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Biomarkers of Exposure in ENDS Users, Smokers, and Dual Users of American Indian Descent

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Abstract

Objectives: We measured biomarkers of exposure among American Indian (AI) ENDS users, smokers, and dual users.

Methods: Urine was analyzed for total nicotine equivalents (TNE) and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL). Expired-air carbon monoxide (CO) was collected. Two analyses were performed. “CO analysis” included smokers and dual users whose CO was ≥ 6 ppm and ENDS users whose CO was < 6 ppm. “NNAL analysis” included smokers and dual users whose NNAL was ≥ 47.3 pg/mg, and ENDS users whose NNAL was < 47.3 pg/mg. Biomarkers were summarized by geometric means (GM) and compared with nonparametric tests.

Results: In both analyses, TNE was no different across the groups, and NNAL and CO were lower in ENDS users. In the NNAL analysis the GM of NNAL was 261.4, 6.1, and 228.0 pg/mg among smokers, ENDS users, and dual users ($p < .001$). Also in the NNAL analysis, the GM of CO was 14.7, 2.4, and 16.8 ppm among smokers, ENDS users, and dual users ($p < .001$).

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Human Subjects Statement

This study was approved by the University of Oklahoma Health Sciences Center (# 6317) and the Oklahoma City Indian Health Service Indian Health Service Institutional Review Boards (# P-16-01-OK).

Conflict of Interest Statement

None declared.

Conclusions: ENDS users did not differ in nicotine and had lower exposure to a lung carcinogen and a cardiovascular toxicant than smokers or dual users. Dual users and smokers did not differ in biomarker levels. Results should be used to inform tribal regulations and to educate the AI community on ENDS.

Keywords

ENDS; e-cigarette; biomarkers; American Indian; biomarkers

Electronic nicotine delivery systems (ENDS) represent a public health challenge. Although there is no standard definition, these devices, also referred to as electronic nicotine delivery systems and e-cigarettes, heat a solution often containing nicotine, the primary addictive chemical in tobacco, and flavorings to generate a vapor.^{1–3} Some can be bought as one-time, disposable products, whereas others can be bought as re-usable kits that use cartridges or e-liquid.^{4,5} As of 2014, there were 466 different brands and 7764 unique flavors of ENDS.⁶

Since 2009, the United States Food and Drug Administration Center for Tobacco Products (US FDA CTP) has been authorized to regulate tobacco products, such as cigarettes, smokeless tobacco, and roll-your-own tobacco, under The Family Smoking Prevention and Tobacco Control Act.⁷ In August of 2016, the US FDA CTP extended its authority to regulate all tobacco products, including ENDS.⁸ However, the variety and the rapid appearance of new products makes the development of regulatory standards for ENDS challenging. Moreover, ENDS use does not always occur in isolation. For example, approximately 60% of ENDS users also smoke cigarettes.⁹ Concurrent use of ENDS and cigarettes is often referred to as “dual use.” Assessments of the acute and long-term health effects of ENDS are needed to inform regulators and public health officials about the effects of ENDS on individual and population-level health.² Assessments should include both exclusive ENDS users and dual users, and be conducted in both general and minority populations. Prior studies, well summarized in systematic reviews,^{10,11} have examined levels of harmful or potentially harmful constituents (HPHC) in ENDS by measuring HPHC uptake in the actual users. Many of these studies have been short-term clinical trials, where cigarette smokers were randomized to ENDS, or among naïve ENDS users. As a result, levels of HPHC were likely influenced by recent exposure to cigarettes and/or inexperience with ENDS. The current investigation aims to expand on 3 earlier cross-sectional studies by quantifying select biomarkers of tobacco exposure among a sample of American Indian (AI) ENDS users, smokers, and dual users. Hecht et al examined nicotine and its major metabolite, cotinine, and several HPHC including 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol and its glucuronides (total NNAL) in the urine of exclusive ENDS users (N = 28).¹² Hecht et al observed lower levels of total NNAL in ENDS users when compared to cigarette smokers in prior studies.¹² Hecht et al also found nicotine and cotinine to be lower in ENDS users when compared to one prior study of cigarette smokers, but this difference was not observed when compared to another study of smokers.¹² Wagener et al examined levels of saliva cotinine, urinary total NNAL, and breath carbon monoxide (CO) in a small sample of smokers (N = 10) and ENDS users (N = 20).¹³ Whereas cotinine levels did not differ between the user groups, NNAL and CO levels were lower among ENDS users than smokers.¹³ Lastly, a study by Shahab et al showed that dual users of ENDS and cigarettes (N

= 36) were exposed to levels of toxins that were no different than exclusive cigarette smokers (N = 37).¹⁴ Despite the strengths of these studies, which include the measurement of HPHC in the actual users of ENDS, a limitation is that participants of all 3 studies were predominately non-Hispanic white or black, thus hindering the generalizability of their findings to other race and ethnic groups.

