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Original Investigation

# Metabolomic Analysis Identified Reduced Levels of Xenobiotics, Oxidative Stress, and Improved Vitamin Metabolism in Smokers Switched to Vuse Electronic Nicotine Delivery System

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## Abstract

**Introduction:** Switching to noncombustible tobacco products presents an opportunity for cigarette smokers to potentially reduce the health risks associated with smoking. Electronic Nicotine Delivery Systems (ENDS) are one such product because the vapor produced from ENDS contains far fewer toxicants than cigarette smoke. To investigate the biochemical effects of switching from smoking to an ENDS, we assessed global metabolomic profiles of smokers in a 7-day confinement clinical study.

**Methods:** In the first 2 days of this clinical study, the subjects used their usual brand of cigarettes and then switched to exclusive ENDS ad libitum use for 5 days. Urine and plasma samples were collected at baseline and 5 days after switching. The samples were analyzed using a mass spectrometry-based metabolomic platform.

**Results:** Random forest analyses of urine and plasma metabolomic data revealed excellent predictive accuracy (>97%) of a 30-metabolite signature that can differentiate smokers from 5-day ENDS switchers. In these signatures, most biomarkers are nicotine-derived metabolites or xenobiotics. They were significantly reduced in urine and plasma, suggesting a decreased xenobiotic load on subjects. Our results also show significantly decreased levels of plasma glutathione metabolites after switching, which suggests reduced levels of oxidative stress. In addition, increased urinary and plasma levels of vitamins and antioxidants were identified, suggesting enhanced bioavailability due to discontinuation of cigarette smoking and switching to Vuse ENDS use.

**Conclusions:** Our results suggest reduced toxicant exposure, reduced oxidative stress, and potential beneficial changes in vitamin metabolism within 5 days in smokers switching to Vuse ENDS.

**Implications:** Switching from smoking to exclusive ENDS use in clinical confinement settings results in significant reduction of nicotine metabolites and other cigarette-related xenobiotics in urine and plasma of subjects. Significantly decreased oxidative stress-related metabolites and increased urinary and plasma levels of vitamin metabolites and antioxidants in 5-day short-term ENDS switchers suggest less toxic physiological environment for consumers of ENDS products and potential health benefits if such changes persist.

## Introduction

Smoking increases the risks of lung cancer, chronic obstructive pulmonary disease, and cardiovascular disease.[1–3] Cigarette smoke contains several thousand chemicals that are generated during the combustion process.[4] Many of these chemicals, designated by the US FDA as harmful and potentially harmful constituents (HPHCs), have been linked to long-term adverse health effects.[5,6] A relative risk continuum across different tobacco and nicotine products has been envisioned,[7] and under this paradigm, cigarette smoking has been recognized as the most harmful form of tobacco use.[7] Complete smoking cessation is the best option to reduce the risk of smoking-related diseases.

Apart from cigarettes, noncombustible tobacco products exist and do not generate combustion-related toxicants. These include smokeless tobacco products such as moist snuff and chewing tobacco, Electronic Nicotine Delivery Systems (ENDS), and heated tobacco products.[8,9] For those smokers (SMK) who are unable to quit, switching to noncombustible tobacco products may significantly reduce their risk of disease.[7] Biomarkers of exposure and biomarkers of potential harm (BoPH) are biological measures that inform of the exposure to tobacco toxicants and the potential harm from the toxicant exposure, respectively. Considering the long latency of smoking-related diseases, biomarkers are important tools to assess the potential long-term adverse health effects of tobacco use.

The inhaled, noncombustible tobacco products such as ENDS generate chemically much simpler aerosol that is devoid or contains substantially lower levels of HPHCs. The e-liquids used in ENDS, while varying from manufacturer to manufacturer, typically contain glycerin, propylene glycol, nicotine and flavors, and are not combusted during product use. Several studies have shown that ENDS use generally results in significant reductions in biomarkers indicative of exposure to several HPHCs.[10–12] Particularly, switching from SMK to exclusive use of Vuse (VS) Digital Vapor Cigarette (ENDS product marketed by R. J. Reynolds Vapor Company) results in substantial reductions in biomarkers of nicotine exposure and the biomarkers of 22 carcinogens and toxicants found in cigarette smoke.[13] According to a 2018 report, "there is substantial evidence that—except for nicotine—exposure to potentially toxic substances from e-cigarettes is significantly lower compared with combustible cigarettes."[14] Furthermore, the report suggested that switching to sole use of noncombustible tobacco products, for example, ENDS, may present a significant harm-reduction opportunity to chronic smokers.[14]

There is limited information on the biological and potential health effects of ENDS usage relative to the adverse effects associated with chronic cigarette smoking. Due to the popularity of ENDS products, however, studies are being conducted to examine the potential human health impact of ENDS usage (reviewed elsewhere[15]). In the absence of strong epidemiologic data associated with the ENDS product category in general, BoPH represent valuable tools in assessing the health effects of ENDS. The generally accepted BoPH associated with cigarette smoking (such as white blood cell count and isoprostanes) require several months of smoking abstinence (or switching to alternate products such as ENDS). In a recent study, we demonstrated that select arachidonic acid metabolites are useful BoPH to assess the short-term effects of switching SMK to noncombustible tobacco products. In particular, we found that urinary leukotriene $E_4$ and 2,3-dinor-thromboxane $B_2$ levels rapidly decline in smokers who switched to VS products to levels seen in nonsmokers.[16]

Global profiling technologies such as metabolomics have been used to assess BoPH and individual health effects in SMK. For example, metabolomic profiling was used to characterize biochemical changes and identify BoPH following smoking cessation or smokeless tobacco use.[17–19] In this study, we investigated global biochemical changes in SMK switching from a combustible tobacco product to either of two VS products over a 5-day period. A baseline was first established during the initial 2 days of usual brand cigarette use, with subjects switching to the exclusive use of an ENDS ad libitum for the remainder of study. The comparative analysis of plasma and urinary metabolomic profiles in SMK switched to VS products revealed a reduced xenobiotic load, lower oxidative stress, and potential beneficial changes in vitamin metabolism within 5 days of switching. Taken together, our data suggest the ability of key metabolites to differentiate combustible and noncombustible tobacco product users. As such, these metabolites might serve as useful BoPH to assess the short-term health effects of ENDS products.

