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Original Investigation
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Original Investigation

Changes in Biomarkers of Cigarette Smoke Exposure After 6 Days of Switching Exclusively or Partially to Use of the JUUL System with Two Nicotine Concentrations: A Randomized Controlled Confinement Study in Adult Smokers

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Abstract

Introduction: Evidence suggests that cigarette smokers who switch to electronic nicotine delivery systems (ENDS) reduce their exposure to harmful toxicants and carcinogens. It is unclear if dual-use is associated with decreases in exposure to toxicants.

Methods: This parallel-group confinement study assessed changes in biomarkers of exposure (BOEs) over six days among healthy adult smokers who were randomized into 1 of 11 study groups: eight JUUL-brand System (JUUL) groups (4 JUUL flavors [Virginia Tobacco, Menthol, Mint, Mango] × 2 nicotine concentrations [5.0% or 3.0% by weight]); Dual-Use group used preferred JUUL flavor (5.0% nicotine) and ≤50% usual brand (UB) cigarettes/day; UB Cigarette group and one group abstained from all tobacco/nicotine product use (Abstinence group). Urine and blood analysis assessed changes in primary BOE endpoints (NNAL, 3-HPMA, MHBMA, S-PMA COHb) and secondary BOE endpoints (NNN, HMPMA, CEMA, 1-OHP, O-toluidine, 2-NA, 4-ABP) among 279 adult smokers.

Results: In JUUL groups, median percent reductions in primary BOEs (Day 6–Baseline) were 90%–≥100% of Abstinence; there were no significant differences between JUUL groups and Abstinence. All reductions in JUUL groups were substantially and statistically significantly greater than reductions in the UB Cigarette group (*p*s < 0.025). Median reductions in primary BOEs in the Dual-Use group were 43%–55% of Abstinence. Similar results were observed for secondary BOEs.

Conclusion: This study suggests that the use of JUUL as a complete or partial substitute (i.e., dual-use with ≥50% reduction in cigarette consumption) for combustible cigarettes can substantially reduce exposure to multiple toxins associated with cigarette smoking.

Implications: This study adds to the growing body of evidence supporting the utility of ENDS products as potentially reduced-harm alternatives to cigarettes for adult smokers. Adult smokers who switched completely from cigarette smoking to use of the JUUL System ("JUUL") in two nicotine concentrations (5.0% and 3.0%) and four flavors significantly reduced their exposure to multiple classes of cigarette-related toxicants. Additionally, smokers who used JUUL and continued smoking but reduced their daily cigarette consumption by ≥50% (dual users) also significantly reduced their toxicant exposure compared to cigarette smoking.

Combustible cigarettes expose smokers to harmful and potentially harmful constituents (HPHCs)[1] known to cause disease.[2] The analogs, degradants, and metabolites of these toxicants (known as biomarkers of exposure [BOEs]) are detectable in the urine and bloodstream of smokers and can serve as intermediate endpoints of disease risk.[3,4] In contrast to combustible cigarettes, electronic nicotine delivery systems (ENDS) deliver nicotine without combusting tobacco, and evidence suggests that completely substituting ENDS for combustible cigarettes reduces smokers' exposure to toxicants and carcinogens.[5,6]

Data from controlled confinement studies in which smokers were randomized to exclusive use of ENDS (vs. continued smoking) demonstrate reduced exposure to toxicants (i.e., BOEs).[7–11] Consistent with these findings, a recent 5-day clinical study[12] and a 6-week randomized trial[13] found that smokers who completely switched from smoking to use of the JUUL System ("JUUL"), a closed-system nicotine-salt-based ENDS, experienced significant reductions in BOEs. However, all JUUL products evaluated in both studies contained 5.0% nicotine by weight (59 mg/mL), and they did not assess exposure implications of using JUUL with lower nicotine concentrations (i.e., 3.0% by weight). Additionally, existing confinement studies did not evaluate Menthol-flavored JUUL e-liquids,[12] which is particularly important because: (1) menthol is one of only two flavors currently permitted in cartridge-based ENDS, such as JUUL, in the US; and (2) recent data suggests menthol-flavored ENDS are widely-used among smokers of mentholated cigarettes.[14]

Concurrent use of ENDS and combustible cigarettes (dual-use) is common among ENDS users.[15,16] Dual use (vs. exclusive smoking), if accompanied by a reduction in cigarettes smoked, may reduce smokers' exposure to tobacco smoke constituents. However, there is also concern that dual use of ENDS and cigarettes may expose smokers to greater toxicant levels and disease risk than cigarette smoking alone.[17–19] Between-subjects observational studies have found that dual use (vs. exclusive cigarette smoking) did not reduce toxicant exposure,[20] and that dual users are exposed to higher levels of certain toxicants and carcinogens (e.g., NNAL, 3-HPMA, MHBMA) than exclusive cigarette smokers.[21] In contrast, results from within-subject and experimental studies suggest that dual users who reduce their cigarette consumption experience significant reductions in levels of BOEs compared to exclusive smokers.[9,22–26] Hence, it is unclear if dual-use is associated with decreases in exposure to toxicants and BOEs.

