| Car (closed windows)              | 3.3                    | 5                    | 1                           | 10                 | 20                      |
| Car (open windows)                | 3.3                    | 1                    | 3.3                         | 3                  | 30                      |

<sup>a</sup> 25% must be fresh air according to the American National Standards Institute and the American Society of Heating, Refrigerating, and Air-Conditioning Engineers (ASHRAE/ANSI Standard 62.1-2016 2016) recommendations.

<sup>b</sup> 22% must be fresh air according to the American National Standards Institute and the American Society of Heating, Refrigerating, and Air-Conditioning Engineers (ASHRAE/ANSI Standard 62.1-2016 2016) recommendations.

<sup>c</sup> Every half hour over a 4 h period, ten puffs each of five second duration with a 30 s interval was used. A total of 800 puffs were taken overall over a 4 h-long session.

Table 3. Mean exhaled breath results used as CFD modeling input. Numbers indicate mean exhaled concentrations in micrograms per puff.

| Constituents         | Virginia tobacco | Mint  | Mango | Menthol |
|----------------------|------------------|-------|-------|---------|
| Glycerin             | 260.9            | 281.9 | 236.1 | 227.0   | 255.7 | 263.7 | 158.7 | 213.4 |
| Propylene glycol     | 3.58             | 1.41  | 1.36  | 1.13    | 1.13  | 1.25  | 0.941 | 1.35  |
| Nicotine             | 1.06             | 0.242 | 0.286 | 0.138   | 0.140 | 0.10  | 0.133 | 0.203 |
| Menthol              | NA               | NA    | 0.605 | 0.564   | NA    | NA    | 0.432 | 0.483 |

NA—not applicable.

non-menthol cigarette group but not the menthol cigarette group. The baseline-adjusted concentration of exhaled menthol was statistically significantly lower (<0.001) in the ENDS groups, including Mint and Menthol flavors, than in the Menthol cigarette group (Group VI). Exhaled menthol concentrations were statistically significantly higher (<0.001) for ENDS Mint and Menthol flavors, in comparison to the non-menthol cigarette group (Group V). No exhaled menthol was detectable in the Virginia tobacco and Mango Product groups (Groups I and II) and the non-menthol cigarette group (Group V).

Although there were statistically significant differences in baseline adjusted average concentrations of some measured constituents in the ten puff collections between the 5.0% and 3.0% nicotine ENDS products, there was no consistent pattern (table 5). Consistent with our hypothesis, there was no statistically significant difference in baseline adjusted concentration of exhaled nicotine between the 5.0% and 3.0% nicotine concentrations in any of the four ENDS products. The baseline adjusted concentration of benzoic acid was only above the limit of detection in the exhaled breath collection from use of Virginia tobacco 5.0%. The baseline adjusted concentration of acrolein was only measured above the limit of detection in the exhaled breath collection from use of Mango (3.0%), but there was no statistically significant difference between the 5.0% and 3.0% products. There was no difference between the 5.0% and 3.0% nicotine mint and menthol Juul products in the baseline adjusted exhaled breath concentration of menthol.

3.3. Adverse events

There were no serious adverse reactions that occurred in this study. Four study emergent and unanticipated adverse reactions were reported (caffeine withdrawal, hypoglycemia, skin irritation, and dizziness) by four separate subjects. All were classified as mild in intensity and resolved during the course of the study. One each was reported in Groups II, IV, V, and VI. The study emergent and unanticipated adverse reactions reported for Groups IV and V were related to
Table 4. Descriptive statistics for baseline exhale breath measurements (µg/10 puff collection unless otherwise noted).

