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Original investigation

# Characterization of Volatile Organic Compound Metabolites in Cigarette Smokers, Electronic Nicotine Device Users, Dual Users, and Nonusers of Tobacco

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## Abstract

**Introduction:** Limited research exists about the possible cardiovascular effects of electronic nicotine delivery systems (ENDS). We therefore sought to compare exposure to known or potentially cardiotoxic volatile organic compounds (VOCs) in ENDS users, smokers, and dual users.

**Methods:** A total of 371 individuals from the Cardiovascular Injury due to Tobacco Use study, a cross-sectional study of healthy participants aged 21–45 years, were categorized as nonusers of tobacco ($n = 87$), sole ENDS users ($n = 17$), cigarette smokers ($n = 237$), and dual users ($n = 30$) based on 30-day self-reported tobacco product use patterns. Participants provided urine samples for VOC and nicotine metabolite measurement. We assessed associations between tobacco product use and VOC metabolite measures using multivariable-adjusted linear regression models.

**Results:** Mean (SD) age of the population was 32 (±6.8) years, 55% men. Mean urinary cotinine level in nonusers of tobacco was 2.6 ng/mg creatinine, whereas cotinine levels were similar across all tobacco product use categories (851.6–910.9 ng/mg creatinine). In multivariable-adjusted models, sole ENDS users had higher levels of metabolites of acrolein, acrylamide, acrylonitrile, and xylene compared with nonusers of tobacco, but lower levels of most VOC metabolites compared with cigarette smokers or dual users. In direct comparison of cigarettes smokers and dual users, we found lower levels of metabolites of styrene and xylene in dual users.

**Conclusion:** Although sole ENDS use may be associated with lower VOC exposure compared to cigarette smoking, further study is required to determine the potential health effects of the higher levels of certain reactive aldehydes, including acrolein, in ENDS users compared with nonusers of tobacco.

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**Implications:** ENDS use in conjunction with other tobacco products may not significantly reduce exposure to VOC, but sole use does generally reduce some VOC exposure and warrants more in-depth studies.

## Introduction

Cardiovascular disease (CVD) is the leading cause of death in tobacco users. Tobacco products contain many reactive, harmful, and potentially harmful constituents that affect different organs systems and physiological processes in a tissue-specific manner.[1,2] A number of volatile organic compounds (VOCs), including acrolein, acrylamide, acrylonitrile, 1,3-butadiene, crotonaldehyde, styrene, and xylene have been shown to be higher in smokers than nonsmokers,[3] and have been proposed to contribute to the CVD risk of tobacco products.[4] Indeed, the World Health Organization considers acrolein an important harmful and potentially harmful constituents for monitoring and regulating tobacco products.[5]

Independent of smoking, exposure to high levels of VOCs has been linked to elevated systolic and diastolic blood pressure, and increased heart rate.[6] Furthermore, exposure to either ambient outdoor air or tobacco products that contain VOCs, has been found to be associated with changes in pulse pressure, increased blood coagulation, inflammation,[7] oxidative stress,[8] and increased hypercholesterolemia,[9] all of which increase CVD risk. Both acute and chronic exposures to VOCs, even at low levels, have been associated with cardiovascular injury, dysfunction, and increased CVD mortality.[10]

Electronic nicotine delivery systems (ENDS) represent a new generation of tobacco products for which the degree of exposure to VOCs and potential for cardiovascular harm remains uncertain. Given the increasing popularity of these products and the lack of data on potential long-term health effects,[11] studies that assess the presence and levels of harmful VOCs in ENDS users, as compared to nonusers of tobacco, are important. Furthermore, a comparison of the levels of VOCs in ENDS users to those in traditional cigarette smokers is critical for a comparative assessment of potential risk of these new products.

Although a few studies have suggested that some VOCs may be present in ENDS, their concentrations in these products remain incompletely described.[12,13] The predominant limitation of these studies is that they are emission studies rather than an assessment of in vivo VOC levels following real-world exposure in human subjects. In addition, in view of the fact that users of both ENDS and traditional cigarettes (dual users) may be exposed to VOCs from both sources, they may represent a different but yet understudied VOC-tobacco exposure phenotype.

We therefore aimed to address these knowledge gaps using data from the Cardiovascular Injury due to Tobacco Use (CITU) study—a cohort of adults with different tobacco exposures including sole ENDS users, cigarette smokers, dual users of both traditional cigarettes, and ENDS, as well as control individuals that use neither of these products. Specifically, we sought to conduct a detailed, controlled, comparative analysis of the degree of exposure to important VOCs across these four groups.

## Methods

### Study Design and Population

Details of the rationale and design of the CITU study have been previously described.[14] Briefly, the CITU study is a two-site (Boston University, Boston, Massachusetts, and University of Louisville, Louisville, Kentucky) observational cross-sectional study designed to evaluate cardiovascular toxicity because of tobacco use, and to assess the role of VOCs in the causation of harm, using biomarkers of subclinical cardiovascular injury.