With a prevalence of cigarette smoking higher than any other major United States (US) racial group,¹⁵ American Indians/Alaska Natives (AI/AN) have high rates of smoking-related disease and death.^{16–19} Research has shown that AI/AN smokers metabolize nicotine more quickly than smokers from other race groups and this may result in greater toxicant and carcinogen exposure.²⁰ Little information exists on ENDS use among the AI/AN population. Few studies have shown that the prevalence of ENDS use is likely higher among AI/AN population than other race groups.^{21,22} To our knowledge, no data exist on levels of HPHC among ENDS users of AI/AN descent. To expand the previous work by Hecht et al, Wagener et al, and Shahab et al, the objective of the present study was to quantify and compare levels of select biomarkers of tobacco exposure among ENDS users, smokers, and dual users of AI descent.

METHODS

Study Overview

Community-based strategies, previously described,²³ were used to recruit adults of AI descent who were in one of 3 groups of current tobacco use: (1) current exclusive cigarette smokers; (2) current exclusive ENDS users; and (3) dual users of cigarettes and ENDS. Inclusion criteria for all participants were: AI race, having at least 2 biological grandparents of AI race, 18 to 65 years of age, and able to speak, read, and write English. Additional inclusion criteria for smokers were: smoked ≥ 5 cigarettes per day (CPD) in the past 3 months and in the past 24 hours, and had not used any other tobacco products in the past 3 months. Additional inclusion criteria for ENDS users were: used an ENDS every day for the past 3 months and in the past 24 hours, and had not used any other tobacco products in the past 3 months. Additional inclusion criteria for dual users included: smoked ≥ 5 CPD in the past 3 months, smoked in the past 24 hours, used an ENDS every day in the past 3 months, used an ENDS in the past 24 hours, and had not used any other tobacco products in the past 3 months. Exclusion criteria for all participants included the following: regular use of medications for seizures, tuberculosis, or cancer, current involvement in a tobacco cessation program; use of nicotine replacement therapy, pregnant or breastfeeding, use of illicit drugs in the 30 days prior to the study, use of alcohol or marijuana on the day of the study.

Data Collection

The informed consent process for all participants was the same and included a description of the nature, purpose, risks and benefits of the study and the participant's right to withdrawal. Only after written consent, participants completed a questionnaire, provided a measurement of carbon monoxide (CO; expressed as parts per million (ppm)) in exhaled breath using a Covita Smokerlyzer®,²⁴ and provided a spot urine sample. Urine collection, storage and shipment were in accordance with instructions provided by the Clinical Pharmacology

Laboratory at the University of California, San Francisco (UCSF) Tobacco Biomarkers Core.²⁵ LC-MS/MS was used to quantify total nicotine equivalents (TNE), which is the molar sum of nicotine and its metabolites (nicotine, nicotine glucuronide, cotinine, cotinine glucuronide, trans-3'-hydroxycotinine, trans-3'-hydroxycotinine glucuronide, nicotine N'-oxide, cotinine N-oxide, nornicotine, and norcotinine). TNE is considered the most comprehensive biomarker of nicotine exposure.^{26,27} Also quantified in urine was 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol and its glucuronides (total NNAL), a biomarker of uptake of the tobacco-specific lung carcinogen 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK). To account for the variability in urinary output, concentrations of TNE (nmol/ml) and NNAL (pg/ml) were adjusted for urine creatinine and expressed, respectively, as nmol/mg creatinine and pg/mg creatinine. Because studies use different units of measurement for total NNAL, we also summarize total NNAL in pmol/mg creatinine.

