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Associations of Cigarettes Smoked Per Day with Biomarkers of Exposure Among US Adult Cigarette Smokers in the Population Assessment of Tobacco and Health (PATH) Study Wave 1 (2013-2014)

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Abstract

Background: The dose-response relationships between number of cigarettes smoked per day (CPD) and health outcomes such as cancer and heart disease are well established, but much less is known about the relationships between CPD and biomarkers of exposure.

Methods: We analyzed biomarker data by CPD from over 2700 adult daily cigarette smokers in Wave 1 of the Population Assessment of Tobacco and Health (PATH) Study. Tobacco use categories consisted of exclusive cigarette smokers, dual cigarette and e-cigarette users, and dual cigarette and smokeless tobacco users.

Results: Biomarker concentrations consistently increased with CPD for each tobacco user group, although concentrations tended to level off at high smoking levels, such as those at and above 20 CPD. Dual cigarette and e-cigarette users had higher levels of some biomarkers such as TNE (Total Nicotine Equivalents)-2 ($p=0.0036$) than exclusive cigarette smokers, and dual cigarette and smokeless tobacco users had higher levels of NNAL ($p<0.0001$) and NNN ($p=0.0236$) than exclusive cigarette smokers.

Conclusions: Among daily smokers, exposure to tobacco toxicants and constituents exhibits a dose-response relationship by number of cigarettes smoked, but the relationship is not necessarily linear in form. Dual users of cigarettes with either e-cigarettes or smokeless tobacco are exposed to higher levels of certain toxicants and carcinogens than exclusive cigarette smokers.

Impact: Availability of biomarker data by CPD may aid in comparisons between cigarette smoking and use of new and potentially reduced exposure tobacco products, which may result in different levels of constituent and toxicant exposure.

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Keywords

cigarette; e-cigarette; smokeless tobacco; biomarker

Introduction

Researchers have consistently found a strong dose-response relationship between cigarettes smoked per day (CPD) and health effects such as increased mortality risk,^{1,2} although the precise nature of this relationship can vary by condition. For example, Doll and Peto³ and Law et al.⁴ observed linear relationships between reported CPD and age-specific lung cancer risks. Kawachi et al.^{5,6} also found that coronary heart disease and stroke risk were strongly associated with CPD among women in the Nurses' Health Study, even though the relationship was not necessarily linear in form. As such, they noted that women smoking only one to four CPD still had double the risk of heart disease compared to never smokers.

Much less is known, however, about the relationship between CPD and levels of exposure to toxic constituents in cigarette tobacco and smoke. This topic is particularly relevant at the present time, given that it has been suggested that some new tobacco products may provide reduced exposure to these constituents compared to cigarettes.^{7,8} Having accurate and up-to-date biomarker of exposure data by CPD could thus help in the assessment of the potential health risks of these products by providing a basis for comparison of how their use and resulting exposure compares to cigarette use at various levels of cigarettes smoked. In addition, the effects of dual use of cigarettes with other tobacco products, including e-cigarettes and smokeless tobacco, continue to be investigated and examined, even though definitions of dual use can vary. Some researchers have suggested that dual users of cigarettes and smokeless tobacco may tend to smoke fewer CPD than exclusive cigarette smokers,⁹ whereas others have emphasized the possibility that these dual users may increase their exposure to tobacco constituents and could thus increase their potential for dependence and harm.¹⁰ Research on dual use of cigarettes and e-cigarettes is more limited, but some published data suggest that these dual users may receive comparable exposure to tobacco-specific nitrosamines (TSNAs) and volatile organic compounds (VOCs) as exclusive cigarette smokers.¹¹ More recent research has suggested that the exposure varies with CPD and that dual users who smoke higher numbers of CPD have higher levels of the TSNA 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL) and the VOC N-Acetyl-S-(2-cyanoethyl)-l-cysteine (CYMA).¹² As such, having accurate biomarker of exposure data by CPD for both exclusive cigarette smokers and dual users could assist in the assessment of constituent and toxicant exposure and thus provide information about possible health risks of these use patterns.

Some previous analyses have examined biomarkers by CPD but for a limited number of smokers or biomarkers. Joseph et al.¹³ analyzed exposure data for carbon monoxide, the nicotine metabolite cotinine, NNAL, and the polycyclic aromatic hydrocarbon (PAH) metabolite 1-hydroxypyrene (1-HOP) by CPD among 400 smokers from clinical studies and recruited cohorts in the Minneapolis area. They found that concentrations of these biomarkers generally increased with CPD, although there was a tendency for concentrations

of some biomarkers such as cotinine and NNAL to level off at very high CPD levels. Rostron¹⁴ analyzed NNAL by CPD and race/ethnicity for over 1500 daily smokers using nationally representative data from the US National Health and Nutrition Examination Survey (NHANES). He also found a general increase with CPD that tended to level off at high levels of CPD. Caraballo et al.¹⁵ found a similar pattern for cotinine using NHANES III data.