## Methods

### Study Design

The Vuse Digital Vapor cigarettes (ENDS) are first-generation cigalike products, and the e-liquid that used this system is composed of propylene glycol, glycerin, nicotine, flavorings, and water.[13] Clinical study design and conduct of the product-switching study were previously described.[13] This study, which was approved by Chesapeake Institutional Review Board (Columbia, Maryland) in November 2014, was conducted in accordance with the principles of the Declaration of Helsinki. It was registered on www.clinicaltrials.gov on December 2014, and its identifier number is NCT02323438. Generally healthy males and females, 21–60 years of age, inclusive, who reported smoking at least 10 combustible, filtered, menthol, or non-menthol cigarettes per day and reported smoking their first cigarette within 30 minutes of waking were included in the study. Written informed consent was obtained from all the subjects in the study.

Briefly, the study was a single-center, randomized, controlled, switching, open-label, parallel cohort study in which SMK were enrolled and randomized to one of three cohorts switched from 2 days of usual brand cigarette use to 5 days of ad libitum use of either VS Original flavor, nicotine gum, or VS Menthol flavor. Both VS brand styles contain approximately 600 µL of a 4.8% nicotine e-liquid or approximately 29 mg of nicotine. Nicorette nicotine polacrilex gum (GlaxoSmithKline Consumer Healthcare, LP, Philadelphia, PA) was commercially available, and a 4-mg strength nicotine gum was chosen in this study. Smokers of non-menthol cigarettes were randomized to VS Original or nicotine gum. Smokers of menthol cigarettes were randomized to VS Menthol or nicotine gum. Cigarettes per day at baseline were similar across the cohorts, ranging from means of 14.0–14.5. The mean daily amounts of e-liquid used by the VS groups increased from day 1 to day 3, and then the amounts used on days 3, 4, and 5 were relatively consistent. By day 4, the mean gram of e-liquid used was approximately 0.43 ± 0.32 for VS Original group and 0.44 ± 0.32 for VS Menthol group.

Plasma and urine samples collected from SMK who switched to ENDS products (VS Original flavor or VS Menthol flavor) were used for metabolomic profiling studies described herein. Plasma was collected at approximately 07:00 AM (before product use began each day) on study days −2 (baseline) and 5 (post-switching) after overnight fasting and abstinence from tobacco product use. Urine samples were collected for 24-hour periods starting at 07:30 PM on days −3 and 4. Plasma and urine samples were stored at −70°C while awaiting shipment to Metabolon Inc (Cary, NC) for metabolomic analysis.

Samples from the subjects who had been switched to nicotine gum were not used in metabolomic profiling because the scope of post hoc analyses was limited to comparison of the effects of cigarette smoking to the usage of Vuse products.

### Metabolomics

Sample processing and analysis was carried out at Metabolon Inc (Cary, NC) as previously described.[20] Briefly, samples were divided into five fractions for nontargeted mass spectrometry analysis using ultra-high-performance liquid chromatography–tandem mass spectrometry. Metabolites were identified by automated comparison of the ion features in the experimental samples to a reference library of chemical standard entries that included retention time, molecular weight (*m/z*), preferred adducts, and in-source fragments as well as associated mass spectrometry spectra and curated by visual inspection for quality control using software developed at Metabolon Inc.[21]

### Statistical Analysis

The matched-pairs *t*-test was used to determine the statistical significance (*p* value) of metabolite mean differences between comparator groups (baselines and post-switching). False discovery rates, estimated by *q* values, were used to control the type I error. In our analysis, the significantly upregulated or downregulated metabolites were defined based on three selections including (1) $p < .05$ and $q < .05$; (2) % filled values (defined as the percentage of the metabolite detected among all the samples using Metabolon platform, eg, 10% filled value means that the metabolite was not detected among 10 samples out of 100 total) ≥ 90%; and (3) fold ratio > 1.1 or < 0.9 (the ratio of relative abundance of the identified metabolites between 5-day post-switching and baselines). Random forest analyses—a supervised classification technique based on an ensemble of decision trees[22]—were performed to develop classification models. ArrayStudio version 5.0 (OmicSoft, Cary, NC) was used to perform *t*-test, and R program (http://cran.r-project.org/) was used to conduct random forest analysis.

## Results

### Global Metabolomic Alterations in Smokers Switched to ENDS

Metabolic profiling was performed using plasma and urine collected from SMK at baseline and 5 days after switching to the ENDS products to characterize the global metabolomic changes in SMK switched to VS products. A total of 698 and 846 metabolites were identified in urine and plasma, respectively (mass-normalized data are included in Supplementary Files S1 and S2). In SMK switched to VS Original, 78 metabolites were significantly upregulated and 48 were downregulated in urine (Supplementary Table S1). In plasma, 72 metabolites were significantly upregulated and 63 were downregulated. In SMK switched to VS Menthol, 96 metabolites were significantly upregulated, whereas 69 metabolites were downregulated in urine. In plasma, 91 metabolites were significantly upregulated, and 62 metabolites were downregulated. These differentiating metabolites belong to amino acid, carbohydrate, cofactors and vitamins, energy, lipid, nucleotide, and peptide metabolic pathways. In addition, many metabolites were mapped to xenobiotic metabolic pathways (Supplementary Table S1).

Random forest classification showed excellent predictions of VS product usage based on the baseline (smoking) and post-switch (ENDS use) metabolomic profiles in both urine and plasma. For VS Original, 36 of 37 (36/37) smoking and 37/37 ENDS use urine samples were accurately identified with 98.65% accuracy and 1.35% out-of-bag error rates using top 30 differentiating metabolites (Table 1, Supplementary Figure S1A). For VS Menthol, 36/38 smoking and 38/38 ENDS use samples were accurately predicted in urine (Supplementary Figure S1B). The corresponding out-of-bag error rate and accuracy for VS Menthol are 2.63% and 97.37%, respectively. Similarly, random forest models were highly predictive for plasma metabolite data for VS Original and VS Menthol (Supplementary Figure S2). Random forest importance plots identified 30 similar metabolites as key contributors to differentiate ENDS use from smoking in urine and plasma (Table 1 for VS Original and Supplementary Table S2 for VS Menthol), with sulfated and nicotine-derived metabolites exerting highest influence on classification regardless of cohort or matrix. We excluded nicotine and its metabolites from the data sets from urine and plasma and repeated the random forest analyses. These analyses led to similar highly predictive models with prediction accuracy > 98% and most of top 30 metabolites were sulfated metabolites, that is, xenobiotics (Table 1, Supplementary Table S2, and Supplementary Figures S1 and S2).