The primary aim of this six-day residential laboratory study was to assess changes in toxicant exposure among smokers following: (1) complete switching from combustible cigarettes to use of JUUL; and (2) partial switching to use of JUUL (dual-use). Comparisons were made to smokers who: (1) continued to smoke their usual brand (UB) combustible cigarettes; and (2) abstained entirely from all tobacco or nicotine product use. Secondary aims were to evaluate differences in BOEs by JUUL nicotine concentration (5.0% vs. 3.0%) and flavor (Virginia Tobacco vs. Menthol vs. Mint vs. Mango) as well as differences in nicotine exposure between study groups.

Methods

Participants

The sample was composed of healthy adult smokers (BMI: 18–40 kg/m^2) aged 21–65 years who smoked ≥10 manufactured (king-size or 100s) combustible cigarettes per day for at least 12 months (urine cotinine ≥200 ng/mL and exhaled carbon monoxide >10 ppm to confirm current smoking) and were willing to use JUUL and be confined to a clinical research setting for the study duration. Exclusion criteria included any existing clinically significant health issues or illnesses (e.g., diabetes, asthma, COPD, cancer), use of non-cigarette nicotine products (e.g., ENDS [including JUUL], cigars, chewing tobacco, hookah) in the past 30 days, use of prescription smoking cessation treatments (e.g., Varenicline, Bupropion) within three months, history of substance abuse in past 24 months, positive urine/breath test for drugs or alcohol at screening or pregnancy.

Participants were recruited to five sites across the US. The study was performed in accordance with the Declaration of Helsinki, and consistent with ICH, Good Clinical Practice (GCP), and applicable regulatory requirements. The study protocol was approved by Advarra Institutional Review Board, all participants provided informed consent and were compensated for their participation. The study was registered at https://clinicaltrials.gov (Identifier: NCT 04107779).

Design

Eligible participants were confined in-clinic for eight days, during which they completed a randomized, open-label, parallel-group study (Supplementary Figure S1). Eligible participants were randomized into 1 of 11 study groups: eight groups exclusively used JUUL (4 flavors [Virginia Tobacco, Menthol, Mint, Mango] × 2 nicotine concentrations [5.0% or 3.0%]); one Dual-Use group used JUUL (preferred JUUL flavor [5.0% nicotine]) concurrently with up to half of their usual brand (UB) cigarettes smoked per day at baseline (as reductions of this magnitude have been considered substantial in previous research[27]); one UB Cigarette group continued exclusively smoking their UB cigarettes, and one group abstained from all tobacco and nicotine product use (Abstinence group). Twice as many participants were randomized to the Dual-Use group as to the other study groups, as it was expected to have greater variance in BOE outcomes due to variability in cigarette consumption. Randomization to study groups was stratified by gender and menthol cigarette preference such that the proportion of males and menthol smokers did not exceed 60%.

Procedure

Screening occurred within 28 days prior to check-in and included a full physical examination (including electrocardiogram and spirometry), urine and breath alcohol and drug screening, and serum pregnancy tests. At screening, participants also completed a 30-minute product trial with JUUL in all four flavors (5.0% nicotine). Participants randomized to the Dual-Use group selected a single JUUL flavor (Virginia Tobacco, Menthol, Mint, or Mango) in 5.0% nicotine to use for six days study (in addition to smoking UB cigarettes). Assignment to the group was not disclosed to participants until Day 1, so as not to influence the number of combustible cigarettes smoked on Day –1. On study Days –2 and –1 participants smoked their UB cigarettes from 07:30 until 23:00.

After randomization, participants used their assigned study product(s) *ad libitum* for six consecutive days (from 07:30 until 23:00 on Days 1–6); study products were provided to participants on each study day as requested. Participants in different groups were restricted to separate areas of the clinics to avoid potential second-hand exposure. Daily JUUL use (pod weight before and after use) and cigarette consumption were assessed.

Blood and 24-hour urine samples were collected on Day –1 and Day 6. Urine was collected from 07:30 on Day –1 until 07:30 on

Day 1 and from 07:30 on Day 6 until 07:30 on Day 7; all urine collected during each 24-hour interval was pooled. Blood samples were collected via direct venipuncture on Days –1 and 6 at approximately 19:00, preceded by a minimum 15-minute abstention from study product use.

Materials

JUUL products (Juul Labs, Inc.) included both the rechargeable device (battery) and disposable pods pre-filled with 0.7 mL of e-liquid in four flavors: Virginia Tobacco, Menthol, Mint, and Mango; each in 5.0% (59 mg/mL) and 3.0% (35 mg/mL) nicotine concentrations by weight, respectively. Participants in the UB Cigarette and Dual-Use groups provided their UB cigarettes in unopened packs at the study start and were reimbursed for the cost.

Measures

At check-in, participants reported demographic and cigarette smoking characteristics and completed the 6-item Fagerström Test of Cigarette Dependence (FTCD, range: 0–10).[28,29]

BOE Evaluation and Quantification

The non-nicotine BOEs assessed (total NNAL, 3-HPMA, MHBMA, S-PMA, COHb, NNN, HMPMA, CEMA, 1-OHP, O-Tol, 2-NA, 4-ABP) represent categories of toxicants commonly found in cigarette smoke which correspond to chemicals that have been classified as HPHCs relevant to major smoking-related diseases by FDA[1] (Supplementary Table S1) and have been reported in previous studies.[7,30–33] Five BOEs were designated as primary endpoints (total NNAL, 3-HPMA, MHBMA, S-PMA, and COHb) because they spanned cigarette smoke-related toxicant chemicals associated with a range of disease classes, and included chemicals found in the gas and particulate phases of smoke. Total nicotine equivalents (TNE) were calculated as the molar sum of nicotine and five metabolites (nicotine-glucuronide, cotinine and its glucuronide, trans-3'-hydroxycotinine, and its glucuronide) excreted in urine over 24 hours.