Data Analysis

For biomarker values below the limit of quantitation (LOQ) we imputed the values as LOQ / 2. As suggested for data with positively skewed distributions,²⁸ values of TNE and NNAL were summarized by geometric means and 95% confidence intervals (CI). Ranges and interquartile intervals (IQI) were also computed. Biomarker values were compared across the 3 nicotine user groups with the nonparametric Kruskal–Wallis test. Statistically significant tests were followed by pairwise testing, with Bonferroni adjustment at an alpha of .017. All analyses were conducted in SAS software version 9.4 during spring 2017. Two analyses were performed to address concerns about misclassification bias due to self-report of smoking and/or ENDS use. A “CO analysis” included smokers and dual users whose CO values were ≥ 6 ppm, along with ENDS users whose CO < 6 ppm. The CO analysis used the CO cutoff levels applied by Hecht et al.¹² A separate “NNAL analysis” included smokers and dual users whose total NNAL ≥ 47.3 pg/mg, along with ENDS users whose total NNAL < 47.3 pg/mg. Goniewicz et al showed this cutoff for total NNAL discriminates between passive and active smoking with a sensitivity of 87.4% and a specificity of 93.9%.²⁹ Based on their CO and total NNAL levels, participants were included in both analyses, excluded from both analyses, or included in one analysis but not the other. Figure 1 provides an overview of the final sample size per user group for each analysis.

RESULTS

Ninety-four individuals participated in this study. The median age of participants was 38.0 years. Age differed across the 3 user groups ($p < .01$). The median ages of smokers, ENDS users, and dual users were 45, 33, and 39 years, respectively. Smokers were significantly older than ENDS users ($p < .01$). Dual users did not differ in age from ENDS users ($p = .099$) or smokers ($p = .074$). Women comprised two-thirds of the sample. Most (60%) were divorced, separated, widowed, or never married. One-half had at least some college or technical school education and slightly more than half (53%) were employed for wages or self-employed. Slightly more than three-fourths of all participants were overweight or obese. Among smokers ($N = 34$) and dual users ($N = 31$), the median ages at which they initiated smoking regularly were 19.0 and 16. Years respectively, median duration of smoking was 26.5 and 22.0 years, and median CPD was 10.0 and 15.0, respectively. Among ENDS ($N =$

29) and dual users (N = 31), the median ages at which they initiated ENDS use regularly were 32.0 and 36.0 years respectively, and median duration of ENDS use was 2.0 and 1.0 years, respectively. Most ENDS and dual users reported < 20 vape sessions per day (72.0% vs 72.0%) with 10 puffs per vape session (70.4% vs 69.2%). Most ENDS (90%) and dual users (94%) reported using a tank or vapor system that was refillable with e-liquid (90%, 100%) and rechargeable (100%, 97%). Most ENDS users (78%) and dual users (65%) reported using a nicotine concentration of 12 mg or less. Table 1 summarizes biomarker values. In the CO analysis, the geometric mean values of TNE were 24.36, 17.64, and 20.31 nmol/mg among smokers, ENDS users, and dual users, respectively (Figure 2). In the NNAL analysis, the geometric mean values of TNE were 26.50, 15.53, and 26.44 nmol/mg among smokers, ENDS users, and dual users, respectively. In both analysis sets, the distribution of TNE was not significantly different across the 3 user groups. Adjusting for age did not change the significance of these results.

Reflecting the lack of a difference in TNE across the user groups, metabolites comprising TNE were not significantly different ($p > .05$) across the user groups for either analysis (Table 2). In the CO analysis, 4 ENDS users and 3 dual users had levels of one or more metabolites comprising TNE below the LOQ. In the NNAL analysis, one smoker, 4 ENDS users, and one dual user had levels of one or more metabolites comprising TNE below the LOQ. Summary statistics were recalculated after excluding participants who had levels of one or more metabolites comprising TNE below the LOQ (Table 3).

In the CO analysis, the geometric mean values of NNAL were 251.46, 7.31, and 177.74 pg/mg among smokers, ENDS users, and dual users, respectively. In pmol/mg, the geometric mean values of NNAL were 1.20 (CI: 82, 1.77), 0.03 (CI: 0.02, 0.06), and 0.85 (CI: 0.51, 1.42) in the CO analysis (Figure 2). In the NNAL analysis, the geometric mean values of NNAL were 261.36, 6.11, and 227.98 pg/mg among smokers, ENDS users, and dual users, respectively. In pmol/mg, the geometric mean value of NNAL was 1.25 (CI: 0.87, 1.79), 0.03 (CI: 0.02, 0.04), and 1.09 (0.79, 1.50) in the NNAL analysis. In both analyses, NNAL was significantly lower in ENDS users when compared to smokers ($p < .001$) and, separately, when compared to dual users ($p < .001$). Smokers and dual users did not differ in NNAL in either the CO ($p = .350$) or NNAL analysis ($p = .411$). Like the analyses looking at TNE, adjusting for age did not change the significance of these results.