### Biochemical Pathways Affected in Smokers Switched to ENDS

As described above, global metabolomic profiling identified a large number of statistically significant metabolites in SMK who had switched to VS Original and VS Menthol products. In the following sections, we present differences in specific biochemical pathways indicative of exposure (nicotine and other exposures) to the VS products and potential biological effects from switching to VS products.

### Exposure to Nicotine and Its Metabolites

Metabolomic profiling detected nicotine and six nicotine metabolites in urine (Table 2). A significant reduction in levels of nicotine and its metabolites was observed in the urine of SMK switched to either VS Original or VS Menthol for 5 days. Relative declines ranged from 35% to 67% in urinary cotinine, hydroxycotinine, cotinine N-oxide, 3-hydroxycotinine glucuronide, norcotinine, nornicotine, and nicotine. Consistent with urinary changes, alterations in nicotine metabolism were observed in the plasma of SMK switched to either VS Original or VS Menthol. Specifically, plasma levels of nicotine metabolites (cotinine, hydroxycotinine, cotinine N-oxide, 3-hydroxycotinine glucuronide) significantly decreased (24%–43%) in SMK switched to either VS Original or VS Menthol. However, nicotine and two nicotine metabolites (nornicotine and norcotinine) were undetectable in plasma.

### Xenobiotic Metabolites Are Decreased in Smokers Switched to VS Products

We examined the impact of short-term switching from combustible to VS ENDS products on the plasma and urinary levels of nonnicotine xenobiotic-derived metabolites. We found that switching SMK to VS for 5 days resulted in significant alterations in the levels of a number of metabolites that were derived from cigarette smoke. Also, given that many of the parent compounds of the differentiating metabolites exist in food or produced through microbial metabolism, the differences in the metabolite levels could reflect those sources as well.

**Table 1.** Top 30 Differentiating Metabolites Identified From Random Forest Analyses in Urine and Plasma of Non-menthol Smokers Switched to VS Original Product

| | Urine | | | | Plasma | | | |
|---|---|---|---|---|---|---|---|---|
| | Random forest of all identified metabolomic data | | Random forest of all metabolomic data excluding nicotine and its metabolites | | Random forest of all identified metabolomic data | | Random forest of all metabolomic data excluding nicotine and its metabolites | |
| No. | Metabolite | MDA[a] | Metabolite | MDA | Metabolite | MDA | Metabolite | MDA |
| 1 | 2-Ethylphenylsulfate | 72.6 | 2-Ethylphenylsulfate | 73.7 | 2-Ethylphenylsulfsate | 69.3 | 2-Ethylphenylsulfate | 69.9 |
| 2 | *o*-Cresol sulfate | 71.2 | *o*-Cresol sulfate | 72.5 | *o*-Cresol sulfate | 64.0 | *o*-Cresol sulfate | 64.5 |
| 3 | S-(3-Hydroxypropyl)-mercaptunic acid | 64.9 | S-(3-Hydroxypropyl)-mercaptunic acid | 66.8 | Isoeugenol sulfate | 62.0 | Isoeugenol sulfate | 62.2 |
| 4 | Isoeugenol sulfate | 56.3 | Isoeugenol sulfate | 57.7 | Methylnaphthyl sulfate (2) | 57.4 | Methylnaphthyl sulfate (2) | 57.9 |
| 5 | Syringol sulfate | 52.3 | Syringol sulfate | 53.0 | Methylnaphthyl sulfate (1) | 52.3 | Methylnaphthyl sulfate (1) | 52.4 |
| 6 | 2,3-Dihydroxyisovalerate | 49.5 | 2,3-Dihydroxyisovalerate | 51.0 | Homocitrulline | 46.0 | Homocitrulline | 46.6 |
| 7 | Umbelliferone sulfate | 48.1 | Umbelliferone sulfate | 49.6 | 4-Hydroxycoumarin | 38.7 | 4-Hydroxycoumarin | 39.3 |
| 8 | Cotinine N-oxide | 41.7 | Argininate | 34.5 | 4-Hydroxyphenyl acetylglutamine | 37.9 | 4-Hydroxyphenyl acetylglutamine | 38.5 |
| 9 | Nornicotine | 40.5 | 4-Hydroxycoumarin | 33.7 | 5-Hydroxylysine | 37.2 | 5-Hydroxylysine | 37.5 |
| 10 | Hydroxycotinine | 40.3 | Galactosylglycerol | 31.5 | Cotinine N-oxide | 30.9 | Octadecanedioylcarnitine (C18-DC) | 28.7 |
| 11 | Norcotinine | 34.2 | Mandelate | 30.7 | Cotinine | 28.8 | Trimethylamine N-oxide | 28.0 |
| 12 | Argininate | 33.1 | Cytosine | 30.3 | Octadecanedioylcarnitine (C18-DC) | 28.2 | 2,3-Dihydroxyisovalerate | 25.8 |
| 13 | Cotinine | 31.9 | Uridine | 28.5 | Trimethylamine N-oxide | 27.7 | Daidzein sulfate (2) | 25.0 |
| 14 | 4-Hydroxycoumarin | 31.6 | 4-Ethylphenol glucuronide | 28.4 | 2,3-Dihydroxyisovalerate | 25.3 | S-Methylcysteine | 24.8 |
| 15 | Mandelate | 30.0 | S-(2-Carboxyethyl)-mercapturic acid | 28.4 | Daidzein sulfate (2) | 24.2 | Linoleoylcarnitine (C18:2) | 23.1 |
| 16 | Galactosylglycerol | 29.5 | 4-Ethylphenyl sulfate | 23.5 | S-Methylcysteine | 23.8 | Mannitol/sorbitol | 22.3 |
| 17 | Cytosine | 28.1 | N-Methylalanine | 22.7 | Linoleoylcarnitine (C18:2) | 23.3 | 2-Aminoheptanoate | 21.6 |
| 18 | Uridine | 27.7 | Succinylglutamine | 22.7 | 2-Aminoheptanoate | 22.0 | Umbelliferone sulfate | 20.8 |
| 19 | S-(2-Carboxyethyl)-mercapturic acid | 27.2 | Naringenin 7-glucuronide | 22.6 | Mannitol/sorbitol | 21.8 | 5-Oxoproline | 20.7 |
| 20 | 3-Hydroxycotinine glucuronide | 26.8 | Anserine | 21.8 | Alliin | 20.2 | Alliin | 20.5 |
| 21 | 4-Ethylphenol gucuronide | 26.6 | 7-Hydroxyindole sulfate | 21.0 | Umbelliferone sulfate | 20.1 | 4-Ethylphenyl sulfate | 18.9 |
| 22 | 4-Ethylphenyl sulfate | 22.8 | 2-Isopropylmalate | 20.9 | 5-Oxoproline | 19.9 | 1-(1-Enyl-palmitoyl)-2-palmitoyl-GPC (P-16:0/18.2) | 18.7 |
| 23 | Succinylglutamine | 21.3 | 4-Vinylphenol sulfate | 20.5 | 4-Ethylphenyl sulfate | 18.7 | 3-Methyl catechol sulfate (2) | 18.1 |
| 24 | N-Methylalanine | 20.9 | N-Octanoylglutamine | 18.2 | 1-(1-Enyl-palmitoyl)-2-palmitoyl-GPC | 18.1 | 3-Methyl catechol sulfate (1) | 18.0 |
| 25 | 4-Vinylphenol sulfate | 20.9 | Hexanoylglutamine | 17.7 | 3-Methyl catechol sulfate (2) | 17.5 | Piperine | 16.5 |
| 26 | Naringenin 7-glucuronide | 20.7 | N-Acetylalliin | 17.4 | 3-Methyl catechol sulfate (1) | 17.3 | Genistein sulfate | 16.5 |
| 27 | Anserine | 20.3 | 4-Acetylphenol sulfate | 17.1 | Genistein sulfate | 16.4 | 2-Oxoarginine | 16.4 |
| 28 | 2-Isopropylmalate | 19.5 | N1-Methyl-2-pyridone-5-carboxamide | 17.1 | Piperine | 16.2 | 3-Methoxytyramine sulfate | 15.7 |
| 29 | 7-Hydroxyindole sulfate | 19.5 | Daidzein | 16.0 | 2-Oxoarginine | 16.0 | 2-Aminooctanoate | 15.4 |
| 30 | Hexanoylglutamine | 18.8 | Furnaeol sulfate | 15.8 | 3-Methoxytyramine sulfate | 15.6 | 4-Vinylphenol sulfate | 15.3 |

