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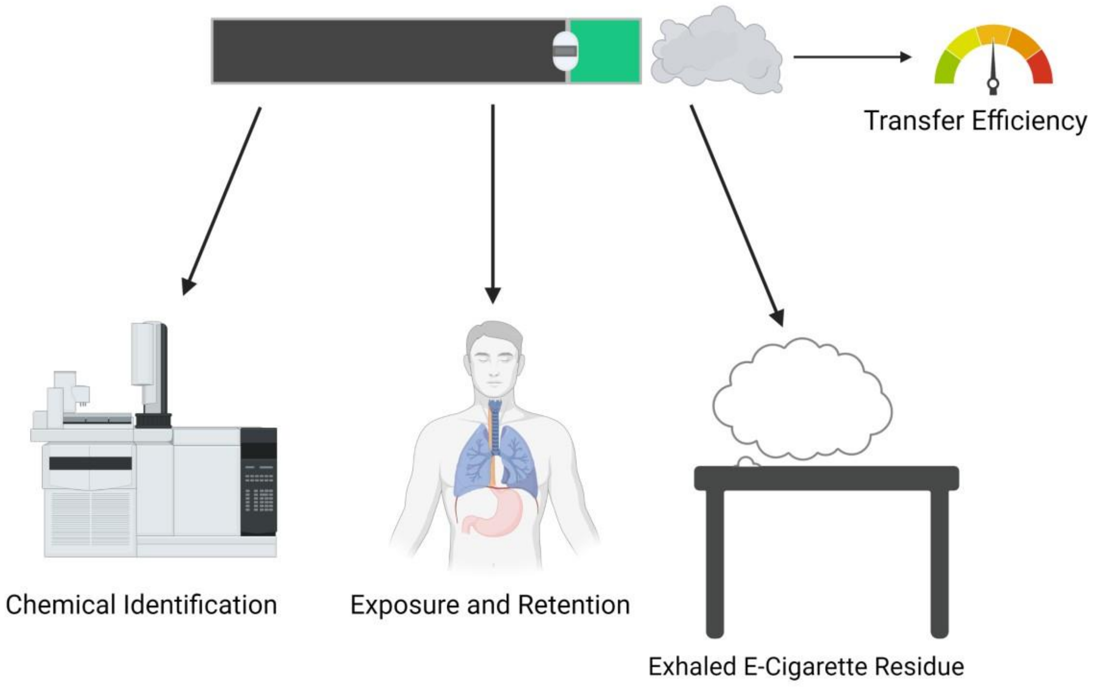
Transfer Efficiency
Chemical Identification
Exposure and Retention
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# Exposure, Retention, Exhalation, Symptoms, and Environmental Accumulation of Chemicals During JUUL™ Vaping

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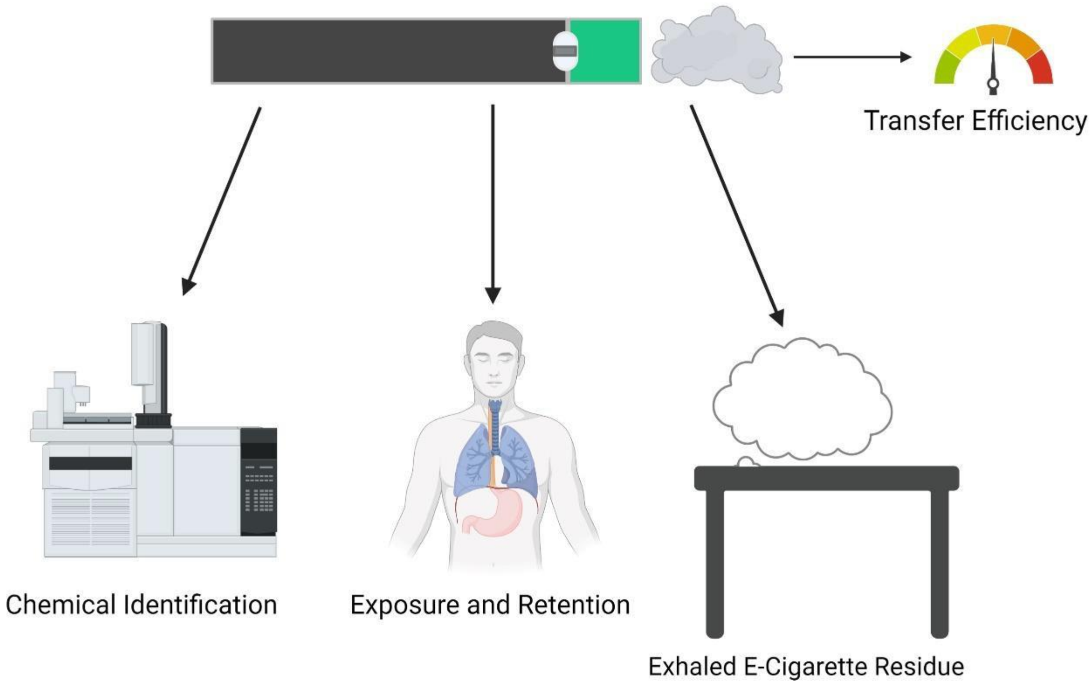

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## ABSTRACT

Little is known about the chemical exposures that electronic cigarette (EC) users receive and emit during JUUL™ vaping and if exposures produce symptoms dose dependently. This study examined chemical exposure (dose), retention, symptoms during vaping, and the environmental accumulation of exhaled propylene glycol (PG), glycerol (G), nicotine, and menthol in a cohort of human participants who vaped JUUL™ "Menthol" ECs. We refer to this environmental accumulation as "EC exhaled aerosol residue" (ECEAR). Chemicals were quantified using gas chromatography/mass spectrometry in JUUL™ pods before and after use, lab-generated aerosols, human exhaled aerosols, and in ECEAR. Unvaped JUUL™ "Menthol" pods contained ~621.3 mg/mL of G, ~264.9 mg/mL of PG, ~59.3 mg/mL of nicotine, ~13.3 mg/mL of menthol, and ~0.1 mg/mL of the coolant WS-23. Eleven experienced male EC users (aged 21-26) provided exhaled aerosol and residue samples before and after vaping JUUL™ pods. Participants vaped *ad libitum* for 20 minutes, while their average puff count (22 ± 6.4) and puff duration (4.4 ± 2.0) were recorded. The transfer efficiency of nicotine, menthol, and WS-23 from the pod fluid into the aerosol varied with each chemical and was generally similar across flow rates (9 - 47 mL/sec). At 21 mL/sec, the average mass of each chemical retained by the participants who vaped 20 minutes was 53.2 ± 39.3 mg for G, 14.0 ± 18.9 mg for PG, 3.3 ± 2.6 mg for nicotine, and 0.5 ± 0.4 mg for menthol, with retention deduced to be ~90% - 100% for each chemical. There was a significant positive relationship between the number of symptoms during vaping and total chemical mass retained. ECEAR accumulated on enclosed surfaces where it could contribute to passive exposure. These data will be valuable to researchers studying human exposure to EC aerosols and agencies that regulate EC products.

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## INTRODUCTION

Electronic cigarettes (ECs) have been used for over 10 years in the United States, during which time they have evolved rapidly into sophisticated devices capable of delivering nicotine and other chemicals to users. JUUL™ products belong to the fourth generation of ECs[1-2] and have been one of the largest selling nicotine vape products in the United States[3]. In 2018, JUUL™ took most of their flavored products off the market after public and government concern over their popularity with high school students and young adults[4-6]. Then, in July 2020, JUUL™ discontinued sales of its “Classic Tobacco” pods, leaving only “Virginia Tobacco” and “Menthol” flavors available to consumers. In the same year, the Food and Drug Administration (FDA) issued an enforcement policy to remove cartridge-based ECs (except for menthol and tobacco flavors) from the market[7]. More recently, in June 2022, the FDA denied JUUL™ authorization to market its products citing lack of sufficient toxicological evidence, but the company appealed the decision and has since been allowed to continue sales[8]. Despite the FDA’s flavor enforcement policy and attempts to regulate EC flavors, JUUL™ “Menthol” products are still readily available and popular both online and in stores.

The dominant chemicals in JUUL™ “Menthol” pods are nicotine (~30 or 60 mg/mL), a solvent comprised of glycerol (G) and propylene glycol (PG) (70:30 ratio), menthol (~13 mg/mL), and benzoic acid (~44.8 mg/mL)[9-12]. These concentrations are higher than normally used in consumer products[9], which has raised concerns about their effects on human health.

This concern is supported by cell, animal, and human studies on JUUL™ ECs. In vitro, JUUL™ e-liquids and dissolved aerosols were cytotoxic to numerous human cell types[9,13-17], an effect that was correlated with nicotine and menthol concentrations in BEAS-2B cells[9]. In Calu-3 and HEK293T cells, JUUL™ “Menthol” and “Mint” pods increased intracellular Ca2+[14,16], the proinflammatory cytokine IL-6, and Annexin V, an early apoptotic marker, with responses being more pronounced in “Mint”[16]. JUUL™ “Mint” aerosols also upregulated genes involved in ROS production, lipid peroxidation, and carcinogen metabolism, while downregulating genes related to cytokine and chemokine signaling in type-2 alveolar epithelial cells[18]. Air liquid interface exposure to JUUL™ “Glacier Mint” aerosols increased cell death and tissue inflammation in human oral epithelium[19]. Menthol can be toxic at high doses in vitro and triggered inflammatory pathways[15]. Exposure to JUUL$^{TM}$ “Menthol” aerosols resulted in an immediate increase in proton leak and decreased coupling efficiency, as well as a decrease in complex I, II, and IV, causing mitochondrial dysfunction in lung epithelial cells[17].

In rodent studies, JUUL™ aerosols produced adverse effects on the vascular, immune, and reproductive systems. In rats, various JUUL™ flavors, including “Menthol”, impaired endothelial function[20,21]. In mice, JUUL™ “Menthol” aerosols (2 weeks, 70 puffs/daily) caused hyperactivation of platelets and shortened thrombus occlusion and bleeding times[22], while 2 weeks of JUUL™ aerosol exposure to a higher dose (192 puffs/day, flavor not reported) disrupted the blood brain barrier, resulting in ischemic stroke[23]. In mice, JUUL™ aerosols also increased inflammation and altered plasma and urinary metabolites, which could increase disease risk[24-25]. JUUL™ “Mango” and “Mint” aerosols induced inflammation of the brain, lungs, heart, and colon[24], and JUUL™ “Mint” significantly increased lung neutrophils and oxidative stress[26]. Exposure of pregnant BALB/c mice to JUUL™ “Mint” aerosol for 1 hour/day x 20 days[27] upregulated genes associated with hypoxia and oxidative stress in the uterus and placenta, while body weights and lengths of the offspring decreased[27].

Human studies have reported on health effects, nicotine dependence, and blood pressure changes associated with JUUL™ use. Symptoms related to the respiratory, neurologic, and cardiovascular systems have been reported on Reddit and Twitter by EC users vaping various JUUL™ products[28-29]. JUUL™ “Menthol” was associated specifically with throat issues (e.g., burning, harshness, and throat hit) on Twitter[28]. In a survey, participants using JUUL™ ECs for the first-time experienced burning in the throat, coughing, wheezing, and headache[30]. Immediately after vaping JUUL™ "Menthol"/"Mint" or "Classic Tobacco", participants reported nausea and dizziness[31]. Additionally, 40.1% of JUUL™ adolescent users who vaped in the past 30 days were likely to have nicotine dependence symptoms (e.g., cravings)[32]. JUUL™ vaping also increased blood pressure and mean arterial pressure in human users[33]. Vaping a ratio of 50/50 PG/G in 30 never-smokers showed that the solvents caused lung inflammation significantly correlated with change in cell counts (cell concentrations, macrophages, and lymphocytes) and cytokines (IL-8, IL-13, and TNFα) in bronchoalveolar lavage samples[34].

EC users exhale clouds of aerosol (which we refer to as "exhale") that settle on indoor surfaces forming "EC exhaled aerosol residue" (ECEAR)[35-36], which could passively expose non-vapers to EC chemicals. ECEAR generated using "Dewberry Cream" and "Churrios" in tank style ECs contained nicotine, nicotine alkaloids, tobacco specific nitrosamines (TSNAs), and flavor chemicals[35]. ECEAR derived from "Churrios" e-liquid induced IL-1α secretion following air liquid interface exposure of EpiDerm™ tissue, a 3D human skin model[37]. Some ECEAR chemicals caused oxidative damage and inflammation to human skin[37]. No data have previously been reported on ECEAR associated with JUUL™ products.

Various lines of evidence show that JUUL™ e-liquids and aerosols can produce adverse effects in cells, animals, and humans and that exhaled aerosols have the potential to passively expose non-vapers to EC chemicals. However, little is known about the actual chemical exposures that humans vaping JUUL™ ECs receive, how doses relate to symptoms, if users experience adverse symptoms during vaping, and if non-vapers are at risk. The purpose of this study was to characterize the exposure to and retention of flavor chemicals, solvents, and nicotine in JUUL™ "Menthol" users, evaluate the residues deposited by exhaled aerosols, and

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correlate the frequency of symptoms reported during JUUL™ vaping to the calculated dose received. JUUL™ “Menthol” was used because it is popular, readily available, and can substitute for JUUL™ "Mint” products, which are no longer marketed. A cohort of male JUUL ™ EC users provided exhale and ECEAR samples after vaping JUUL ™ “Menthol”. Topography (puff duration and total puffs seconds) was evaluated for each user. The solvents (PG and G), nicotine, menthol, and flavor chemicals were quantified in the exhale and ECEAR after a 20-minute vaping session. Data on acute symptoms were collected before, during, and after vaping, and correlated with mass retained for the dominant chemicals. This is the first comprehensive study on JUUL™ “Menthol” that includes data on topography, exposure, retention, self-reported symptoms and their frequency before, during, and after vaping. These data will be important in future studies that evaluate human health effects caused by JUUL™ vaping and in designing experiments with relevant exposures to the chemicals in JUUL ™ products. Supplementary Table 1 introduces abbreviations and terms used in this study.

## METHODS

### *Recruitment of Human Subjects*

An advertisement for participants was placed in the University of California, Riverside listserv. The study inclusion criteria were: (1) at least 6 months of JUUL™ use, (2) ability to use JUUL™ “Menthol” containing 5% (50 mg/mL) of nicotine, (3) 21 years or older, (4) lung inhalers (users who take aerosol into their lungs not just their mouth)[38], and (5) no pre-existing health conditions. The exclusion criteria were: (1) insufficient experience using JUUL™ products, (2) less than 21 years of age, (3) inability to use nicotine products with 50 mg/mL of nicotine, (4) mouth inhalers (those who inhale only into the mouth)[38], and (5) individuals with pre-existing conditions.