| Constituent          | Baseline statistics | Group I | Group II | Group III | Group IV | Group V | Group VI |
|----------------------|---------------------|---------|----------|-----------|----------|---------|----------|
|                      | N(n*)               | 5.0%    | 3.0%     | 5.0%      | 3.0%     | 5.0%    | 3.0%     | 5.0%    | 3.0%     | 5.0%    | 3.0%    | 5.0%    | 3.0%    | 5.0%    | 3.0%    | 5.0%    | 3.0%    |
| Propylene glycol     | Median (SD)         | 3.87 (2.58) | 5.17 (2.27) | 5.27 (3.73) | 5.61 (3.06) | 3.87 (2.58) | 5.17 (2.27) | 5.27 (3.73) | 5.61 (3.06) |
|                      | N(n*)               | 21 (21) | 21 (21)  | 23 (23)   | 25 (25)   | 23 (23)  | 25 (25)   | 23 (23)  | 25 (25)  |
|                      | Median (SD)         | 2.93 (2.85) | 2.93 (2.85) | 2.93 (2.93) | 2.93 (2.93) | 2.93 (2.85) | 2.93 (2.85) | 2.93 (2.93) | 2.93 (2.93) |
| Glycerin             | Median (SD)         | 1.38 (2.128) | 1.76 (1.52) | 1.54 (1.44) | 1.50 (1.36) | 1.38 (2.128) | 1.76 (1.52) | 1.54 (1.44) | 1.50 (1.36) |
|                      | N(n*)               | 21 (21) | 21 (21)  | 23 (23)   | 25 (25)   | 23 (23)  | 25 (25)   | 23 (23)  | 25 (25)  |
| Nicotine             | Median (SD)         | 1.06 (0.06) | 1.06 (0.06) | 1.06 (0.06) | 1.06 (0.06) | 1.06 (0.06) | 1.06 (0.06) | 1.06 (0.06) | 1.06 (0.06) |
|                      | N(n*)               | 21 (21) | 21 (21)  | 23 (23)   | 25 (25)   | 23 (23)  | 25 (25)   | 23 (23)  | 25 (25)  |
| Benzoic acid         | Median (SD)         | 0.39 (0.033) | 0.33 (0.033) | 0.33 (0.033) | 0.33 (0.033) | 0.39 (0.033) | 0.33 (0.033) | 0.33 (0.033) | 0.33 (0.033) |
|                      | N(n*)               | 21 (21) | 21 (21)  | 23 (23)   | 25 (25)   | 23 (23)  | 25 (25)   | 23 (23)  | 25 (25)  |
| Formaldehyde         | Median (SD)         | 0.43 (0.17) | 0.38 (0.16) | 0.42 (0.23) | 0.39 (0.31) | 0.43 (0.17) | 0.38 (0.16) | 0.42 (0.23) | 0.39 (0.31) |
|                      | N(n*)               | 21 (21) | 21 (21)  | 23 (23)   | 25 (25)   | 23 (23)  | 25 (25)   | 23 (23)  | 25 (25)  |
| Acetaldehyde         | Median (SD)         | 0.33 (0.033) | 0.33 (0.033) | 0.33 (0.033) | 0.33 (0.033) | 0.33 (0.033) | 0.33 (0.033) | 0.33 (0.033) | 0.33 (0.033) |
|                      | N(n*)               | 21 (21) | 21 (21)  | 23 (23)   | 25 (25)   | 23 (23)  | 25 (25)   | 23 (23)  | 25 (25)  |
| Acrolein             | Median (SD)         | 0.33 (0.033) | 0.33 (0.033) | 0.33 (0.033) | 0.33 (0.033) | 0.33 (0.033) | 0.33 (0.033) | 0.33 (0.033) | 0.33 (0.033) |
|                      | N(n*)               | 21 (21) | 21 (21)  | 23 (23)   | 25 (25)   | 23 (23)  | 25 (25)   | 23 (23)  | 25 (25)  |
| Carbon monoxide (ppm)| Median (SD)         | 1.67 (1.28) | 2.33 (2.33) | 2.00 (2.00) | 1.67 (1.67) | 1.67 (1.28) | 2.33 (2.33) | 2.00 (2.00) | 1.67 (1.67) |
|                      | N(n*)               | 21 (21) | 21 (21)  | 23 (23)   | 25 (25)   | 23 (23)  | 25 (25)   | 23 (23)  | 25 (25)  |
|                      | Mean (SD)           | 3.07 (2.30) | 3.42 (3.72) | 3.13 (4.06) | 2.65 (2.04) | 3.07 (2.30) | 3.42 (3.72) | 3.13 (4.06) | 2.65 (2.04) |
Table 5. LS-mean baseline adjusted constituent statistical comparison for Groups I–IV. Number of subjects = sample number otherwise noted. Bold values are statistically significantly different.