[a]MDA is the mean decrease in accuracy computed from random forest analysis. The larger MDA is, the more important the variable is for classification of the data.

**Table 2.** Relative Levels of Nicotine and Its Metabolites in Smokers Switched to VS Original and VS Menthol Products

| | Non-menthol smokers switched to VS Original | | | | Menthol smokers switched to VS Menthol | | | |
|---|---|---|---|---|---|---|---|---|
| | Urine | | Plasma | | Urine | | Plasma | |
| Biochemical name | Adjusted *p* | Percentage change from baseline | Adjusted *p* | Percentage change from baseline | Adjusted *p* | Percentage change from baseline | Adjusted *p* | Percentage change from baseline |
| Cotinine | <.001 | −36.2 | <.001 | −33.8 | <.001 | −38.2 | <.001 | −33.4 |
| Hydroxycotinine | <.001 | −45.3 | <.001 | −24.1 | <.001 | −49.1 | <.001 | −27.9 |
| Cotinine N-oxide | <.001 | −35.4 | <.001 | −38.3 | <.001 | −47.8 | <.001 | −42.9 |
| 3-Hydroxycotinine glucuronide | <.001 | −42.6 | <.001 | −29.3 | <.001 | −48.2 | <.001 | −23.6 |
| Norcotinine | <.001 | −48.6 | NA | NA | <.001 | −67.0 | NA | NA |
| Nornicotine | <.001 | −46.2 | NA | NA | <.001 | −55.4 | NA | NA |
| Nicotine | .023 | −18.5 | NA | NA | <.001 | −39.9 | NA | NA |

NA = not applicable because some metabolites were not identified in plasma samples that were collected following overnight abstinence from tobacco product use by metabolomic profiling.

The levels of the biomarkers of tobacco exposure, S-(3-hydroxypropyl) mercapturic acid (S-3-HPMA) and S-(2-carboxyethyl) mercapturic acid (metabolites of acrolein), were significantly reduced (67% and 45%, respectively) in the urine of VS Original users after switching. The decrease in HPMA levels was also substantially lower in the urine of VS Menthol users (72% and 55%, respectively). The levels of several benzoate metabolites and plant-derived metabolites showed declines upon switching SMK to VS products (Table 3). Significant declines in 2-ethylphenylsulfate (>90%), *o*-cresol sulfate (>80%), isoeugenol sulfate (>70%), and 4-hydroxycoumarin (>25%) were detected in urine and plasma of VS Original and VS Menthol users at 5 days of product use, relative to the baseline. Several of the benzoate and plant-derived metabolites showed consistent decreases in plasma samples from baseline to 5 days of VS product use, consistent with the urine data.

Among the metabolites that showed significant increases upon switching to VS products was plasma thymol sulfate in non-menthol SMK (but not in menthol SMK), which is derived from thymol used as a food additive. Interestingly, its increase in plasma is accompanied by significant decrease in urinary levels. In addition, significant increase in urinary levels of 3-(3-hydroxyphenyl) propionate sulfate (HMDB ID: 0000375), which is a plant-derived metabolite, was observed in the SMK who were switched to VS products.

### BoPH Changes in Vitamin Metabolism and Oxidative Stress Metabolism

Cigarette smoking is associated with depletion of micronutrients, eg, vitamins and free-radical scavenging metabolites essential to the body's antioxidant defense mechanisms.[23–25] Examination of plasma metabolome following product-switching revealed alterations in circulating levels of several micronutrients (Table 4). For example, the metabolomic profile in SMK switched to VS Original indicated increased urinary levels of vitamin $B_2$, vitamin C (dehydroascorbate and oxalate), and vitamin $B_6$ (pyridoxate). Plasma metabolomic profiles of VS Original users and VS Menthol users also showed increases in vitamin C (threonate) and vitamin A (retinol and carotene diols) levels. Improvements in plasma glutathione metabolism and purine metabolism (hypoxanthine and urate) were also evident in the VS Original users relative to baseline smoking.

