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Nicotine, Carcinogen, and Toxin Exposure in Long-Term E-Cigarette and Nicotine Replacement Therapy Users

A Cross-sectional Study

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Background: Given the rapid increase in the popularity of e-cigarettes and the paucity of associated longitudinal health-related data, the need to assess the potential risks of long-term use is essential.

Objective: To compare exposure to nicotine, tobacco-related carcinogens, and toxins among smokers of combustible cigarettes only, former smokers with long-term e-cigarette use only, former smokers with long-term nicotine replacement therapy (NRT) use only, long-term dual users of both combustible cigarettes and e-cigarettes, and long-term users of both combustible cigarettes and NRT.

Design: Cross-sectional study.

Setting: United Kingdom.

Participants: The following 5 groups were purposively recruited: combustible cigarette–only users, former smokers with long-term (≥6 months) e-cigarette–only or NRT-only use, and long-term dual combustible cigarette–e-cigarette or combustible cigarette–NRT users (n = 36 to 37 per group; total n = 181).

Measurements: Sociodemographic and smoking characteristics were assessed. Participants provided urine and saliva samples and were analyzed for biomarkers of nicotine, tobacco-specific *N*-nitrosamines (TSNAs), and volatile organic compounds (VOCs).

Results: After confounders were controlled for, no clear between-group differences in salivary or urinary biomarkers of nicotine intake were found. The e-cigarette–only and NRT-only users had significantly lower metabolite levels for TSNAs (including the carcinogenic metabolite 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol [NNAL]) and VOCs (including metabolites of the toxins acrolein; acrylamide; acrylonitrile; 1,3-butadiene; and ethylene oxide) than combustible cigarette–only, dual combustible cigarette–e-cigarette, or dual combustible cigarette–NRT users. The e-cigarette–only users had significantly lower NNAL levels than all other groups. Combustible cigarette–only, dual combustible cigarette–NRT, and dual combustible cigarette–e-cigarette users had largely similar levels of TSNA and VOC metabolites.

Limitation: Cross-sectional design with self-selected sample.

Conclusion: Former smokers with long-term e-cigarette–only or NRT-only use may obtain roughly similar levels of nicotine compared with smokers of combustible cigarettes only, but results varied. Long-term NRT-only and e-cigarette–only use, but not dual use of NRTs or e-cigarettes with combustible cigarettes, is associated with substantially reduced levels of measured carcinogens and toxins relative to smoking only combustible cigarettes.

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E-cigarettes (1), which produce an aerosol by heating a solvent (e-liquid) usually containing nicotine through a battery-powered heating element, are becoming increasingly popular. Unlike smoked tobacco, e-cigarettes can deliver nicotine to the respiratory tract without combustion (2). Despite this possible advantage, health concerns for e-cigarettes remain about potential cytotoxicity; delivery of carcinogens (3), including carbonyls (4, 5), tobacco-specific *N*-nitrosamines (TSNAs) (6), and heavy metals (4); effects on cardiovascular and respiratory function and inflammatory effects (7); and nicotine delivery (8). Data on the long-term effects of e-cigarettes are needed to accurately assess risk and inform health professionals encountering e-cigarette users (9).

Most studies to date have examined toxin concentrations in e-liquids or aerosols (4, 6) using cell-line or animal models (7). However, these models may not provide accurate information because user characteristics, together with device characteristics and their interactions, determine actual body-level exposure and thus potential health consequences (10). Three studies that have assessed such exposure found lower levels for carcinogens, including TSNAs, in recent former smokers of e-cigarettes than in a historic sample of smokers of combustible cigarettes (11); these studies also found reductions in toxins over a 2- or 4-week period in smokers switching to e-cigarettes with or without concurrent use of combustible cigarettes (12, 13). However, none of the studies involved long-term users, which is important given observed learning effects in e-cigarette use (14, 15), or included real-world control groups to reduce the risk for confounding when interpreting the results of observational studies.

Users of nicotine replacement therapy (NRT) (which includes chewing gum and adhesive patches), would be an appropriate control. Dual use of combustible cigarettes and either e-cigarettes or NRT is common, and long-term use of both types of products has been reported (16, 17). They have been advocated to reduce the harms and risks associated with combustible tobacco (18). However, unlike e-cigarettes, the NRT safety

profile is well-established (19) and NRT effectiveness for smoking cessation through initial partial (20) or complete substitution (21) has been shown. Therefore, NRT is recommended as a harm reduction strategy in several countries (22).

Although longitudinal cohort studies and randomized, controlled trials will provide the best data to answer questions about the safety and efficacy of e-cigarettes for smoking cessation, these designs are time- and resource-intensive. In the absence of long-term data, a more pragmatic approach is to compare smokers and former smokers with or without concurrent e-cigarette use in real-life settings. This study aimed to address the gap in the literature by measuring biomarker levels in long-term e-cigarette users compared with an appropriate control—NRT users. Specifically, this study assessed whether long-term e-cigarette–only, NRT-only, dual combustible cigarette–e-cigarette, or dual combustible cigarette–NRT use is associated with differences in metabolites of nicotine, TSNAs, and volatile organic compounds (VOCs) compared with combustible cigarette–only use.

METHODS

Study Design and Procedure

This cross-sectional study was done in London, United Kingdom, from January 2014 to June 2014. It evaluated the range of toxin levels measured in smokers and former smokers with or without concurrent long-term use of e-cigarettes or NRT. The study methodology has been described elsewhere (23). Briefly, participants visited the laboratory for a single session, lasting 30 minutes, after abstaining from eating, drinking, or using combustible cigarettes or other nicotine products for an hour before their visit to standardize assessment. At the laboratory, after providing written consent, participants completed a short questionnaire assessing sociodemographic, smoking, and product use characteristics and provided breath, saliva, and urine samples. Exhaled air was assessed for carbon monoxide with a breathalyzer (Micro IV Smokerlyzer, Bedfont Scientific). In addition, 2 saliva samples were collected with sterile dental rolls (Salivette, Sarstedt) that participants were asked to gently chew for about 2 minutes or until saturated. Urine was collected in a sealable, sterilized cup by participants on site and transferred by staff into cryovials. Urine and saliva samples were then kept frozen at −20 °C until they were shipped in dry ice to laboratories at Roswell Park Cancer Institute (Buffalo, New York) and the Centers for Disease Control and Prevention (Atlanta, Georgia) for analysis. All participants were reimbursed for time and travel (£25). The study was approved by the University College London Ethics Committee (project ID 0483/002).

Participants

Participants were purposively recruited in the greater London area using various methods to increase sample diversity, including newspapers and online advertisements, posters in pharmacies, and the use of marketing companies. They had to be ever smokers and to meet the following eligibility criteria: Current smokers had to smoke an average of 5 or more combustible cigarettes per day for at least 6 months, and former smokers had to have stopped using tobacco products (including combustible cigarettes, water pipes, cigars, and such smokeless products as snus or chewing tobacco) for at least 6 months. Because we sought to evaluate the effect of long-term use of noncombustible nicotine delivery devices (NRT and e-cigarettes), smokers (that is, dual combustible cigarette–e-cigarette or combustible cigarette–NRT users) and former smokers (that is, e-cigarette–only or NRT-only users) had to have been using these products at least weekly for 6 months or more (users of nicotine-free products, such as e-liquid without nicotine, were excluded). In practice, however, participants used products daily as indicated by latency to last product use across groups (combustible cigarettes–only users, 1.4 hours; combustible dual cigarette–NRT users, 4.3 hours; combustible dual cigarette–e-cigarette users, 1.3 hours; NRT-only users, 24 hours; and e-cigarette–only users, 5.4 hours). Product use was verified by asking participants to bring in the NRT or e-cigarette that they were currently using, and smoking status was verified with carbon monoxide readings (10-ppm cutoff) (24). We excluded persons who used both NRT and e-cigarettes as well as those who were younger than 18 years; were pregnant; had a history of heart or lung disease; or had bleeding gums, illness, or an active infection within 24 hours of their scheduled appointment.