Participants were recruited through print materials and social media advertising. The study enrolled a total of 465 healthy participants aged 21–45 years from July 2014 to July 2016. Study participants included both nonusers of tobacco, and users of tobacco products including cigarette smokers, ENDS users, as well as users of other tobacco products including hookah, pipe, cigarillo, cigar, smokeless tobacco.

Study visits were scheduled after an 8-hour food fast and a 6-hour tobacco fast. Study visits involved collection of detailed surveys via structured interviews, anthropometric measures, and vascular reactivity testing, and then collection of bio specimens. Participants were excluded if they had been diagnosed with chronic diseases, including hypertension, diabetes mellitus, thyroid disease, or chronic kidney disease.[14] Individuals who reported use of medications such as diuretics and steroids were also excluded from the study. Specific details of other exclusion criteria applied in the CITU study are described elsewhere.[14] The CITU study was approved by both Boston University and University of Louisville institutional review boards and all participants provided written consent.

In this analysis, we used data on 371 participants of the CITU study who had complete information on tobacco product use, and had urine samples for measurement of VOC metabolites and tobacco metabolites. Participants who had missing tobacco exposure measurements were excluded from this analysis.

### Definition of Tobacco Product Use Categories and Patterns

Detailed self-reported tobacco-use history was collected using a modified version of the National Health Interview Survey on tobacco use.[15] The survey was modified to include information on ENDS and nontraditional tobacco products.

Participants were grouped into four tobacco product use categories (nonusers of tobacco, sole ENDS users, cigarette smokers, and dual users) based on tobacco product usage over the previous 30 days. Nonusers of tobacco were defined as individuals who reported no use of tobacco products (including cigarettes and ENDS), over the past 30 days and who had minimal exposure to secondhand smoke (based on urinary cotinine levels <10 ng/mg creatinine[16]). A total of 47 participants who were not tobacco users but had moderate or greater secondhand exposure (urinary cotinine levels >10 ng/mg creatinine[16]) were excluded.

Current cigarette smokers were defined as those who reported smoking cigarettes for at least 7 of the past 30 days or who had urinary cotinine levels more than 500 ng/mg creatinine.[4]

Sole ENDS users were defined as those who reported use of any ENDS product at least 7 of the previous 30 days without concurrent cigarette smoking. Dual users were defined as those who met criteria for both current smoking and current ENDS use.

To preserve adequate sample size and to better approximate polytobacco use patterns,[17,18] information on occasional use of other tobacco products (eg, hookah, smokeless tobacco, pipe) was collected. Participants' use of tobacco products other than cigarettes and ENDS was defined as number of other products used and additionally by frequent of other product use (none, some days, everyday).

For all participants, time since use of last tobacco product (in hours) was derived as the difference between the time of urine collection and self-reported time of last use of the designated tobacco product.

### VOC Metabolite Measurements

Standard clean catch urine specimens were obtained from participants and stored at 4°C. The samples were transported for long-term storage and mass spectrometric analysis at the University of Louisville.

A total of 23 urinary metabolites of tobacco-induced aldehydes and other VOCs where quantified with a modified version of the mass spectrometry method developed by the Centers of Disease Control and Prevention (CDC)[19] and described in detail by Lorkiewicz et al.[20] The analysis was performed on ACQUITY UPLC core system and a Quattro Premier XE triple quadrupole mass spectrometer coupled with an electrospray source (Waters, Inc, MA). Urine (25 µL) was mixed with 15 mM ammonium acetate (975 µL) containing a mixture of internal standards and filtered through a 0.2 mm polytetrafluoroethylene membrane. Two microliters of the sample was applied on ACQUITY UPLC HSS T3 column (150 mm × 2.1 mm, 1.8 µm; Waters, Inc) maintained at 40°C and preequilibrated with ammonium acetate (15 mM, pH 6.8; solvent A) at a flow rate of 0.45 mL/min. The binary gradient started with 3% solvent A at 0 minute, and was linearly increased to 5% solvent B (acetonitrile) at 1.3 minutes, 10% B at 2.0 minutes, 30% B at 3.35 minutes, and 40% at 4.36 minutes. The gradient was then decreased to 15% B at 4.7 minutes, 10% B at 5.0 minutes, and 3% B at 5.36 minutes. The samples were analyzed, both in positive and negative ion modes. For each analyte, three multiple reaction monitoring obtained for each analyte: one for quantification, one for confirmation, and one for stable isotope labeled analogous internal standard. At least 12 data points across the peaks were used for the quantitation of peak area. Analytes in urine samples were quantified using peak area ratio (analyte to internal standard) based on 10 point-standard curves that were run before and after the urine samples. TargetLynx quantification application manager software (Waters, Inc) was used for peak integration, calibration, and quantification. The concentration ranges determined for this method are comparable to those reported in the CDC method for the VOC metabolites.[19,20] Similarly, the reproducibility of the method was satisfactory, with relative SDs below 8% for VOC metabolites and alkaloids (5.5% for cotinine). Additional validation shows comparability in terms of sensitivity, accuracy, and precision as compared to the CDC.[20]

The concentration values of analytes obtained from ultra performance liquid chromatography- tandom mass spectrometer were normalized to creatinine level, which was measured on a COBAS MIRA-plus analyzer (Roche, NJ) with Infinity Creatinine Reagent (Thermo Fisher Scientific, MA). If a metabolite failed to reach the lower limit of detection in more than 85% of participants, it was excluded from further analysis. A total of five metabolites (MHBMA1, 12DCVMA, 22DCVMA, TCVMA, and DPMA) were thus excluded from further analysis.