| Constituent Statistics | Group 1 | Group II | Group III | Group IV |
|------------------------|---------|----------|-----------|----------|
| Nicotine %             | 5.0%    | 3.0%     | Diff.     | 5.0%     | 3.0%     | Diff.     | 5.0%     | 3.0%     | Diff.     | 5.0%     | 3.0%     | Diff.     |
| Number of subjects     | 21      | 21       | NA        | 23       | 23       | NA        | 26       | 26       | NA        | 23       | 23       | NA        |
| Nicotine (µg)          | 6.47    | −1.79    | −8.26     | 1.15     | 0.75     | −0.40     | 2.89     | 1.36     | −1.54     | 1.33     | 2.01     | 0.68      |
| Glycerin (µg)          | 6.73    | 1723     | 1951      | 227      | 1517     | 1604      | 87.3     | 1273     | 1187      | 85.7     | 2090     | 566       |
| Propylene glycol (µg)  | 148     | −303, 1125| −853, 1103| −50.0, 5.05| 7.94, 11.7| 9.20, 13.0| −1.11, 3.64| 5.18, 12.5| 2.60, 9.89| −7.26, 2.11| 3.87, 10.8| 8.22, 15.2| −0.26, 8.96|
| P value<sup>a</sup>     | NA      | NA       | 0.174     | NA       | NA       | 0.369     | NA       | NA       | 0.355     | NA       | NA       | 0.119     |
| P value<sup>b</sup>     | NA      | NA       | 0.128     | NA       | NA       | 0.640     | NA       | NA       | 0.620     | NA       | NA       | 0.119     |
| Benzoic acid (µg)      | 0.50    | 0.57     | 0.44      | 0.95     | 0.18     | 0.05      | 0.05     | 0.13     | 0.05      | 0.15     | 0.05     | 0.15      |
| Menthol (µg)           | 0       | 0        | 0         | 0        | 0        | 0         | 5.74     | 5.37     | −0.37     | 4.46     | 5.09     | 0.62      |
| Formaldehyde (µg)      | −0.001  | 0.050    | 0.051     | −0.043   | 0.081    | 0.12      | −0.026   | 0.045    | 0.071     | −0.033   | 0.023    | 0.056     |
| P value<sup>a</sup>     | NA      | NA       | NA        | NA       | NA       | NA        | NA       | NA       | NA        | NA       | NA       | 0.250     |
| P value<sup>b</sup>     | NA      | NA       | NA        | NA       | NA       | NA        | NA       | NA       | NA        | NA       | NA       | 0.728     |

(Continued.)
Table 5. (Continued.)