These BoPH also showed marked improvements in VS Menthol users compared with the levels found at baseline smoking (Table 4). Urinary levels of vitamin $B_2$ and the vitamin C metabolites, ascorbate and oxalate, were higher upon switching to VS Menthol. Plasma levels of oxalate and threonate (ascorbate metabolism) were higher in VS Menthol users, whereas the plasma levels of glutathione metabolism and purine metabolism were lower compared with baseline smoking.

By comparison, urinary levels of vitamin $B_2$ (riboflavin), vitamin C-related metabolites (dehydroascorbate and oxalate), and the vitamin $B_6$-associated metabolite (pyridoxate) were higher following product switching to either VS Original or VS Menthol. Moreover, switching to either VS product culminated in reduced urinary levels of hypoxanthine and urate (purine metabolites), which exhibit free-radical scavenging activity.

## Discussion

ENDS are a diverse and relatively new category of tobacco products, and there is limited information on the biological/health effects of their use by consumers. In this study, we have used metabolomic profiling to differentiate the effects of short-term use of two VS ENDS products (VS Original and VS Menthol) from chronic cigarette smoking. Key findings from these analyses are as follows: (1) reduced exposure to nicotine and its metabolites, (2) reduced xenobiotic exposure to other cigarette smoke constituents, and (3) improved vitamin metabolism and reduced oxidative stress.

Global urine and plasma profiles revealed several statistically significant changes upon switching SMK to VS products (Supplementary Table 1). These differentiating metabolites were able to differentiate smoking from VS use in random forest analyses (Table 1, Supplementary Table S2, and Supplementary Figures S1 and S2). Metabolomic profiling detected nicotine and six of its metabolites in urine and four metabolites were detected in plasma, reflecting their short half-lives and fasting conditions under which blood collections were made (Table 2). Significant declines in nicotine and its metabolites were observed in VS Original and VS Menthol use, relative to baseline smoking. These results are consistent with the quantitative measurements of nicotine exposure from a previous study.[13]

Several investigators also have reported significant declines in HPHC biomarkers in SMK who switched to ENDS use.[11,12,26]

**Table 3.** Relative Levels of Non-nicotine-Derived Xenobiotic Metabolites in Smokers Switched to VS Original and VS Menthol Products

| | | Non-menthol smokers switched to VS Original | | | | Menthol Smokers switched to VS Menthol | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Urine | | Plasma | | Urine | | Plasma | |
| Biochemical name | Metabolic pathway | Adjusted *p* | Percentage change from baseline | Adjusted *p* | Percentage change from baseline | Adjusted *p* | Percentage change from baseline | Adjusted *p* | Percentage change from baseline |
| 2-Ethylphenylsulfate | Benzoate metabolism | <.001 | −93.6 | <.001 | −90.7 | <.001 | −95.1 | <.001 | −90.7 |
| *o*-Cresol sulfate | Benzoate metabolism | <.001 | −80.1 | <.001 | −81.4 | <.001 | −84.8 | <.001 | −87.8 |
| Isoeugenol sulfate | Food component/ plant | <.001 | −74.5 | <.001 | −80.5 | <.001 | −70.7 | <.001 | −74.7 |
| Umbelliferone sulfate | Food component/plant | <.001 | −56.9 | .006 | −2.9 | <.001 | −55.1 | .096 | 29.6 |
| 2,3-Dihydroxyisovalerate | Food component/plant | <.001 | −41.0 | <.001 | −25.3 | <.001 | −20.0 | .039 | −1.6 |
| 3-Methyl catechol sulfate (2) | Benzoate Metabolism | <.001 | −36.2 | <.001 | −38.9 | <.001 | −55.5 | <.001 | −48.7 |
| 3-Methyl catechol sulfate (1) | Benzoate metabolism | <.001 | −33.5 | <.001 | −40.1 | <.001 | −49.8 | <.001 | −55.1 |
| 4-Hydroxycoumarin | Tobacco smoke metabolism | <.001 | −27.2 | <.001 | −53.6 | <.001 | −42.9 | <.001 | −40.0 |
| 4-Allylphenol sulfate | Other xenobiotic | <.001 | −24.8 | .312 | 5.4 | <.001 | −22.2 | .26 | 13.1 |
| 4-Vinylguaiacol sulfate | Other xenobiotic | .001 | −22.4 | <.001 | −25.6 | .002 | −7.5 | .005 | 18.1 |
| Pyrraline | Other xenobiotic | .003 | −15.9 | .295 | 22.5 | <.001 | −18.5 | .29 | 20.0 |
| Thymol sulfate | Other xenobiotic | .003 | −12.5 | .025 | 1134.6 | <.001 | −37.2 | .46 | 110.7 |
| 2,8-Quinolinediol sulfate | Other xenobiotic | .017 | −12.2 | .306 | 33.6 | .028 | −7.8 | .41 | 31.3 |
| 3-Hydroxypyridine sulfate | Chemical | .019 | −9.8 | <.001 | −26.0 | <.001 | −26.3 | <.001 | −21.6 |
| 3-Methoxycatechol sulfate (2) | Benzoate metabolism | .021 | −6.7 | .015 | −7.8 | <.001 | −26.9 | .029 | −9.7 |
| 2,3-Dihydroxypyridine | Other xenobiotic | .024 | 2.4 | .007 | −13.3 | <.001 | −24.3 | .012 | −11.5 |
| 4-Vinylphenol sulfate | Benzoate metabolism | .055 | 3.7 | .236 | 33.9 | <.001 | −27.6 | .003 | 5.5 |
| Hydroquinone sulfate | Topical agents | .256 | 4.5 | .049 | 8.4 | .043 | −3.9 | .014 | 15.4 |
| 3-(3-Hydroxyphenyl)propionate sulfate | Benzoate metabolism | .033 | 84.8 | .378 | 71.9 | .003 | 94.2 | .45 | 103.5 |
| 3-Acetylphenol sulfate | Other xenobiotic | .095 | 161.4 | .011 | −9.9 | .155 | 64.8 | <.001 | −25.1 |
| S-(3-Hydroxypropyl)mercapturic acid | Tobacco smoke | <.001 | −67 | NA | NA | 0 | −72 | NA | NA |
| S-(2-Carboxyethyl)mercapturic acid | Tobacco smoke | <.001 | −45 | NA | NA | <.001 | −55 | NA | NA |

NA = not applicable because the metabolite is not identified in urine or plasma.