BOE assays used validated methods[34] based on FDA's Guidance to Industry for Bioanalytical Method Validation (2001), Good Laboratory Practices (per 21 CFR Part 58), and EMEA Guideline on Bioanalytical method validation (EMEA/CHMP/EWP/192217/2009 Rev. 1 Corr.2).[12]

Measured urine biomarker concentrations were converted to total mass excreted per 24 hours by multiplying the concentration by the total urine volume of the interval. All values reported as below the limit of quantitation (BLQ) were set to $1/\sqrt{2}$ times the limit of quantitation (LOQ) for summarization and analysis. Absolute and percent changes from baseline (Day 6–Day -1) were derived. Non-creatinine-adjusted BOE values are presented throughout.[35]

Safety Endpoints

Safety endpoints included incidence and intensity (or severity in MedDRA classifications) of adverse events (AEs). Study-emergent AEs (SEAEs), defined as an AE which occurred post-randomization, were summarized. Frequencies of SEAEs were summarized by severity and relationship to study product.

Data Analysis

This study was prospectively powered to detect a significant decrease in BOEs between smokers who switched from UB combustible cigarettes to JUUL (JUUL groups) and those who continued to smoke (UB Cigarette group). Power calculations based on a previous study using a similar design[12] determined that 21 participants per group were required to achieve 80% power for the 40 primary statistical comparisons (5 primary BOEs×4 JUUL flavors×2 JUUL nicotine concentrations). Statistical comparisons of secondary BOE endpoints and among other study groups were not powered, and are therefore considered exploratory.

Descriptive analyses assessed median percent changes (Day 6–Baseline) in primary and secondary BOE endpoints in each of the 11 study groups, as the data were not normally distributed. Median percent changes in non-nicotine BOEs in the eight JUUL groups were also aggregated (i.e., combined to create one JUUL group). Median percent changes in BOEs in the JUUL, Dual-Use, and UB Cigarette groups were compared to reductions in the Abstinence group to determine reductions in BOEs relative to those observed in the Abstinence group (e.g., JUUL/Abstinence).

Primary analyses utilized analysis of covariance (ANCOVA) models with a mean absolute change in BOE (Day 6–baseline) as the dependent variable and study group as the independent variable; gender, preference for mentholated (vs. nonmentholated) cigarettes, and a statistical interaction term for study group × menthol preference were included as covariates. Post-hoc pairwise comparisons among the eight JUUL groups were descriptive and no statistical tests were conducted.

All 40 primary comparisons were one-tailed; the Holm-Bonferroni method was used to control family-wise Type 1 error rate at 2.5% (alpha = 0.025/40; adjusted *P*-values reported for primary endpoints). Secondary comparisons were two-tailed with Type 1 error rate of 5.0% with no adjustment for multiplicity. All statistical analyses were performed using SAS version 9.4 (Cary, NC) or R version 2.15.0.

Results

Participant Accrual and Sample Characteristics

Out of 686 participants screened, 65.3% (N = 448) enrolled, 300 were randomized (67.0% of enrolled) and 279 (93.0% of randomized) provided data required to assess changes in BOEs at Day 6 (Supplementary Figure S2). Between 22–27 participants were randomized to each JUUL group; 24, 29 and 48 participants were randomized to the UB Cigarette, Abstinence, and Dual-Use groups, respectively.

In the analytic sample (N = 279), the mean age was 40.2 (SD = 11.0) years, 48.0% were female, 55.2% identified as Caucasian, 43.4% identified as Black or African American. Slightly over half (52.0%) of the sample smoked mentholated cigarettes and, on average, participants smoked 18.0 cigarettes per day (SD = 5.6), smoked for 21.5 years (SD = 12.2), and reported moderate levels of nicotine dependence on the FTCD (Mean[SD] = 5.47[1.77]). Sample characteristics by study group are displayed in Supplementary Table S2. In the Dual-Use group, 60.0% of participants (N = 27) used Mango, 17.8% (N = 8) used Mint, 15.6% (N = 7) used Virginia Tobacco and 6.7% (N = 3) used Menthol JUULpods.

Baseline Cigarette Smoking and Test Product Consumption

On Day -1, when all participants smoked UB cigarettes, the analytic sample (N = 279) averaged 13.5 cigarettes/day (SD = 4.0); there was

minimal variation among study groups (mean cigarettes/day, range: 12.5–13.8).