| Constituent                        | Group 1 | Group II | Diff. | Group III | Group IV | Diff. | Group V | Group VI | Diff. |
|-----------------------------------|---------|----------|-------|-----------|----------|-------|---------|----------|-------|
| Nicotine %                        | 5.0%    | 3.0%     | Diff. | 5.0%      | 3.0%     | Diff. | 5.0%    | 3.0%     | Diff. |
| Number of subjects                | 21      | 21       | NA    | 23        | 23       | NA    | 26      | 26       | NA    |
| Acetaldehyde (µg)                 | LS-mean | 0.67     | 0.85  | 0.18      | 0.79     | 0.574 | 0.038   | 0.011    | 0.049 |
|                                  | 90% CI  | 0.53, 0.81 | 0.71, 0.99 | 0.008, 0.35 | 0.48, 1.10 | 0.61, 1.23 | 0.12 | 0.011 | 0.049 |
|                                  | P value<sup>a</sup> | NA   | NA     | 0.086    | NA       | 0.574 | NA      | NA       | 0.049 |
|                                  | P value<sup>b</sup> | NA   | NA     | 0.086    | NA       | 0.574 | 0       | 0        | 0     |
| Acrolein (µg)                     | LS-mean | 0       | 0     | −0.003   | 0.003    | 0.005 | 0       | 0        | 0     |
|                                  | 90% CI  | NE, NE  | NE, NE| NE, NE   | −0.008, 0.002 | −0.002, 0.012 | NE, NE | NE, NE | NE, NE |
|                                  | P value<sup>a</sup> | NA   | NA     | NE       | NA       | 0.196 | NA      | NA       | 0.32  |
|                                  | P value<sup>b</sup> | NA   | NA     | NE       | NA       | NE    | NA      | NA       | NE    |
| Carbon monoxide (ppm)             | LS-mean | −0.019  | 0.36  | 0.27     | −0.005   | −0.27 | −0.30   | 0.59     | 0.62  |
|                                  | 90% CI  | −0.26, 0.22 | 0.12, 0.59 | 0.051, 0.70 | −0.053, 0.58 | −0.32, 0.31 | −0.69, 0.15 | −0.37, 0.31 | −0.25, 0.93 | 0.15, 1.09 | −0.19, 0.19 | 0.13, 0.51 | 0.073, 0.58
|                                  | P value<sup>a</sup> | NA   | NA     | 0.059    | NA       | 0.278 | NA      | NA       | 0.033 |
|                                  | P value<sup>b</sup> | NA   | NA     | 0.059    | NA       | 0.714 | NA      | NA       | 0.074 |

<sup>a</sup> P value from the primary analysis model.
<sup>b</sup> P value from the sensitivity analysis.
<sup>c</sup> Except for carbon monoxide N = 25 samples.
<sup>d</sup> N = 22 for Carbonyl collection and carbon monoxide.
NE = not estimable; NA = not applicable.
Table 6. Summary of CFD predicted average maximum indoor concentrations of glycerin, propylene glycol, nicotine, and menthol for ten exposure scenarios using the average measured minimum and maximum per puff values in exhaled breath.

| Exposure scenarios                          | Nicotine | Propylene glycol | Glycerin | Menthol |
|--------------------------------------------|----------|------------------|----------|---------|
|                                            | Max      | Min              | Max      | Min     | Max     | Min     |
| Environmental chamber—residential ventilation | 1.93     | 0.18             | 6.52     | 1.71    | 513.3   | 288.8   | 1.17    | 0.08    |
| Environmental chamber—office ventilation   | 1.34     | 0.13             | 4.53     | 1.19    | 357.0   | 200.9   | 0.81    | 0.05    |
| Environmental chamber—hospitality ventilation | 0.85     | 0.08             | 2.87     | 0.75    | 225.8   | 127.1   | 0.51    | 0.03    |
| Office—0.35 ACH; 30 min                    | 0.44     | 0.04             | 1.47     | 0.39    | 116.0   | 65.3    | 0.26    | 0.02    |
| Office 1 ACH; 30 min                       | 0.43     | 0.04             | 1.45     | 0.38    | 114.4   | 63.5    | 0.26    | 0.02    |
| Office—0.35 ACH; 60 min                    | 0.41     | 0.04             | 1.39     | 0.37    | 109.5   | 61.8    | 0.25    | 0.02    |
| Restaurant                                 | 0.72     | 0.07             | 2.42     | 0.64    | 190.3   | 107.1   | 0.43    | 0.03    |
| Car (closed windows for 10 min)            | 0.39     | 0.32             | 11.45    | 3.01    | 902.0   | 508.3   | 2.05    | 0.14    |
| Car (closed windows for 3 min)             | 4.02     | 0.38             | 13.56    | 3.56    | 1068.0  | 601.0   | 2.43    | 0.16    |
| Car (open windows)                         | $1.7 \times 10^{-3}$ | $1.6 \times 10^{-4}$ | $5.7 \times 10^{-3}$ | $1.5 \times 10^{-3}$ | $4.5 \times 10^{-1}$ | $2.5 \times 10^{-1}$ | $1.0 \times 10^{-3}$ | $6.8 \times 10^{-3}$ |