### Other Covariates

Sociodemographic characteristics including age, gender, race/ethnicity, and education and income levels were assessed using standard questionnaires. In addition, detailed self-reported tobacco product use characteristics including age of smoking initiation, frequency of use of tobacco products, and whether ENDS used was nicotine-containing were assessed.

### Statistical Analysis

We summarized baseline sociodemographic and tobacco use habits data by tobacco product use categories, reporting means ± SDs for continuous measures and proportions for categorical variables. Differences across groups were tested using one-way analysis of variance and simple chi-square statistics for continuous and categorical variables, respectively.

To evaluate differences in the levels of VOC metabolites by tobacco product use category, we first summarized mean values of each VOC metabolite measure and tested differences in the levels of VOC metabolites across tobacco product use categories using one-way analysis of variance statistics. We then assessed the relationship between tobacco products and log-transformed VOC metabolite measures using multivariable-adjusted linear regression models with nonusers of tobacco as the reference group. Models were adjusted for age, sex, race, and other tobacco product use. Results were reported as β-coefficients with 95% confidence intervals, and to facilitate interpretation, additionally as percent change in the geometric mean of individual VOCs, which is accomplished by exponentiation of the β-coefficients from the linear regression models. To enable direct comparison between the groups, additional multivariable identical models were repeated for pairwise comparisons between tobacco use groups (ie, sole ENDS users vs. cigarette smokers; sole ENDS users vs. dual users; and cigarette smokers vs. dual users).

In addition, we assessed for a dose–response relationship between smoking intensity and VOC metabolites among cigarette smokers (including dual users). After dividing cigarette smokers into four groups (<5 cigarettes per day [CPD], 5–10 CPD, 11–15 CPD, and >15 CPD), we tabulated the mean values for each VOC metabolite across each of these defined dose groups and then conducted multivariable linear regressions adjusted for age, sex, race, electronic cigarette use, and use of other tobacco products, reporting statistical tests for trends across increasing intensity groups.

We also conducted several sensitivity analyses. First, to determine if observed differences in VOC metabolite measures across groups persisted after controlling for degree of nicotine exposure, multivariable linear regression models were repeated with further adjustment for (log) cotinine. Second, to determine if observed differences in VOC metabolite measures across groups persisted after adjusting for time since last tobacco product use, multivariable linear regression models were repeated with further adjustment time since last use (in hours).

All analyses were performed using STATA version 14 (College Station, TX). A two-sided $p$ value of ≤.05 was considered statistically significant for all analyses.

## Results

### Baseline Characteristics

Table 1 shows the distribution of sociodemographic characteristics by product use category. Overall, the mean (SD) age of the study population was 32 (±6.8) years, and participants were mostly men (55%). Sole ENDS users and tobacco nonusers were younger than cigarette smokers and dual users. There were notable sex differences, with sole ENDS users and dual users significantly more likely to be men as compared to cigarette smokers. Sole ENDS users were predominantly white (88.2%), whereas cigarette smokers were mostly blacks (52.3%). Nonusers of tobacco were more educated and had higher annual income than users of tobacco products (Table 1). Of a total of 237 cigarette smokers, 17 (7.3%) reported smoking, on average, less than 5 CPD, as compared to 13.8% of dual users.

**Table 1.** Baseline Characteristics by Product Use Categories

| | Total population | Nonusers of tobacco ($n$ = 87) | Cigarette smokers ($n$ = 237) | Sole ENDS users ($n$ = 17) | Dual users ($n$ = 30) | $p$ Value |
|---|---|---|---|---|---|---|
| Age (mean ± SD) | 31.5 ± 6.8 | 28.8 ± 6.5 | 32.5 ± 6.8 | 28.2 ± 4.8 | 33.2 ± 7.2 | **.306** |
| Gender ($n$, %) | | | | | | |
| Females | 164, 44.3 | 48, 55.2 | 102, 43.2 | 2, 11.8 | 12, 40.0 | **.008** |
| Males | 206, 55.7 | 39, 44.8 | 134, 56.8 | 15, 88.2 | 18, 60.0 | |
| Race | | | | | | |
| Blacks | 168, 45.5 | 31, 35.6 | 123, 52.3 | 0, 0 | 14, 46.7 | **<.001** |
| Whites | 170, 46.1 | 42, 48.3 | 97, 41.3 | 15, 88.2 | 16, 53.3 | |
| Other | 31, 8.4 | 14, 16.1 | 15, 6.4 | 2, 11.8 | 0, 0 | |
| Education ($n$, %) | | | | | | **<.001** |
| Some HS/HS Grad | 162, 43.7 | 8, 9.2 | 135, 57.0 | 5, 29.4 | 14, 46.7 | |
| Some college | 102, 27.4 | 23, 26.4 | 60, 25.3 | 9, 52.9 | 10, 33.3 | |
| 2–4 y degree | 85, 22.9 | 39, 44.8 | 38, 16.0 | 3, 17.7 | 5, 16.7 | |
| Masters/doctorate | 22, 5.9 | 17, 19.5 | 4, 1.7 | 0, 0 | 1, 3.3 | |
| Income ($n$, %) | | | | | | |
| <$20 000 | 128, 37.9 | 21, 25.6 | 92, 43.6 | 2, 12.5 | 13, 44.8 | **<.001** |
| $20 000–$45 000 | 138, 40.8 | 29, 35.7 | 87, 41.2 | 10, 62.5 | 12, 40.8 | |
| >$45 000 | 72, 21.3 | 32, 39.0 | 32, 15.2 | 4, 25.0 | 4, 21.3 | |