Mean JUUL consumption during the six-day period (measured in pod equivalents [PE]/day; 0.77g) ranged from ~0.62 ± 0.35 PE/day (Menthol 5.0%) to ~0.85 ± 0.40 PE/day (Virginia Tobacco 5.0%) in the four JUUL 5.0% groups and from ~0.77 ± 0.39 (Virginia Tobacco 3.0%) to ~1.03 ± 0.58 PE/day (Mango 3.0%) in the four 3.0% groups (Supplementary Table S3). JUUL consumption in the Dual-Use group was lower than in all eight individual JUUL groups (~0.39 ± 0.30 PE/day). The Dual-Use group smoked, on average, 5.9±2.2 cigarettes/day during the six-day period compared to 13.6 ± 4.2 cigarettes/day at baseline (mean reduction = 56.6%; participants smoked fewer than maximum allowable cigarettes on 33% of all study days). The UB Cigarette group smoked, on average, 11.0 ± 3.0 cigarettes/day during the 6-day period, compared to 13.3 ± 3.5 cigarettes at baseline.

Changes in Primary BOE Endpoints

Figure 1 displays changes in BOEs from baseline to Day 6 in the eight JUUL groups. BOE values were aggregated to examine the overall pattern in JUUL-only groups compared to the UB Cigarette, Abstinence, and Dual-Use groups. Figures 2 and 3 display changes in BOEs in the eight JUUL groups individually. Across all six days, 5.6% (N = 517) of individual biomarker values per participant were BLQ, 96.5% (N = 499) of which occurred on Day 6.

In the combined JUUL group, levels of primary BOE endpoints (total NNAL, 3-HPMA, MHBMA, S-PMA, COHb) substantially decreased across the six-day study period, with aggregate median percent reductions at Day 6 ranging from approximately 66%-95% of baseline values across biomarkers (Figure 1). In contrast, median changes from baseline in primary BOE endpoints among participants in the UB Cigarette group ranged from 17% increase to 6% reduction (Figure 1). Reductions in BOEs from baseline to Day 6 in the eight individual JUUL groups were significantly greater than reductions in the UB Cigarette group for all primary BOE endpoints (Ps < 0.001; Supplementary Table S4).

In the Abstinence group, median reductions ranged from 64% to 96% across the primary BOE endpoints with reductions in NNAL and COHb at the lower end of the range (Figure 1). Changes in primary BOE endpoints in the eight JUUL groups did not significantly differ from the Abstinence group (Ps > 0.05; Supplementary Table S4). Across all JUUL groups (5.0% and 3.0% inclusive), reductions in NNAL ranged from 61%-69% (corresponding to 95% to ≥100% of reductions in the Abstinence group), 3-HPMA 77%–86% (95% to ≥100% of reductions in the Abstinence group), MHBMA 86%–95% (91% to ≥100% of reductions in Abstinence group), S-PMA 93%–96% (97% to ≥100% of reductions in Abstinence group) and COHb 67%–74% (98% to ≥100% of reductions in Abstinence group; Figure 2 and Table 1). Changes among participants in the UB Cigarette group ranged from –18% to 8% of reductions observed in Abstinence group (Table 1).

In the Dual Use group, median reductions in primary BOEs were intermediate between the JUUL groups and UB Cigarette group, with reductions from baseline ranging from 31% to 44% (43%–53% of Abstinence group; Figure 1 and Table 1) across biomarkers. Reductions in all primary BOE endpoints in the Dual Use group were: (1) significantly greater than reductions in the UB Cigarette group (Ps ≤ 0.001 except NNAL [P = 0.04]); and (2) significantly less than reductions in the Abstinence group (Ps ≤ 0.001; Supplementary Table S4).