ACH—air exchanges per hour; Min—minimum; Max—maximum.

product use, the one reported for Group VI was possibly related to product use and the one reported for Group II was not related to product use. The study emergent and unanticipated adverse reaction reported for Group IV resulted in the subject withdrawing from the study.

3.4. CFD predictions

The CFD predicted average maximum indoor air concentration using the maximum and minimum exhaled breath concentration for glycerin, propylene glycol, nicotine, and menthol showed substantial differences among the indoor simulations (table 6).

The CFD predicted airflow within the exposure chamber and the indoor air concentration over the 4 h time period for glycerin, propylene glycol, and nicotine at the lowest ventilation level (Residential, supplemental figures 1(B) and (C)) resulted in the highest indoor constituent concentrations (see supplemental figures 2–4 for office and 5–7 for hospitality ventilation levels). The saw tooth pattern reflects the puffing regimen. The environmental chamber reached an equilibrium pattern after the fourth puffing episode with subsequent maximum glycerin, propylene glycol, and nicotine concentrations varying by less than 52, 0.64, and 0.1 µg m$^{-3}$, respectively.

Contrary to the environmental chamber, the private office with a smaller volume, fewer occupants, lower air exchanges per hour (ACH) and shorter puffing duration still shows a higher nicotine concentration around the ENDS user 27 min after puffing ceased (figure 1-top). CFD predicted concentrations of glycerin, propylene glycol, and nicotine reach a maximum shortly after puffing stops and then gradually drop over the 30 min simulation period (figure 1-bottom). The average concentration over the 30 min simulation for glycerin, propylene glycol, and nicotine ranged from 57–102 µg m$^{-3}$, 0.34–1.3 µg m$^{-3}$ and 0.036–0.38 µg m$^{-3}$, respectively. If the ventilation level is tripled, the average constituent concentrations of glycerin, propylene glycol, and nicotine decrease slightly over the 30 min period to 52–93 µg m$^{-3}$, 0.31–1.2 µg m$^{-3}$ and 0.033–0.35 µg m$^{-3}$, respectively. (See supplemental figures 8–10). Decreasing the puffing frequency to one-third of the other office scenarios, increasing the duration of puffing from 3 to 30 min, and following the exposure for a total of 60 min results in similar
average constituent concentrations (see supplemental figures 11–13).

In the restaurant scenario (figure 2), the increased nicotine concentration immediately surrounding the five ENDS users significantly dissipates within 10 min and more completely in 20 min (figure 2-top). This pattern applies to glycerin, propylene glycol, and any other exhaled constituents. Unlike the office, but similar to the environmental chamber, an equilibrium in constituent concentration is reached after 60 min (figure 2-bottom). Over the 2 h exposure scenario, the average concentration for glycerin, propylene glycol and nicotine ranged between 59–105 µg m⁻³, 0.35–1.33 µg m⁻³ and 0.038–0.4 µg m⁻³, respectively.