Measures

Biomarkers of Exposure

Level of nicotine exposure was measured to assess effectiveness of nicotine delivery products by using 2 methods. Saliva samples were analyzed for nicotine, and its major metabolite, cotinine, using an established gas chromatography method (25, 26). Urine samples were analyzed for main nicotine metabolites to derive total nicotine equivalents and for minor tobacco alkaloids using validated tandem mass spectrometry (27, 28).

Levels of urinary TSNA and VOC metabolites were measured using either liquid chromatography/atmospheric pressure ionization/tandem mass spectrometry (29) or ultra-high performance liquid chromatography coupled with electrospray ionization and tandem mass spectrometry (30) to assess the potential risks of nicotine delivery products. Although we assessed a comprehensive battery of metabolites (**Appendix Table 1**, available at Annals.org), we focus here on well-established metabolites of compounds that are known to contribute significantly to smoking-related toxicologic and carcinogenic risks (31–39) (**Table 1**). All urinary and salivary biomarkers were analyzed by the Centers for Disease Control and Prevention and Roswell Park Cancer Institute, respectively.

Covariates

Sociodemographic characteristics (age, sex, ethnicity, education, and marital status) were assessed in addition to self-reported recently resolved physical illness (chest infection, cold or flu, sore throat, or fever) and subjective well-being (happiness and satisfaction, both assessed with established single-item measures) (40). Salivary C-reactive protein level was used as a marker of inflammation (and thus potential health problems) and analyzed with an enzyme-linked immunosorbent assay (Salimetrics Europe) (41). Smoking characteristics, including current and past daily combustible cigarette consumption as a measure of dependence for smokers and former smokers, respectively; age at which participants had started smoking; and the proportion of family members or friends who smoke were assessed to gauge environmental tobacco smoke exposure.

Statistical Analysis

Because this was a cross-sectional study, exposure biomarkers, including metabolites of known tobacco-related carcinogens and toxins, were used as proxies for future disease risk. Previous research on the association of the carcinogenic metabolite 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL) with lung cancer suggests that medium to large reductions in NNAL levels (Cohen $f = 0.25$ to 0.40) would result in an appreciable reduction in risk (42) and could thus be considered clinically meaningful in magnitude and warrant further investigation (43). A priori power calculation showed that 180 participants (36 per group) would provide 90% power to detect between-group differences of a medium effect size (Cohen $f = 0.3$) in NNAL levels when comparing 5 groups by using analysis of variance (44). However, this calculation did not account for multiple outcomes being tested, and based on 35 biomarker outcomes reported here, power to detect such an effect size across all biomarkers would have been reduced to 54%. The sample size therefore only provided sufficient power (≥80%) to detect effects at the upper range of the estimate (Cohen $f \geq 0.36$) when multiple comparisons were accounted for.

Analyses were conducted with SPSS, version 22.0 (IBM). In initial analysis of between-group differences on covariates, 1-way analysis of variance was used for continuous covariates and chi-square analysis was used for categorical covariates. Before the main analysis, urinary metabolites were standardized algebraically to account for individual differences in urine concentration by dividing metabolite data by the ratio of observed urinary metabolites to age-, sex-, and ethnicity-adjusted creatinine levels. Creatinine (measured by standard colorimetric method at Roswell Park Cancer Institute) was also included as a covariate in the analysis (45). Due to nonnormal distribution of data, generalized linear models with a log link and γ distribution were used to assess between-group differences in outcome measures, which were adjusted for all covariates and latency to product use. B coefficients were exponentiated to calculate the percentage of change in biomarker levels in all groups compared with combustible cigarette–only smokers. For prespecified tests of the main effects of a group, type I errors were controlled for by using the false discovery rate (46) separately for sociodemo-

Table 1. Major Toxicants and Carcinogens Related to Tobacco Use

Parent Compound	Biomarker/Metabolite	Rationale for Inclusion
Tobacco-specific *N*-nitrosamines		
4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone	4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol	A potent lung carcinogen (40) and major contributor to cancer risk (34); IARC group 1 carcinogen (39)*; and 1 of 9 toxins recommended for mandated reduction in tobacco smoke on the WHO TobReg list (36)
Volatile organic compounds		
Acrolein	*N*-acetyl-S-(3-hydroxypropyl)-L-cysteine	A major contributor to respiratory effects (34, 35); IARC group 3 carcinogen (41)†; and 1 of 9 toxins recommended for mandated reduction in tobacco smoke on the WHO TobReg list (36)
Acrylamide	*N*-acetyl-S-(2-carbamoylethyl)-L-cysteine	IARC group 2A carcinogen (37)‡; a neurotoxin
Acrylonitrile	*N*-acetyl-S-(2-cyanoethyl)-L-cysteine	A major contributor to cancer risk (34) and highly specific volatile organic compound biomarker for tobacco use (33); IARC group 2B carcinogen (37)§; and 1 of 9 toxins considered high priority for disclosure and monitoring on the WHO TobReg list (36)
1,3-butadiene	*N*-acetyl-S-(4-hydroxy-2-buten-1-yl)-L-cysteine‖	A major contributor to cancer risk (34, 35); IARC group 1 carcinogen (42)*; and 1 of 9 toxins recommended for mandated reduction in tobacco smoke on the WHO TobReg list (36)
Ethylene oxide	*N*-acetyl-S-(2-hydroxyethyl)-L-cysteine¶	IARC group 1 carcinogen (37)*

IARC = International Agency for Research on Cancer; WHO TobReg = World Health Organization Study Group on Tobacco Product Regulation.
* Carcinogenic to humans.
† Not classifiable with regard to carcinogenicity to humans.
‡ Probably carcinogenic to humans.
§ Possibly carcinogenic to humans.
‖ More selective metabolite of parent compound than *N*-acetyl-S-(3,4-dihydroxybutyl)-L-cysteine (33).
¶ A major urinary metabolite of ethylene oxide exposure and a minor metabolite of acrylonitrile and vinyl chloride exposure (toxic tobacco smoke constituents).