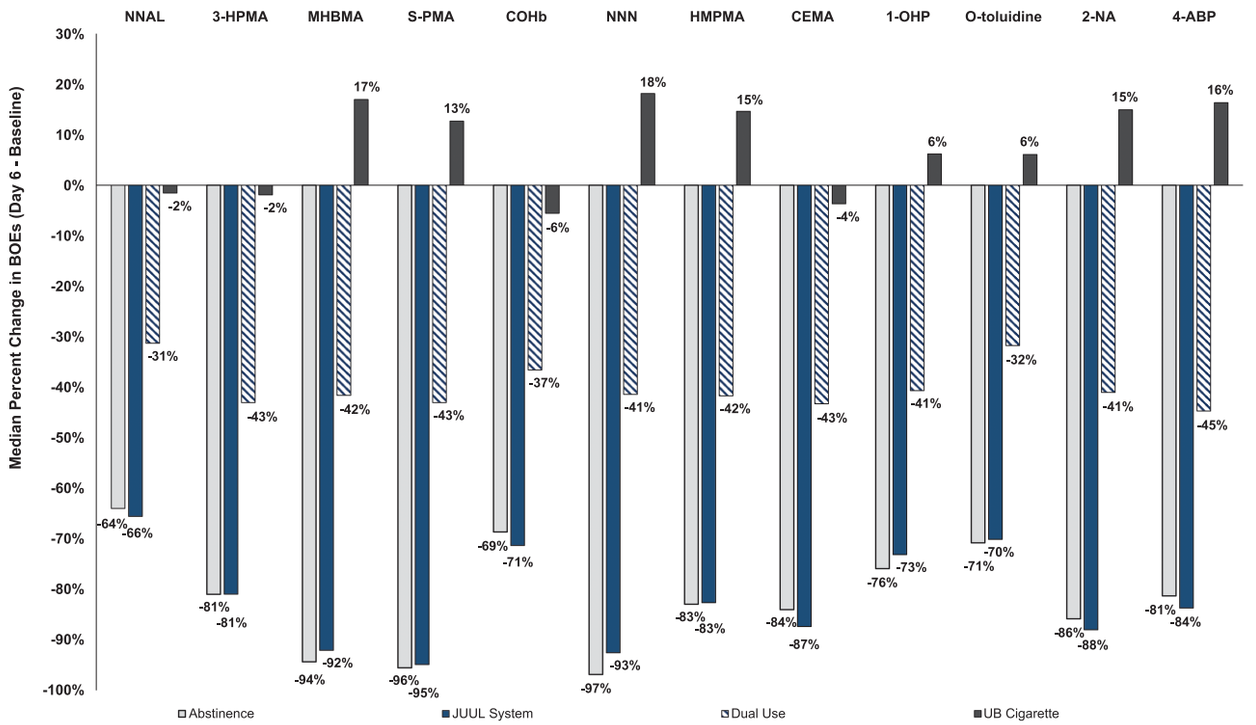

Figure 1. Median percent change in non-nicotine biomarkers of exposure (day 6 – baseline), aggregating across the eight JUUL system groups. JUUL System, N = 186–188; Abstinence, N = 22–23; Dual Use, N = 45; UB Cigarette, N = 23. Abbreviations: Nic. Equiv., nicotine equivalents.. JUUL System includes eight individual products (4 flavors [Virginia Tobacco, Menthol, Mint, Mango] each in 5.0% and 3.0% nicotine concentrations).

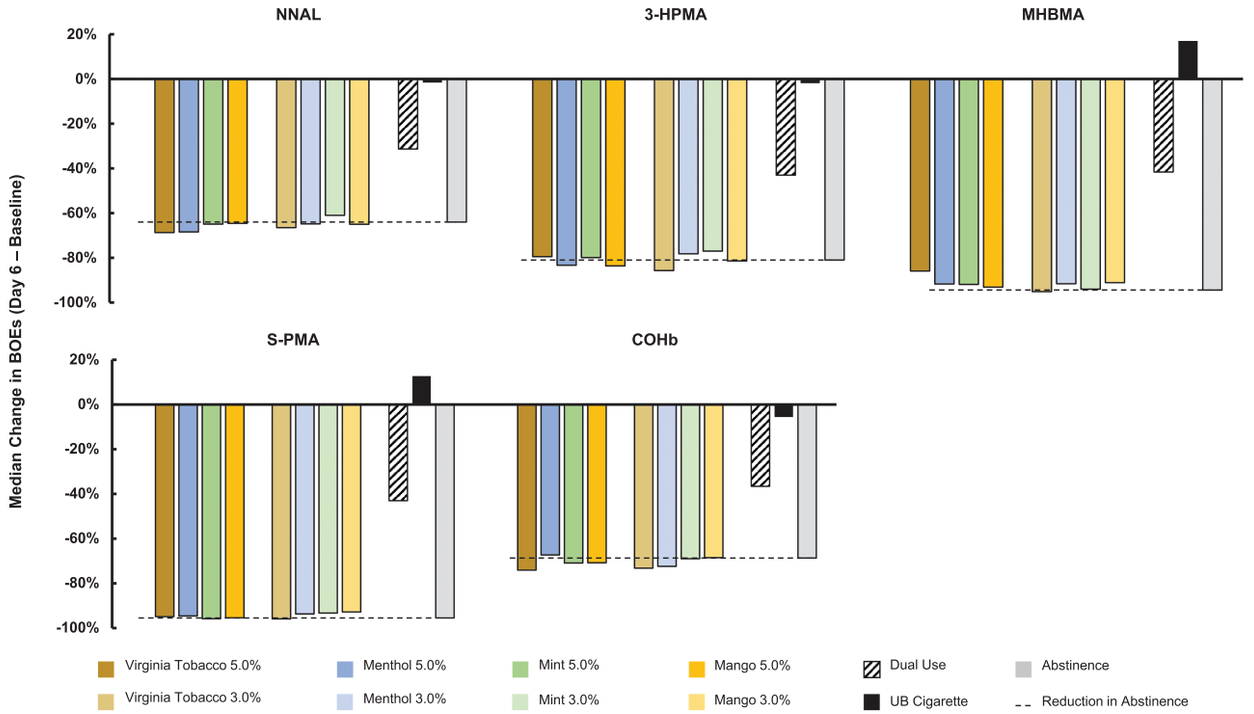

Figure 2. Median percent change in primary biomarkers of exposure endpoints after 6 days of product use. JUUL VT 5.0%, *N* = 23; JUUL VT 3.0%, *N* = 24; JUUL Mint 5.0%, *N* = 25; JUUL Mint 3.0%, *N* = 22; JUUL Menthol 5.0%, *N* = 22; JUUL Menthol 3.0%, *N* = 24; JUUL Mango 5.0%, *N* = 24; JUUL Mango 3.0%, *N* = 24; Dual Use, *N* = 48; UB Cigarette, *N* = 23; Abstinence, *N* = 23.