ENDS = electronic nicotine delivery systems.
Bold values indicate statistically significant ($p$ = 0.05).

However, comparable to cigarette smokers, 80% of dual users reported smoking cigarettes at least 29 days of the previous 30 days. Conversely, although sole ENDS users reported a high frequency of daily electronic cigarette use (64.7%), only 20% of dual users reported everyday ENDS use (Supplementary Table 1).

Half of dual users also reported additional occasional use of other tobacco products, including cigars, hookah, bidi, snuff, and chewing tobacco, compared to 37% of cigarette smokers and 29% of sole ENDS users. The predominant type of other tobacco product used among cigarette smokers (26.2%) and dual users (26.7%) was cigars, whereas sole ENDS users who also used other products predominantly used hookah (23.5%) (Supplementary Table 1).

Among nonusers of tobacco, 12.6% reported former smoking and 8.1% reported former ENDS use. Approximately 88% of sole ENDS users were former smokers (Supplementary Table 1).

### Tobacco Products and Nicotine Metabolites

The mean (SD) cotinine level in the nonuser of tobacco category was 2.6 (2.4) ng/mL. There were moderate differences in the raw levels of cotinine across product categories, with cigarette smokers having a slightly higher cotinine level than sole ENDS users or dual users. Sole ENDS users however had comparable mean levels of cotinine to dual users (Table 2). In models accounting for age, sex, race, and pattern of use of other tobacco products, however, pairwise comparisons of cigarette smokers, dual users and sole ENDS users showed no significant differences in the association of nicotine or its metabolites across product categories (data not shown).

### Relationship Between Tobacco Products and VOC Metabolites

Nonusers of tobacco had lower mean levels of most VOC metabolites compared to users of tobacco products (Table 2). Specific VOC metabolites that were not significantly different between nonusers of tobacco and users of tobacco products include MU, BPMA, and BMA (Table 2).

In multivariable linear regression models testing the associations between tobacco product use categories and log-transformed VOC metabolite levels, cigarette smokers and dual users had significantly elevated levels of all VOC metabolites except MU, BPMA, and BMA, whereas cigarette smokers alone also had elevated 2HPMA, when compared to nonusers of tobacco (Table 3).

Among sole ENDS users, levels of CEMA, AAMA, CYMA, 2MHA, and 3MHA + 4MHA were noted to be significantly elevated compared to nonusers of tobacco (Table 3). Multivariable-adjusted pairwise comparisons of tobacco product use categories however showed sole ENDS users to have significantly lower levels of 3HPMA, AAMA, CYMA, HEMA, DHBMA, MHBMA3, HPMMA, HPMMA, AMCC, 2HPMA, PHEMA, MA, 2MHA, and 3MHA + 4MHA compared to cigarette smokers, despite similar cotinine levels (Table 4). A similar comparison of sole ENDS users to dual users also showed significantly lower levels of the same metabolites as smokers except for PHEMA and 2MHA in sole ENDS users (Table 4). Conversely, dual users and cigarette smokers had similar levels of all VOC metabolites except PGA, PHEMA, and 3MHA + 4MHA, which were all significantly higher in cigarette smokers than in dual users (Table 4). Anabasine levels were significantly lower in sole ENDS users that in cigarette or dual users, though no other tobacco alkaloids differed (Table 4).

Sensitivity analyses additionally accounting for time since last tobacco product use ($N$ = 218) showed similar associations as in our primary analysis (Supplementary Table 2). In addition to the metabolites noted in the primary analysis, however, 3HPMA and MHBMA3 were found to be significantly elevated in sole ENDS users compared to nonsmokers in the supplementary analysis, whereas statistical significance was lost for 3MHA + 4MHA (Supplementary Table 2).

A separate sensitivity analysis comparing sole ENDS users and dual users against a reference of cigarette smokers additionally adjusting for cotinine (to normalize nicotine exposure) showed no significant differences from our primary results (Supplementary Table 3).