In the car scenario with closed windows, the highest constituent concentration is obtained with the higher puffing frequency. The CFD predicted temporal and spatial nicotine distribution (figure 3-top) shows that a more uniform distribution takes at least 30 min. Similar to the office scenario, maximum constituent concentrations occur shortly after puffing stops (figure 3-bottom). When the puffing frequency is reduced to once/min for 10 min, the maximum concentration of glycerin, propylene glycol, and nicotine are 150, 2 and 0.5 µg m⁻³, respectively (see supplemental figures 14–16). As might be expected, when the car windows are open, constituent concentrations rise with each puff, but return to almost zero between puffs. The average 3 min constituent concentration in the car with the windows open scenario ranged from 0.078–0.138 µg m⁻³, 0.000 46–0.0018 µg m⁻³ and 0.000 049–0.00 052 µg m⁻³ for glycerin, propylene glycol, and nicotine, respectively (see supplemental figures 17–19).

4. Discussion

4.1. Clinical study

Consistent with our clinical hypothesis, there was no statistically significant difference in baseline adjusted concentration of exhaled nicotine between the four 5.0% and 3.0% ENDS products. Only baseline adjusted exhaled breath concentrations of glycerin, propylene glycol, nicotine, and menthol were used in the CFD simulations since the exhaled breath concentrations of formaldehyde, acetaldehyde, acrolein, benzoic acid, and CO were not above the limit of detection for all or most of the ENDS products in our clinical trial. The substantial variability in baseline adjusted exhaled breath concentrations of glycerin (1.8 times), propylene glycol (3.8 times), nicotine (10 times), and menthol (1.4 times) from use of the ENDS products, resulted in a broad range of CFD predicted concentrations of these constituents. Papaefstathiou et al (2020) also noted a wide range in volatile organic compounds in the exhaled breath of the 26 subjects that used 16 different ENDS products.

There is limited published data for comparison with our measured baseline and baseline adjusted levels of exhaled breath constituents from our clinical study. Similar to our measured baseline levels of formaldehyde and acetaldehyde, Long (2014) reported most ENDS users (N = 10) had below detection limit (LOD = 0.1 and 0.39 µg/session, respectively) levels in their exhaled breath. Samburova et al (2018) reported a lower range of baseline formaldehyde (below LOD to 0.012 µg/breath) and acetaldehyde (0.002–0.035 µg/breath) levels in 12 subjects than our baseline levels. Consistent with our measured baseline levels, Edmiston et al (2021) measured exhaled breath from 32 subjects using similar analytical methods, but four different ENDS products and reported that all baseline values (called sham) for formaldehyde, acetaldehyde, and acrolein were below their minimum detectable level and the majority (87.5%) of values were below their minimum detectable level for nicotine, glycerol, propylene glycol, and menthol.

Similar to the data of Long (2014) the baseline adjusted exhaled breath level of acetaldehyde for a majority of subjects was below the limit of detection. Unlike Marco and Grimalt (2015) we detected glycerin and propylene glycol in the exhaled breath of each group of ENDS users. Samburova et al (2018) measured carbonyl levels in exhaled breath after use of three different ENDS products in 19 different sessions, with mean exhaled breath levels of formaldehyde ranging from −0.001 to 0.408 µg/breath and for acetaldehyde ranging from −0.007 to 0.555 µg/breath. Our LS mean baseline adjusted exhaled breath levels of formaldehyde and acetaldehyde were consistent ranging from −0.01–0.23 and −0.02–0.99, respectively. The range of exhaled breath levels (µg per breath basis) of nicotine (8.9–19.7), glycerin (537–648), and propylene glycol (119–335.4) reported by Edmiston et al (2021) were higher compared to our LS mean values, while those for menthol (0.017–3.1) had a wider range than the ranges reported in this study.

4.2. CFD modeling

As anticipated, the car with the open window scenario produced the lowest CFD predicted concentrations of every constituent, usually 2–3 orders of magnitude less than predicted concentrations in the other indoor scenarios. As the ventilation level increased within the exposure chamber or office, CFD predicted exposure concentrations decreased. In the office at the same ventilation rate, when the same number of puffs were taken (puffing frequency time duration) in 3 min instead of 30 min, a similar predicted maximum exposure concentration required only half as long (30 min vs 60 min). In the car with closed windows and the same ventilation rate, taking the same number of puffs in 3 min vs 10 min resulted in the predicted maximum exposure concentration being reached in 20 min vs 30 min. In all scenarios, each exhaled breath resulted in a predicted increase in
each constituent’s concentration with the magnitude primarily dependent on the ventilation level and total volume of the indoor space.