Table 2. Sociodemographic, Smoking, Physical Health, and Subjective Well-Being Characteristics of Study Participants

Characteristic	Total Participants (*n* = 181)	Smokers			Former Smokers		*P* Value*
		Cigarette-Only Users (*n* = 37)	Dual Cigarette–NRT Users (*n* = 36)	Dual Cigarette–EC Users (*n* = 36)	NRT-Only Users (*n* = 36)	EC-Only Users (*n* = 36)	
Mean age (SD), *y*	37.8 (11.8)	34.4 (14.0)	36.4 (8.5)	39.3 (13.1)	40.3 (11.1)	38.5 (11.1)	0.27
Female, *n (%)*	71 (39.2)	16 (43.2)	22 (61.1)	11 (30.6)	15 (41.7)	7 (19.4)	0.024
White, *n (%)*	131 (72.4)	30 (81.1)	21 (58.3)	27 (75.0)	23 (63.9)	30 (83.3)	0.111
High school, *n (%)*	140 (77.3)	25 (67.6)	30 (83.3)	29 (80.6)	28 (77.8)	28 (77.8)	0.56
Single, *n (%)*	97 (53.6)	26 (70.3)	21 (58.3)	18 (50.0)	13 (36.1)	19 (52.8)	0.104
Mean age started smoking (SD), *y*	17.8 (4.3)	16.6 (3.2)	18.2 (3.4)	17.3 (3.1)	20.3 (6.4)	16.6 (3.2)	0.012
Mean cigarettes per day (SD), *n*†	13.3 (8.7)	13.9 (9.0)	10.8 (4.6)	11.9 (9.6)	14.7 (10.3)	15.9 (8.3)	0.104
Mean proportion of friends/family who smoke (SD)	35.6 (27.5)	50.9 (23.6)	39.8 (24.1)	38.0 (32.4)	33.2 (27.7)	15.6 (15.2)	<0.001
Recent illness, *n (%)*	42 (23.2)	14 (37.8)	3 (8.3)	7 (19.4)	10 (27.8)	8 (22.2)	0.104
Geometric mean salivary C-reactive protein level (SD), *nmol/L*‡	0.017 (3.32)	0.020 (2.99)	0.013 (3.48)	0.016 (3.15)	0.018 (3.20)§	0.021 (3.78)	0.47
Mean global life satisfaction (SD)‖	3.9 (1.0)	4.1 (0.9)	3.8 (1.1)	3.7 (1.1)	3.9 (0.9)	3.9 (1.1)	0.54
Mean happiness levels (SD)¶	5.0 (1.5)	4.6 (1.7)	5.6 (1.1)	4.7 (1.7)	5.3 (1.3)	5.0 (1.6)	0.104

Cigarette = combustible cigarette; EC = e-cigarette; NRT = nicotine replacement therapy.
* Omnibus test result, adjusted for the reported comparisons in this table using the false discovery rate (46).
† Former smokers were asked about their typical past consumption levels.
‡ Statistical comparison conducted on log-transformed values (not shown).
§ Data are missing for 1 participant.
‖ Assessed by asking, "All things considered, how satisfied are you with your life as a whole?" Response options ranged from "very dissatisfied" (1) to "very satisfied" (5).
¶ Assessed by asking, "Some people are very generally very happy. They enjoy life regardless of what is going on, getting the most out of everything. To what extent does this characterization describe you?" Response options ranged from "not at all" (1) to "a great deal" (7).

graphic comparisons (*n* = 13) and biomarker comparisons (*n* = 35). Where overall omnibus effects were considered significant, the Sidak correction was used in post hoc analysis to determine which (if any) between-group differences persisted. Biomarker values below the limit of detection (LOD) were imputed using standard methods (LOD divided by the square root of 2) (47), and biomarkers with 50% or more values below the LOD were not analyzed.

Role of the Funding Source

The funding source had no role in the study design, data collection, data analysis, data interpretation, or writing of the report. Dr. Shahab had full access to all study data and had final responsibility for the decision to submit the manuscript for publication.

RESULTS

Overall, participants were relatively young, were mainly men, were white, and had at least a high school education; about half of them were single (Table 2). On average, participants had started smoking nearly 1 pack of cigarettes per day in their late teens, and a substantial proportion (16% to 51%) of their family members or friends also smoked. Salivary C-reactive protein levels were within the range observed for healthy adults (0.05 to 64.3 μg/L) (48), and the reported level of well-being was similar to that of representative population samples (40). Between-group differences included that the proportion of women varied from 19.4% in e-cigarette–only users to 61.1% in dual combustible cigarette–NRT users, fewer e-cigarette–only users were women, NRT-only users started smoking the latest, and e-cigarette–only users had the lowest proportion of family members or friends who smoked. Considerable variation in ethnicity, marital status, combustible cigarette consumption, recent illness, and reported happiness levels were also found (Table 2).

As previously reported, length of product use was broadly similar across groups at around 17 months, and mean daily NRT and e-cigarette use, measured by self-reported nicotine dose, was higher for NRT-only and e-cigarette–only users than for dual combustible cigarette–NRT and combustible cigarette–e-cigarette users (23). For the product type used, first-generation "cig-a-likes," with replaceable or disposable cartridges, were most popular among dual combustible cigarette–e-cigarette users (60.0%). Third- or fourth-generation advanced personal vaporizers were most popular among e-cigarette–only users (47.2 %). Refillable pen-style, second-generation e-cigarettes were equally popular among dual combustible cigarette–e-cigarette (31.4%) and e-cigarette–only (36.1%) users. For both dual combustible cigarette–NRT and NRT-only users, gum (44.4% and 33.3%, respectively) and patches (both 33.3%) were the most popular NRTs, and a similar proportion (27.8%) used more than 1 NRT.

Nicotine Levels

Nicotine intake among the products was roughly similar (Figure 1), with some variation across groups (Appendix Table 1). For urinary biomarkers, users of all products had levels of total nicotine equivalents at least as high as combustible cigarette–only users in adjusted analysis (Table 3). Findings related to salivary biomarkers varied. Dual combustible cigarette–NRT users had relatively low nicotine and cotinine levels, and e-cigarette–only users had relatively low nicotine levels—at around half that of combustible cigarette–only users—with other groups obtaining levels slightly less or more than those from combustible cigarette–only users (Table 3). The minor tobacco alkaloids anabasine and anatabine, which are specific to tobacco as opposed to nicotine exposure, were clearly distinguished between smokers and former smokers, with significantly lower levels than combustible cigarette–only, dual combustible cigarette–NRT, or dual combustible cigarette–e-cigarette users (Appendix Table 1).