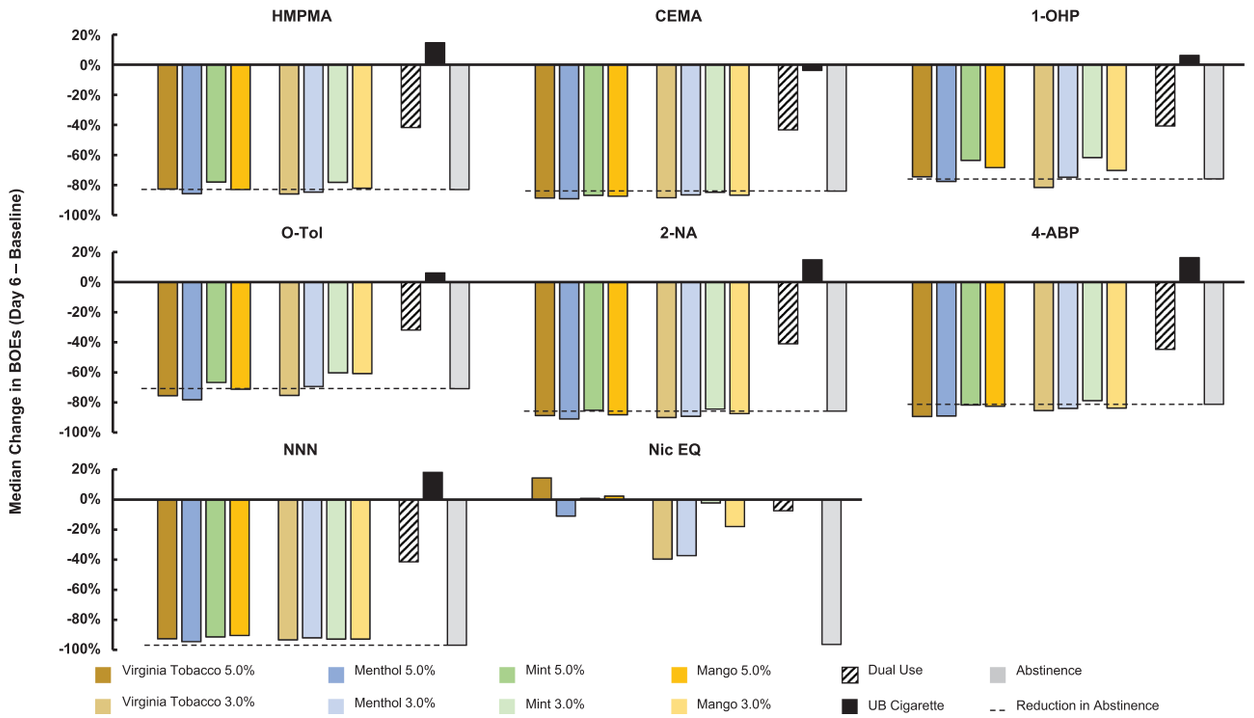

Figure 3. Median percent change in secondary biomarkers of exposure endpoints after 6 days of product use. JUUL VT 5.0%, *N* = 23; JUUL VT 3.0%, *N* = 24; JUUL Mint 5.0%, *N* = 25; JUUL Mint 3.0%, *N* = 22; JUUL Menthol 5.0%, *N* = 22; JUUL Menthol 3.0%, *N* = 24; JUUL Mango 5.0%, *N* = 24; JUUL Mango 3.0%, *N* = 24; Dual Use, *N* = 48; UB Cigarette, *N* = 23; Abstinence, *N* = 23.

Table 1. Reductions in Non-Nicotine BOE per Study Group, Relative to Reductions Observed in Abstinence Group

BOE	Nic. %	VT	Menthol	Mint	Mango	Dual Use	UB Cigarette
NNAL	5.0%	≥100%	≥100%	≥100%	≥100%	48.9%	2.4%
	3.0%	≥100%	≥100%	95.3%	≥100%	—	—
3 HPMA	5.0%	98.2%	≥100%	98.7%	≥100%	53.1%	2.4%
	3.0%	≥100%	96.6%	95.1%	≥100%	—	—
MHBMA	5.0%	91.0%	97.2%	97.4%	98.6%	44.1%	–18.0%
	3.0%	≥100%	97.1%	99.6%	96.6%	—	—
S-PMA	5.0%	99.3%	99.0%	≥100%	99.8%	45.0%	–13.3%
	3.0%	≥100%	98.1%	97.6%	97.1%	—	—
COHb	5.0%	≥100%	98.0%	≥100%	≥100%	53.3%	8.1%
	3.0%	≥100%	≥100%	≥100%	99.8%	—	—
NNN	5.0%	95.6%	97.5%	94.3%	93.3%	42.8%	–18.7%
	3.0%	96.3%	95.0%	95.8%	95.8%	—	—
HMPMA	5.0%	99.6%	≥100%	94.1%	≥100%	50.3%	–17.6%
	3.0%	≥100%	≥100%	94.3%	98.9%	—	—
CEMA	5.0%	≥100%	≥100%	≥100%	≥100%	51.5%	4.4%
	3.0%	≥100%	≥100%	≥100%	≥100%	—	—
1-OHP	5.0%	98.1%	≥100%	83.8%	90.1%	53.6%	–8.2%
	3.0%	≥100%	98.5%	81.3%	92.6%	—	—
O-Tol	5.0%	≥100%	≥100%	94.3%	≥100%	44.8%	–8.6%
	3.0%	≥100%	98.1%	85.2%	85.9%	—	—
2-NA	5.0%	≥100%	≥100%	99.2%	≥100%	47.8%	–17.4%
	3.0%	≥100%	≥100%	98.3%	≥100%	—	—
4-ABP	5.0%	≥100%	≥100%	≥100%	≥100%	55.0%	–20.1%
	3.0%	≥100%	≥100%	97.0%	≥100%	—	—

Abbreviations: VT, Virginia Tobacco.