Figure 2 also shows levels of CO across the 3 user groups. The geometric mean values of CO were 16.58 ppm in smokers, 2.21 ppm in ENDS users, and 17.38 ppm in dual users. In the NNAL analysis, the geometric mean values of CO were 14.72, 2.36, and 16.75 among smokers, ENDS users, and dual users, respectively. In both analyses, ENDS users had significantly lower levels of CO when compared to smokers ($p < .001$) and dual users ($p < .001$). There was no difference in CO when comparing smokers to dual users in the CO analysis ($p = .800$) or NNAL analysis ($p = .539$). Adjusting for age did not change the significance of these results.

DISCUSSION

The present study is the first to examine select tobacco-related biomarker levels in ENDS users, cigarette smokers, and dual users of AI descent. Extending previous findings to a minority population with high rates of tobacco use, we demonstrate that although levels of nicotine did not differ in the 3 tobacco use groups, levels of total NNAL and CO were lower in exclusive ENDS users than in exclusive smokers or dual users of ENDS and cigarettes. Moreover, consistent with data from Shahab et al,¹⁴ dual users did not differ in levels of metabolites of a potent lung carcinogen or a cardiovascular toxicant when compared to exclusive smokers. This finding is particularly concerning given reports that most ENDS users are dual users,^{22,30} who may be incorrectly assuming that they are reducing their exposure to harmful tobacco-related toxicants. The finding supports that ENDS users need to stop smoking cigarettes to achieve significant reduction of exposure to tobacco-related toxicants. Although long-term risk data on ENDS are not yet available, our study and the mounting evidence from others suggest that ENDS are considerably less harmful than cigarettes. Given the high-rates of smoking-related disease and death among the AI population, there could be a major public health gain if current smokers of AI descent completely switched to ENDS and dual users quit cigarettes.

Proper classification of ENDS users was important to the study's validity, and prior studies have reported higher than expected levels of NNAL among some self-reported ENDS users.^{12,14} To assess potential misclassification, we examined exposure levels in 2 separate analyses. Like prior studies, our first analysis included participants based on CO level. Specifically, a CO cutoff level of 6 ppm was employed to discriminate non-smokers (ENDS users) from smokers and dual users. In a study of 41 outpatients attending a respiratory clinic and 24 healthy participants, a CO > 6 ppm had a sensitivity of 94% and specificity of 96% for distinguishing between smokers and non-smokers.³¹ Furthermore, Hecht et al used this cutoff level for CO to reduce misclassification of cigarette smoking status among ENDS users.¹² Using this CO cutoff level, one self-reported exclusive ENDS user had a total NNAL value higher than expected – 260.93 pg/mg. Although a measurement of CO is an easy and noninvasive method for the assessment of cigarette smoking status, it has limitations. CO has a short half-life (2–3 hours), which limits its utility in identifying light or non-daily smokers.³² Moreover, it is not specific to cigarette smoking as there are many environmental sources of CO.³² To address this limitation, we conducted a second analysis that enforced a NNAL cutoff level of 47.3 pg/mg for discriminating ENDS users from smokers and dual users. One self-reported ENDS user had a low NNAL level (4.22 pg/ml) and reported no secondhand smoke exposure, but nevertheless, had a value of CO slightly higher than expected – 9 ppm. In comparison to the CO analysis, the NNAL analysis, which relied on Goniewicz et al's NNAL cutoff level,²⁹ may classify ENDS exposure more accurately. Discordances between the CO and NNAL analysis sets were also observed among the dual users. Two dual users with CO values > 6 ppm (7 and 20 ppm) had lower than expected levels of NNAL (1.5 and 15.0 pg/ml). These individuals may have smoked a cigarette to participate in the research study, resulting in a high CO but low NNAL. Thus, the NNAL analysis, which excluded these 2 participants, likely produced a more accurate picture of exposure in dual users as well as in ENDS users. The HPHC carbon monoxide