TSNA Levels

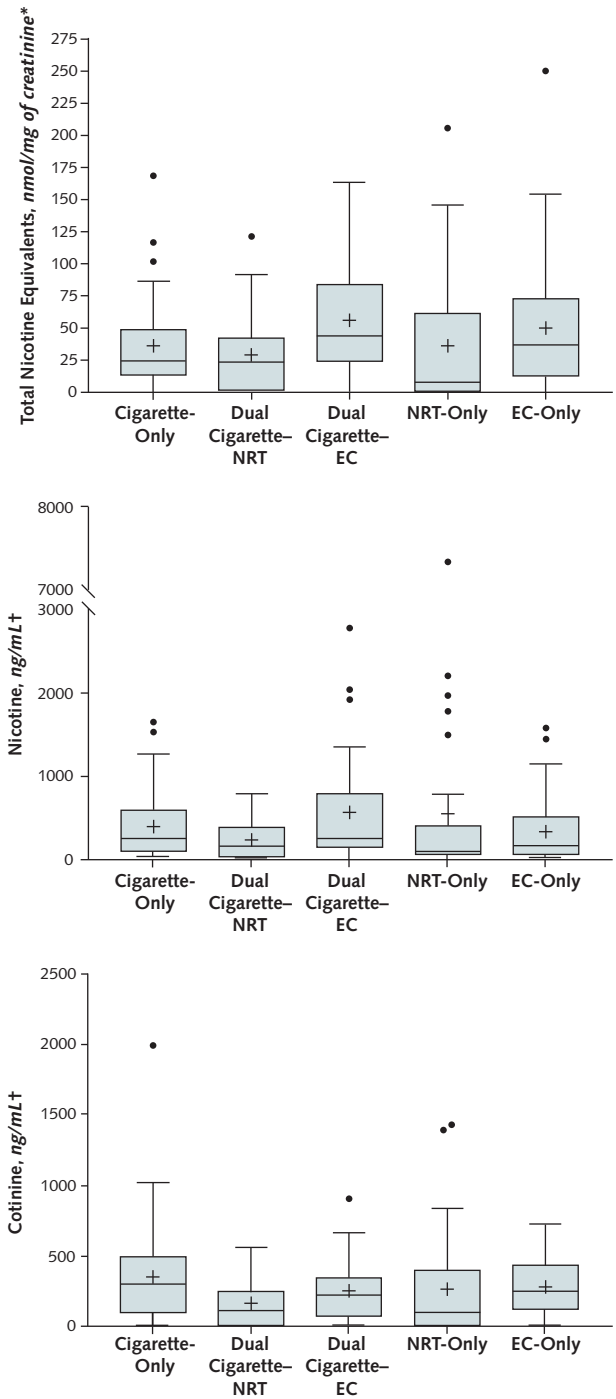
There were clear between-group differences in NNAL levels (Figure 2). The NRT-only and e-cigarette–only users had markedly lower NNAL levels than combustible cigarette–only, dual combustible cigarette–NRT, and dual combustible cigarette–e-cigarette users ($P < 0.001$); e-cigarette–only users had significantly lower NNAL levels than all other groups—equivalent to a 97% reduction compared with the levels of combustible cigarette–only users (Table 3). Compared with combustible cigarette–only users, there were no large differences in NNAL levels for dual combustible cigarette–e-cigarette users but dual combustible cigarette–NRT users had somewhat lower NNAL levels. Results followed a similar, albeit less pronounced, pattern for the other TSNAs measured (Appendix Table 1).

VOC Levels

Of the major urinary VOC metabolites, e-cigarette–only users had the lowest levels overall, with acrylonitrile levels as low as 2.9% for combustible cigarette–only users; further, NRT-only users had the second lowest levels overall, with acrylonitrile levels as low as 10.5% for combustible cigarette–only users (Table 3). By contrast, dual combustible cigarette–NRT, dual combustible cigarette–e-cigarette, and combustible cigarette–only users all had very similar urinary VOC metabolite levels (Figure 2). Compared with all other groups, NRT-only and e-cigarette–only users had at least half of the reference values of combustible cigarette–only users (Table 3) and had significantly lower levels of all major metabolites of selected toxic and carcinogenic VOCs (all $P < 0.001$) (Appendix Table 1).

Results were largely confirmed by reviewing other VOC metabolites that were assessed. E-cigarette–only users generally had the lowest levels, followed by NRT-only users, with no detectable differences among dual combustible cigarette–NRT, dual combustible cigarette–e-cigarette, and combustible cigarette–only users (Appendix Table 1). The only exceptions were metab-

Figure 1. Urinary and salivary nicotine metabolite levels, by group.



Boxplots show the median with interquartile range (25th percentile, 75th percentile). Error bars show Tukey's whiskers, and crosses indicate arithmetic means (geometric means are provided in Appendix Table 1). Circles indicate outliers. Cigarette = combustible cigarette; EC = e-cigarette; NRT = nicotine replacement therapy.

* Measured in urine. Data are raw values divided by the ratio of observed urinary metabolites to covariate-adjusted creatinine levels. Values below the limit of detection were imputed by the limit of detection divided by square root of 2.

† Measured in saliva. There were no significant between-group differences.

Table 3. Adjusted Biomarker Levels by Group as a Proportion of Cigarette-Only Smoker Levels*

Parent Compound	Biomarker/Metabolite	Smokers		Former Smokers	
		Dual Cigarette–NRT Users (n = 36)	Dual Cigarette–EC Users (n = 36)	NRT-Only Users (n = 36)	EC-Only Users (n = 36)
Alkaloids					
Nicotine	Total nicotine equivalents†	104.2 (64.3–168.9)	156.8 (105.1–233.8)	121.6 (62.5–236.8)	126.9 (82.1–196.2)
	Nicotine‡	64.2 (39.2–104.9)	152.2 (90.7–255.1)	135.1 (68.1–268.0)	60.4 (35.8–101.8)
	Cotinine‡	46.8 (26.3–83.3)	69.7 (42.1–115.3)	82.1 (42.9–157.3)	75.1 (45.3–124.4)
Tobacco-specific *N*-nitrosamines					
4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone	4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol	57.1 (33.1–98.4)	81.2 (49.7–132.8)	11.6 (6.3–21.3)	2.5 (1.5–4.2)
Volatile organic compounds					
Acrolein	*N*-acetyl-S-(3-hydroxypropyl)-L-cysteine	107.1 (71.8–159.7)	91.2 (60.2–138.2)	35.3 (23.5–53.0)	33.3 (20.9–53.1)
Acrylamide	*N*-acetyl-S-(2-carbamoylethyl)-L-cysteine	80.2 (57.9–111.1)	115.9 (80.8–166.1)	45.4 (32.4–63.5)	42.9 (31.1–59.2)
Acrylonitrile	*N*-acetyl-S-(2-cyanoethyl)-L-cysteine	85.6 (48.7–150.4)	102.7 (63.7–165.6)	10.5 (5.4–20.6)	2.9 (1.7–4.7)
1,3-butadiene	*N*-acetyl-S-(4-hydroxy-2-buten-1-yl)-L-cysteine	101.9 (64.6–160.7)	115.0 (73.2–180.6)	19.9 (12.8–30.7)	11.0 (7.5–16.1)
Ethylene oxide, acrylonitrile, and vinyl chloride	*N*-acetyl-S-(2-hydroxyethyl)-L-cysteine	86.6 (58.7–127.8)	104.0 (73.9–146.4)	54.2 (38.4–76.5)	43.5 (30.8–61.3)

Cigarette = combustible cigarette; EC = e-cigarette; NRT = nicotine replacement therapy.
* Levels as a proportion of cigarette-only smoker levels are estimated from a model that adjusted for all variables in Table 2, latency to product use, and creatinine levels. For urinary metabolites, inputs to the model were divided by the ratio of observed to covariate-adjusted creatinine levels. Values are percentages (95% CIs).
† Sum of cotinine, nicotine, *trans*-3'-hydroxycotinine, cotinine N-oxide, nicotine 1'-oxide, norcotinine, and nornicotine levels measured in urine.
‡ Measured in saliva (all other metabolites were measured in urine).

olites of benzene (*N*-acetyl-S-(phenyl)-L-cysteine [PMA] and muconic acid [MU]), carbon disulfide (2-thioxothiazolidine-4-carboxylic acid [TTCA]), and styrene (*N*-acetyl-S-(1- and 2-phenyl-2-hydroxyethyl)-L-cysteine [PHEMA] and phenylglyoxylic acid [PGA]). Dual combustible cigarette–e-cigarette users had somewhat higher PMA, MU, and PHEMA levels, and dual combustible cigarette–NRT and combustible cigarette–e-cigarette users had somewhat higher PGA levels than other groups (**Appendix Table 1**). There were no appreciable between-group differences in TTCA levels. However, these metabolites were either nonspecific to the parent VOC measured (MU and TTCA have dietary contributions, and PGA is a metabolite of ethylbenzene and styrene exposure) or had low detection rates (PMA and PHEMA) (**Appendix Table 2**, available at Annals.org).