**Table 4.** Relative Levels of Metabolites Involved in Vitamin Metabolism and Oxidative Stress in Smokers Switched to VS Original and VS Menthol Products

| | | Non-menthol smokers switched to VS Original | | | | Menthol smokers switched to VS Menthol | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Urine | | Plasma | | Urine | | Plasma | |
| Metabolic pathway | Biochemical name | Adjusted *p* | Percentage change from baseline | Adjusted *p* | Percentage change from baseline | Adjusted *p* | Percentage change from baseline | Adjusted *p* | Percentage change from baseline |
| Riboflavin metabolism | Riboflavin (Vitamin B2) | <.001 | 108.0 | NA | NA | <.001 | 117.0 | NA | NA |
| Ascorbate and aldarate metabolism | Ascorbate (Vitamin C) | .32 | 1168.0 | NA | NA | .121 | 1688.0 | NA | NA |
| | Dehydroascorbate | .008 | 311.0 | NA | NA | .003 | 1048.0 | NA | NA |
| | Oxalate (ethanedioate) | .035 | 65.0 | .195 | 107.0 | .013 | 53.0 | .03 | 21.0 |
| | Threonate | .36 | 4.0 | .023 | 21.0 | .407 | 2.0 | <.001 | 32.0 |
| Vitamin B6 metabolism | Pyridoxate | .025 | 34.0 | .213 | 13.0 | .029 | 34.0 | .207 | 22.0 |
| Vitamin A metabolism | Retinol (vitamin A) | NA | NA | .042 | 7.0 | NA | NA | <.001 | 13.0 |
| | Carotene diol (1) | NA | NA | <.001 | 18.0 | NA | NA | <.001 | 25.0 |
| | Carotene diol (2) | NA | NA | .246 | 4.0 | NA | NA | .046 | 13.0 |
| | Carotene diol (3) | NA | NA | <.001 | 130.0 | NA | NA | <.001 | 31.0 |
| Glutathione metabolism | 2-Aminobutyrate | NA | NA | .016 | −9.0 | NA | NA | .008 | −10.0 |
| | Cysteinylglycine | .265 | 62.0 | .055 | −6.0 | .17 | 18.0 | .001 | −15.0 |
| | 5-Oxoproline | .337 | 1.0 | <.001 | −14.0 | .093 | −4.0 | .027 | −6.0 |
| | Cysteine-glutathione disulfide | .265 | 62.0 | .012 | −9.0 | .127 | 18.0 | .008 | −15.0 |
| Y-Glutamyl amino acid | Y-Glutamylglutamate | NA | NA | .027 | −8.0 | NA | NA | .089 | −4.0 |
| | Y-Glutamylglutamine | NA | NA | .011 | −7.0 | NA | NA | .004 | −7.0 |
| | Y-Glutamylglycine | .080 | −4.0 | <.001 | −12.0 | .131 | −2.0 | <.001 | −10.0 |
| Purine metabolism | Hypoxanthine | .012 | 33.0 | .002 | −17.0 | .087 | 18.0 | <.001 | −23.0 |
| | Urate | .007 | 11.0 | <.001 | 7.0 | .057 | 8.0 | .004 | 6.0 |

NA = not applicable because the metabolite is not identified in urine or plasma.

Consistent with these results, metabolomic profiling also revealed that metabolites of several cigarette smoke constituents/toxicants also decreased after 5 days of VS use. These included metabolites of acrolein, 4-hydroxycoumarin, and other smoke constituents. Although a majority of the metabolites declined following 5 days of VS product use, two compounds—thymol sulfate and 3-(3-hydroxyphenyl) propionate sulfate—increased significantly (Table 3). Thymol is a natural compound and food additive, whereas 3-(3-hydroxyphenl) propionate sulfate is a metabolite derived from 3-(3-hydroxyphenyl) propanoic acid (HMDB ID: 0000375) (a metabolite of caffeic acid, present in coffee, vegetables, and fruits). Pyrraline (HMDB ID: 0033143), which appears to be food-derived product, also declined following VS usage. Thus, metabolomic profiling demonstrates declines in several xenobiotic-derived metabolites in short-term switching SMK to VS products. Targeted analyses of biomarkers of exposure revealed that switching SMK to the VS products significantly reduces HPHC exposure.[13] Collectively, our findings indicate an overall reduced toxicant/xenobiotic exposure in VS Original and VS Menthol users.

The differentiating metabolites between baseline smoking and 5-day usage of VS products belonged to several different classes of biochemicals (Supplementary Table 1). In addition to xenobiotics, several lipid metabolites including glycerophoshphocholines and acyl carnitines were also important in differentiating the effects of smoking and ENDS use in Random Forest analyses (Table 1 and Supplementary Table 2). Carnitine plays a central role in transport of fatty acids to mitochondria for β-oxidation[27] and increased levels of acyl carnitines are reported to be associated with increased risk of cardiovascular death and other diseases.[28] Lipidomic analyses also have revealed differences in lung tissue lipid profiles in mice exposed to different tobacco products,[29] and serum of current smokers, former smokers, and chronic obstructive pulmonary disease patients.[30]

Chronic cigarette smoking has been shown to cause depletion of micronutrients such as vitamins, which contributes to increased oxidative stress in SMK. Metabolomic profiling of chronic SMK, moist snuff consumers, and nonsmokers also revealed differences in vitamin levels and select metabolites indicative of oxidative stress status.[18]

Vitamin C, a key antioxidant micronutrient, is consistently reduced in SMK compared with nonsmokers.[31] In the current study, there was an increase in circulating and excreted levels of vitamin C-related metabolites. Specifically, plasma threonate and oxalate levels were elevated in SMK switched to VS Menthol, and plasma threonate levels were increased in SMK switched to VS Original. Urinary dehydroascorbate and oxalate were increased in SMK switched to either VS Original or VS Menthol.