Interested volunteers were directed to an online Qualtrics survey that asked questions covering study eligibility and tobacco product use. Participants were screened based on their responses to the questions. Participants that specified EC and minimal tobacco and substance use

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were preferred. Selected volunteers were asked to come into the lab to review and sign informed consent forms, approved by the UCR Human Research Review Board, and agree to study protocols and vape product use. Participants had their initial puffing topography recorded to assess whether they inhaled into the mouth only or inhaled into their lungs.

Control participants who did not use ECs or tobacco cigarettes, who were at least 18 years old, and had no pre-existing health conditions were also recruited from the University. The controls came on separate days from the EC users to provide exhale samples.

### ***Purchase of JUUL™ Products***

JUUL™ “Menthol” pods (5.0% nicotine) and batteries were purchased from local convenience stores in Riverside, CA and used within 2-3 months. To avoid possible use of counterfeit ECs[39], products were authenticated by contacting JUUL™ to confirm the batch numbers on the products. Vaped pods were stored at room temperature in 50 mL conical tubes until shipped to Portland State University for fluid analysis.

### ***Study Design***

Exhale and ECEAR samples were collected from participants before and after vaping for 20 minutes on two separate days (Day 1 and Day 2) (Supplementary Figure 1). Before coming into the lab, participants were asked to abstain from vaping, other tobacco products, recreational drugs, alcohol, and caffeine for at least 10 hours before the study. When participants arrived, they were asked to rinse their mouths thoroughly with water for at least 30 seconds. Participants were given fresh JUUL™ “Menthol” pods to use during the two study days, along with a fully charged JUUL™ battery. JUUL™ “Menthol” pods were weighed before and after vaping. Controls provided a single exhale sample during one session while breathing room air.

### ***ECEAR Collection from Control and JUUL™ Participants (Day 1)***

On Day 1, participants vaped for 20 minutes *ad libitum*, and the exhaled aerosol was allowed to settle for 30 minutes before collection and subsequent extraction. Puff duration, which was measured using a stopwatch, was defined as the interval between activation of the light on the

JUUL™ and removal of the EC from the participants' mouth. Puff count was recorded manually by researchers. On Day 2, participants were asked to take the same number of puffs. To collect the ECEAR, a paper towel was placed on the bottom of a 3 cubic foot acrylic box with a detachable top. Boxes had 1 cm holes on each side to allow aeration. After each puff, participants exhaled into a three-foot Tygon tube to deposit aerosol in the tank. After each session, there was a 30-minute waiting period before the paper towels were collected and placed into Ziploc bags for immediate extraction. Tubing for each participant was washed with water, ethanol, water, followed by ethanol, water, ethanol, followed by water only and then dried overnight.

Paper towels with ECEAR were cut into small pieces (1.3 x 1.4 inches) and soaked in IPA (isopropyl alcohol) (0.1 g of paper towel/mL of IPA) for an hour. The tubing was washed with IPA and combined with paper towel extracts, put into 1 ml GC/MS vials, and shipped to Portland State University for GC/MS flavor chemical analysis.

The total surface area of the acrylic box and paper towel that the ECEAR was collected on was determined. The ECEAR concentration measured in the paper towel cut out was multiplied by the total surface area of the acrylic box to obtain an estimate of the total mass (mg) of ECEAR deposited. Previous lab experiments showed that ECEAR collected in the lab-made acrylic box was fairly evenly distributed (not shown).

### *Exhale Collection (Day 2)*

On Day 2, participants returned to the lab, and pre and post vape exhale samples were collected. Two impingers in tandem, each with 25 mL of IPA, were used to capture participants' exhale, as previously described[38]. Participants exhaled into a 1-foot Tygon tube that connected to the first impinger. Control (from the non-EC users) and pre-vape exhale was deposited directly into the impinger without vaping. Participants were given signals during the pre and post vape session so that they exhaled the same number of times as they puffed on Day 1. Controls were asked to provide one exhale sample/minute during a 20-minute session.

### *JUUL™ Aerosol Production at Various Flow Rates*

Triplicate JUUL™ "Menthol" aerosol samples were generated in the lab at various flow rates (9, 11, 13, 21, 41, and 47 mL/sec) for 20 minutes (1 puff/minute at 4.3 seconds/puff), as described in previous methods[40-41]. To collect the aerosols, two glass impingers were connected in tandem and attached to a peristaltic pump. A smoking machine[42] was used to count and time the puffs, and generate aerosols, as described in detail previously[41]. Based on prior data[40], a 4.3 second puff was taken every minute. Pods were weighed before and after vaping sessions. Based on prior data[43], a range of flow rates was selected for transfer efficiency calculations.

### *Identification and Quantification of Nicotine, Flavor Chemicals, and Solvents in Samples Using GC/MS*

The nicotine and flavor chemical concentrations in pod e-liquids, exhale, and ECEAR were analyzed by GC/MS at Portland State University. Internal standard-based calibration procedures similar to those described elsewhere were used[9,44], and analyses for nicotine and 180 flavor-related target analytes were performed with an Agilent 7693 autosampler (Santa Clara, California, USA), Agilent 7890A GC and Agilent 5975C MS. The capillary column used was Rxi-624Sil MS (30 m × 250 µm × 1.4 µm film thickness). The relatively high film thickness was chosen to provide the column with adequate capacity for compounds present at high concentrations and retention for relatively volatile flavor compounds. For each e-liquid sample, 50 µL was dissolved in 950 µL of isopropanol (Fisher Scientific, Fair Lawn, New Jersey, USA). 20 µL of internal standard solution (2 µg/µL of 1,2,3-trichlorobenzene in isopropyl alcohol) was added into 1 mL of diluted refill, exhale, and ECEAR extract samples before analysis; 1 µL was injected into the GC/MS at 235 °C with a 10:1 split. The GC temperature program was: 40 °C hold for 2 min; 10 °C/min to 100 °C; then 12 °C/min to 280 °C and hold at 280 °C for 8 min, then 10 °C/min to 220 °C. The MS was operated in electron impact (EI) ionization mode (70eV) and positive ion detection mode. The ion source temperature was 220°C and the quadrupole temperature was 150°C. The scan range was from 34 to 400 amu. Each target analyte was quantitated using: (a) authentic standard material; (b) its specific quantitation ion; and (c) internal-standard (1,2,3-trichlorobenzene)-normalized multipoint calibration based on peak area. When the concentrations were less than the limit of quantitation, they were estimated based on the response factor generated from the calibration standards.

To estimate the PG and G concentration in all samples previously analyzed for flavor chemicals, an external standard calibration curve was applied. PG and G concentrations often overloaded the MS during analysis and thus are likely underestimated at high sample concentrations.

Control air samples collected from the fume hood and IPA rinses of the Tygon tubing were collected and analyzed by GC/MS to confirm that chemicals did not come from these sources.

### *Calculating Transfer Efficiency for Nicotine, Menthol, and Solvents (PG and G) at Various Flow Rates*

The transfer efficiency is the percent of the chemical in the pod liquid that transfers to the aerosol during vaping. To calculate the transfer efficiency at our six flow rates, the aerosol concentration of the chemical (µg/g) was multiplied by the pod liquid density (g/mL) and divided by the total concentration (µg/mL) of the chemical in the pod liquid before aerosolization. The percent transfer was calculated by multiplying this result by 100%.

$$\%\,Transfer\,Efficiency = \frac{aerosol\,concentration\,of\,the\,chemical\,x\,pod\,fluid\,density}{total\,concentration\,of\,chemical\,in\,pod\,fluid}\,x\,100\%$$

### *Calculating Mass Transfer Derived from Transfer Efficiency Data*

The mass of chemical (nicotine, menthol, PG, and G) that transferred from pod fluid to aerosol was calculated at six flow rates. The mass that transferred to the aerosol was calculated

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by multiplying the concentration of the chemical in the aerosol (ng/mL) by the total volume of IPA it was captured in (mL). The mass that transferred to the aerosol was converted to mg by dividing by $10^6$. The average and standard deviation for the mass consumed during the vaping session was determined.

To confirm that the mass transfer was calculated correctly, it needed to be in agreement with the percent transfer efficiency. The total chemical consumed during vaping was calculated by multiplying the unvaped chemical concentration (mg/mL) by total weight (g) divided by the density of the pod fluid (mg/uL = g/mL). The total chemical consumed was converted to mg by dividing by 1000. The mass transfer was divided by the total chemical consumed during vaping multiplied by 100%. This percent value was compared to our transfer efficiency to confirm that the numbers matched and that the mass transfer was accurate.

$$Mass\,Transfer\,(mg) = \frac{total\,mass\,chemical\,quantified\,x\,total\,IPA\,volume}{conversion\ factor\,(depending\,on\,units\,for\,mass)}$$

### *Calculating Exposure, Mass Retained, and Percent Retention of Nicotine, Menthol, and Solvents in JUUL™ Users*

The maximal exposure to nicotine, menthol, PG, and G was calculated for each vaped pod by determining the total pod liquid (mL) consumed multiplied by the concentration (µg/mL) of the chemical. To calculate the actual exposure for each chemical, the maximal exposure was multiplied by the transfer efficiency (%) for each chemical. To determine the mass retained (mg) for each chemical, the mass of each chemical in the exhale was subtracted from the actual exposure. To calculate the percent retention, the mass retained was divided by the actual exposure and multiplied by 100%.

### *Exposure Estimates for a Single Puff, a Session, and a Whole Day*

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Using the mass retention data for a single session for the dominant JUUL™ chemicals, we estimated how much users retained in a single puff and whole day. Single puff retention was calculated by dividing the total retention in a session by the total number of puffs taken in the session. For the whole day estimates we used the average number of puffs estimated for human EC users (~140 puffs per day)[45]. The nicotine equivalency to cigarette use was also calculated for a single session (1 cigarette = 1.1 mg; whole pack = 22 mg)[46] and estimated for the whole day.

### *Symptoms Reported by JUUL™ Users*

Surveys were administered to both JUUL™ users and controls. The surveys contained questions on symptoms commonly reported by EC users. The surveys asked users to select symptoms experienced before, during, and after their exhale session on Day 2. The results were recorded, and the number of symptoms reported was correlated to the total mass retained for the dominant JUUL™ "Menthol" chemicals calculated for the participants during vaping.

### *Statistical Analysis*

A one-way analysis of variance (ANOVA) was performed to analyze the difference between the transfer efficiencies of nicotine, menthol, WS-23, PG, and G at various flow rates. A correlation analysis was performed on the relationship between the transfer efficiency and mass of the e-liquid consumed during aerosolization.

For symptom data, an outlier analysis was performed for each user. The ROUT method on GraphPad Prism was used to detect outliers while fitting a curve with nonlinear regression. This outlier detection method is based on the false discovery rate, to decide which points are far enough from the prediction of the model to be called outliers. Following the identification of an outlier, a correlation analysis and significance testing was performed on the symptoms and chemical mass retained data for each user (except LO who was determined to be an outlier) to understand the dose response relationship. All statistical analyses were done using GraphPad Prism (GraphPad, San Diego).

## RESULTS

### *Demographics of Recruited Participants*

Eleven male participants with at least 6 months experience using JUUL™ products were recruited from the University listserv. The participants included the following ethnicities: Asian/Asian-American and Pacific Islander (N = 6), African American (N = 2), Middle Eastern (N = 1), Hispanic (N = 1), and White/Caucasian (N = 1). Three of these participants identified as mixed race. Three participants started smoking cigarettes socially after using EC products. Three other participants were previous smokers. The age of the users ranged from 21 to 26 years. Some of the participants reported occasional use of tetrahydrocannabinol (N = 6), alcohol (N = 5), and cocaine (N = 2).

The non-e-cigarette users (controls) included the following ethnicities: Asian-American (N = 9), Hispanic (N = 1), and mixed race (N = 1). None of the controls were previous smokers and reported no prior drug use. The age of the controls ranged from 18 -34.

On the modified Fagerstrom Test[47], participants indicated that they used their EC products 10-19 session/day. The average nicotine dependence was 4 using the Fagerstrom index, which indicates a low to moderate level of addiction.

### *Chemical Composition of JUUL™ Menthol Pods*

JUUL™ “Menthol” pods contain four dominant chemicals, which were detected at concentrations above 1 mg/mL (Supplementary Figure 2A, B). The average concentrations of the four chemicals quantified from three unvaped pods were: nicotine ~59 mg/mL, menthol ~13 mg/mL, PG ~265 mg/mL, and G ~621 mg/mL. The relative abundance of each chemical was: solvents > 91%, nicotine 6%, and menthol 1% (Supplementary Figure 2B). This figure does not take into account benzoic acid, which was not measured in this study.

### *Total Mass of Pod Liquid Consumed During Vaping Sessions*

The total mass of the JUUL™ "Menthol" pod liquid consumed during the vaping sessions was recorded for all participants in Days 1 and 2 (Supplementary Figure 3). The mass of the consumed liquids ranged from 10 to 180 mg. The average mass consumed was 73.6 ± 34.1 mg for Day 1 and 70.9 ± 53.6 mg for Day 2, and these means were not significantly different ($p = 0.78$). Most users consumed more than 40 mg during the vaping sessions, and three consumed more than 100 mg.

### *Flavor Chemicals in JUUL™ "Menthol" Pod Fluid that were $\leq$ 1 mg/mL*

GC-MS analysis identified 19 flavor chemicals in two unvaped JUUL™ "Menthol" pods and in the vaped pods from all participants (Supplementary Table 2). Of the 19, eight were above the limit of quantification (LOQ) (10 µg/mL), but < 1 mg/mL (WS-23, benzyl alcohol, hydroxyacetone, caffeine, p-menthone, β-damascone, neomenthol, and isopulegol). The concentrations of WS-23, benzyl alcohol, and hydroxyacetone were similar in fluids before and after vaping. The concentrations of the other chemicals decreased after vaping.

### *Puff Duration, Puff Number, and Total Puffs Seconds for JUUL™ Users*

The total number of puffs and average puff duration were recorded for each participant during Day 1 (ECEAR) and Day 2 (Exhale) sessions (Supplementary Figure 4A). The total puff number ranged from 11-36 for both days (Supplementary Figure 4A) and averaged 22 ± 6.4. Participants were asked to puff the JUUL™ for the same number of puffs during the Day 2 session. Puff duration for each individual was similar from day to day and averaged 4.1 ± 1.6 seconds on Day 1 and 4.7 ± 2.1 seconds on Day 2, which is in good agreement with prior topography studies[40,48-49]. The insert in Supplementary Figure 4A shows the puff duration and puff number for one individual (JE) who provided data on three separate occasions. Puff duration did not differ significantly ($p > 0.05$) across the three days (Supplementary Figure 4A insert),

The total puff seconds (TPS) (puff duration in seconds times total puff number) was calculated for each participant during each session (Supplementary Figure 4B). The TPS gives a quantitative measure of how much each user vaped during one session. As can be seen in Supplementary Figure 4B, TPS varied considerably among participants. For Day 1 (ECEAR) and Day 2 (Exhale), the TPS ranged from ~19 - 135 and from ~12 - 188, respectively. The average TPS for both days was ~ 93.3 ± 33.7 seconds. Except for one individual (BO), who had a puff duration that was difficult to evaluate, TPS values for any given individual were similar on Days 1 and 2.