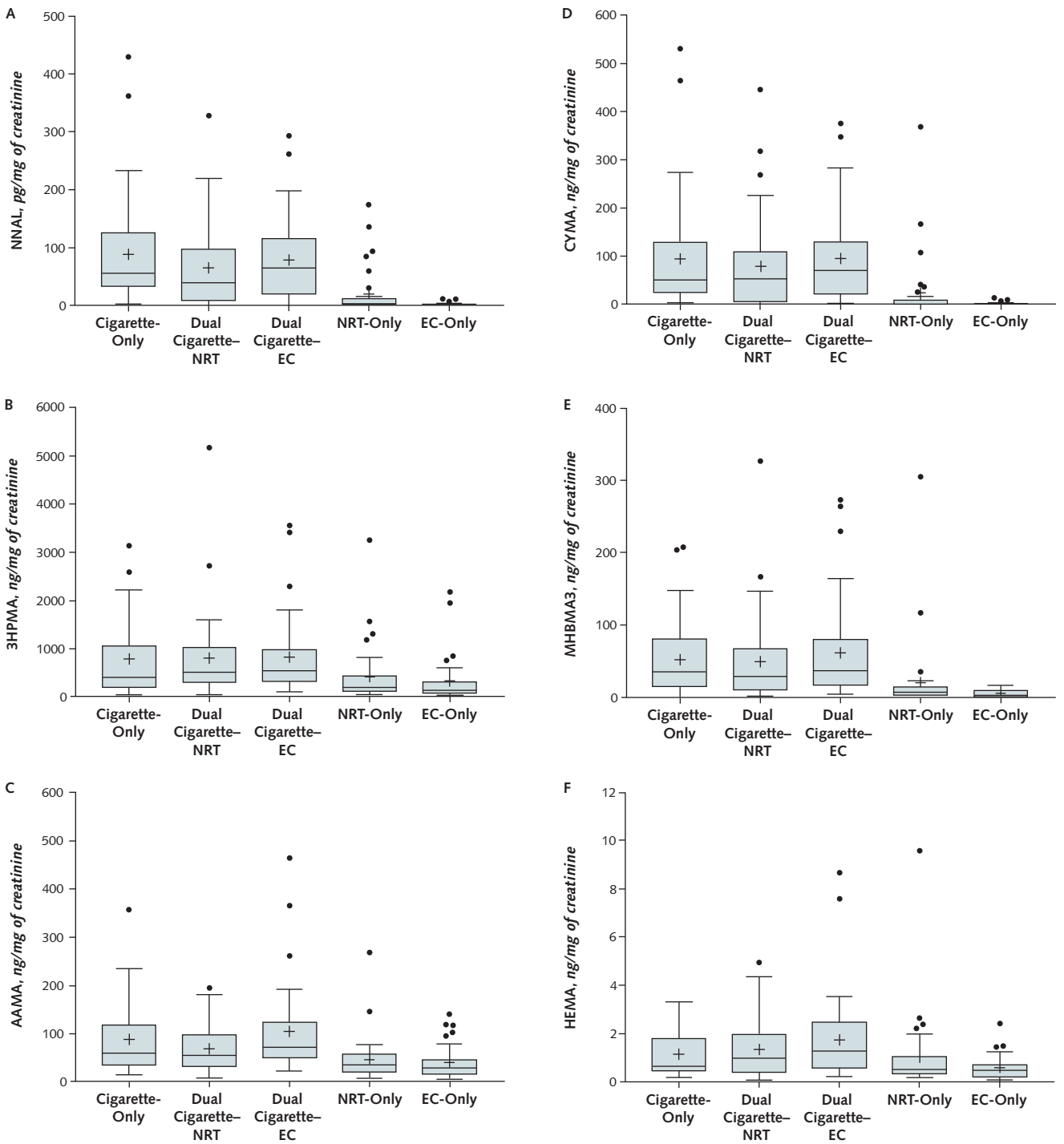
DISCUSSION

To our knowledge, this is the first direct comparison of the metabolite levels of nicotine and important carcinogens and toxins in long-term e-cigarette or NRT users. We found that former smokers who had switched to e-cigarette–only or NRT-only use obtained roughly similar levels of nicotine compared with combustible cigarette–only smokers, but results varied. Long-term NRT-only use and especially e-cigarette–only use, but not dual use of NRTs or e-cigarettes with combustible cigarettes, were associated with lower levels of known tobacco-related carcinogens and toxins measured in this study compared with combustible cigarette–only use.

The finding that NRT-only or e-cigarette–only use is associated with roughly similar nicotine intake compared with that of combustible cigarette–only use supports the view that users seek a particular level of nicotine intake, regardless of the delivery system (49), and adjust product use accordingly (50). This finding is consistent with more recent (51) but not older (8) studies on nicotine delivery from e-cigarettes, which may reflect the improved design of newer generations of e-cigarettes (52), and highlights the importance of focusing on experienced, long-term users rather than naive, short-term users. Similarly, efficient nicotine intake from NRT-only use has been observed in long-term (53) but not short- or intermediate-term NRT users (54). Nicotine intake was largely similar for both groups, which suggests that greater craving reductions observed in e-cigarette–only users than in NRT-only users (23, 55) may be due to factors other than nicotine delivery, such as the greater behavioral similarity of e-cigarette use (unlike NRT use) with smoking. This is consistent with research on nonnicotine sensory factors that have been shown to influence tobacco withdrawal (56). However, this study was not powered to detect anything other than relatively large effects, so results about smaller differences in nicotine intake between e-cigarettes and NRTs are indeterminate.

The lower levels of carcinogens and toxins associated with NRT-only and e-cigarette–only use in this study confirm the known low risk for complications from long-term NRT use (57). This finding also underscores the translation of greatly reduced concentrations of some carcinogens and toxins from e-liquids

Figure 2. Urinary metabolite levels for selected toxins and carcinogens, by group.



Data are raw values divided by ratio of observed urinary metabolites to covariate-adjusted creatinine levels. The levels below the limit of detection were imputed by the limit of detection divided by square root of 2. Boxplots show the median with interquartile range (25th percentile, 75th percentile). Error bars show Tukey's whiskers, and cross indicate arithmetic means (geometric means are provided in Appendix Table 1). Circles indicate outliers. Significant pairwise comparisons are presented in Appendix Table 1. Cigarette = combustible cigarette; EC = e-cigarette; NRT = nicotine replacement therapy. **A.** Tobacco-specific *N*-nitrosamine. NNAL = 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol. **B.** Acrolein. 3HPMA = *N*-acetyl-S-(3-hydroxypropyl)-L-cysteine. **C.** Acrylamide. AAMA = *N*-acetyl-S-(2-carbamoylethyl)-L-cysteine. **D.** Acrylonitrile. CYMA = *N*-acetyl-S-(2-cyanoethyl)-L-cysteine. **E.** 1,3-butadiene. MHBMA3 = *N*-acetyl-S-(4-hydroxy-2-buten-1-yl)-L-cysteine. **F.** Ethylene oxide. HEMA = *N*-acetyl-S-(2-hydroxyethyl)-L-cysteine.