Relative reduction calculated as (median percent reduction in BOE observed in JUUL, Dual Use or UB Cigarette group [Day 6 – baseline]) / (median percent reduction in BOE observed in Abstinence group [Day 6 – baseline]). In other words, e.g., a tabled value of 99.8% indicates that the reduction in that condition was equal to 99.8% of the reduction observed in the abstinence condition.

*Instances where reduction was greater than abstinence condition are reported as ≥100%

**Negative value means that BOE level increased rather than decreased

Changes in Non-Nicotine Secondary BOE Endpoints

The pattern of results for secondary non-nicotine BOE endpoints (NNN, HMPMA, CEMA, 1-OHP, O-Tol, 2-NA, 4-ABP) was consistent with the primary BOE endpoints. Across non-nicotine BOEs designated as secondary endpoints, median percent reductions, aggregated across the eight JUUL groups, ranged from 70% to 93% of baseline values (Figure 1). Reductions in secondary BOE endpoints in the JUUL groups were significantly greater than changes in the UB Cigarette group for all BOEs except NNN in the Menthol JUUL groups and O-Tol in the Mint 5.0% group (Supplementary Table S4). In addition, reductions in the JUUL groups either did not statistically differ or were significantly greater than the Abstinence group.

Reductions in the Dual-Use group followed a similar pattern to that observed for the primary endpoints. The magnitude of reductions in all secondary BOE endpoints was intermediate between the changes associated with continued smoking (UB Cigarette group) and changes associated with no tobacco product use (Abstinence group; Figure 1). With the exception of NNN, the reductions in all secondary BOE endpoints were significantly greater than those in the UB Cigarette group, and significantly less than all secondary BOE endpoints in the Abstinence group (Supplementary Table S4).

Differences in Changes in BOEs among JUUL Nicotine and Flavor Groups

There were minimal differences in the magnitude of reductions in primary and non-nicotine secondary BOE endpoints by nicotine concentration (5.0% vs. 3.0%) or flavor (Virginia Tobacco vs. Menthol vs. Mint vs. Mango) among the eight JUUL groups; all JUUL groups were generally within ±5% of each other (Figures 2 and 3).

Changes in TNE

When aggregated across flavors, median percent changes in TNE (Day 6–baseline) in the 3.0% JUUL groups (–28%) were substantially greater in magnitude than the 5.0% JUUL groups (–4%; Supplementary Figure S3). Across all eight JUUL groups, median percent changes in TNE ranged from –40% (Virginia Tobacco 3.0%) to 14% (Virginia Tobacco 5.0%); TNE in the Dual-Use group (–16%) and Abstinence group (–96%) decreased. JUUL 5.0% nicotine concentration groups did not significantly differ from the UB Cigarette group. TNE in all JUUL 3.0% groups, except Mint ($P = 0.155$), were significantly lower than UB Cigarette group ($Ps < 0.005$).

Adverse Events

No serious or severe SEAEs were observed and the majority of reported events were judged to be unrelated or unlikely to be related to JUUL use (Supplementary Table S5). In the eight JUUL groups, 47 total participants experienced SEAEs (23.6%). In comparison, SEAE rates in Dual Use, UB Cigarette, and Abstinence groups were 25.0%, 20.8%, and 27.6% respectively. Five participants (10.6% of SEAEs) in the JUUL groups experienced an SEAE that was classified as related or likely related to the use of the study product (Supplementary Table S5).

Discussion

In this residential confinement study of adult smokers, switching completely or partially (i.e., dual-use) from cigarette smoking to use of JUUL for six days resulted in substantial and statistically significant decreases in each of the primary BOEs evaluated in this study. Reductions in BOEs among complete switchers were similar in magnitude to reductions in smokers who abstained completely from all tobacco products, and there were no statistically significant differences in primary BOEs. Additionally, the Dual-Use group (i.e., participants who were required to reduce their smoking by ≥50%) showed significant reductions in BOEs compared to UB Cigarette group (median reductions 44%–53% of complete abstinence).

The median percent reductions of all non-nicotine BOEs observed in the JUUL groups are consistent with the magnitude of reductions observed in previous studies of other ENDS products for five days.[7,36] Findings from these confinement studies are also supported by the results of clinical trials[13,37] and longer-term observational studies[38] in which smokers demonstrated reductions in BOEs after switching from cigarette smoking to use of ENDS. In sum, these results support the fundamental premise of tobacco harm reduction,[39] and suggest that cigarette smokers who switch to the use of JUUL reduce their exposure to harmful cigarette smoke-related toxicants and carcinogens.