### ***Transfer Efficiency and Mass Transfer of Nicotine, Menthol, WS-23, and Solvents at Various Flow Rates***

For each dominant chemical as well as WS-23, the transfer efficiency (percent of a chemical that transferred from the fluid to the aerosol) and the actual mass that transferred (mg) are shown in Figure 1 for various flow rates. The transfer efficiency (percent) of nicotine, menthol, WS-23, PG, and G was determined for aerosols generated in our lab on a smoking machine at flow rates between 9-47 mL/sec (Figure 1 A-F) and statistical analyses were performed on the percent transfer efficiency data. The percent transfer efficiency was affected by flow rate. At flow rates of 9 mL/sec, there were no statistically significant differences between chemicals in percent transfer efficiency (Figure 1A). As flow rates increased, significant differences in the transfer efficiencies of individual chemicals were observed (Figure 1 B-F). At flow rates of 41 and 47 mL/sec, the transfer efficiencies were significantly different for each chemical (Figure 1 E,F). Specifically, PG and G transferred the most efficiently (~100%), followed by nicotine (~73%), menthol (~58%), and WS-23 (~45%) (Figure 1F).

The mass (mg) of each chemical that transferred into the aerosol is shown in the colored bars in Figure 1A-F for each flow rate. In general, the mass that transferred varied for each chemical (at 47 mL/sec the average masses were G = 62.7 mg; PG = 26.2 mg; nicotine = 4.2 mg; menthol = 0.7 mg; WS-23 = 0.004 mg). These data show the actual mass of each chemical that a user would inhale at different flow rates. In general, mass increased when transfer efficiency and flow rate increased, except at 41 mL/sec when a decrease in mass was observed (Figure 1E). This decrease may be due to an unidentified technical variation, such as greater loss of aerosol chemicals in the tubing and capture system in this experiment.

The effect of flow rate on transfer efficiency of each chemical was also examined (Figure 2). The flow rate did not affect the percent transfer for nicotine, menthol, and WS-23, which were not statistically significant across all flow rates ($p > 0.05$). However, for PG and G, the transfer efficiency increased at the higher flow rates (e.g., PG = 55.5% at 9 mL/sec; PG = 101.9% at 47 mL/sec) (Figure 2D,E). The mass transferred at 41 mL/sec (e.g., PG = 15.5 mg) was lower than the masses at 21 mL/sec (e.g., PG = 24.4 mg) and 47 mL/sec (e.g., PG = 26.2 mg), perhaps due to an unidentified technical variation in this experiment (Figure 2E).

### *Estimated Maximal and Actual Exposure for Each Dominant Chemical*

The maximal exposure to the dominant JUUL™ chemicals varied among the participants and depended on the concentration of each chemical in the pod fluid and how much fluid was consumed (Figure 3). For nicotine, the maximal exposure ranged from 0.6 – 10.9 mg, whereas menthol ranged from 0.1 – 2.4 mg. The maximal exposure for PG and G ranged from 2.7 – 48.6

mg and 6.3 – 114 mg, respectively. The average maximal exposures in mg were 4.2 ± 3.2 for nicotine, 0.95 ± 0.72 for menthol, 18.9 ± 14.31 for PG, and 44.3 ± 33.6 for G.

The actual exposure to the dominant JUUL™ chemicals considers the transfer efficiency of each chemical. For nicotine, the actual exposure ranged from 0.4 – 8.7 mg, whereas menthol ranged from 0.08 - 1.5 mg. The actual exposure for PG was the same as the maximal exposure because its transfer efficiency was 100%. The actual exposure for G (7.6 – 137 mg) was slightly greater than the maximal exposure. This could be explained by the fact the concentrations of G were estimated, which may have produced transfer efficiencies that were greater than 100%.. The average actual exposures in mg were 3.4 ± 2.6 for nicotine, 0.6 ± 0.4 for menthol, 18.9 ± 14.3 for PG, and 53.2 ± 40.3 for G.

### *Nicotine, Menthol, Solvents, and Other Chemicals Detected in Participants' Exhale After JUUL™ Vaping*

Nicotine, menthol, PG, and G were quantified in JUUL™ users' exhale samples (Figure 4 A,B). Data in Figures 7A and B show the total mass for each chemical captured in both impingers was <1 mg in all but one exhale sample. The total exhaled mass of nicotine after vaping ranged from 0 to ~1 mg (Figure 4A), whereas menthol ranged from 0 to 0.1 mg. Four patterns of exhale for nicotine and menthol were identified across the participants. Two users exhaled more nicotine than menthol; four users exhaled more menthol than nicotine; four users exhaled menthol only; and one did not exhale either menthol or nicotine. Control participants did not exhale nicotine or menthol, except for one participant who exhaled trace levels of nicotine.

Very little PG (0 to < 0.01 mg) and G (0 to 0.01 mg) were detected in the exhaled aerosol of JUUL™ users. In some cases, there was less solvent than nicotine or menthol in the exhale. None of these chemicals were found in exhale of the participants before vaping. All control exhale samples were negative for PG and G.

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Other flavor chemicals detected in users' exhale included benzaldehyde, p-menthone, acetophenone, and triacetin (Supplementary Figure 5A-C). Only one user had detectable p-menthone in their post-vape exhale sample (not shown). Benzaldehyde was not detected in five samples and was below the limit of quantification (500 ng/mL) in six samples. Triacetin and acetophenone were detected in most pre-vape samples and were higher in concentration in nine post-vape samples. The control participants were negative for p-menthone and triacetin. Five controls had benzaldehyde in their exhale and most controls had detectable concentrations of acetophenone that ranged from 0.0002 - 0.0003 mg and 0.0003 - 0.001 mg, respectively

### *Retention of Nicotine, Menthol, PG, and G by Study Participants Calculated at a Flow Rate of 21 mL/sec*

The total mass retained and the percent retention for nicotine, menthol, PG, and G were calculated at six flow rates (9, 11, 13, 21, 41, and 47 mL/sec) for each user as described in the Materials and Methods (Supplementary Table 3). For the 21 mL/sec flowrate, the mass retained for each chemical was variable among participants and ranged from ~0.05 – 8.7 mg for nicotine, ~0.05 – 1.4 mg for menthol, ~2.7 – 48.6 mg for PG, and ~7.6 – 136.7 mg for G (Figure 5 A,C,E,G).

At the 21 mL/sec flow rate, the percent retention for all users, excluding BL, ranged from 90-100% for nicotine and 86-100% for menthol (Figure 5 B,D). All users retained between 99-100% of the PG and G. BL was not included in the nicotine and menthol ranges since he was likely a mouth inhaler rather than a lung inhaler[38]. The results for mass retained and percent retention were similar for the low (9-13 mL/sec) and high (21-47mL/sec) flowrates (Supplementary Table 3).

### *Symptoms Reported During JUUL™ Vaping*

Various symptoms were reported before, during, and after vaping JUUL™" Menthol" pods (Figure 6A, C). While most participants generally did not report symptoms before vaping

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JUUL “Menthol”, most experienced symptoms during the 20-minute vaping session. These symptoms included: lightheadedness (N = 7/11), increased heart rate (N = 3/11), dry throat (N = 2/11), dizziness (N = 2/11), coughing (N = 2/11), nausea (N = 1/11), and shortness of breath (N = 1/11), After the 20-minute vaping session, some symptoms (such as lightheadedness, shortness of breath, and increased heart rate) persisted in some of the JUUL ™ users. Most symptoms were related to the neurological, respiratory, and cardiovascular systems.

When data were examined for individuals within the population, most did not report symptoms before vaping; however, all except for one individual (BO) experienced symptoms during vaping, and these persisted in all individuals except two when vaping stopped (Figure 6 B).

To explore a possible relationship between dose and the number of symptoms, the total mass retained for the dominant chemicals (nicotine, menthol, and the solvents) was graphed for each participant (Figure 6C). The mass retained for the dominant chemicals ranged from 10.8 mg to 195.4 mg based on our 21 mL/sec data during a 20-minute vaping session. The number of symptoms was correlated to the mass retained mass retained ($R^{2=}$0.7) to the dose and was significant (p = 0.004 (Figure 6D).

### *Estimated Exposure to Nicotine, Menthol, and the Solvents for a Single Puff, Session, or Whole Day Exposure to JUUL™ “Menthol” Aerosol and Cigarette Equivalency*

The nicotine, menthol, PG, and G mass retained (dose) were estimated for a single puff, a single session, and a whole day for all participants based on the 21 mL/sec flow rate (Supplementary Table 4). For all users, a single puff of JUUL™ “Menthol” delivered less than 0.5 mg of nicotine (0.002-0.33 mg) or menthol (0.002-0.05 mg). In contrast, a single puff delivered more than 1 mg of PG (0.22 – 1.87 mg) and G (0.62-5.26 mg) (total PG + G = 0.85-7.1mg) for eight of eleven users. The mass retained and percent delivered in a single session for each chemical is shown in Supplementary Table 3. For the whole day estimates, nicotine

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and menthol ranged from 0.3 - 46.2 mg and 0.02 – 7 mg, respectively. For the whole day estimates, PG (30.8 - 261.8 mg) and G (86.8 - 736.4 mg) were delivered at much higher masses than the other chemicals, and the combined solvent (119 - 998.2 mg) estimates exceeded 100 mg for all users.

To compare the extrapolated nicotine exposure during JUUL™ vaping to cigarette smoking (FTC standard protocol for Marlboro Red filtered hard pack), 1.1 mg was selected to as equivalent to 1 cigarette/day and 22 mg was equivalent to 1 pack/day[46] (Supplementary Table 4). For the session data, the nicotine dose for JUUL™ users was equivalent to < 1 to ~8 cigarettes. For whole day estimates, five users were exposed to the nicotine equivalent to ~1 to ~2 packs/day.

### ***Nicotine. Menthol, and Solvents Deposited as ECEAR After JUUL™ Vaping***

Nicotine and menthol were detected in ECEAR extracts of paper towels that had been exposed to exhale in an acrylic box (Figure 7 A,B). The total mass of nicotine and menthol in the ECEAR extracts was between < 0 to 0.2 mg and < 0 to 0.9 mg, respectively (Figure 7A). Nine of 11 participants had more menthol than nicotine in their ECEAR. For the solvents, 0 – 3.8 mg of PG and 0 – 67.4 mg of G were detected in ECEAR (Figure 7B), and the relative amounts of each solvent varied with the participants. Three participants deposited both PG and G, three deposited G only, and six deposited neither solvent in ECEAR.

Other flavor chemicals that were detected in the ECEAR included: hexanol, benzyl alcohol, isoamyl isovalerate, p-tolualdehyde, acetophenone and triacetin (Supplementary Figure 6). In some participants, chemicals, such as hexanol, p-tolualdehyde, and triacetin, were elevated in the ECEAR after vaping.

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## *DISCUSSION*

The puffing topography, transfer efficiency of dominant chemicals, exposure, retention, and ECEAR deposition were examined during 20-minute vaping sessions, providing comprehensive data on JUUL™ "Menthol" use. Acute symptoms were also evaluated relative to the mass retained of the dominant JUUL™ chemicals, and the nicotine equivalency per single puff and day was estimated (Figure 8). Topographies varied among users but were similar for a given individual on different days. Transfer efficiencies for nicotine, menthol, WS-23, and the solvents were generally 49 - 115% at 21 mL/sec. At six flow rates, high levels of PG and G and moderate levels of nicotine and menthol were delivered to and retained by the participants. When the data were extrapolated to whole day exposures, half of the JUUL™ users received nicotine doses > 1 pack of Marlboro Red cigarettes, with one individual receiving the equivalent of 2 packs. Symptoms reported by participants during vaping had a significant correlation to the total mass of the dominant chemicals that were retained. Overall, JUUL™ "Menthol" ECs delivered high levels of chemicals and produced symptoms during vaping that sometimes persisted after the session ended.

Our concentrations of nicotine, menthol, and PG/G agree with previous studies on JUUL™ ECs[9-11], indicating that the chemical formulation for JUUL™ "Menthol" e-liquid has not changed in the past 3 years. Menthol concentration in JUUL™ "Menthol" pods was ~13 mg/mL, which is higher than the concentration in mentholated tobacco products (< 0.002 - 7 mg/cigarette)[51], but low compared to the concentrations of nicotine and the solvents (PG and G) in JUUL™ e-liquids. Prior studies emphasized the importance of the "high" concentrations of nicotine and flavor chemicals in e-liquids[9,41,50]; however, the solvents comprise over 90% of the chemical mixture in JUUL™ "Menthol" pods and are the major chemical that vapers receive. Although benzoic acid was not examined in the present study, it is found at 44.8 ± 0.6 mg/mL in JUUL™ pods[12].

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Puff duration can be affected by the EC model, efficiency of nicotine delivery[1], and the desire to create large clouds. In contrast to conventional cigarette smokers (~2.4 ± 0.8) [40,52], the mean puff duration across all generations of EC models is 3.2 seconds[38,40,48-49,53-55,]. In a JUUL™ study, puff duration was 3.0 ± 1.4 seconds[49], which is in reasonably good agreement with our average puff of 4.4 ± 2.0 seconds. Longer puff duration can increase the transfer efficiency of chemicals to aerosols[38], and in EC tank models, the level of toxicants, such as acetaldehyde, acrolein, and formaldehyde, that are inhaled[56]. Some reaction products, such as hydroxyacetone, were not detected in our study, and formaldehyde and acrolein were at or below the LOQ in another JUUL™ study[13] and may be less of a concern than the dominant chemicals.

TPS, which considers puff duration and puff count, is a simple rapid method for estimating total exposure during a session. Two users (VN and BL) had TPS values < 80 on both days and were subsequently found to have lower mass retained (exposure) than other participants in our vaping data. In contrast, participants with high TPS received higher total chemical exposure during vaping.

The chemical transfer efficiency from an e-liquid to an aerosol can be influenced by various factors and affects the actual exposure (dose) that a vaper receives. The transfer efficiency of nicotine, menthol, other flavor chemicals, PG, G, and benzoic acid have been reported using various EC models and operating conditions. In older models, including disposables, the transfer efficiency of nicotine was < 50 - 60 %[17,57-58]. In newer models, efficiency increased with ranges between 63 - 82% for tanks[38] and ~50 - 80% for JUUL™ products[9,-10]. Omaiye et al (2019) reported the transfer efficiency of menthol in JUUL™ "Menthol" pods was ~69% at flow rates between 10 −13 mL/s[9], which is slightly higher than our range (40-60%) for flow rates of 9-47 mL/sec. At either 3V or 5V, SMOK ECs transferred menthol with an efficiency close to 100%[41], perhaps because the pure menthol/PG mixture had a lower boiling point than e-liquid mixtures that usually contain PG and G.