and aerosols (4, 6, 58) to body-level exposure, contrary to worries that long-term e-cigarette use would result in

substantial harmful exposure (59). Given the involvement of these TSNAs and VOCs with cancer, cardiovascular diseases, and pulmonary diseases (42, 60), our results suggest that complete substitution of combustible cigarettes with e-cigarettes may reduce disease risk and support the assertion that e-cigarette use may be less harmful than smoking (2, 61–63). We found no evidence that long-term e-cigarette–only use was associated with greater levels of carcinogens or toxins than NRT-only use; on some measures, e-cigarette–only use was associated with lower levels. Although this could be due to occasional combustible cigarette smoking lapses by long-term NRT-only users, it is unlikely to have made a substantial contribution, given very low levels of tobacco-specific (as opposed to nicotine-specific) biomarkers for acrylonitrile, anabasine, and anatabine (64, 65) in this group. Alternatively, these differences may reflect typical low-level contamination in these products (for example, with TSNAs from tobacco-derived nicotine) (66), nonspecificity of the metabolite for the toxin (for example, muconic acid for benzene) (67), or non–smoking-related environmental sources of toxin exposure (for example, for styrene) (68). Contrary to findings from a recent short-term switching study (12), dual combustible cigarette–NRT or combustible cigarette–e-cigarette use was not associated with appreciable reductions in carcinogen and toxin levels. This may be because participants in our study may have been even heavier smokers before starting concurrent e-cigarette or NRT use, thus masking the benefit of potential partial substitution in our cross-sectional study, or because dual users used noncombustible products to bridge times of nonsmoking and thus did not actually reduce combustible cigarette consumption. Alternatively, lack of notable reductions in carcinogens and toxins after dual use may reflect either differences in study design (for example, different use pattern in long-term vs. short-term users) or our study's relatively low power to detect smaller, yet meaningful, effects. Further longitudinal research is needed to differentiate among these explanations.

Our findings have several implications. Although complete, long-term switching to e-cigarettes may produce a net benefit for the health outcomes of the smoking population because e-cigarette–only use significantly reduced exposure to known tobacco-related carcinogens and toxins, we found that dual use of e-cigarettes with combustible cigarettes did not reduce exposure appreciably. Therefore, e-cigarettes are likely to be beneficial only if complete cessation of combustible cigarette smoking is achieved. Thus, dual users should be encouraged to cease using combustible products to reduce long-term health risks. Our results also indicate that machine-derived and actual body-level exposure to toxins can be very different, as shown, for example, by greatly reduced aldehyde levels in e-cigarette users in this study compared with reportedly high levels in e-cigarette aerosols under certain laboratory conditions (5, 69). Of note, although e-cigarette–only and NRT-only use was associated with marked reductions in carcinogens and toxins compared with combustible cigarette–only use, use of these products did not eliminate exposure (and thus possible health risks) completely. Full cessation of all nicotine products remains the best option to avoid harm.

The study had several limitations. Although participants were recruited through diverse methods, resulting in a sample broadly similar to the population of NRT and e-cigarette users (16, 70), and we controlled for important confounders, between-group differences may not generalize and reflect self-selection. The sample was too small to allow more sophisticated analyses to evaluate the association of different types of e-cigarettes or NRTs (and other characteristics, such as e-cigarette flavors) with intake, and we may not have picked up on small but important differences in exposure levels. In particular, the lack of between-group differences in nicotine intake has to be interpreted cautiously given the low power to detect smaller effects and the variability across different urinary and salivary measures. Lastly, we did not assess indirect exposure and the analysis was limited by the number of biomarkers available and spot sampling, which can only provide a snapshot of exposure. However, given the lack of long-term data, we chose this pragmatic design to quickly evaluate potentially important associations of e-cigarette use with intake of carcinogens and toxins to inform further longitudinal work. Moreover, the relatively slow pharmacokinetics of the assessed metabolites provides stable estimates of recent exposure and should militate against variations associated with different patterns of use for different products. Future work should sample a larger range of biomarkers over a longer period, including those of actual harm, such as lung function measures, and evaluate the effect of potential interactions of users with device characteristics on the delivery of toxins to users and bystanders.

In conclusion, long-term NRT-only or e-cigarette–only use among former smokers is associated with substantially reduced levels of selected carcinogens and toxins compared with combustible cigarette smoking; however, concurrent use of NRTs or e-cigarettes with combustible cigarettes does not seem to offer this benefit. We found no evidence that e-cigarette–only use compared with NRT-only use is associated with greater levels of carcinogens and toxins. Nicotine delivery of e-cigarettes and NRTs, although variable, is roughly similar to combustible cigarettes, but smaller meaningful differences may exist.

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Appendix Table 1. Urinary and Saliva Biomarker Levels, by Group*