## Discussion

In this real-world cross-sectional study of differential exposures to VOCs across ENDS and conventional cigarettes, we found that despite similar cotinine levels sole ENDS users had significantly lower levels of most VOC metabolites compared to smokers and dual users,

**Table 2.** Mean VOC Levels by Tobacco Product Category

Mean normalized values ng/mg creatinine

| Parent compound | Analyte | Total ($N$ = 371) | Nonusers of tobacco ($n$ = 87) | CIG only ($n$ = 237) | ENDS only ($n$ = 17) | Dual users ($n$ = 30) | $p$ Value |
|---|---|---|---|---|---|---|---|
| Volatile organic compounds | | | | | | | |
| Acrolein | CEMA | 154.3 ± 129.4 | 79 ± 62.5 | 180.1 ± 134.8 | 120.8 ± 90.3 | 188.1 ± 160 | **<.001** |
| | 3HPMA | 576.6 ± 643.1 | 223 ± 149.4 | 724.4 ± 735.1 | 338.6 ± 206.4 | 569.5 ± 450.8 | **<.001** |
| Acrylamide | AAMA | 157.3 ± 132.7 | 67.8 ± 60.5 | 191.9 ± 136.1 | 88.5 ± 43.6 | 181.8 ± 157.4 | **<.001** |
| | GAMA | 38.7 ± 32.2 | 25.4 ± 21.2 | 43.6 ± 34.8 | 36.5 ± 24.7 | 39 ± 30.8 | **<.001** |
| Acrylonitrile | CYMA | 92.8 ± 116.9 | 3.0 ± 12.3 | 129.8 ± 126 | 29.3 ± 31.3 | 97 ± 78.6 | **<.001** |
| Acrylonitrile, vinyl chloride, ethylene oxide | HEMA | 3.5 ± 4.7 | 1.7 ± 1.3 | 4.2 ± 5.2 | 1.1 ± 0.9 | 4.5 ± 5.4 | **<.001** |
| Benzene | MU | 139.4 ± 110 | 138.8 ± 92.3 | 132.4 ± 102.7 | 211 ± 179.3 | 156.2 ± 147.6 | .59 |
| 1-Bromopropane | BPMA | 17.9 ± 23.4 | 13 ± 12.3 | 20.6 ± 27.2 | 6.0 ± 4.8 | 17.5 ± 16.5 | .17 |
| 1,3-Butadiene | DHBMA | 361.1 ± 182.2 | 283.2 ± 104.5 | 389.9 ± 194.4 | 262.7 ± 107.7 | 415.6 ± 209 | **<.001** |
| | MHBMA3 | 15.1 ± 15.4 | 3.6 ± 2.5 | 19.5 ± 15.4 | 6.8 ± 7.9 | 18.7 ± 20.7 | **<.001** |
| Crotonaldehyde | HPMMA | 371.2 ± 367.5 | 138.7 ± 49.5 | 462.4 ± 398.6 | 179.1 ± 112.4 | 433.5 ± 399.8 | **<.001** |
| *N,N*-Dimethlyformamide | AMCC | 275.5 ± 218.9 | 127.1 ± 90.5 | 327.7 ± 226.3 | 169.7 ± 105.4 | 354 ± 251.6 | **<.001** |
| Ethylbenzene, Styrene | PGA | 286.5 ± 163.5 | 186.2 ± 66.9 | 330.2 ± 177.3 | 191.2 ± 52.3 | 286.3 ± 139.3 | **<.001** |
| Propylene oxide | 2HPMA | 63.7 ± 114.4 | 84.4 ± 214.7 | 60.1 ± 57.1 | 34.8 ± 27.6 | 48.6 ± 33.6 | **<.001** |
| Styrene | PHEMA | 1.8 ± 2.2 | 0.9 ± 0.9 | 2.3 ± 2.5 | 1.0 ± 0.7 | 1.5 ± 1.1 | **<.001** |
| | MA | 238.2 ± 216.7 | 126.8 ± 52.2 | 284.9 ± 251.8 | 146.8 ± 70.5 | 244.6 ± 110.7 | **<.001** |
| Toluene | BMA | 11.5 ± 24.7 | 10.6 ± 14.0 | 12.8 ± 29.5 | 5.0 ± 2.5 | 8.3 ± 6.3 | .86 |
| Xylene | 2MHA | 26 ± 31.5 | 8.6 ± 14.6 | 33.3 ± 35.2 | 17.1 ± 12.7 | 23.8 ± 21.7 | **<.001** |
| | 3MHA + 4MHA | 291.7 ± 281.3 | 115.9 ± 129.3 | 365.7 ± 305.8 | 165.3 ± 104.7 | 289.1 ± 213.5 | **<.001** |
| Tobacco alkaloids | | | | | | | |
| Anabasine | ANB | 4.8 ± 5.3 | 2.7 ± 2.7 | 5.6 ± 5.6 | 3.1 ± 3.0 | 6.2 ± 7.7 | **<.001** |
| Antabine | ANTB | 4.1 ± 6.1 | 0.9 ± 1.0 | 5.3 ± 6.7 | 2.4 ± 3.6 | 4.7 ± 7.2 | **<.001** |
| Nicotine | NIC | 349.3 ± 687.7 | 10.2 ± 15 | 453.4 ± 771.7 | 434.5 ± 769.5 | 462.1 ± 639.2 | **<.001** |
| | COT | 690.6 ± 844.3 | 2.6 ± 2.4 | 910.9 ± 868.3 | 855.8 ± 958.9 | 851.6 ± 770.9 | **<.001** |
| | 3HC | 2197.3 ± 2326.8 | 6.4 ± 13.7 | 2887.6 ± 2237 | 3204.1 ± 2865.3 | 2527.8 ± 2196.4 | **<.001** |

CIG = cigarette; ENDS = electronic nicotine delivery systems.
Bold values indicate statistically significant ($p$ = 0.05).