(CO) is a major constituent of cigarette smoke and has received considerable attention for its role as a risk factor for cardiovascular disease.¹⁵ In the present study, ENDS users had significantly lower levels of CO than smokers or dual users. TSNA's such as NNK and NNN (N'-nitrosonornicotine) are powerful carcinogens with NNK showing higher carcinogenicity.^{33–35} The vast majority (95%) of NNK is metabolized to NNAL, which is eliminated in the urine with a half-life of 18–45 days.^{33,36} Prospective cohort studies have demonstrated that urinary NNAL levels are associated with a dose-dependent risk of lung cancer in humans.³⁷ Consistent with prior studies,^{12–14} the geometric mean value of NNAL in ENDS users was 2%–3% of the concentration observed in smokers and dual users. Therefore, ENDS users' exposure to a substantially lower carcinogenic dose likely diminishes their risk of lung cancer compared with that of exclusive cigarette smokers or dual users.

Although lower when compared to smokers and dual users, levels of NNAL in ENDS users were not negligible and warrant discussion. In non-smokers, exposure to NNAL via secondhand smoke has been linked causally to an increased risk of lung cancer.³⁸ Studies of nonsmokers with exposure to secondhand smoke reported mean levels of total NNAL between 5 and 10 pg/ml,^{29,39,40} values that are comparable to the level of total NNAL among ENDS users in the present study (6.11 pg/mg). Levels of NNAL in ENDS users could be a result of misreporting of combustible tobacco abstinence, contamination of e-liquid with NNK, or secondhand smoke exposure. Forty percent of the ENDS users in the present study self-reported exposure over the past 7 days to secondhand smoke. The geometric mean of total NNAL among ENDS users who reported exposure to secondhand smoke was more than twice the mean among ENDS users who reported no exposure (10.05 pg/mg vs 4.43 pg/mg). Thus, secondhand smoke exposure may be contributing to the non-negligible levels of NNAL observed among ENDS users in the present study. Studies of secondhand smoke exposure measured by biomarkers have had a limited number of AI participants, and thus, been unable to report on the prevalence of secondhand smoke exposure among AI. A future study needs to examine levels of NNAL among non-smoking AI to estimate the prevalence and impact of secondhand smoke exposure in this population.

Although TNE in ENDS users was nearly half the level observed in smokers and dual users, this difference was not statistically significant. The interquartile interval (IQI), a measure of the extent to which the central 50% of values are dispersed, of TNE was 2.76–220.84 in ENDS users, 16.60–45.95 in smokers, and 14.64–50.24 in dual users. Thus, some ENDS users had relatively high levels of TNE whereas others had low and even undetectable levels. Four ENDS users had values of one or more of the metabolites comprising TNE below the LOQ. Because all ENDS users self-reported using nicotine in their devices, undetectable levels of nicotine may be a result of the e-liquid not containing nicotine or perhaps reflect users' inexperience with the ENDS. After excluding ENDS users with values below the LOQ, the geometric mean TNE among ENDS users doubled to approximately 30 nmol/mg, a value similar to what was observed among smokers and dual users. American Indians have high rates of lung cancer.^{16–18,41,42} Cigarette smoking and secondhand smoke exposure cause the majority of lung cancer cases and an estimated 80% to 90% of lung cancer deaths.¹⁵ High rates of lung cancer reflect the high prevalence of cigarette smoking among AI compared to other race groups. Thus, NNAL levels are not necessarily expected to be higher among AI compared to other racial groups, but rather one would expect to see a higher

incidence of elevated NNAL among AI. This study did not confirm the latter, but supports the former because levels of NNAL in this sample of AI smokers (geometric mean 261.36: pg/mg or 1.25 pmol/mg) and dual users (geometric mean: 227.98 pg/mg or 1.09 pmol/mg) were similar to a nationally representative sample of smokers (geometric mean: 285.2 pg/mL; 95% CI, 241–337; N = 1,393)³⁹ and smaller studies comprised of predominately white and black smokers (~1.2–1.5 pmol/ml).^{12,13}