Parent Compound	Biomarker/Metabolite	Smokers			Former Smokers		*P* Value†
		Cigarette-Only Users (*n* = 37)	Dual Cigarette–NRT Users (*n* = 36)	Dual Cigarette–EC Users (*n* = 36)	NRT-Only Users (*n* = 36)	EC-Only Users (*n* = 36)	
Tobacco alkaloids (saliva), *ng/mL*							
Nicotine	Nicotine‡	260.3 (189.1–358.4)	147.2 (102.1–212.0)	299.4 (193.2–464.0)	158.5 (97.1–258.6)	184.4 (125.2–271.6)	0.003§
	Cotinine‡	174.8 (105.1–290.8)	67.1 (39.1–115.1)	149.2 (95.8–232.3)	83.9 (45.8–153.7)	179.6 (118.1–273.0)	0.134
Tobacco alkaloids (urine)							
Nicotine, *nmol/mg of creatinine*	Total nicotine equivalents‡	21.1 (14.0–31.8)	8.5 (3.9–18.4)	28.8 (16.6–49.8)	6.3 (2.9–14.1)	25.0 (14.8–42.0)	0.204
	trans-3′-Hydroxycotinine	8.5 (5.1–14.3)	3.2 (1.4–7.4)	10.9 (6–19.8)	2.8 (1.2–6.3)	11.4 (6.5–19.9)	0.442
	Cotinine	5.9 (3.8–9.3)	1.8 (0.7–4.4)	8.2 (4.6–14.8)	1.4 (0.6–3.5)	7.5 (4.5–12.4)	0.188
	Nicotine	1.9 (1.2–3.3)	1.2 (0.5–2.5)	4 (2.3–7.1)	0.8 (0.3–1.7)	2.5 (1.5–4.2)	0.088
	Cotinine N-oxide	0.6 (0.4–1.0)	0.2 (0.1–0.5)	0.8 (0.5–1.4)	0.2 (0.1–0.4)	0.8 (0.5–1.3)	0.254
	Nicotine 1′-oxide	0.7 (0.4–1.1)	0.4 (0.2–0.8)	1.3 (0.7–2.2)	0.2 (0.1–0.6)	0.9 (0.5–1.6)	0.166
	Norcotinine	0.2 (0.1–0.3)	0.1 (0.1–0.2)	0.3 (0.2–0.5)	0.1 (0.1–0.1)	0.2 (0.1–0.3)	0.161
	Nornicotine	0.2 (0.1–0.2)	0.1 (0.1–0.2)	0.2 (0.1–0.4)	0.1 (0.1–0.1)	0.1 (0.1–0.2)	0.022§
Anabasine, *pmol/mg of creatinine*	Anabasine	17.0 (11.2–25.8)‖¶	11.1 (6.3–19.4)	25.5 (16.3–40.1)‖¶	5.5 (3.5–8.7)**††	6.2 (4.1–9.5)**††	<0.001
Anatabine, *pmol per milligram of creatinine*	Anatabine	26.0 (16.3–41.4)‖¶	14.9 (7.6–29.2)‖	36.0 (22.0–59.1)‖¶	3.8 (2.4–6.2)**††‡‡	4.6 (2.8–7.6)**††	<0.001
Tobacco-specific *N*-nitrosamines, *pg/mg of creatinine*							
4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK)	4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL)‡	53.4 (36.6–77.8)‖¶	24.4 (13.2–45.1)‖¶	44.5 (28.5–69.4)‖¶	4.83 (2.79–8.34)¶**††‡‡	1.47 (1.02–2.12)‖**††‡‡	<0.001
N′-nitrosoanabasine	*N*′-nitrosoanabasine (NAB)	6.17 (4.31–8.82)‖¶	3.64 (2.20–6.02)‖¶	6.02 (4.15–8.73)‖¶	1.52 (1.09–2.12)**††‡‡	1.07 (0.79–1.47)**††‡‡	<0.001
N′-nitrosoanatabine	*N*′-nitrosoanatabine (NAT)	32.8 (20.5–52.5)‖¶	11.8 (5.77–24.0)‖¶	30.8 (18.5–51.1)‖¶	2.95 (1.81–4.81)**††‡‡	1.79 (1.21–2.67)**††‡‡	<0.001
Volatile organic compounds, *ng/mg of creatinine*							
Acrolein	*N*-acetyl-S-(2-carboxyethyl)-L-cysteine (CEMA)	119.8 (88.2–162.9)‖¶	136.1 (100.7–184)‖¶	141.8 (106.7–188.4)‖¶	67.8 (49.3–93.2)**††‡‡	54.6 (41.7–71.4)**††‡‡	<0.001
	N-acetyl-S-(3-hydroxypropyl)-L-cysteine (3HPMA)‡	488.4 (345.1–691.2)‖¶	499.7 (350–713.5)‖¶	574.5 (429.1–769.2)‖¶	236.1 (168.1–331.6)**††‡‡	175.3 (124–247.8)**††‡‡	<0.001
Acrylamide	*N*-acetyl-S-(2-carbamoylethyl)-L-cysteine (AAMA)‡	65.6 (50.6–85.1)‖¶	52.5 (40.4–68.4)‖¶	82.4 (66.1–102.8)‖¶	33.6 (25.8–43.7)**††‡‡	29.3 (22.3–38.3)**††‡‡	<0.001
	N-acetyl-S-(2-carbamoyl-2-hydroxyethyl)-L-cysteine (GAMA)	18.5 (14.7–23.3)‖¶	16.8 (13.1–21.5)	24.3 (19.6–30.2)‖¶	12.1 (9.5–15.5)**††	10.0 (7.6–13.2)**††	<0.001
Acrylonitrile	*N*-acetyl-S-(2-cyanoethyl)-L-cysteine (CYMA)‡	49.2 (32.9–73.6)‖¶	28.4 (15.6–51.9)‖¶	51.6 (33.6–79.2)‖¶	3.7 (2.1–6.5)**††‡‡	1.4 (1.1–1.9)**††‡‡	<0.001
Benzene	*trans,trans*-muconic acid (MU)	78.6 (58.2–106.2)	106.8 (72.7–157.0)	135.0 (102.3–178.1)¶	131.8 (94.1–184.5)	55.2 (42.3–71.9)††	0.002
	N-acetyl-S-(phenyl)-L-cysteine (PMA)	0.64 (0.48–0.84)††	0.44 (0.30–0.63)††	1.43 (1.11–1.83)¶**‡‡	0.52 (0.37–0.71)	0.74 (0.55–0.98)††	<0.001
1,3-butadiene	*N*-acetyl-S-(3,4-dihydroxybutyl)-L-cysteine (DHBMA)	202.7 (162.8–252.3)¶	204.3 (162.3–257.3)¶	294.9 (242.9–358.0)¶	204.2 (156.9–265.9)	156.3 (126.0–193.8)**††‡‡	<0.001
	N-acetyl-S-(4-hydroxy-2-buten-1-yl)-L-cysteine (MHBMA3)‡	29.8 (19.9–44.8)‖¶	23.9 (15.1–37.9)‖¶	36.6 (25.4–52.6)‖¶	7.67 (5.08–11.6)**††‡‡	4.44 (3.42–5.78)**††‡‡	<0.001

Continued on following page

***Appendix Table 1*–Continued**

Parent Compound	Biomarker/Metabolite	Smokers			Former Smokers		*P* Value†
		Cigarette-Only Users (*n* = 37)	Dual Cigarette-NRT Users (*n* = 36)	Dual Cigarette-EC Users (*n* = 36)	NRT-Only Users (*n* = 36)	EC-Only Users (*n* = 36)	
Carbon disulfide	2-thioxothiazolidine-4-carboxylic acid (TTCA)	6.03 (4.40–8.27)	13.8 (8.79–21.7)	9.95 (6.85–14.5)	13.4 (9.07–19.7)	6.84 (4.33–10.8)	0.015§
Crotonaldehyde	*N*-acetyl-S-(3-hydroxypropyl-1-methyl)-L-cysteine (HPMMA)	804.2 (563.8–1147.1)‖¶	735.3 (495.2–1091.7)‖¶	1199.5 (881.9–1631.6)‖¶	366.3 (266.0–504.5)**††‡‡	235.9 (179.1–310.7)**††‡‡	<0.001
Cyanide	2-aminothiazoline-4-carboxylic acid (ATCA)	91.2 (69.6–119.5)¶	107.1 (79.4–144.5)‖¶	132.3 (97.8–179.0)‖¶	102.0 (72.6–143.4)	55.3 (41.0–74.5)**††‡‡	0.013
N,N-dimethylformamide	*N*-acetyl-S-(N-methylcarbamoyl)-L-cysteine (AMCC)	162.2 (120.6–218.1)¶	138.5 (95.4–201.2)¶	176.3 (129.1–240.5)¶	100.2 (72.4–138.7)	60.8 (44.4–83.3)**††‡‡	<0.001
Ethylene oxide, acrylonitrile, vinyl chloride	*N*-acetyl-S-(2-hydroxyethyl)-L-cysteine (HEMA)‡	0.81 (0.61–1.07)‖¶	0.81 (0.55–1.18)	1.15 (0.84–1.57)‖¶	0.64 (0.48–0.84)**††	0.42 (0.32–0.55)**††‡‡	<0.001
Propylene oxide	*N*-acetyl-S-(2-hydroxypropyl)-L-cysteine (2HPMA)	41.1 (30.4–55.6)¶	47.3 (35.6–63.0)¶	68.9 (52.6–90.4)‖¶	37.4 (28.7–48.9)††	29.3 (21.9–39.3)**††‡‡	<0.001
Styrene	Mandelic acid (MA)	188.6 (147.4–241.2)¶	198.7 (153.8–256.7)¶	227.2 (181.1–284.9)¶	173.0 (127.3–235.3)	100.8 (78.2–129.9)**††‡‡	<0.001
	N-acetyl-S-(1 and 2-phenyl-2-hydroxyethyl)-L-cysteine (PHEMA)	0.75 (0.57–0.98)	0.82 (0.56–1.18)	1.09 (0.8–1.48)¶	0.75 (0.55–1.00)	0.48 (0.36–0.63)††	0.001
Styrene, ethylbenzene	Phenylglyoxylic acid (PGA)	88.0 (62.6–123.8)	129.9 (92.1–183.3)¶	124.5 (91.1–170.0)	88.1 (60.6–128.2)	71.1 (53.7–94.1)‡‡	0.007
Xylene	2-methylhippuric acid (2MHA)	41.9 (30.1–58.4)‖¶	36.3 (23.9–55.2)‖¶	56.9 (41.8–77.4)‖¶	19.6 (13–29.7)**††‡‡	10.5 (7.80–14.2)	<0.001
	3- + 4-methylhippuric acids (34MHA)	266.5 (182.1–390.1)‖¶	181.1 (119.7–274.0)‖¶	273.2 (201.1–371.0)‖¶	76.3 (48.8–119.4)**††‡‡	51.4 (38.5–68.6)**††‡‡	<0.001