Since transfer efficiencies for menthol and nicotine were < 100% in our study, actual exposures (dose) received by EC participants were lower than for the solvents (~100% efficiency at flow rates $\geq$ 21 mL/sec) and lower than the concentration of the chemical in the e-liquid. Therefore, when computing actual exposures and retention, it is necessary to consider transfer efficiency for each chemical and the conditions used to generate aerosols. Transfer efficiency for nicotine, menthol, and WS-23 did not increase at flow rates greater than 21 mL/sec, perhaps because their maximum efficiency is affected by the mixture of chemicals in the JUUL™ e-liquid or the high-performance pump head. To fully understand the effect of vapor pressure on transfer efficiency, it would be necessary to know the vapor pressure of each chemical during heating in a mixture of chemicals in an atomizer.

Puff duration, EC wattage/power level, flow rate (13 and 41 mL/sec), individual chemicals, chemical vapor pressure, vaping protocol, and the pump head affected transfer efficiencies for laboratory generated aerosols made using a SMOK Alien EC[38]. In general, transfer efficiency increased with increasing puff duration, wattage, flow rate, and vapor pressure. Our results establish transfer efficiencies for JUUL™ "Menthol" ECs and are in good agreement with the SMOK Alien study. In our study, both PG and G transferred with very high efficiency (~100%) at flow rates between 21 - 47 mL/sec. Poorer transfer at lower flow rates may be due to incomplete heating of the filament, poorer efficiency of the low-performance pump head[38], trapping of chemicals in the EC atomizer or pods, and greater loss of chemicals in tubing and other parts of the aerosol collection system at the lower flow rates. The high transfer efficiency of G is unexpected based on its low vapor pressure (0.0002 mm HG at 25°C). However, inclusion of water and nicotine in G solutions lowers its boiling point[59], which would improve its transfer into an aerosol.

Regardless of the model, human EC users exhale low concentrations of chemicals[38,60-63], which are lower than concentrations exhaled from combustible cigarettes[60-61]. The exhale of JUUL™ users had 99% less formaldehyde and carbon monoxide than that of traditional smokers[63]. Participants using blu™ disposable "Classic Tobacco" and "Menthol" ECs and other cig-a-like and sub-ohm models had little solvent and nicotine in their exhale[60,62]. Phenolics and carbonyls were not detectable[60], but some TSNAs and copper (< LOQ-2.92 ng) were present[62]. We found that the exhale from JUUL™ "Menthol" users had very low concentrations of solvents (PG and G), nicotine, and menthol.

Exhale content is influenced by whether a user inhales into their lungs (lung inhaler) or their mouth (mouth inhaler)[38]. For mouth inhalers, but not lung inhalers, chemical concentration in the exhale was significantly correlated with longer puff durations[38]. The 10 lung inhalers in our study had a low total mass of chemicals (< 1 mg) in their exhale, except for one participant (BL), whose total exhaled mass (~1 mg) was in the range of mouth inhalers using tank models[38].

Minor chemicals, such as benzaldehyde, triacetin, and acetophenone, were detected in participants' exhale. Triacetin, a PG and G related chemical, was found only in EC users' exhaled aerosol and was elevated shortly after vaping, suggesting rapid formation in the oral cavity. Acetophenone and benzaldehyde were identified in both EC user and control breath, and they are common chemicals found in human breath[64,65]. The presence of triacetin in the exhaled aerosol of vapers suggests it may be an exposure biomarker related to JUUL™ use.

Nicotine rapidly enters the plasma of EC users, but little is known about its actual retention (dose). Chemical retention can be estimated using the transfer efficiency of a chemical, the exposure a user receives, and the concentration of the chemical in the exhale. Retention data characterize the chemical dose the oral cavity and lungs receive. Lung inhalers retained 80 - 100% of the nicotine and flavor chemicals that they took in when using a tank style EC[38]. For 10 of 11 participants in our study, 86 -100% of the inhaled nicotine and menthol were retained, and 99 - 100% of the solvents were retained at 21 mL/sec. Although we did not report on benzoic acid, JUUL™ Crème Brulé aerosols contained 86.9 µg/puff of benzoic acid[17]. Based on our average of ~20 puffs/session, a user in our study would have been exposed to ~1.7 mg of benzoic acid in a session. Due to its low volatility, it is likely benzoic acid was retained by the participants.

Our study is the first to demonstrate that symptoms increase in JUUL™ users with increasing chemical dose. The main ingredients in JUUL™ "Menthol" (nicotine, benzoic acid, and solvents) can produce the reported symptoms. Nicotine can cause nausea, dizziness, headache, and increased heart rate[66]. PG and G are associated with nausea, vomiting, headache, dizziness, lightheadedness, and skin/eye/lung irritation[67,68]. Benzoic acid is a respiratory irritant that can cause coughing and sore throat[69]. The overlap between the symptoms associated with nicotine, PG, G, and benzoic acid exposure suggest that the adverse effects reported by users were caused by a combination of chemicals rather than an individual chemical. In addition, there was a direct correlation between the dose a participant received, and the number of symptoms reported. This is the first-time exposures were correlated to symptoms during JUUL™ vaping. The long-term effects of persistent direct inhalation of EC aerosols are unknown but studies suggest that exposure to the solvents cause inflammatory effects[34].

Thirdhand smoke (THS), the chemical residue deposited on indoor surfaces after cigarette smoking has stopped, has been studied extensively[70-72]. THS contains high concentrations of nicotine and related alkaloids, including carcinogens[73,74] and accumulates with increased cigarette use[75]. A similar relationship has been reported in a vape shop in southern California, where ECEAR (the EC counterpart of THS) accumulated over time reaching 3.6 mg of nicotine per gram of fabric by 1 month of sampling[36]. THS has caused adverse health effects in humans following dermal and inhalation exposure[76,77]; however, little is known about the effects of ECEAR on human health.

Because retention of inhaled EC chemicals was high in most JUUL™ users, the concentrations of exhaled nicotine, menthol, PG, and G were low and accordingly their contributions to ECEAR were a small percent of the total chemicals in an EC puff. The ratio of exhaled nicotine/menthol varied among participants, indicating individual variation in the relative amounts of these chemicals retained. JUUL™ ECs produce a thin wispy aerosol, often facilitating their stealth use. In contrast, many ECs produce larger clouds than JUUL™, and these likely contain higher concentrations of chemicals than we observed with JUUL™ users.

The JUUL™ ECEAR in our study after aging for 30 minutes produced additional chemicals that were not in the exhaled aerosol, such as p-tolualdhehyde (a skin irritant) and hexanol (central nervous system toxicant)[78,79]. Vapers and non-vapors occupying Indoor environments containing ECEAR would be passively exposed, mainly dermally and via inhalation, to the chemicals in ECEAR. While adverse health effects have not yet been linked directly to ECEAR, a better understanding of ECEAR exposures is an important knowledge gap to be filled.

**Study Limitations**

Our data were derived from a cohort of male JUUL™ users from various ethnicities. JUUL™ users were chosen because this was the most popular EC brand at the time the study was started. Future studies could be extended to third generation and other fourth generation EC brands, which are currently popular. Our data are based on JUUL™ ECs, which deliver relatively small clouds of aerosol. Third generation products, which deliver large aerosol volumes, may result in larger quantities of chemicals being retained and exhaled. We limited our study to male participants, since EC use is more prevalent among males than females[80]. The retention and exhale data were similar across the 11 participants in our study, suggesting these data are representative of JUUL™ users in general. Future studies could be extended to a larger population that includes representatives from more ethnicities and females.

## Conclusion Paragraph

We provide a comprehensive overview of exposure, retention, and ECEAR deposition for JUUL™ "Menthol" users. Nicotine, menthol, PG, and G were transferred with variable efficiency to EC aerosols and well retained by participants. The total mass retained (dose) in a 20-minute vaping session (calculated at 21 mL/sec) ranged from 10.8 to 195 mg for the dominant chemicals. Retention of PG and G was close to 100% in most participants, and as a consequence relatively low levels of chemicals appeared in ECEAR. Most users reported adverse symptoms, such as -nausea, dizziness and lightheadedness, during the 20-minute vaping session, and there was a significant correlation between the dose and symptom count. The potential doses JUUL™ users receive of nicotine, PG, and G are concerning, especially the solvents which are understudied. Additionally, the potential for higher delivery and higher deposition of ECEAR may exist in other products, such as EC tanks/mods. Although there are few chemicals in JUUL™ pods, the potential for chemical transfer is high and there is the possibility for the formation of toxic byproducts in ECEAR that can contribute to passive exposure over time. Going forward, it will be important to conduct similar studies using other EC products and to follow the long-term health effects of both JUUL™ use and passive exposure of non-users to ECEAR.

**Supporting Information:** Additional experimental details, data, and terms/abbreviations are included (PDF).

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## FIGURE LEGENDS

**Figure 1: Mass Transfer and Percent Efficiency of Nicotine, Menthol, and the Solvents in JUUL™ "Menthol" Pods at Various Flow Rates. (A-F)** The transfer efficiencies of nicotine, menthol, propylene glycol, and glycerol were estimated at six flow rates 9. 11, 13, 21, 41, and 47 mL/sec. The mass (mg) of the chemical transferred from liquid to aerosol during aerosolization is shown in their respective flow rate bars. The averaged transfer efficiency and mass (mg) transferred are shown for nicotine, menthol, WS-23, propylene glycol, and glycerol at the six flow rates. * = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$; **** = $p < 0.0001$.

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**Figure 2: Mass Transfer and Percent Efficiency of Nicotine, Menthol, WS-23 and the Solvents in JUUL™ "Menthol" Pods Plotted at Various Flow Rates. (A-E)** The transfer efficiency of nicotine, menthol, WS-23, propylene glycol, and glycerol were plotted separately at various flow rates 9. 11, 13, 21, 41, and 47 mL/sec. The mass (mg) of the chemical transferred from liquid to aerosol during aerosolization is shown in their respective flow rate bars. * = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$.

**Figure 3: Maximal and Actual Exposure for Each Dominant Chemical.** The estimated maximal and actual exposures (A-D) and their averages ± SD were calculated for nicotine, menthol, PG, and G as described in the Materials & Methods. Light Red = maximal exposure; Red = actual exposure.

**Figure 4: Total Exhale Quantified for Each Participant During Day 2 Session.** (A) The total exhale (mg) for nicotine, menthol, and WS-23 quantified for each user. (B) The total exhale (mg) for propylene glycol and glycerol quantified for each user.

**Figure 5: Mass and Percent Retained Calculated for Nicotine, Menthol, PG, and G at 21 mL/sec for Each User.** The estimated mass delivered (A, C, E, G) and percent retention (B, D, F, H) was computed by calculating the amount of nicotine, menthol, PG, and G consumed and subtracting from this from the amount of nicotine, menthol, PG, and G exhaled.

**Figure 6: Acute Symptoms Observed for Users During Exhale Session.** (A-B) The most reported symptoms before, during, and after vaping are shown along with the average number of symptoms reported during Day 1 and 2 sessions. (C) Total exposure calculated for vapers from all four chemicals. (D) The correlation between the number of symptoms (Y-axis) and the total chemical mass retained (X-axis).

**Figure 7: Total ECEAR Concentrations for Each Participant During Day 1 Session.** (A) The total ECEAR mass (mg) in the acrylic box for nicotine and menthol for each user. (B) The total ECEAR mass (mg) in the acrylic box for PG and G for each user.

**Figure 8: Summary of Major Results.**

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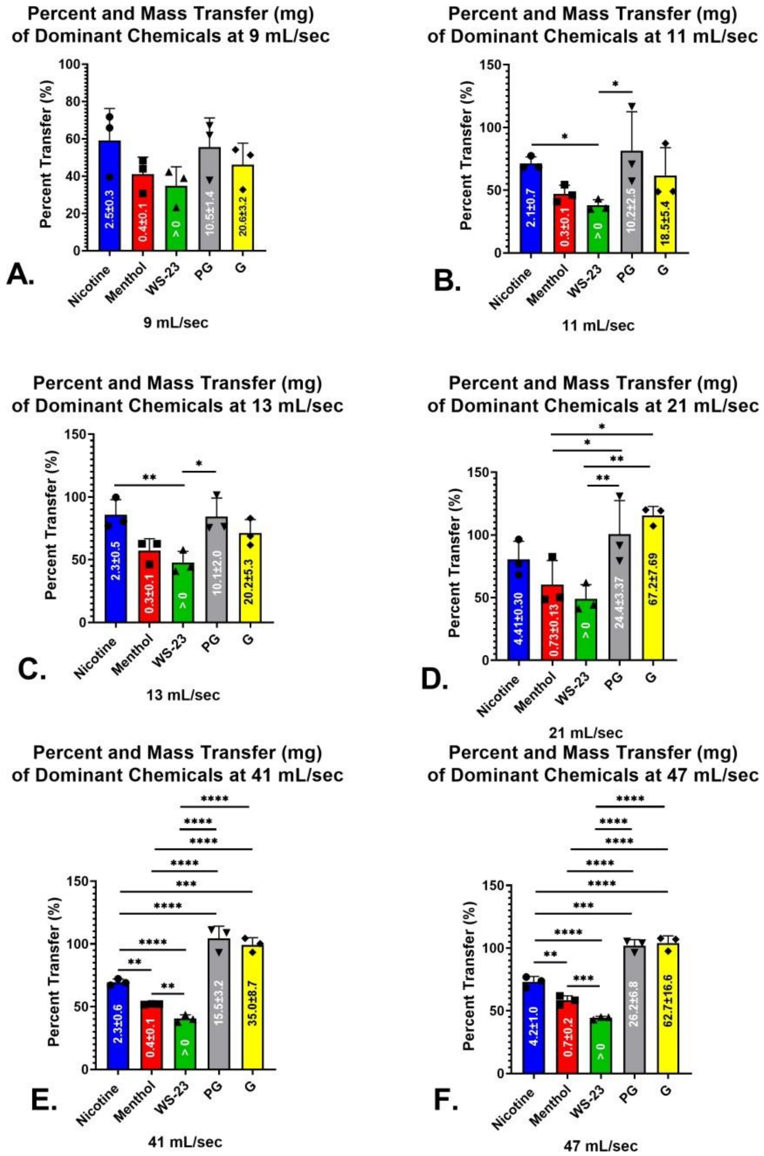

Figure 1: Mass Transfer and Percent Efficiency of Nicotine, Menthol, and the Solvents in JUUL™ "Menthol" Pods at Various Flow Rates. (A-F) The transfer efficiencies of nicotine, menthol, propylene glycol, and glycerol were estimated at six flow rates 9. 11, 13, 21, 41, and 47 mL/sec. The mass (mg) of the chemical transferred from liquid to aerosol during aerosolization is shown in their respective flow rate bars. The averaged transfer efficiency and mass (mg) transferred are shown for nicotine, menthol, WS-23, propylene glycol, and glycerol at the six flow rates. * = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$; **** = $p < 0.0001$.