This study is not without limitations. Its focus was not on testing of hypotheses, but rather, on estimating biomarkers of exposure among cigarette smokers, ENDS users, and dual users of AI descent. As a result, we did not incorporate a formal sample size calculation to address this study and consequently, the estimates have wide confidence intervals. However, our sample size numbers are in line with previous studies.^{12–14} Second, the eligibility criteria may have resulted in participants with tobacco use behaviors and other characteristics that do not reflect the overall population of AI smokers, ENDS users, and dual users. For example, the study included only dual users who smoke at least 5 CPD on average and used ENDS daily. Given the eligibility criteria and that participants were recruited via convenience-based sampling, a future study with a representative sample of AI dual users and smokers who use varying levels of CPD as well both daily and sporadic or non-daily ENDS users are needed to improve understanding of exposure levels among these user groups. Third, we did not collect information on the voltage or power of the ENDS, which has been shown to influence ENDS use behavior and nicotine exposure.¹³ Lastly, this study examined a limited number of biomarkers at a single timepoint. Future studies, such as the ongoing Population Assessment of Tobacco Use and Health study, should include a wide array of biomarkers and an examination over time. In conclusion, this study extends the current body of research seeking to understand the role of ENDS by measuring select tobacco-related toxicant levels in AI ENDS users and comparing them to cigarette smokers and dual users. Whereas user groups did not differ in their levels of TNE, levels of NNAL and CO were significantly lower in ENDS users when compared with either smokers or dual users. Findings suggests that ENDS, when used exclusively, exposes the user to similar levels of nicotine yet lower levels of metabolites of a potent lung carcinogen and a cardiovascular toxicant than cigarettes, whether they are used alone or in combination with ENDS. Additionally, this study demonstrated the utility of an NNAL cutoff level over CO and self-report for discriminating ENDS users from smokers.

IMPLICATIONS FOR TOBACCO REGULATION

American Indian/Alaska Native tribes have an inalienable and inherent right to self-government. Through collaboration, consultation, and communication, tribal governments and the FDA CTP work together to protect the health of tribal members via implementation and enforcement of The Family Smoking Prevention and Tobacco Control Act.⁴³ The results of this study, in addition to prior studies among non-AI ENDS users, demonstrate that ENDS have a more favorable toxicant and carcinogenic profile than cigarettes and dual users need to stop smoking to reduce harmful exposure levels. These results should be used to inform tribal regulatory decisions on ENDS and tobacco control messaging, and to educate the AI community.

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Figure 1.

Overview of Inclusion Criteria for CO and NNAL Analysis Sets

Note.

Based on a given participant's CO and NNAL levels, they may be included in both analyses, excluded in both analyses, or included in one analysis but not the other.

Figure 2.
Geometric Mean and 95% Confidence Interval of TNE, total NNAL, and CO by User Group

Table 1
Urinary TNE, Urinary Total NNAL, and Expired Breath Carbon Monoxide (CO) by User Group

	Cigarette smokers		ENDS users		Dual users	p
CO Analysis	N = 27		N = 23		N = 28	
TNE (nmol/mg)						.929
GM (95% CI)	24.36 (16.43, 36.10)		17.64 (7.48, 41.63)		20.31 (10.47, 39.40)	
Range	1.35–89.13		0.33–220.84		0.13–182.24	
Total NNAL (pg/mg) ^{a, b}						< .001
GM (95% CI)	251.46 (170.57, 370.71)		7.31 (4.42, 12.10)		177.74 (106.30, 297.19)	
Range	39.21–1398.96		1.50–260.93		1.46–1311.92	
CO (ppm) ^{a, b}						< .001
GM (95% CI)	16.58 (13.49, 20.37)		2.21 (1.89, 2.58)		17.38 (13.70, 22.03)	
Range	6.0–39.0		1.0–4.0		6.0–57.0	
NNAL Analysis	N = 27		N = 23		N = 27	
TNE (nmol/mg)						.831
GM (95% CI)	26.50 (19.13, 36.73)		15.53 (6.52, 37.01)		26.44 (15.71, 44.49)	
Range	2.89–89.13		0.33–220.84		0.14–182.24	
Total NNAL (pg/mg) ^{a, b}						< .001
GM (95% CI)	261.36 (182.64, 374.01)		6.11 (4.20, 8.90)		227.98 (165.73, 313.60)	
Range	50.56–1398.96		1.50–39.08		47.30–1311.92	
CO (ppm) ^{a, b}						< .001
GM (95% CI)	14.72 (11.38, 19.05)		2.36 (1.93, 2.88)		16.75 (12.76, 22.00)	
Range	4.0–39.0		1.0–9.0		3.0–57.0	
All Participants	N = 34		N = 29		N = 31	
TNE (nmol/mg)						.715
GM (95% CI)	14.18 (8.24, 24.40)		16.96 (8.55, 33.64)		15.79 (7.98, 31.24)	
Range	0.34–89.13		0.33–220.84		0.13–182.24	
Total NNAL (pg/mg) ^{a, b}						< .001
GM (95% CI)	132.13 (69.12, 252.57)		12.19 (6.62, 22.47)		147.00 (86.89, 248.70)	
Range	0.60–1398.96		1.50–419.50		1.46–1311.92	