Vitamin B family members with antioxidant activity—folic acid, vitamin B2 (riboflavin), and vitamin B6 (pyridoxine)—are reduced in SMK compared with nonsmokers.[32,33] In this study, urinary levels of Vitamin B2- and B6-associated metabolite (pyridoxate) were increased following product switching to either VS Original or VS Menthol. Vitamin B2 acts as a cofactor for glutathione (GSH) reductase.[34] In addition, Vitamin B6 contributes to cellular antioxidant defense as a cofactor for enzymes that convert homocysteine to cysteine, the rate-limiting substrate for GSH synthesis.[35] SMK switched to either VS Original or VS Menthol were found to have reduced levels of circulating GSH metabolites. These data are consistent with the finding that GSH-related urinary metabolites, which are markers of oxidative stress, are reduced in SMK switched to ENDS products. Carotenoids, which are precursors of Vitamin A, are also depleted in cigarette smokers.[36] In SMK switched to VS Original and VS Menthol products, we found increased plasma levels of Vitamin A (retinol) and its carotene diol metabolites; however, no differences in urinary levels were detected.

Currently, there is limited information on the BoPH for ENDS use. A recent publication evaluated BoPH in the lungs of ENDS users, conventional combusted cigarette smokers and never tobacco users in a cross-sectional study.[37] Using bronchioalveolar lavage and brushings, lung BoPH were assessed by cell counts, cytokines, transcriptomics, and global methylation. Overall, the authors concluded that e-cigarettes are associated with less toxicity than cigarettes for smoking-related pathways.

The strengths of this study are the assessment of global plasma and urine metabolomic changes within subjects at baseline and after exclusive use of VS products. Global profiling results are consistent and reveal reductions in the levels of select HPHC and several other cigarette smoke toxicants upon switching to the ENDS products, and are consistent with the findings from previous work.[13]

This study also has several limitations. First, this study lacks a comparator group of a smoking abstinence cohort, which is necessary to determine whether smoking abstinence and product switching would lead to qualitatively and quantitatively similar changes. Second, the short duration of Vuse ENDS use, following switching from smoking, needs to be considered while interpreting the metabolomic data, as the smokers adapt to using the Vuse products. And, a comprehensive diet history of study subjects prior to enrollment (baseline smoking) was not available, and additionally, diet was not controlled in the study; subjects were provided non-mutagenic diet during the clinical confinement. In this article, because we considered only those metabolites with ≥90% fill (metabolites detected in 90% or more of the study subjects), it is less likely that diet history would be a major factor in confounding metabolite profiles reported herein.

Given the diversity of ENDS products, some have reported increased inflammation in ENDS users, relative to nontobacco users.[38] Furthermore, there was an outbreak of e-cigarette, or vaping, product use-associated lung injury, which was largely associated with the use of tetrahydocannabinol.[39] It is important to emphasize that our findings are specific to the investigated study products, and generalization across the ENDS category requires careful consideration of the product characteristics.

In conclusion, short-term switching of SMK to VS Original and VS Menthol was characterized by reduced toxicant/xenobiotic load and potential improvement in vitamin metabolism and antioxidant defense pathways.

## 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.

**Supplementary Table S1.** Summary of significantly different metabolites identified in urine and plasma of smokers switched to VS Original and VS Menthols products.

**Supplementary Table S2.** Top 30 differentiating metabolites identified from random forest analyses in urine and plasma of menthol smokers switched to VS Menthol product.

**Supplementary Figure S1. Random forest analysis of urinary metabolomics profiles upon switching from smoking to ENDS use.** The predictive accuracy of the random forest model built on the metabolomics data with (A) or exclusion of nicotine and its metabolites (C) for those switched to VS Original

product. The predictive accuracy unchanged with the inclusion or exclusion of nicotine metabolites. The predictive accuracy for VS Menthol with (B) and without nicotine and its metabolites (D) is comparable.

**Supplementary Figure S2. Random forest analysis of plasma metabolomics profiles upon switching from smoking to ENDS use.** The predictive accuracy of the random forest models built on plasma metabolomics with (A) or without nicotine and its metabolites (C) for VS Original users. The predictive accuracy for VS Menthol users remains comparable with (B) or without (D) the inclusion of nicotine and its metabolites in the analyses.

**Supplementary File S1.** List of all identified metabolites and their intensity data in urine samples collected from 5-day Vuse switching studies.

**Supplementary File S2.** List of all identified metabolites and their intensity data in plasma samples collected from 5-day Vuse switching studies.

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## Declaration of Interests

CJL and GLP are full-time employees of RAI Services Company. GL is a former employee of RAI Services Company. CRY is a full-time employee of Quinn Pharms. None of the authors have any other conflict of interest to declare.