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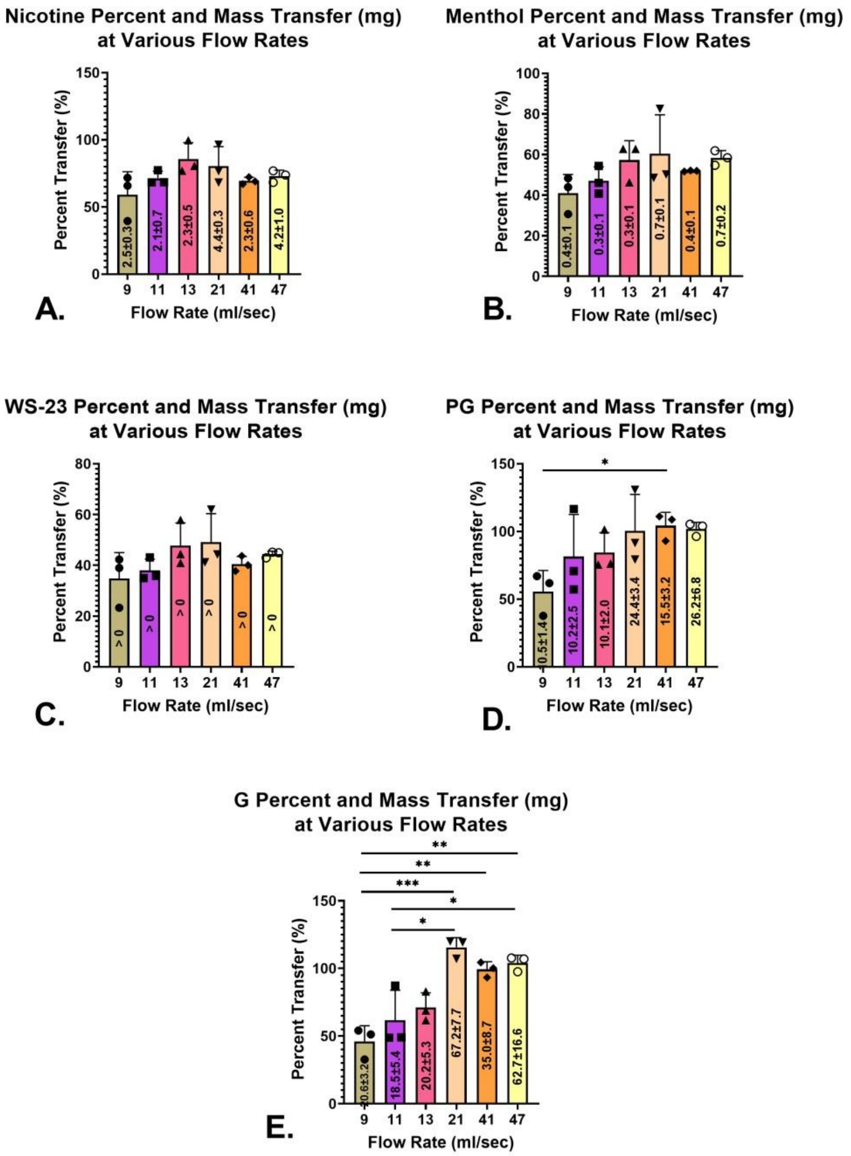

Figure 2: Mass Transfer and Percent Efficiency of Nicotine, Menthol, WS-23 and the Solvents in JUUL™ “Menthol” Pods Plotted at Various Flow Rates. (A-E) The transfer efficiency of nicotine, menthol, WS-23, propylene glycol, and glycerol were plotted separately at various flow rates 9. 11, 13, 21, 41, and 47 mL/sec. The mass (mg) of the chemical transferred from liquid to aerosol during aerosolization is shown in their respective flow rate bars. * = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$.

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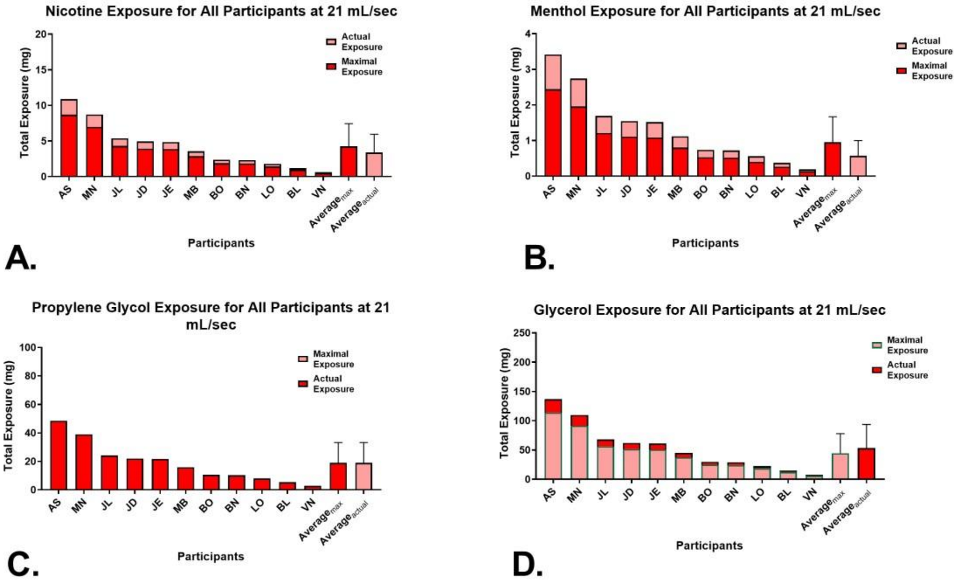

Figure 3: Maximal and Actual Exposure for Each Dominant Chemical. The estimated maximal and actual exposures (A-D) and their averages ± SD were calculated for nicotine, menthol, PG, and G as described in the Materials & Methods. Light Red = maximal exposure; Red = actual exposure.

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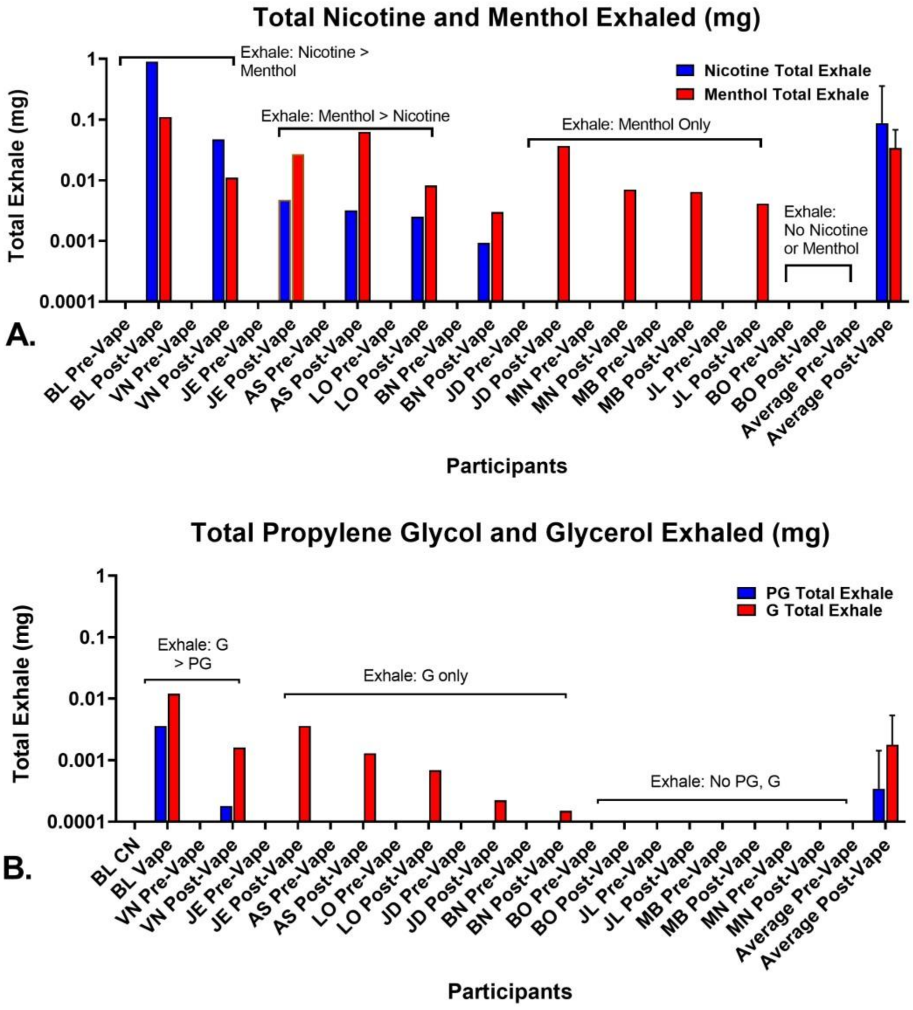

Figure 4: Total Exhale Quantified for Each Participant During Day 2 Session. (A) The total exhale (mg) for nicotine, menthol, and WS-23 quantified for each user. (B) The total exhale (mg) for propylene glycol and glycerol quantified for each user.

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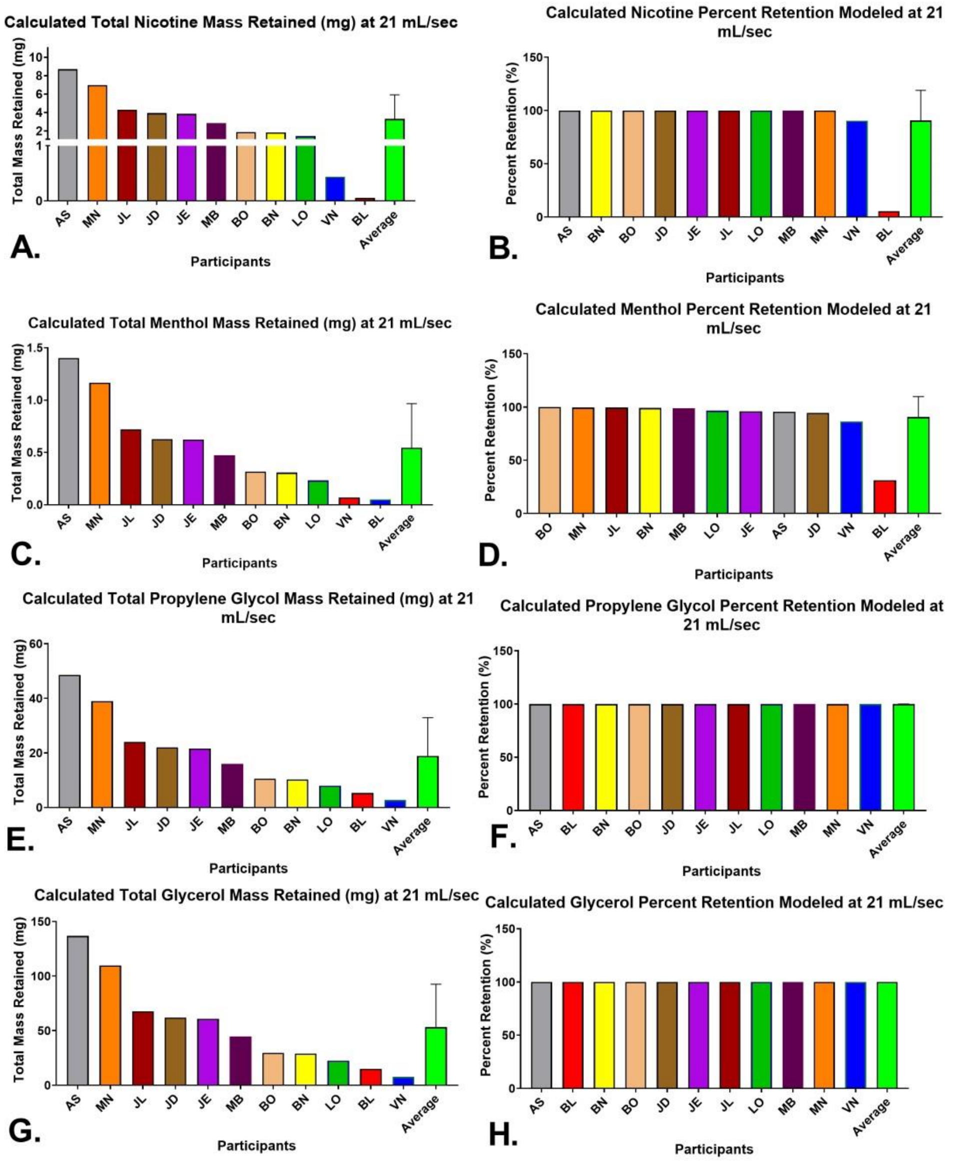

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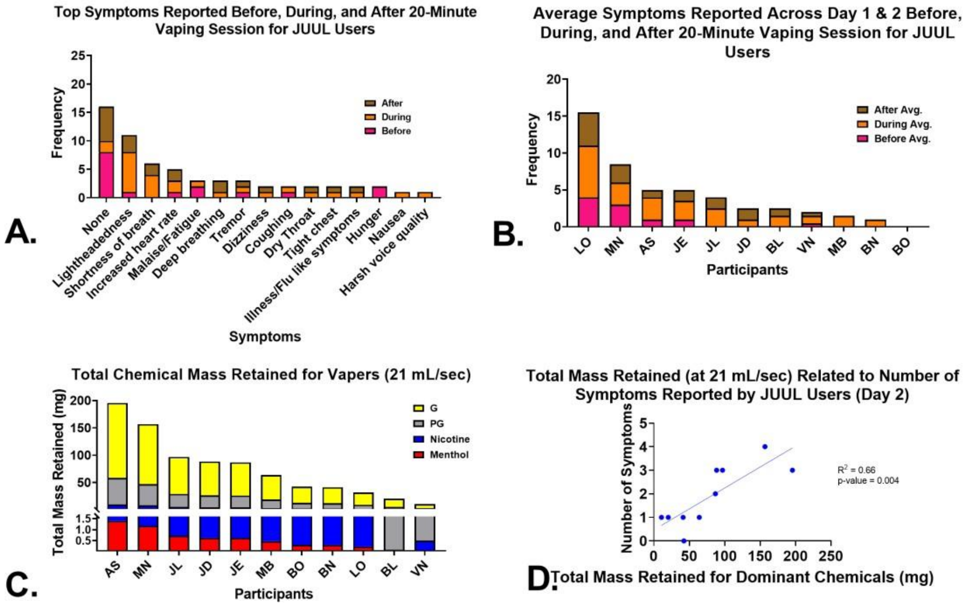