Determining the risk profile of dual use of cigarettes and ENDS is a critical question facing public health, as dual-use is common and it is unclear whether dual use increases or decreases exposure to toxicants. Consistent with several studies,[9,22–26] the results of the current study suggest that dual users who decrease cigarette consumption by ≥50% experience substantial reductions in cigarette-related toxicant exposure compared to cigarette smokers. These findings contrast with a recent analysis of data from the Population Assessment of Tobacco and Health (PATH) Study which reported that dual users are exposed to higher levels of toxicants than exclusive smokers.[21] However, further analysis of PATH biomarker data suggests that cigarette smoking is the primary driver of toxicant exposure among dual users, and that use of ENDS minimally affects BOE levels.[40,41] Dual users in the current study were required to reduce their cigarette consumption by ≥50%, which reflects real-world patterns of dual-use: a recent observational study of adult smokers who purchased JUUL found that after 12 months approximately 60% of those who used JUUL and smoked self-reported reducing their cigarette consumption by ≥50%, with average reductions over 80%.[42]

Nicotine intake was similar in the Dual-Use and UB Cigarette groups, suggesting that Dual Users compensated for their reduction in nicotine intake from cigarettes via JUUL use. These results concord with a study that found dual users' cotinine levels did not differ from exclusive smokers',[26] and a clinical trial in which smokers randomized to use JUUL for six weeks did not differ in cotinine levels from those who continued smoking.[13] Nicotine intake was generally lower in the JUUL 3.0% compared to JUUL 5.0% groups, as three of the four JUUL 3.0% groups significantly differed from the UB Cigarette group (vs. none of the 5.0% groups). However, consistent with prior literature that suggests the use of ENDS with lower nicotine concentrations may be associated with compensatory use behavior,[43–45] e-liquid consumption was generally greater in the JUUL 3.0% compared to 5.0% groups.

Across the eight JUUL groups, there were minimal differences in exposure to non-nicotine BOEs by flavor (Virginia Tobacco, Menthol, Mint, Mango). Similarly, there were few differences in changes in BOEs by JUUL nicotine concentration (5.0% vs. 3.0%). These findings suggest that flavors and nicotine concentration did not materially affect BOEs and non-nicotine BOES, respectively, over the six-day study; future research may further elucidate potential differences in exposure by these ENDS product characteristics over longer periods of time.

The reduction in assessed BOEs should be interpreted in light of their pharmacokinetics. The smallest reductions in BOEs, approximately two-thirds of baseline levels, were observed for NNAL and COHb. NNAL has a half-life of 10–16 days; thus it would not be expected to be completely eliminated after six days.[46–48] COHb levels drop quickly following smoking abstinence, but COHb is not specific to smoking (non-smokers show levels approximately one-third those of smokers)[49]; thus complete (100%) reductions were not expected.[50] Accordingly, six days of smoking abstinence resulted in only about two-thirds reductions in NNAL and COHb compared to baseline. Additionally, changes in NNN may be affected by endogenous nitrosation of nicotine.[12] These examples highlight the utility of framing the observed reductions in BOEs following switching to use of JUUL as a proportion of reductions following complete abstinence, as reductions in NNAL, COHb, and NNN were similar in magnitude to, and did not statistically significantly differ from, smokers who completely abstained from smoking.

Strengths of the study include the sample size and resultant statistical power necessary to test primary hypotheses, inclusion of a dual-use group, controlled evaluation of multiple BOEs over a six-day period, and assessment of JUUL in four flavors and two nicotine concentrations. The residential laboratory confinement design ensured compliance with the assigned use condition, and randomization eliminated differences in self-selection and compliance that might otherwise confound interpretation. However, participants and their access to their respective test products were maintained in a controlled environment that may not reflect real-world consumption patterns or participant preferences. By design, this study evaluated a short-term (six-day) switch from combustible cigarettes to JUUL or abstinence in a controlled setting, and future research should assess changes in BOEs in the natural ecology over longer periods of time.

This study evaluated a single, pod-based ENDS, and the results may not generalize to other ENDS products. While this study assessed changes in biomarkers of tobacco and smoke exposure, it did not assess exposure to ENDS-specific compounds—future research is needed to address this question. Participants in the Dual-Use group were allotted no more than 50% of their baseline cigarettes, thus the findings regarding dual-use likely do not generalize to dual users who do not decrease their cigarette consumption by at least this amount. Additionally, comparisons of secondary BOE endpoints and primary endpoints between JUUL groups were not fully powered.

Conclusions

The findings of this tightly controlled six-day study suggest that smokers who switch completely from cigarette smoking to use of JUUL reduce their exposure to multiple toxins associated with cigarette smoking, to levels approximating those in abstainers who neither smoke nor use JUUL. Additionally, the data suggest that when dual-use is accompanied by a reduction in cigarette consumption of at least 50% it can result in substantially reduced toxicant exposure. This study adds to the growing body of evidence supporting the

utility of ENDS products as potentially reduced-harm alternatives to cigarettes for adult smokers.

Supplementary Material

A Contributorship Form detailing each author's specific involvement with this content, as well as any supplementary data, are available online at https://academic.oup.com/ntr.

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Declaration of Interests

Authors GC, NIG and PCB are full-time employees of Juul, Labs, Inc.; SC was a full-time employee of Juul Labs when the study was conducted and manuscript was developed. SS is a Senior Scientific Advisor to Pinney Associates, Inc. Pinney Associates provides consulting services on tobacco harm reduction on an exclusive basis to Juul Labs, Inc. Within the last two years, Pinney Associates has consulted for British American Tobacco and Reynolds American Inc and subsidiaries on tobacco harm reduction. SS co-holds a patent for a novel nicotine medication that has not been developed or commercialized.

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