**Table 3.** Percentage Change in Levels of VOC Metabolites, Comparing Tobacco Product Use Categories to Nontobacco Users

| Parent compound | Analyte | Cigarette smokers, % (95% CI) | Sole ENDS users, % (95% CI) | Dual users, % (95% CI) |
|---|---|---|---|---|
| VOC metabolites | | | | |
| Acrolein | CEMA | **136 (95 to 200)** | **68 (16 to 144)** | **125 (65 to 200)** |
| | 3HPMA | **200 (143 to 267)** | 45 (–1 to 110) | **141 (79 to 232)** |
| Acrylamide | AAMA | **200 (151 to 267)** | **42 (2 to 97)** | **172 (27 to 164)** |
| | GAMA | **92 (52 to 139)** | 54 (–1 to 139) | **84 (27 to 164)** |
| Acrylonitrile | CYMA | **8901 (6569 to 12051)** | **1002 (505 to 1909)** | **6569 (3945 to 10895)** |
| Acrylonitrile, vinyl chloride, Ethylene oxide | HEMA | **112 (65 to 172)** | –20 (–51 to 32) | **151 (67 to 267)** |
| Benzene | MU | –10 (–28 to 12) | 14 (–26 to 75) | –9 (–36 to 31) |
| 1-Bromopropane | BPMA | 27 (–3 to 67) | –19 (–52 to 37) | 20 (–23 to 86) |
| 1,3-Butadiene | DHBMA | **38 (23 to 55)** | –5 (–24 to 19) | **43 (20 to 73)** |
| | MHBMA3 | **447 (348 to 568)** | 46 (–1to 114) | **348 (232 to 505)** |
| Crotonaldehyde | HPMMA | **172 (127 to 232)** | 13 (–22 to 62) | **141 (79 to 232)** |
| *N,N*-Dimethlyformamide | AMCC | **200 (143 to 232)** | 27 (–7 to 75) | **167 (108 to 232)** |
| Ethylbenzene, styrene | PGA | **73 (54 to 95)** | –3 (–24 to 22) | **46 (20 to 77)** |
| Propylene oxide | 2HPMA | **54 (25 to 90)** | –20 (–46 to 21) | 31 (–7 to 82) |
| Styrene | PHEMA | **129 (84 to 200)** | 13 (–27 to 73) | **54 (8 to 120)** |
| | MA | **103 (77 to 136)** | 17 (–11 to 55) | **92 (52 to 141)** |
| Toluene | BMA | –8 (–27 to 17) | –10 (–43 to 42) | –15 (–41 to 25) |
| Xylene | 2MHA | **569 (348 to 802)** | **200 (62 to 505)** | **348 (172 to 717)** |
| | 3MHA + 4MHA | **232 (183 to 305)** | **52 (7 to 114)** | **151 (90 to 232)** |

Estimates were derived using the formula (eβ – 1) × 100%, where β represents the β-coefficient of the multivariable-adjusted association between product use categories and ln (VOC metabolite levels). Models adjusted for age, sex, race, and pattern of use of other products. Bolded values are statistically significant at $p < 0.05$. CI = confidence interval; ENDS = electronic nicotine delivery systems; VOC = volatile organic compound.