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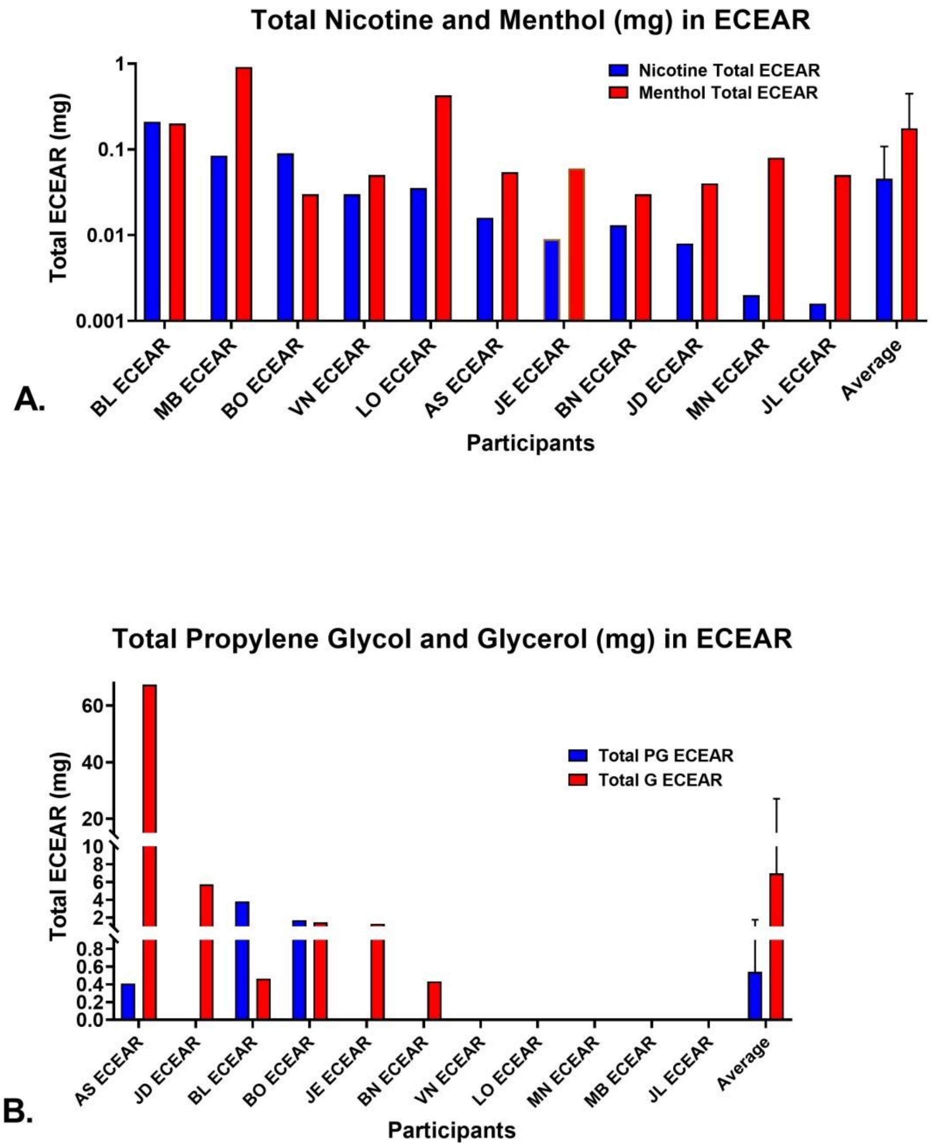

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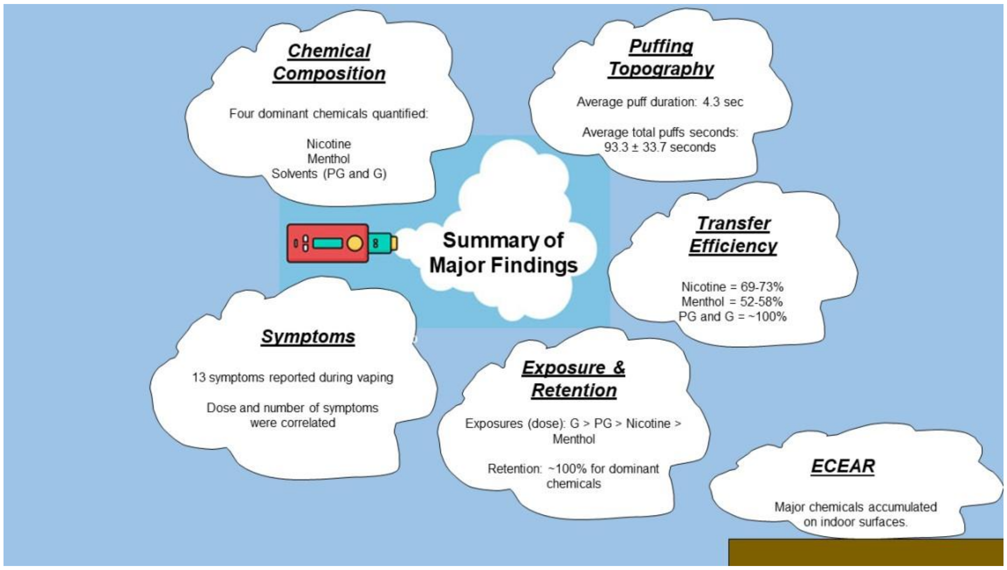

Figure 8: Summary of Major Results.

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# Exposure, Retention, Exhalation, Symptoms, and Environmental Accumulation of Chemicals During JUUL™ Vaping

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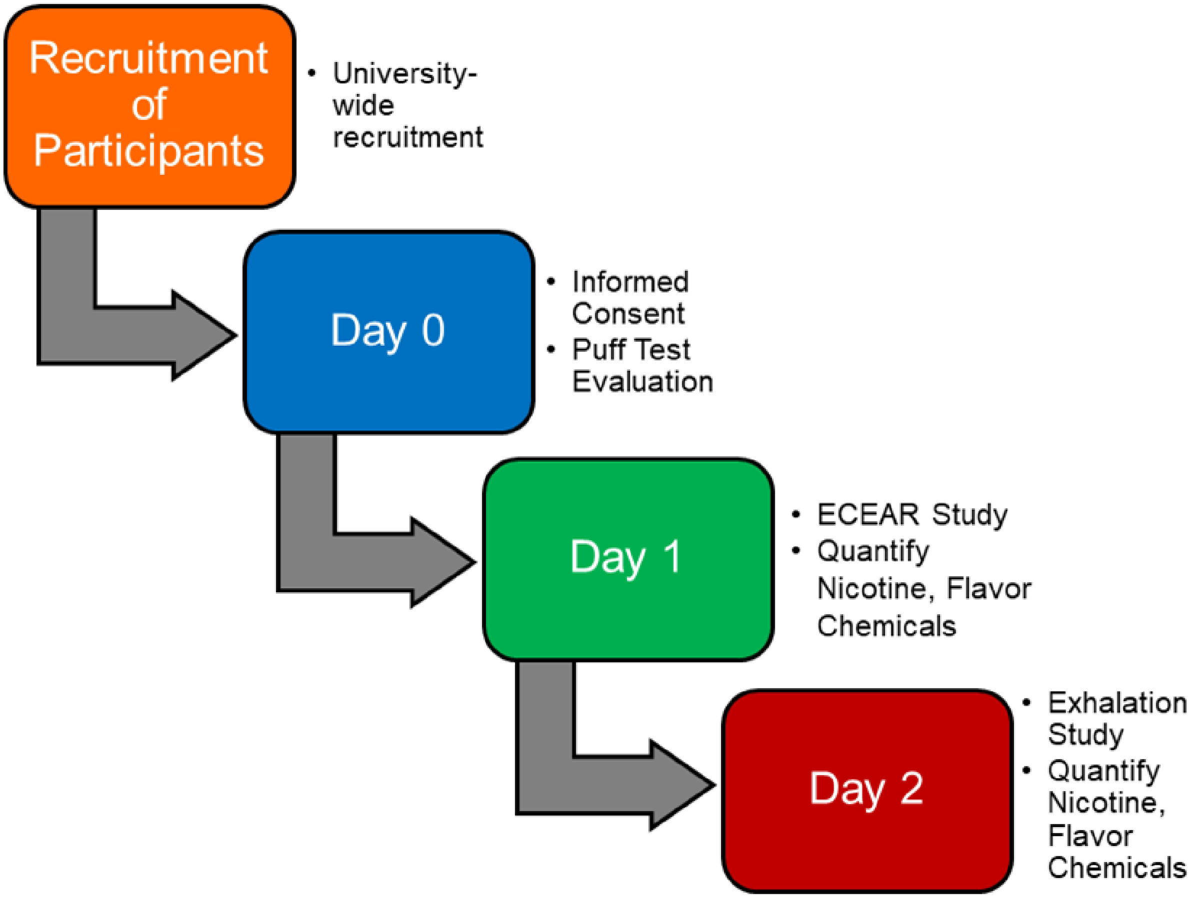

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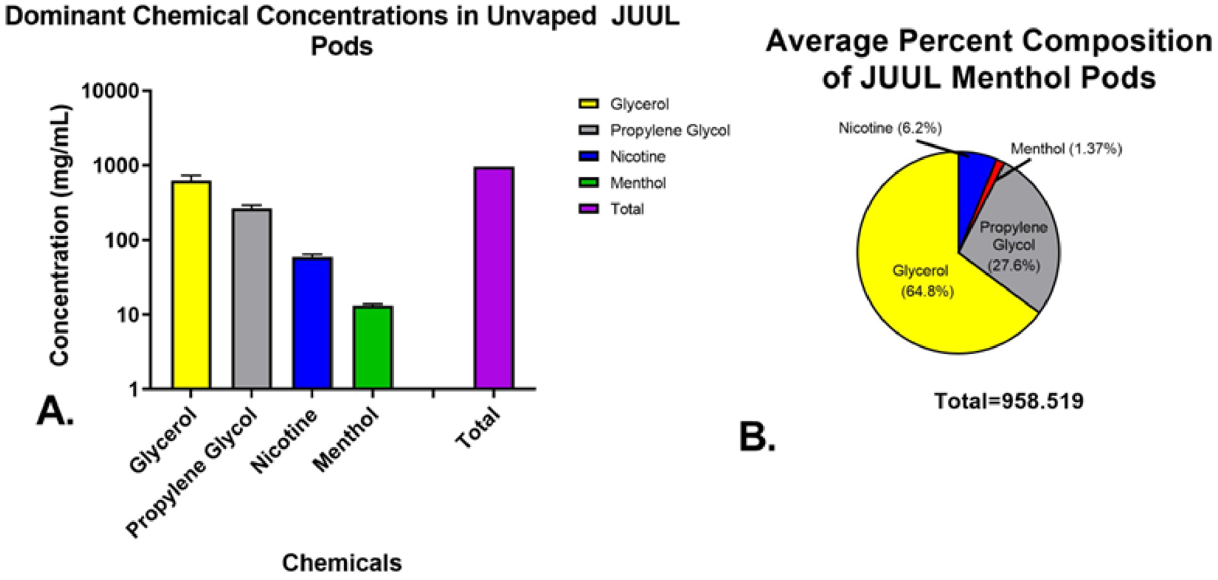

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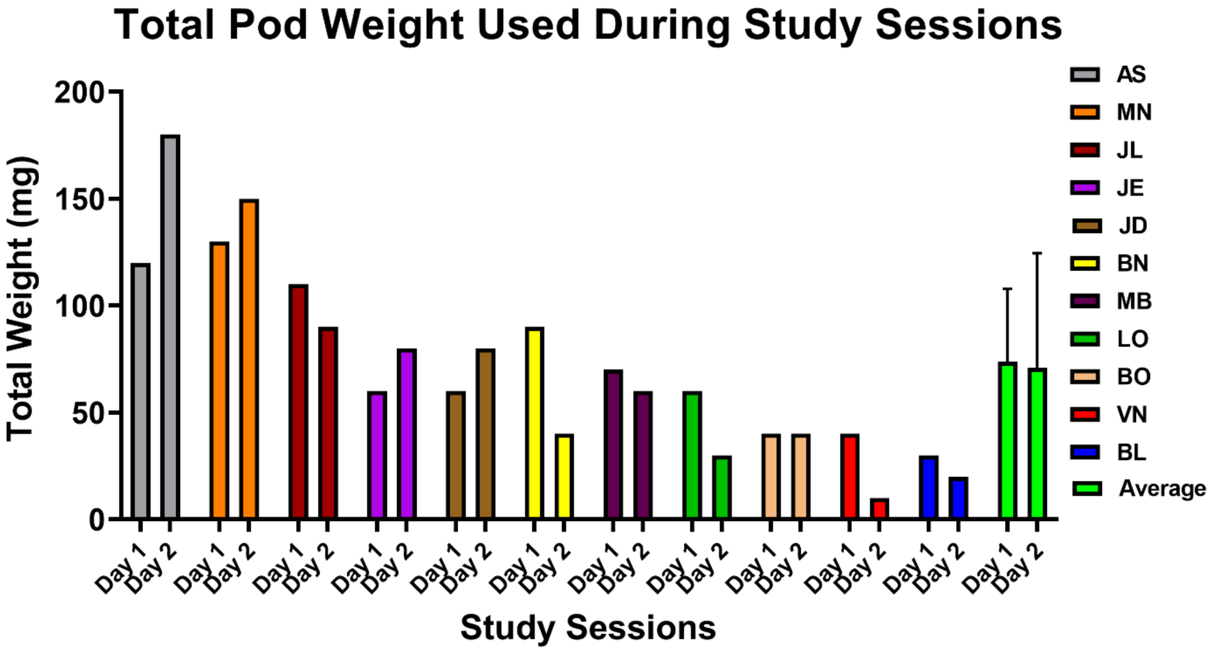