	Cigarette smokers	ENDS users	Dual users	p
CO (ppm) ^{a, b}				< .001
GM (95% CI)	12.18 (9.27, 13.00)	3.05 (2.30, 4.04)	14.60 (10.90, 19.57)	
Range	2.0–39.0	1.0–25.0	2.0–57.0	
Note.				
ENDS: electronic nicotine delivery system user; GM: Geometric mean; CI: confidence interval; IQI: Interquartile interval				
^a : Significantly different (p < .0001) when comparing ENDS users to cigarette smokers				
^b : Significantly different (p < .001) when comparing ENDS users to dual users				

Table 2
Metabolites Comprising Urinary Total Nicotine Equivalents (TNE) by User Group

Cigarette Smokers (N = 27) GM (95% CI)							ENDS Users (N = 23) GM (95% CI)		Dual Users (N = 28) GM (95% CI)		p
CO Analysis		N = 27			N = 23			N = 28			
Total NIC (ng/mg)	664.32 (357.13,49, 1235.73)				417.09 (157.30, 1105.90)			515.33 (260.24,1020.43)			.808
Total NIC (nmol/mg)	4.10 (2.20, 7.63)				2.57 (0.97, 6.83)			3.18 (1.61, 6.30)			
Total COT(ng/mg)	622.33 (380.69, 1017.35)				388.07 (158.17, 952.13)			504.79 (248.24, 1026.51)			.964
Total COT (ng/mg)	3.55 (2.16, 5.78)				2.20 (0.90, 5.41)			2.87 (1.41, 5.83)			
Total 3HC (ng/mg)	1835.64 (1222.16, 2757.06)				1402.86 (503.69, 3907.17)			1473.57 (671.37, 3234.28)			.813
Total 3HC (nmol/mg)	9.56 (6.37, 14.36)				7.31 (2.62, 20.35)			7.67 (3.50, 16.85)			
Total NNO (ng/mg)	475.29 (318.68, 708.87)				288.50 (144.24, 577.01)			351.87 (191.13, 634.48)			.739
Total NNO (nmol/mg)	2.67 (1.79, 3.98)				1.62 (0.81, 3.24)			1.20 (1.09, 3.56)			
Total CNO (ng/mg)	237.68 (154.26, 366.20)				111.29 (43.77, 282.96)			170.65 (86.66, 336.01)			.780
Total CNO (nmol/mg)	1.24 (0.80, 1.91)				0.58 (0.22, 1.47)			0.89 (0.45, 1.75)			
Total NNIC (ng/mg)	33.76 (20.58, 55.37)				18.15 (7.80, 42.21)			27.53 (14.77, 51.32)			.733
Total NNIC (nmol/mg)	0.23 (0.14, 0.37)				0.13 (0.05, 0.29)			0.19 (0.10, 0.35)			
Total NCOT (ng/mg)	91.38 (60.23, 138.65)				42.76 (17.22, 106.14)			62.97 (31.19, 127.16)			.780
Total NCOT (nmol/mg)	0.56 (0.37, 0.86)				0.26 (0.11, 0.66)			0.39 (0.19, 0.78)			
NNAL Analysis		N = 27			N = 23			N = 27			
Total NIC (ng/mg)	607.13 (322.49, 1143.01)				361.60 (129.47, 1009.98)			557.55 (286.70, 1084.28)			.847
Total NIC (nmol/mg)	3.75 (1.99, 7.06)				2.23 (0.80, 6.23)			3.44 (1.77, 6.69)			
Total COT (ng/mg)	662.60 (440.67, 996.29)				329.52 (133.69, 812.20)			691.60 (408.86, 1169.87)			.679
Total COT (nmol/mg)	3.76 (2.50, 5.66)				1.87 (0.76, 4.61)			3.93 (2.32, 6.65)			
Total 3HC (ng/mg)	1987.56 (1413.38, 2795.00)				1249.23 (449.56, 3471.34)			2101.72 (1144.90,3858.16)			.691
Total 3HC (nmol/mg)	10.35 (7.36, 14.56)				6.51 (2.34, 18.08)			10.95 (5.96, 20.09)			
Total NNO (ng/mg)	455.36 (306.84, 675.78)				250.49 (122.92, 510.46)			426.65 (263.45, 690.96)			.527
Total NNO (nmol/mg)	2.56 (1.72, 3.80)				1.41 (0.70, 2.87)			2.40 (1.48, 3.88)			
Total CNO (ng/mg)	265.74 (201.06, 351.23)				98.37 (38.87, 248.95)			218.25 (122.05, 390.28)			.428
Total CNO (nmol/mg)	1.38 (1.05, 1.83)				0.51 (0.20, 1.30)			1.14 (0.64, 2.03)			
Total NNIC (ng/mg)	36.26 (24.48, 53.72)				15.91 (6.78, 37.32)			35.21 (21.84, 56.78)			.428
Total NNIC (nmol/mg)	0.24 (0.17, 0.36)				0.11 (0.05, 0.25)			0.24 (0.15, 0.38)			