Cigarette = combustible cigarette; EC = e-cigarette; NRT = nicotine replacement therapy.
* Data presented are log-transformed raw values (for urinary metabolites also standardized for creatinine). Statistical comparisons were carried out on nontransformed data and adjusted for all variables in **Table 2**, latency to product use, and creatinine levels. Values are geometric means (95% CIs).
† Omnibus test result, adjusted for the number of reported comparisons in this table using the false discovery rate (46).
‡ Non–log-transformed data shown in **Figures 1** and **2**.
§ Overall differences but no significant (Sidak-corrected) difference in post hoc test.
‖ Indicates statistically significant (Sidak-corrected) difference ($P < 0.05$) for NRT-only users.
¶ Indicates statistically significant (Sidak-corrected) difference ($P < 0.05$) for EC-only users.
** Indicates statistically significant (Sidak-corrected) difference ($P < 0.05$) for cigarette-only smokers.
†† Indicates statistically significant (Sidak-corrected) difference ($P < 0.05$) for dual cigarette–EC users.
‡‡ Indicates statistically significant (Sidak-corrected) difference ($P < 0.05$) for dual cigarette–NRT users.

Appendix Table 2. Proportion of Samples Below Limit of Detection, by Group and Across All Samples*

Biomarker/Metabolite†	Limit of Detection	All Samples	Smokers			Former Smokers	
			Cigarette-Only Users (*n* = 37)	Dual Cigarette–NRT Users (*n* = 36)	Dual Cigarette–EC Users (*n* = 36)	NRT-Only Users (*n* = 36)	EC-Only Users (*n* = 36)
Nicotine‡	10 ng/mL	1.1	0.0	0.0	2.8	2.8	0.0
Cotinine‡	10 ng/mL	14.4	13.5	16.7	5.6	27.8	8.3
trans-3'-hydroxycotinine	0.03 ng/mL	0.0	0.0	0.0	0.0	0.0	0.0
Cotinine	0.03 ng/mL	0.0	0.0	0.0	0.0	0.0	0.0
Nicotine	10.5 ng/mL	11.0	2.7	13.9	5.6	30.6	2.8
Cotinine N-oxide	2 ng/mL	7.7	0.0	13.9	2.8	19.4	2.8
Nicotine 1'-oxide	2.5 ng/mL	8.8	0.0	13.9	2.8	25.0	2.8
Norcotinine	2.5 ng/mL	11.6	0.0	22.2	5.6	27.8	2.8
Nornicotine	1.1 ng/mL	17.7	5.4	30.6	11.1	33.3	8.3
Anabasine	0.5 ng/mL	29.3	10.8	36.1	13.9	55.6	30.6
Anatabine	0.4 ng/mL	29.3	5.4	27.8	11.1	61.1	41.7
N-acetyl-S-(2-carboxyethyl)-L-cysteine (CEMA)	8 ng/mL	2.8	0.0	2.8	0.0	5.6	5.6
N-acetyl-S-(3-hydroxypropyl)-L-cysteine (3HPMA)	13 ng/mL	0.0	0.0	0.0	0.0	0.0	0.0
N-acetyl-S-(2-carbamoylethyl)-L-cysteine (AAMA)	2.2 ng/mL	0.0	0.0	0.0	0.0	0.0	0.0
N-acetyl-S-(2-carbamoyl-2-hydroxyethyl)-L-cysteine (GAMA)	9.4 ng/mL	30.9	16.2	25.0	19.4	41.7	52.8
N-acetyl-S-(2-cyanoethyl)-L-cysteine (CYMA)	0.5 ng/mL	2.2	0.0	0.0	0.0	2.8	8.3
trans,trans-muconic acid (MU)	20 ng/mL	6.6	2.7	2.8	2.8	5.6	19.4
N-acetyl-S-(phenyl)-L-cysteine (PMA)	0.6 ng/mL	56.9	37.8	94.4	30.6	86.1	36.1
N-acetyl-S-(3,4-dihydroxybutyl)-L-cysteine (DHBMA)	5 ng/mL	0.0	0.0	0.0	0.0	0.0	0.0
N-acetyl-S-(4-hydroxy-2-buten-1-yl)-L-cysteine (MHBMA3)	0.6 ng/mL	0.0	0.0	0.0	0.0	0.0	0.0
2-thioxothiazolidine-4-carboxylic acid (TTCA)	3.5 ng/mL	28.2	29.7	22.2	41.7	13.9	33.3
N-acetyl-S-(3-hydroxypropyl-1-methyl)-L-cysteine (HPMMA)	2 ng/mL	0.0	0.0	0.0	0.0	0.0	0.0
2-aminothiazoline-4-carboxylic acid (ATCA)	15 ng/mL	7.2	0.0	8.3	5.6	5.6	16.7
N-acetyl-S-(*N*-methylcarbamoyl)-L-cysteine (AMCC)	5.5 ng/mL	0.6	0.0	0.0	2.8	0.0	0.0
N-acetyl-S-(2-hydroxyethyl)-L-cysteine (HEMA)	0.6 ng/mL	48.6	32.4	41.7	27.8	61.1	80.6
N-acetyl-S-(2-hydroxypropyl)-L-cysteine (2HPMA)	1.3 ng/mL	0.0	0.0	0.0	0.0	0.0	0.0
Mandelic acid (MA)	12 ng/mL	1.1	0.0	0.0	0.0	2.8	2.8
N-acetyl-S-(1 and 2-phenyl-2-hydroxyethyl)-L-cysteine (PHEMA)	0.7 ng/mL	61.3	48.6	58.3	55.6	63.9	80.6
Phenylglyoxylic acid (PGA)	12 ng/mL	9.9	10.8	11.1	8.3	11.1	8.3
2-methylhippuric acid (2MHA)	5 ng/mL	0.0	0.0	0.0	0.0	0.0	0.0
3- + 4-methylhippuric acids (34MHA)	8 ng/mL	1.7	0.0	0.0	0.0	2.8	5.6
4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL)	0.6 pg/mL	6.6	0.0	2.8	0.0	8.3	22.2
N'-nitrosoanabasine (NAB)	4.0 pg/mL	47.0	8.1	38.9	25.0	80.6	83.3
N'-nitrosoanatabine (NAT)	1.6 pg/mL	43.1	5.4	41.7	13.9	75.0	80.6

Cigarette = combustible cigarette; EC = e-cigarette; NRT = nicotine replacement therapy.
* Values are percentages.
† Urinary biomarkers unless otherwise indicated.
‡ Measured in saliva.