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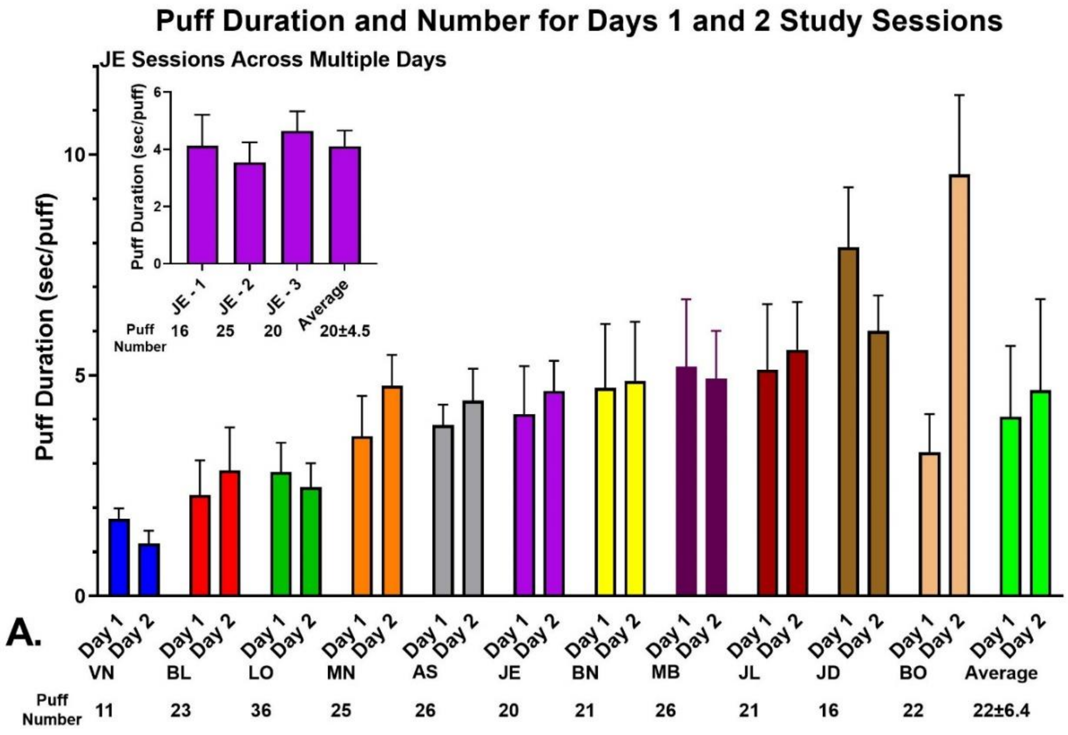
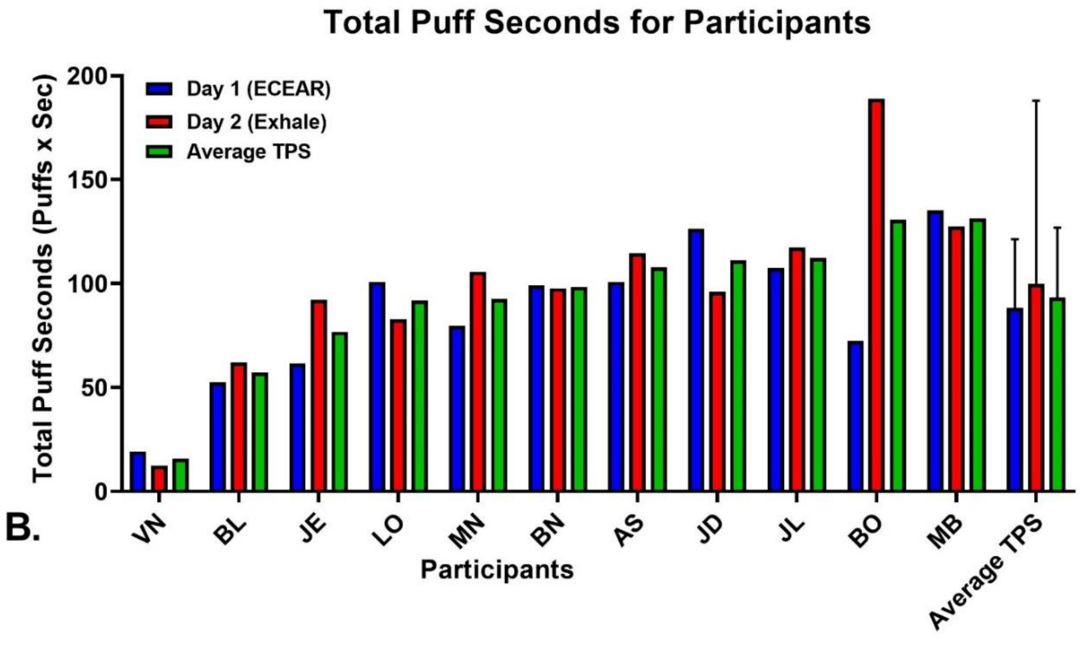

Puff Duration and Number for Days 1 and 2 Study Sessions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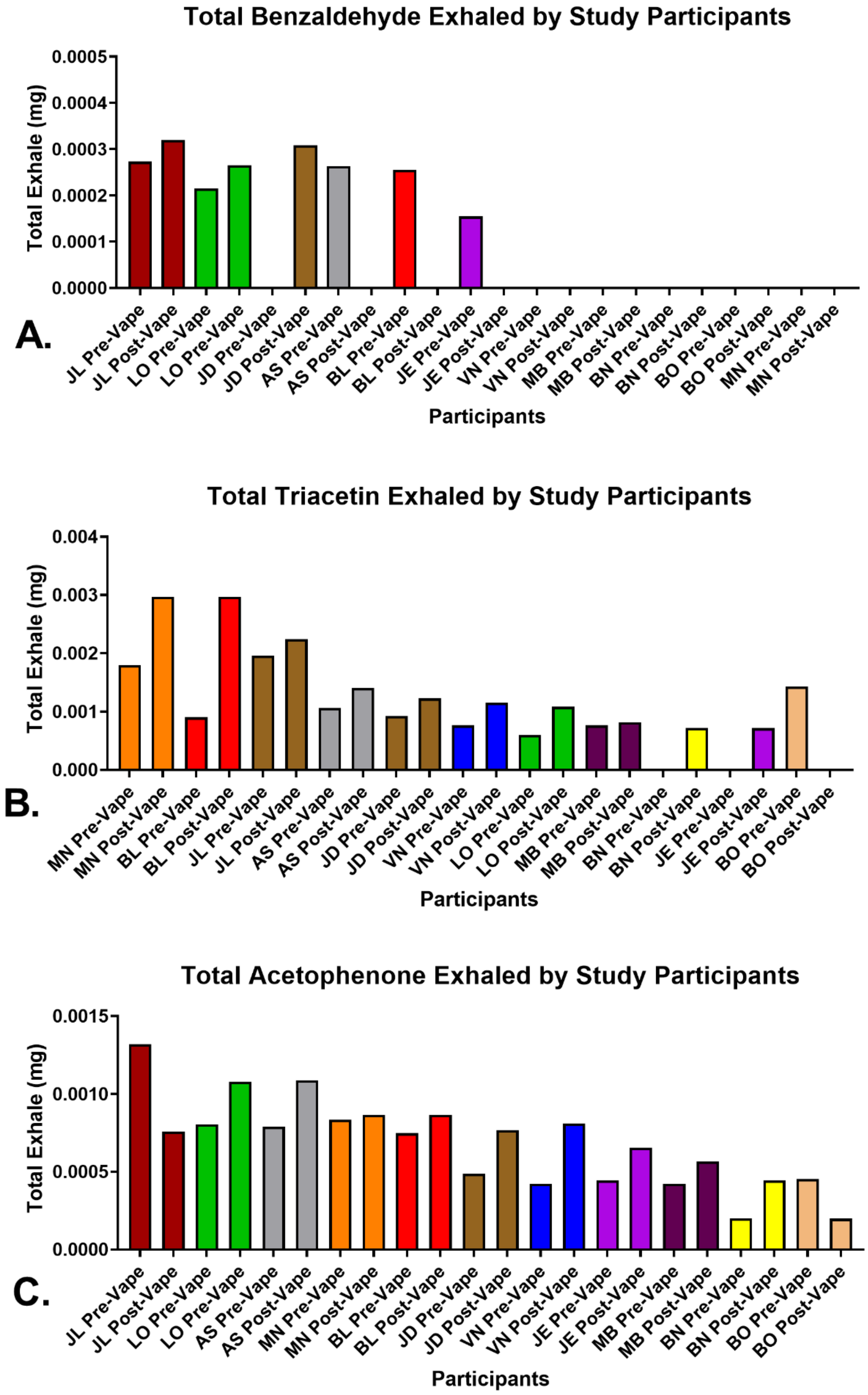

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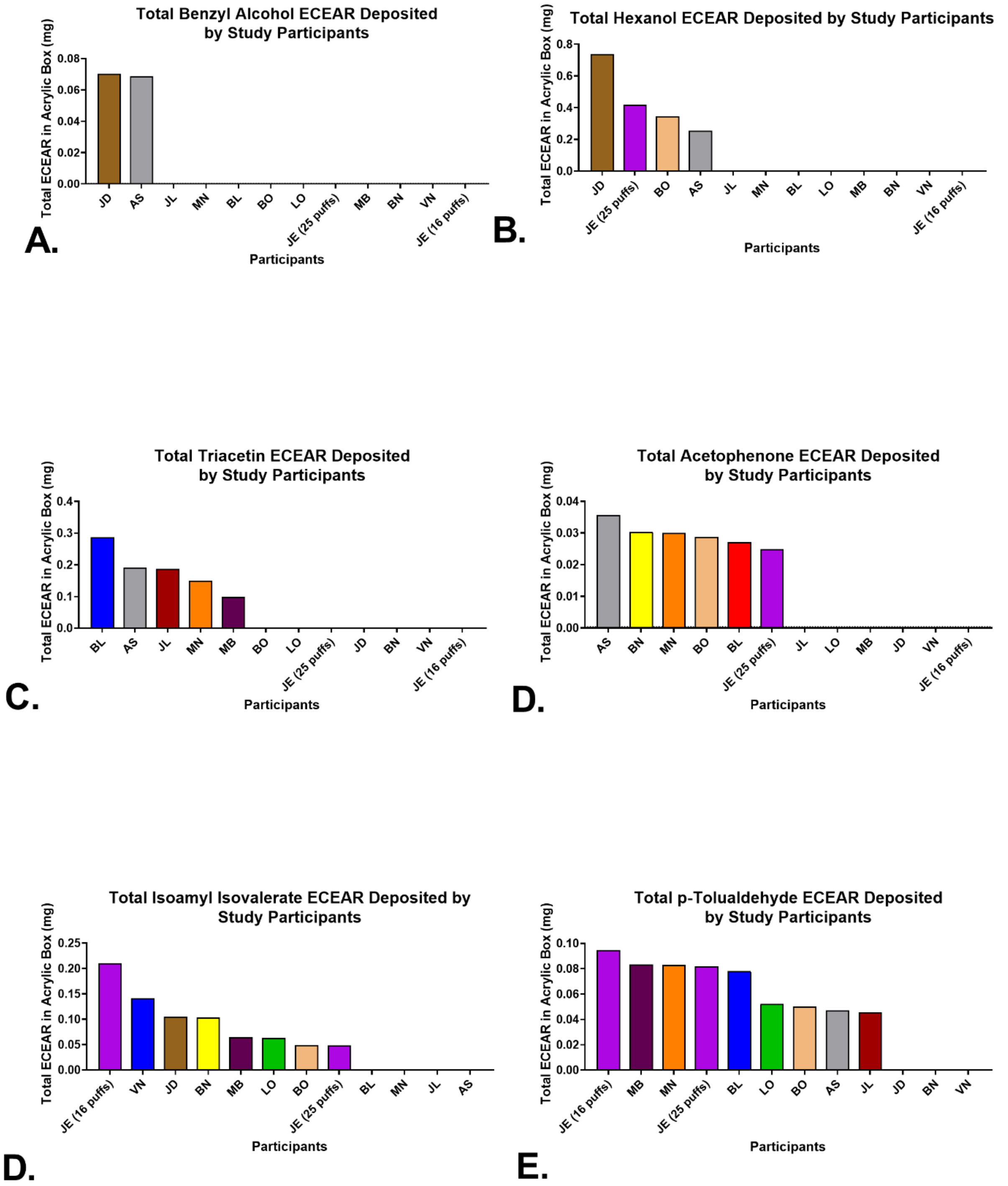

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## Supplementary Table 1. Abbreviations and Terms

### Abbreviations

ANOVA = analysis of variance

EC = electronic cigarette

ECEAR = electronic cigarette exhaled residue

FDA = Food and Drug Administration

GC/MS = gas chromatography/mass spectrometry

G = glycerol

IPA = isopropyl alcohol

LOQ = limit of quantification

PG = propylene glycol

THS = thirdhand smoke

TSNAs = tobacco specific nitrosamines

TPS = total puffs seconds

WS-23 = 2-Isopropyl-N,2,3-trimethylbutyramide (a synthetic coolant found in some e-liquids)

### Terms

Actual Exposure: the dose users receive that takes into account the transfer efficiency (%) for each chemical.

Dominant Chemicals: chemicals that were quantified in JUUL™ Menthol pod fluid and had concentrations > 1 mg/mL (nicotine, menthol, PG, G).

Mass transfer: the mass of a chemical transferred during aerosolization.

Mass Retained: the mass of a chemical retained by e-cigarette users after exhaling.

Maximal Exposure: the dose calculated from the total pod liquid (mL) consumed by users and multiplied by the concentration (µg/mL) of the chemical. It does not take into account the transfer efficiency (%) for each chemical.

Non-Dominant Chemicals: chemicals that were quantified in JUUL™ Menthol pod fluid and had concentrations < 1 mg/mL.

Percent Retention: the percentage of a chemical retained after vaping.

Transfer efficiency: the percent of chemical transferred from refill fluid to aerosol during vaping.

## Supplementary Table 2. Flavor Chemicals Detected (≤1 mg/mL) in Unvaped and Vaped JUUL™ "Menthol" Pods[1]

| | WS-23 | Benzyl Alcohol | Hydroxyacetone | β-Demascone | p-Menthone | Neomenthol | Caffeine | Isopulegol |
|---|---|---|---|---|---|---|---|---|
| Unvaped Pods | 106.55 | 75.5 | 22.6 | 19.7 | 17.9 | 18.7 | 11.6 | 22.65 |
| JD | 101.85 | 65.7 | 30.45 | 3.9 | 3.25 | 2.1 | 11.45 | |
| BN | 100.3 | 63.8 | 29.85 | 3.55 | 3.3 | 1.8 | 11 | |
| VN | 97.25 | 62.25 | 29 | 4.55 | 4.3 | 2.45 | 11.1 | |
| JE | 104.1 | 66.85 | 30.05 | 4.65 | 4.35 | 2.45 | 12.05 | |
| BO | 95.25 | 60.3 | 30.4 | 3.85 | 2.85 | 1.65 | 11.05 | |
| LO | 105 | 66.75 | 30.3 | 4 | 3.2 | 2.1 | 12.7 | |
| AS | 111.85 | 67.85 | 36.05 | 6 | 4.85 | 2.6 | 11.3 | |
| MB | 100.75 | 64.90 | 33.15 | 4.5 | 3.4 | 2.2 | 11.7 | |
| JL | 108.35 | 67.35 | 32.75 | 4.9 | 5.1 | 2.8 | 11.65 | |
| MN | 118.05 | 70.15 | 36.2 | 6.25 | 5.3 | 2.6 | 11.25 | |
| BL | 113.05 | 67 | 31.9 | 7.05 | 6.95 | 3.05 | 11.1 | |
| JE | 102.1 | 65.10 | 33.05 | 3.45 | 3.3 | 2.2 | 11.95 | |

[1] Only chemicals greater than the limit of quantification (>10 μg/mL) are shown.