Comparisons of the range of CFD predicted concentrations of glycerin, propylene glycol, and nicotine in our scenarios are generally consistent with measured concentrations reported in the literature, but also highlight important variables that must be considered. For example, Oldham et al (2021) reported concentrations of glycerin, propylene glycol, and nicotine in the breathing zone for the exposure chamber, ventilation levels and prescribed ENDS use modeled in this study. Ten smokers used two ENDS products, including one used in this work (Virginia tobacco 5.0% nicotine by weight). The average (±standard deviation) 4 h cumulative nicotine concentration reported from prescribed use of the Virginia tobacco 5.0% ENDS in the residential, office and hospitality ventilation levels were: 4.01 ± 1.63 µg m⁻³; 1.37 ± 0.37 µg m⁻³; and 0.75 ± 0.2 µg m⁻³, respectively. The CFD predicted range (minimum to maximum) of room averaged 4 h cumulative nicotine concentrations using the exhaled breath data from daily ENDS users were: 0.122–1.29 µg m⁻³; 0.0809–0.858 µg m⁻³; and 0.0417–0.442 µg m⁻³, respectively. Overall, this shows good agreement between the average measured 4 h cumulative and CFD predicted room averaged 4 h cumulative nicotine concentrations. Both the measured and CFD predicted nicotine concentrations follow the expected pattern due to the difference in ventilation conditions. As the ventilation increases (supplemental figure 1A), a more homogenous airflow distribution within the exposure chamber occurs resulting in better agreement between measured and CFD predicted room averaged 4 h cumulative nicotine concentrations. Better agreement between measured and CFD predicted room averaged 4 h cumulative concentrations for both glycerin and propylene glycol also occurred as the ventilation level increased. CFD predicted glycerin concentrations exceeded measurements by 7–12 times while measured propylene glycol concentrations exceeded CFD predicted concentrations by 2–6 times depending on ventilation level. This comparison highlights the importance of several experimental and computational variables (not exhaustive), including how well air sampler placement in experiments represents the room average as calculated by the CFD predictions, potential differences between groups of users (smokers vs daily ENDS users), complete glycerin evaporation (one of our CFD modeling assumptions), and variability in human exhaled breath concentrations, their exhaled direction, and velocity of exhaled breath. The velocity of exhaled breath plumes from ENDS products was recently reported by Sussman et al (2021).

Liu et al (2017) also conducted an environmental chamber (114 m²) study using a residential ventilation level to measure 4 h cumulative amounts of chemicals including glycerin, propylene glycol and nicotine from prescribed and ad libitum use of two different ENDS products by groups of ten smokers. Different concentrations (4 h cumulative samples) of glycerin, propylene glycol, and nicotine from use of two ENDS products were reported, as well as differences between prescribed and ad libitum use for one of the ENDS products (Liu et al 2017). The 4 h cumulative room air concentration of glycerin, propylene glycol and nicotine from prescribed use of one of the ENDS products was, 67.89 ± 16.81 µg m⁻³, 44.86 ± 3.84 µg m⁻³, and 0.48 ± 0.16 µg m⁻³ respectively and for the other ENDS product were, 126.73 ± 12.71 µg m⁻³, 211.51 ± 14.23 µg m⁻³, and 2.83 ± 0.44 µg m⁻³, respectively. Considering that a similar size exposure chamber (132 m³), the same ventilation level and number of ENDS users were studied, but different ENDS products (2.5% nicotine by weight), our CFD predicted room averaged 4 h cumulative nicotine concentration range of 0.122–1.29 µg m⁻³ can be considered consistent with values reported by Liu et al (2017). Our CFD predicted room averaged 4 h cumulative glycerin concentration range of 193–344 µg m⁻³ and propylene glycol concentration range of 1.15–4.36 µg m⁻³ confirm however, that the specific product used is an important variable that must be modeled.