## References

1. Gordon T, Kannel WB. Multiple risk functions for predicting coronary heart disease: the concept, accuracy, and application. *Am Heart J*. 1982;103(6):1031–1039.
2. Mattson ME, Pollack ES, Cullen JW. What are the odds that smoking will kill you? *Am J Public Health*. 1987;77(4):425–431.
3. Park KY, Chung CS, Lee KH, Kim GM, Kim YB, Oh K. Prevalence and risk factors of intracranial atherosclerosis in an asymptomatic Korean population. *J Clin Neurol*. 2006;2(1):29–33.
4. Rodgman A, Perfetti TA. *The Chemical Components of Tobacco and Tobacco Smoke*. Boca Raton, FL: CRC Press; 2016.
5. IARC Working Group on the Evaluation of Carcinogenic Risks to Humans. Personal habits and indoor combustions. Volume 100 E. A review of human carcinogens. *IARC Monogr Eval Carcinog Risks Hum*. 2012;100(Pt E):1–538.
6. Ganti S, Taylor SL, Kim K, et al. Urinary acylcarnitines are altered in human kidney cancer. *Int J Cancer*. 2012;130(12):2791–2800.
7. Zeller M, Hatsukami D. The strategic dialogue on tobacco harm reduction: a vision and blueprint for action in the US. *Tob Control*. 2009;18(4):324.
8. Benowitz NL. Emerging nicotine delivery products. Implications for public health. *Ann Am Thorac Soc*. 2014;11(2):231–235.
9. Yanbaeva DG, Dentener MA, Creutzberg EC, Wesseling G, Wouters EF. Systemic effects of smoking. *Chest*. 2007;131(5):1557–1566.
10. Lorkiewicz P, Riggs DW, Keith RJ, et al. Comparison of urinary biomarkers of exposure in humans using electronic cigarettes, combustible cigarettes, and smokeless tobacco. *Nicotine Tob Res*. 2019;21(9):1228–1238.
11. Goniewicz ML, Smith DM, Edwards KC, et al. Comparison of nicotine and toxicant exposure in users of electronic cigarettes and combustible cigarettes. *JAMA Netw Open*. 2018;1(8):e185937.
12. O'Connell G, Graff DW, D'Ruiz CD. Reductions in biomarkers of exposure (BoE) to harmful or potentially harmful constituents (HPHCs) following partial or complete substitution of cigarettes with electronic cigarettes in adult smokers. *Toxicol Mech Methods*. 2016;26(6):443–454.
13. Round EK, Chen P, Taylor AK, Schmidt E. Biomarkers of tobacco exposure decrease after smokers switch to an e-cigartette or nicotine gum. *Nicotine Tob Res*. 2019;21(9):1239–1247.
14. National Academies of Sciences, Engineering, and Medicine. *Public Health Consequences of E-Cigarettes*. Washington, DC: The National Academies Press; 2018.
15. Pisinger C, Døssing M. A systematic review of health effects of electronic cigarettes. *Prev Med*. 2014;69:248–260.
16. Makena P, Liu G, Chen P, Yates CR, Prasad GL. Urinary leukotriene E4 and 2,3-dinor thromboxane B2 are biomarkers of potential harm in short-term tobacco switching studies. *Cancer Epidemiol Biomarkers Prev*. 2019;28(12):2095–2105.
17. Goettel M, Niessner R, Mueller D, Scherer M, Scherer G, Pluym N. Metabolomic fingerprinting in various body fluids of a diet-controlled clinical smoking cessation study using a validated GC-TOF-MS metabolomics platform. *J Proteome Res*. 2017;16(10):3491–3503.
18. Prasad GL, Jones BA, Schmidt E, Chen P, Kennedy AD. Global metabolomic profiles reveal differences in oxidative stress and inflammation pathways in smokers and moist snuff consumers. *J Metabolomics*. 2015;1(1):2.
19. Liu G, Lee DP, Schmidt E, Prasad GL. Pathway analysis of global metabolomic profiles identified enrichment of caffeine, energy, and arginine metabolism in smokers but not moist snuff consumers. *Bioinform Biol Insights*. 2019;13. doi:10.1177/1177932219882961
20. Evans A, Bridgewater B, Liu Q, et al. High resolution mass spectrometry improves data quantity and quality as compared to unit mass resolution mass spectrometry in high- throughput profiling metabolomics. *Metabolomics*. 2014;4(2):132.
21. Dehaven CD, Evans AM, Dai H, Lawton KA. Organization of GC/MS and LC/MS metabolomics data into chemical libraries. *J Cheminform*. 2010;2(1):9.
22. Breiman L. Random Forests. *Mach Learn*. 2001;45(1):5–32.
23. Alberg A. The influence of cigarette smoking on circulating concentrations of antioxidant micronutrients. *Toxicology*. 2002;180(2):121–137.
24. Gu F, Derkach A, Freedman ND, et al. Cigarette smoking behaviour and blood metabolomics. *Int J Epidemiol*. 2016;45(5):1421–1432.
25. Eiserich JP, van der Vliet A, Handelman GJ, Halliwell B, Cross CE. Dietary antioxidants and cigarette smoke-induced biomolecular damage: a complex interaction. *Am J Clin Nutr*. 1995;62(6 Suppl):1490S–1500S.
26. Pulvers K, Emami AS, Nollen NL, et al. Tobacco consumption and toxicant exposure of cigarette smokers using electronic cigarettes. *Nicotine Tob Res*. 2018;20(2):206–214.
27. Sharma S, Black SM. Carnitine homeostasis, mitochondrial function, and cardiovascular disease. *Drug Discov Today Dis Mech*. 2009;6(1–4):e31–e39.
28. Strand E, Pedersen ER, Svingen GF, et al. Serum acylcarnitines and risk of cardiovascular death and acute myocardial infarction in patients with stable angina pectoris. *J Am Heart Assoc*. 2017;6(2):e003620.
29. Titz B, Szostak J, Sewer A, et al. Multi-omics systems toxicology study of mouse lung assessing the effects of aerosols from two heat-not-burn tobacco products and cigarette smoke. *Comput Struct Biotechnol J*. 2020;18:1056–1073.
30. Titz B, Luettich K, Leroy P, et al. Alterations in serum polyunsaturated fatty acids and eicosanoids in patients with mild to moderate chronic obstructive pulmonary disease (COPD). *Int J Mol Sci*. 2016;17(9):1583.
31. Wei W, Kim Y, Boudreau N. Association of smoking with serum and dietary levels of antioxidants in adults: NHANES III, 1988–1994. *Am J Public Health*. 2001;91(2):258–264.
32. Skeie E, Strand E, Pedersen ER, et al. Circulating B-vitamins and smoking habits are associated with serum polyunsaturated fatty acids in patients

with suspected coronary heart disease: a cross-sectional study. *PLoS One*. 2015;10(6):e0129049.
33. Ulvik A, Ebbing M, Hustad S, et al. Long- and short-term effects of tobacco smoking on circulating concentrations of B vitamins. *Clin Chem*. 2010;56(5):755–763.
34. Schulz GE, Schirmer RH, Pai EF. FAD-binding site of glutathione reductase. *J Mol Biol*. 1982;160(2):287–308.
35. Stocker P, Lesgards JF, Vidal N, Chalier F, Prost M. ESR study of a biological assay on whole blood: antioxidant efficiency of various vitamins. *Biochim Biophys Acta*. 2003;1621(1):1–8.
36. Preston AM. Cigarette smoking-nutritional implications. *Prog Food Nutr Sci*. 1991;15(4):183–217.
37. Song MA, Freudenheim JL, Brasky TM, et al. Biomarkers of exposure and effect in the lungs of smokers, nonsmokers, and electronic cigarette users. *Cancer Epidemiol Biomarkers Prev*. 2020;29(2): 443–451.
38. Singh KP, Lawyer G, Muthumalage T, et al. Systemic biomarkers in electronic cigarette users: implications for noninvasive assessment of vaping-associated pulmonary injuries. *ERJ Open Res*. 2019;5(4). doi: 10.1183/23120541.00182-2019
39. Krishnasamy VP, Hallowell BD, Ko JY, et al.; Lung Injury Response Epidemiology/Surveillance Task Force. Update: characteristics of a nationwide outbreak of e-cigarette, or vaping, product use-associated lung injury – United States, August 2019–January 2020. *MMWR Morb Mortal Wkly Rep*. 2020;69(3):90–94.