## Supplementary Table 3. Total Mass and Percent Retained for Nicotine, Menthol, Propylene Glycol, and Glycerol Calculated at Various Flow Rates (9, 11, 13, 41, and 47 mL/sec)

| Mass or Percent Retained (Flow Rate)[1] | AS | MN | JL | JD | JE | MB | BO | BN | LO | BL | VN | Average ± SD |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| N-MR (mg) (9) | 6.4 | 5.1 | 3.2 | 2.9 | 2.9 | 2.1 | 1.4 | 1.4 | 1.1 | 0 | 0.3 | 2.4 ± 2.0 |
| N-PR (%) (9) | 100 | 100 | 100 | 100 | 99.8 | 100 | 100 | 99.9 | 99.8 | 0 | 86.8 | 87.1 ± 38.5 |
| N-MR (mg) (11)[2] | 7.7 | 6.2 | 3.8 | 3.5 | 3.4 | 2.5 | 1.7 | 1.6 | 1.3 | 0 | 0.4 | 3.0 ± 2.4 |
| N-PR (%) (11) | 100 | 100 | 100 | 100 | 99.9 | 100 | 100 | 99.9 | 99.8 | 0 | 89.1 | 89.3 ± 32 |
| N-MR (mg) (13) | 9.4 | 7.5 | 4.6 | 4.2 | 4.2 | 3.1 | 2.0 | 2.0 | 1.5 | 0.1 | 0.5 | 3.6 ± 2.9 |
| N-PR (%) (13) | 100 | 100 | 100 | 100 | 99.9 | 100 | 100 | 100 | 100 | 12 | 91 | 91.1 ± 26.4 |
| N-MR (mg) (41) | 7.5 | 6.0 | 3.7 | 3.4 | 3.3 | 2.5 | 1.6 | 1.6 | 1.2 | 0 | 0.4 | 2.8 ± 2.3 |
| N-PR (%) (41) | 100 | 100 | 100 | 100 | 99.9 | 100 | 100 | 99.9 | 99.8 | 0 | 88.7 | 89 ± 32.9 |
| N-MR (mg) (47) | 7.9 | 6.4 | 3.9 | 3.6 | 3.5 | 2.6 | 1.7 | 1.7 | 1.3 | 0 | 0.4 | 3.0 ± 2.4 |
| N-PR (%) (47) | 100 | 100 | 100 | 100 | 99.9 | 100 | 100 | 99.9 | 99.8 | 0 | 89.4 | 89.6 ± 31.1 |
| M-MR (mg) (9) | 0.9 | 0.8 | 0.5 | 0.4 | 0.4 | 0.3 | 0.2 | 0.2 | 0.2 | 0 | 0.04 | 0.4 ± 0.3 |
| M-PR (%) (9) | 93.7 | 99.1 | 99.2 | 91.8 | 93.9 | 98 | 100 | 98.6 | 95 | 0 | 80.2 | 86.3 ± 29.4 |
| M-MR (mg) (11) | 1.1 | 0.9 | 0.6 | 0.5 | 0.5 | 0.4 | 0.2 | 0.2 | 0.2 | 0.02 | 0.05 | 0.4 ± 0.3 |
| M-PR (%) (11) | 94.5 | 99.2 | 99.3 | 92.9 | 94.7 | 98.3 | 100 | 98.8 | 95.6 | 12.2 | 82.8 | 88 ± 25.6 |
| M-MR (mg) (13) | 1.3 | 1.1 | 0.7 | 0.6 | 0.6 | 0.5 | 0.3 | 0.3 | 0.2 | 0.04 | 0.07 | 0.5 ± 0.4 |
| M-PR (%) (13) | 95.5 | 99.4 | 99.4 | 94.1 | 95.6 | 98.6 | 100 | 99 | 96.4 | 27.6 | 85.8 | 90.1 ± 21.1 |
| M-MR (mg) (41) | 1.2 | 1.0 | 0.6 | 0.5 | 0.5 | 0.4 | 0.3 | 0.3 | 0.2 | 0.03 | 0.06 | 0.5 ± 0.4 |
| M-PR (%) (41) | 95 | 99.3 | 99.3 | 93.6 | 95.3 | 98.5 | 100 | 98.9 | 96.1 | 20.7 | 84.4 | 89.2 ± 23.1 |
| M-MR (mg) (47) | 1.4 | 1.1 | 0.7 | 0.6 | 0.6 | 0.5 | 0.3 | 0.3 | 0.2 | 0.04 | 0.07 | 0.5 ± 0.4 |
| M-PR (%) (47) | 95.5 | 99.4 | 99.4 | 94.2 | 95.7 | 98.6 | 100 | 99 | 96.5 | 28.9 | 86 | 90.3 ± 20.8 |
| PG-MR (mg) (9) | 29.2 | 23.4 | 14.4 | 13.2 | 13 | 9.6 | 6.3 | 6.2 | 4.8 | 3.2 | 1.6 | 11.3 ± 8.6 |
| PG-PR (%) (9) | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 99.9 | 100 | 100 ± 0.03 |
| PG-MR (mg) (11) | 38.9 | 31.2 | 19.3 | 17.6 | 17.3 | 12.7 | 8.4 | 8.2 | 6.4 | 4.2 | 2.2 | 15.1 ± 11.4 |
| PG-PR (%) (11) | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 99.9 | 100 | 100 ± 0.03 |
| PG-MR (mg) (13) | 38.9 | 31.2 | 19.3 | 17.6 | 17.3 | 12.7 | 8.4 | 8.2 | 6.4 | 4.2 | 2.2 | 15.1 ± 11.4 |
| PG-PR (%) (13) | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 99.9 | 100 | 100 ± 0.03 |
| PG-MR (mg) (41) | 48.6 | 39 | 24.1 | 22 | 21.6 | 15.9 | 10.5 | 10.3 | 8 | 5.3 | 2.7 | 18.9 ± 14.3 |
| PG-PR (%) (41) | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 99.9 | 100 | 100 ± 0.02 |
| PG-MR (mg) (47) | 48.6 | 39 | 24.1 | 22 | 21.6 | 15.9 | 10.5 | 10.3 | 8 | 5.3 | 2.7 | 18.9 ± 14.3 |
| PG-PR (%) (47) | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 99.9 | 100 | 100 ± 0.02 |
| G-MR (mg) (9) | 52.4 | 42 | 26 | 23.7 | 23.3 | 17.2 | 11.4 | 11.1 | 8.6 | 5.7 | 2.9 | 20.4 ± 15.4 |
| G-PR (%) (9) | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 99.8 | 99.9 | 100 ± 0.06 |
| G-MR (mg) (11) | 69.5 | 55.8 | 34.5 | 31.5 | 30.9 | 22.8 | 15.1 | 14.7 | 11.4 | 7.6 | 3.9 | 27.1 ± 20.5 |
| G-PR (%) (11) | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 99.8 | 100 | 100 ± 0.05 |
| G-MR (mg) (13) | 80.9 | 64.9 | 40.1 | 36.7 | 36 | 26.5 | 17.6 | 17.1 | 13.3 | 8.8 | 4.5 | 31.5 ± 23.8 |
| G-PR (%) (13) | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 99.9 | 100 | 100 ± 0.04 |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| G-MR (mg) (41) | 112.8 | 90.5 | 55.9 | 51.1 | 50.2 | 37 | 24.5 | 23.9 | 18.5 | 12.3 | 6.3 | 43.9 ± 33.2 |
| G-PR (%) (41) | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 ± 0.03 |
| G-MR (mg) (47) | 118.5 | 95.1 | 58.7 | 53.7 | 52.7 | 38.8 | 25.7 | 25.1 | 19.5 | 13 | 6.6 | 46.1 ± 34.9 |
| G-PR (%) (47) | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 99.9 | 100 | 100 ± 0.03 |

[1] N = nicotine; M = menthol; PG = propylene glycol; G = glycerol; MR = mass retained; PR = percent retained.

[2] Numbers in parentheses indicate the flow rate for the calculation.

## Supplementary Table 4. Dominant Chemical Exposures for JUUL™ Users and Estimated Cigarette Equivalency Based on Nicotine[1]

| User | Number of Puffs Taken Per Session | Nicotine Retained (mg) in Single Puff (sp)[2], Single Session (ss)[3], and Whole Day (wd)[4] | Menthol Retained (mg) in Single Puff (sp), Single Session, and Whole Day (wd) | PG Retained (mg) in Single Puff (sp), Single Session (ss), and Whole Day (wd) | G Retained (mg) in Single Puff (sp), Single Session (ss), and Whole Day (wd) | Total Solvent Retained (mg) in Single Puff (sp), Single Session (ss), and Whole Day (wd) | Nicotine Retained Comparison to Smokers (1.1 mg/Cigarette)[5,6] in a Single Session and Whole Day (22 mg for ppd)[7] |
|---|---|---|---|---|---|---|---|
| AS | 26 | 0.3 (sp); 8.7 (ss); 46.2 (wd) | 0.05 (sp); 1.4 (ss); 7.5 (wd) | 1.9 (sp); 48.6 (ss); 261.6 (wd) | 5.3 (sp); 136.7 (ss); 736.4 (wd) | 7.1 (sp); 185.3 (ss); 997.9 (wd) | ~8 cigarettes (ss);<br>~2.1 ppd (wd) |
| JD | 16 | 0.3 (sp); 3.9 (ss); 34.5 (wd) | 0.04 (sp); 0.6 (ss); 5.5 (wd) | 1.4 (sp); 22 (ss); 192.6 (wd) | 3.9 (sp); 62 (ss); 542.1 (wd) | 5.2 (sp); 84 (ss); 734.7 (wd) | ~3.6 cigarettes;<br>~1.6 ppd (wd) |
| MN | 25 | 0.3 (sp); 7.0 (ss); 39.1 (wd) | 0.05 (sp); 1.2 (ss); 6.5 (wd) | 1.6 (sp); 39 (ss); 218.2 (wd) | 4.4 (sp); 109.7 (ss); 614.2 (wd) | 5.9 (sp); 148.6 (ss); 832.4 (wd) | ~6.4 cigarettes;<br>~1.8 ppd (wd) |
| JL | 21 | 0.2 (sp); 4.3 (ss); 28.8 (wd) | 0.03 (sp); 0.7 (ss); 4.8 (wd) | 1.1 (sp); 24.1 (ss); 160.5 (wd) | 3.2 (sp); 67.8 (ss); 451.9 (wd) | 4.4 (sp); 91.9 (ss); 612.4 (wd) | ~4 cigarettes (ss);<br>~1.3 ppd (wd) |
| JE | 20 | 0.2 (sp); 3.9 (ss); 27.1 (wd) | 0.03 (sp); 0.6 (ss); 4.4 (wd) | 1.1 (sp); 21.6 (sp); 151.2 (wd) | 3 (sp); 60.8 (ss); 425.9 (wd) | 4.1 (sp); 82.5 (ss); 577.2 (wd) | ~3.5 cigarettes;<br>~1.2 ppd (wd) |
| MB | 26 | 0.1 (sp); 2.9 (ss); 15.4 (wd) | 0.02 (sp); 0.5 (ss); 2.5 (wd) | 0.6 (sp); 15.9 (ss); 85.7 (wd) | 1.7 (sp); 44.80 (sp); 241.2 (wd) | 2.3 (sp); 60.7 (ss); 327 (wd) | ~2.6 cigarettes (ss);<br>~0.7 ppd (wd) |
| BO | 22 | 0.09 (sp); 1.9 (ss); 12.0 (wd) | 0.01 (sp); 0.3 (ss); 2.0 (wd) | 0.5 (sp); 10.5 (ss); 67.1 (wd) | 1.3 (sp); 29.7 (ss); 188.8 (wd) | 1.8 (sp); 40.2 (ss); 255.8 (wd) | ~1.7 cigarettes;<br>~0.5 ppd (wd) |
| BN | 21 | 0.09 (sp); 1.8 (ss); 12.3 (wd) | 0.01 (sp); 0.3 (ss); 2.0 (wd) | 0.5 (sp); 10.3 (sp); 68.5 (wd) | 1.4 (sp); 28.94 (ss); 192.9 (wd) | 1.9 (sp); 39.2 (ss); 261.5 (wd) | ~1.7 cigarettes;<br>~0.6 ppd (wd) |
| LO | 36 | 0.04 (sp); 1.4 (ss); 5.5 (wd) | 0.006 (sp); 0.2 (ss); 0.9 (wd) | 0.2 (sp); 8.0 (ss); 31 (wd) | 0.6 (sp); 22.5 (ss); 87.3 (wd) | 0.8 (sp); 30.4 (ss); 118.4 (wd) | ~1.3 cigarettes;<br>~0.3 ppd (wd) |
| VN | 11 | 0.04 (sp); 0.4 (ss); 5.6 (wd) | 0.006 (sp); 0.07 (ss); 0.9 (wd) | 0.2 (sp); 2.7 (ss); 34.3 (wd) | 0.7 (sp); 7.6 (ss); 96.8 (wd) | 0.9 (sp); 10.3 (ss); 131.2 (wd) | <1 cigarette;<br>~0.3 ppd (wd) |
| BL | 23 | 0.002 (sp); 0.05 (ss); 0.3 (wd) | 0.002 (sp); 0.05 (ss); 0.3 (wd) | 0.2 (sp); 5.3 (ss); 32.3 (wd) | 0.6 (sp); 14.9 (ss); 90.9 (wd) | 0.9 (sp); 20.2 (ss); 123.2 (wd) | <1 cigarette;<br>~0.01 ppd (wd) |

[1] = JUUL™ calculations were based on 21 mL/sec flow rate

[2] = Single puffs (sp) data were calculated from total actual exposure data divided by the total number of puffs users took in a session.

[3] = Single session (ss) data were estimated by multiplying the total number of puffs recorded for each user during a session by the single puff data.

[4] = Whole day (wd) exposures were calculated by multiplying the single puff data by 140 puffs/day. 140 puffs/day was chosen based on previously reported data[1].

[5] = To obtain the estimated cigarette equivalency, the amount of nicotine delivered by a JUUL™ "Menthol" EC in a 20-minute session was divided by the amount delivered by one Marlboro Red filtered cigarette (1.1 mg/cigarette)[2].

[6] = The estimated cigarette equivalency for a single session was calculated by dividing the users' individual single session nicotine data by 1.1 mg.

[7] = The estimated cigarette equivalency for a whole day was calculated by dividing the whole day exposure data by nicotine delivered in a single cigarette (1.1 mg) or pack of cigarettes (22 mg). If the users' whole day nicotine exposure was ≥ 22mg it was divided by 22 mg.

## References

1. Dautzenberg B, Bricard D. Real-time characterization of e-cigarettes use: the 1 million puffs study. J. Addict. Res. Ther. 2015;6(229.10):4172.
2. Federal Trade Commission. “Tar,” nicotine, and carbon monoxide of the smoke of 1294 varieties of domestic cigarettes for the year 1998, 2000.