An additional study by Schober et al (2014) was conducted including nine occasional smokers (<10 cigarettes/week), three different ENDS formulations with and without nicotine and was performed in a room larger than our office scenario with a higher ventilation rate. The reported range of glycerin, propylene glycol and nicotine concentrations were <0.04–81.0 µg m⁻³, <0.04–395.0 µg m⁻³, and <0.04–4.6 µg m⁻³, respectively. Considering the differences in room size (45 m² vs 24 m³), ventilation rate (0.56 vs 0.35 ACH), number of ENDS users (9 vs 1), product use, (ad libitum vs prescribed), and products used (refillable tank vs Juul pod), the reported concentrations of glycerin, propylene glycol, and nicotine are remarkably consistent with our office scenario (figure 2).

The exhaled breath data from non-menthol and menthol cigarette use was not used for predictions of room air concentrations because the predominant source of environmental tobacco smoke is side stream smoke generated between cigarette puffs rather than exhaled breath (Baker and Proctor 1990, Guerin et al 1992). Due to indoor smoking bans in many states and localities that started several decades ago, recent environmental tobacco smoke concentration data does not exist. A comprehensive review/synthesis of past indoor environmental tobacco smoke concentrations (EPA 1992) reported nicotine ranges of 0.5–33 µg m⁻³, 0.5–70 µg m⁻³, and 8–83 µg m⁻³, for offices, restaurants, and cars, respectively. For offices and restaurants, our CFD predicted average

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maximum nicotine concentration range (table 5) is between 12–77 and 7–97 times lower, respectively than the measured values from environmental tobacco smoke. For cars, using our worst case (windows closed) the CFD predicted average maximum nicotine concentration (table 5) ranges between 20 and 28 times lower than the measured values from environmental tobacco smoke. These reported nicotine values from environmental tobacco smoke are also consistent with the differences found between exhaled breath concentrations of nicotine from ENDS use and non-menthol and menthol cigarette use in the clinical study (figure 5).

Results from this study must be viewed considering the variables already mentioned and the study limitations. Although there was substantial variability in baseline adjusted concentrations of exhaled breath constituents that were measured above their limit of detection, human variability was likely underestimated due to our small clinical sample size (N = 80; N = 20/ENDS group), use of a single abstinence period prior to product use, limited product use scenarios (single puffing regimen) and analytical method limits of detection. In addition the lack of more precise control of inhalation volume and therefore exhaled volume or its measurement was also a factor in variability of baseline adjusted concentrations of exhaled breath constituents. Another limitation was the use of two simplifying assumptions in the CFD modeling work that over predicted room concentrations (complete constituent evaporation & no constituent loss to walls and surfaces). Additionally, none of the CFD modeling work incorporated movement of the ENDS users or differences in the direction or velocity of exhaled breath that normally occurs in real life situations that would affect short term spatial and temporal distribution of the exhaled constituents. These more realistic scenarios can be incorporated in the CFD modeling work but must be mathematically defined for each ENDS user.

In summary, although there was substantial variability in the range of exhaled breath data from use of eight different ENDS products, except for propylene glycol and glycerin, CFD predicted room air constituent concentrations were substantially less from use of ENDS products studied compared to combustible cigarettes.

Data availability statement

All data that support the findings of this study are included within the article (and any supplementary files).

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Conflict of interest

MIO, PCB, and QL are employees of Juul Labs, Inc. NC, AS, and AAR are employees of Altria Client Services, LLC, who was contracted to perform the CFD work. Altria Client Services, LLC, is a wholly owned subsidiary of Altria.

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