**Table 4.** Multivariable-Adjusted Pairwise Comparisons of VOC Metabolites Across Tobacco Product Use Categories

| Parent compound | Analyte | Sole ENDS users vs. cigarette smokers % difference (95% CI) | Dual users vs. cigarette smokers % difference (95% CI) | Sole ENDS users vs. dual users % difference (95% CI) |
|---|---|---|---|---|
| VOC metabolites | | | | |
| Acrolein | CEMA | −29 (−50, 1) | −5 (−27 to 24) | −25 (−51 to 14) |
| | 3HPMA | **−51 (−66 to −30)** | −18 (−37 to 7) | **−40 (−61 to −8)** |
| Acrylamide | AAMA | **−52 (−65 to −34)** | −6 (−26 to 19) | **−49 (−65 to −26)** |
| | GAMA | −20 (−47 to 22) | −4 (−30 to 31) | −16 (−49 to 38) |
| Acrylonitrile | CYMA | **−87 (−93 to −78)** | −27 (−53 to 12) | **−83 (−91 to −66)** |
| Acrylonitrile, vinyl chloride, ethylene oxide | HEMA | **−62 (−77 to −39)** | 18 (−17 to 68) | **−68 (−82 to −43)** |
| Benzene | MU | 27 (−16 to 92) | 2 (−25 to 38) | 25 (−23 to 104) |
| 1-Bromopropane | BPMA | −36 (−62 to 6) | −6 (−35 to 38) | −32 (−63 to 24) |
| 1,3-Butadiene | DHBMA | **−31 (−45 to −15)** | 4 (−11 to 22) | **−34 (−49 to −15)** |
| | MHBMA3 | **−73 (−81 to −60)** | −16 (−36 to 11) | **−67 (−79 to −49)** |
| Crotonaldehyde | HPMMA | **−59 (−71 to −42)** | −12 (−32 to 15) | **−53 (−69 to −29)** |
| *N*,*N*-Dimethlyformamide | AMCC | **−55 (−67 to −40)** | −6 (−25 to 17) | **−52 (−67 to −32)** |
| Ethylbenzene, Styrene | PGA | **−44 (−55 to −30)** | **−16 (−29 to −1)** | **−34 (−49 to −13)** |
| Propylene oxide | 2HPMA | **−48 (−64 to −23)** | −15 (−36 to 13) | **−38 (−61 to −2)** |
| Styrene | PHEMA | **−51 (−67 to −26)** | **−33 (−51 to −9)** | −27 (−55 to 20) |
| | MA | **−43 (−56 to −25)** | −6 (−23 to 14) | **−39 (−55 to −16)** |
| Toluene | BMA | −2 (−37 to 50) | −8 (−33 to 28) | 5 (−37 to 77) |
| Xylene | 2MHA | **−52 (−74 to −11)** | −28 (−54 to 15) | −34 (−68 to 39) |
| | 3MHA + 4MHA | **−55 (−68 to −38)** | **−26 (−42 to −5)** | **24 (−59 to −11)** |
| Tobacco alkaloids | | | | |
| Anabasine | ANB | **−64 (−80 to −36)** | −5 (−38 to 48) | **−63 (−81 to −24)** |
| Antabine | ANTB | 30 (−41, 186) | 24 (−31 to 124) | 4 (−59 to 169) |
| Nicotine | NIC | 12 (−40 to 107) | −26 (−53 to 17) | 51 (−27 to 215) |
| | COT | 32 (−36 to 172) | −29 (−59 to 21) | 87 (−22 to 344) |
| | 3HC | −29 (−50 to 1) | −5 (−27 to 24) | −25 (−51 to 14) |

Estimates were derived using the formula $(e\beta - 1) \times 100\%$, where β represents the β-coefficient of the multivariable-adjusted association between product use categories and ln (VOC metabolite levels). Models adjusted for age, sex, race, and pattern of use of other products. Bolded values are statistically significant at $p < .05$. CI = confidence interval; ENDS = electronic nicotine delivery systems; VOC = volatile organic compound.

whereas cigarette smokers and dual users had similar levels of VOC metabolites. However, despite lower levels of VOC metabolites compared with cigarette smokers and dual users, sole ENDS users had elevated levels metabolites of acrolein (CEMA), acrylamide (AAMA), acrylonitrile (CYMA), and Xylene (2MHA and 3MHA + 4MHA), when compared to nontobacco using controls. Though these metabolites can be ubiquitous and we did not control for dietary intake, the elevation in VOC metabolites is similar to that seen in with dual users and has been found in ENDS vapor in prior studies.[21,22]

We found that in comparison with nonusers of tobacco, individuals who smoked had up to 8901% higher adjusted levels of VOC metabolites and, as expected, up to 15–20 times higher levels of unadjusted tobacco metabolites in the urine. Dual users had similar levels with up to 6569% adjusted levels of the VOC metabolites and 16 times the nicotine metabolites levels as compared to nonusers of tobacco. Interestingly, sole ENDS users had a similar fold increase in tobacco metabolites (up to 16 times) as cigarette and dual users in comparison to nonsmokers, but a much smaller, approximately 1000%, increase in adjusted VOC metabolites in comparison to nonsmokers.

Similar to data reported here, previous studies have quantified VOCs in the mainstream cigarette smoke of 50 US brand cigarettes showing that smoking increases VOC exposure up to 6-fold.[15] ENDS aerosol has contained nicotine, particles, and VOCs, although the VOCs were detected in only a small number of ENDS products and under controlled testing conditions,[23] not real-world conditions. Moreover, it has been hypothesized that under most conditions VOC concentrations in ENDS are significantly lower than traditional cigarettes.[21] This is supported by preliminary evidence showing that those who switch from combustible cigarettes to ENDS, do not decrease their nicotine exposure, but do reduce their exposure to some VOCs.[22,24] However, even though overall VOC metabolite levels are lower in sole ENDS users than those who use cigarettes, it remains unclear, but likely, that these lower levels may still uniquely contribute to CVD risk.[25]

To extend prior work, our study measured urinary metabolites of VOC exposure, rather than relying on machine yields directly from tobacco devices.[26] Overall our study contributes to the body of literature that suggests habitual daily smokers had significantly higher levels of VOC metabolites than those who used ENDS only, but not dual users. Importantly, our study also showed elevations in specific metabolites of acrolein in sole ENDS users. In animal models, we have found that exposure to reactive aldehydes, such as acrolein, is linked to dyslipidemia and increased CVD risk.[27,28] Of particular importance, it has been hypothesized that much of the tobacco-induced cardiovascular injury is mediated by aldehydes such as acrolein and crotonaldehyde. Furthermore, our study showed an elevation of benzene metabolite MU in dual users similar to previous work.[22] Exposure to benzene has been linked to CVD in animal and population studies.[29]