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	Cigarette Smokers (N = 27) GM (95% CI)	ENDS Users (N = 23) GM (95% CI)	Dual Users (N = 28) GM (95% CI)	p
Total NCOT (ng/mg)	101.78 (73.74, 140.47)	37.25 (14.91, 93.03)	86.12 (50.47, 146.94)	.434
Total NCOT (nmol/mg)	0.63 (0.46, 0.87)	0.23 (0.09, 0.57)	0.53 (0.31, 0.91)	

Note.

ENDS: electronic nicotine product; GM: geometric mean; NIC: nicotine; COT: cotinine; 3HC: 3-hydroxycotinine;

NNO: nicotine-n-oxide; CNO: cotinine-n-oxide; NNIC: normicotine; NCOT: norcotinine

Urinary TNE, Urinary Total NNAL, and Expired Breath Carbon Monoxide (CO) by User Group Excluding Individuals with Nicotine and/or One of its Metabolites below the Limit of Quantification (LOQ)

Table 3

	Cigarette smokers N = 27	ENDS users N = 19	Dual users N = 25	P
CO Analysis Excluding Below the LOQ for TNE				
TNE (nmol/mg)				.265
GM (95% CI)	24.36 (16.43, 36.10)	35.17 (18.48, 66.94)	33.60 (24.03, 46.97)	
Range	1.35–89.13	1.63–220.84	5.44–182.24	
Total NNAL (pg/mg) ^{a, b}				< .001
GM (95% CI)	251.46 (170.57, 370.71)	7.12 (3.99, 12.70)	244.01 (175.44, 339.39)	
Range	39.21–1398.96	1.50–260.93	47.30–1311.92	
CO (ppm) ^{a, b}				< .001
GM (95% CI)	16.58 (13.49, 20.37)	2.21 (1.90, 2.57)	18.25 (14.17, 23.50)	
Range	6.0–39.0	1.0–4.0	6.0–57.0	
NNAL Analysis Excluding Below the LOQ for TNE				
TNE (nmol/mg)				.651
GM (95% CI)	27.60 (19.87, 38.22)	30.15 (14.97, 60.72)	32.30 (23.19, 44.99)	
Range	2.89–89.13	1.63–220.84	5.44–182.24	
Total NNAL (pg/mg) ^{a, b}				< .001
GM (95% CI)	277.21 (195.10, 393.87)	5.73 (3.84, 8.55)	235.95 (170.69, 326.17)	
Range	50.55–1398.96	1.50–39.08	47.30–1311.92	
CO (ppm) ^{a, b}				< .001
GM (95% CI)	15.48 (12.10, 19.79)	2.39 (1.93, 2.97)	17.02 (12.84, 22.56)	
Range	4.0–39.0	1.0–9.0	3.0–57.0	

Note.

GM: geometric mean

CI: confidence interval

^a: Significantly different ($p < .001$) when comparing ENDS users to cigarette smokers

^b: Significantly different ($p < .001$) when comparing ENDS users to dual users