Although previous work supports the idea that exposure to VOCs can increase the risk of CVD,[2,30,31] it is unclear how exposure is related to such outcomes and whether there is a threshold at which the toxicity of VOC appears. Nevertheless, current literature suggests

that chronic exposure to low-level VOC can lead to an increase in CVD risk.[32] Further complicating ENDS related VOC exposure is the variability in devices, electronic liquids[33] and use patterns.[34,35] VOC emission during ENDS use is likely based on the coil, battery, electronic liquid composition, and inhalation patterns associated with use,[12,36] mainly because these characteristics and use patterns affect the temperature at which electronic liquids are vaporized. Moreover, these variables result in a variable exposure of VOCs. Consequently, our studies of real-world cohorts are a critical in advancing the understanding of ENDS-related VOC exposure. The results of our real-world study support the literature suggesting that sole ENDS use, not dual use, results in a decreased exposure to many VOCs, but not all, at similar nicotine levels to conventional cigarette use.

Nicotine is the addictive constituent present in tobacco. When ENDS originally emerged in the marketplace, they were marketed as cessation devices. In addition, ENDS use was originally perceived to be associated with little to no risk, despite the lack of scientific evidence to support these conclusions. Current literature demonstrates that most individuals become dual users of cigarettes and ENDS[17,37–39] and studies suggest that the majority of those who use ENDS, report no intent-to-quit cigarettes altogether.[40] Our study population reflects these reported trends, as 60% of our participants who use ENDS were dual users. We found that among dual users, VOC metabolites were similar to cigarette users. Given the reported use of ENDS as cessation and harm-reduction devices, with little to no health risks, dual users in particular may be unaware of the magnitude of the health impact associated with continued use of both cigarettes and ENDS. Furthermore, the most predominant pattern of ENDS use is dual use.[41] These findings suggest that a reduction in the consumption of cigarettes smoked per day may not reduce an individual's exposure to VOCs and consequently CVD.[42] Of particular concern is the fact that the risk for CVD in smokers is nonlinear and as few as three cigarettes confer a majority of CVD risk associated with smoking two packs a day.[43] In this study 81.7% of dual users reported using more than 5 CPD.

ENDS-only users tend to report themselves as former smokers,[44] and often perceive beneficial changes to their health after they quit smoking.[45,46] This perception of low health risk with ENDS use may lead ENDS-only users to continue using these products long term. Though conclusions surrounding health effects of ENDS are currently unclear based on inconsistence findings, recent studies suggest that ENDS share some of the same risks as cigarettes, including VOC production as demonstrated here and increased cardiovascular risk as reflected by changes in heart rate, heart variability, and flow-mediated dilation.[47–49] Our study showing the wide range of VOC metabolites in users of different tobacco products could be useful in assessing future disease risk.

### Limitations

Despite many strengths, our study has limitations. Our study is limited by the size of our ENDS-only cohort. However, given the previous reported tendency toward dual users,[50] the scarcity of the ENDS-only phenotype is not surprising, and the distribution of use patterns in CITU is similar to national statistics arguing against substantial selection bias. Our study also required individuals to fast prior to the study visit. Although the protocol used a standardized fast, the time from last cigarette smoked varied among individuals, however not significantly across tobacco user groups. Though many VOC metabolites have long half-lives, the metabolic rate of each VOC is different, which could create a difference in certain VOC metabolite measures based on their elimination rate. However, our results were similar after adjusting for time since last cigarette. While reflecting true real-world use patterns, our study is limited by the use of other forms of tobacco (ie, cigars) across our use groups. We used multiple strategies for adjusting for other tobacco use, and the results were robust to multiple different modeling approaches. Furthermore, our cohort of ENDS users mainly reported second- and third-generation ENDS use, matching typically reported national trends,[50] but limiting the generalizability of these findings to other generations of ENDS.

## Conclusions

Tobacco use continues to be a major public health concern, both in the United States and globally. With the advent of new tobacco products such as ENDS, it is imperative to develop a better understanding of the risks associated with both traditional and new tobacco use. Although the contribution VOCs to tobacco-induced disease is unclear, the observation that VOC metabolites are elevated ENDS users, suggest that the use of these products results in VOC exposure that should be investigated for potentially higher risk of cardiovascular injury than nonusers. Understanding the risks associated with the individual compounds found in the tobacco products will allow targeted regulation of specific compounds for harm reduction. Regulatory agencies may also be able undertake a more targeted toxicological screen prior to allowing new products on the market. Finally, a greater understanding of VOC exposure associated with specific products could provide individuals with a greater understanding of their exposure to harmful and potentially harmful constituents.

## Supplementary Material

Supplementary data are available at *Nicotine and Tobacco Research* online.

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## Declaration of Interests

*None declared.*

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