This analysis examines a much larger and comprehensive set of biomarkers of exposure by CPD among over 2700 US adult daily cigarette smokers in the nationally representative Population Assessment of Tobacco and Health (PATH) Study. It includes a wide array of biomarkers including indicators of exposure to nicotine, TSNAs, PAHs, VOCs, metals, and arsenic. It examines biomarker concentrations among exclusive cigarette smokers as well as in dual users who use e-cigarettes or smokeless tobacco in addition to cigarettes. The study represents the first analysis of the dose-response relationship between biomarkers of exposure and CPD using the extensive resources provided by the PATH Study.

Materials and Methods

The PATH Study is a nationally representative, longitudinal cohort study of tobacco use and health outcomes in the US conducted by the National Institutes of Health and the Food and Drug Administration.¹⁶ Wave 1 survey data and biospecimen collection occurred between September 2013 and December 2014. All adult Wave 1 PATH interview participants were asked to provide urine and blood samples. Biospecimens from a stratified probability sample of 11,522 respondents who provided a urine sample were selected for biomarker analysis. These individuals were chosen to ensure that respondents represented diverse tobacco product use patterns including users of multiple tobacco products and never users of any tobacco product. Among these participants, 7,159 also provided a blood sample. Biospecimens were analyzed for relevant biomarkers at laboratories of the National Center for Environmental Health (NCEH), Centers for Disease Control and Prevention (CDC).

Cotinine and trans-3'-hydroxycotinine, were analyzed in all samples, and their molar sum was calculated as TNE (Total Nicotine Equivalents)-2. Nicotine and its six major metabolites were analyzed in urine samples with cotinine levels at or above 20 ng/mL, and their molar sum was calculated as TNE-7. Serum cotinine and hydroxycotinine and the minor alkaloids anabasine and anatabine were also analyzed as were four TSNAs including NNAL, the principal metabolite of the lung carcinogen NNK,¹⁷ and NNN (N'-nitrosonornicotine), a carcinogen known to cause tumors in the esophagus and oral cavity.¹⁸ Seven monohydroxylated metabolites of PAHs including 1-HOP and 2-hydroxyfluorene (2-FLU) were measured. Twenty biomarkers of exposure to VOCs were analyzed including N-acetyl-S-3-hydroxypropylcysteine (HPMA), a metabolite of acrolein, N-Acetyl-S-(2-cyanoethyl)-l-cysteine (CYMA), a metabolite of acrylonitrile, and (MHB3), a metabolite of 1,3-Butadiene. It has been suggested that 1,3-Butadiene and acrylonitrile are the particular constituents in cigarette smoke that represent the largest cancer risks and that acrolein represents the largest non-cancer risk, particularly for respiratory effects.¹⁹ Eight metals including cadmium and lead were analyzed, and total inorganic arsenic was calculated as the sum of arsenous acid, arsenic acid, dimethylarsinic acid, and monomethylarsonic acid.

Creatinine was analyzed in urine samples as a measure of hydration. Individuals with creatinine values outside the normal range of 10 – 370 mg/dL were excluded from this analysis. In cases where biomarker concentrations were found to be below the Limit of Detection (LOD), imputed values equal to the LOD divided by the square root of 2 were used in analyses. All results met the Division of Laboratory Sciences, CDC/NCEH quality assurance and control criteria for accuracy and precision, similar to the Westgard rules.²⁰ Quality assurance and control protocols also met the mandates of the 1988 Clinical Laboratory Improvement Act. The accuracy of biomarker measurements was assessed as the percentage of a target concentration and imprecision expressed as the coefficient of variation (CV). Reported intra-day imprecision generally had a CV between 5% and 10%, with some values approaching 15% for sample concentrations near the LOD. Full details of the biospecimen collection and biomarker analysis are provided as Supplementary Methods Material.

Tobacco user groups consisted of exclusive cigarette smokers, who currently used cigarettes every day; dual cigarette and e-cigarette users, who currently used cigarettes every day and e-cigarettes every day or some days; and dual cigarette and smokeless tobacco users, who used cigarettes every day and either smokeless tobacco or snus every day or some days. The analysis was restricted to daily cigarette smokers to ensure consistency in exposure. Study participants were excluded from these groups if they currently used filtered cigars, cigarillos, traditional cigars, pipes, hookah, or dissolvables every day or some days or had used nicotine replacement therapy products in the past three days. Exclusive cigarette smokers and dual cigarette and e-cigarette users also could not currently use smokeless tobacco or snus, and exclusive cigarette smokers and dual cigarette and smokeless tobacco users could not currently use e-cigarettes. Current daily cigarette smokers in the PATH Study were asked, “On average, about how many cigarettes do you now smoke each day?” CPD was categorized as 0-4, 5-9, 10-14, 15-19, 20-24, and 25 or more cigarettes per day. Participants were excluded from the analysis if their recorded CPD exceeded 60 because of the possibility of misreporting. Among eligible daily cigarette smokers, 99.3% reported smoking 0 to 60 CPD.

We examined the distribution of important demographic characteristics by tobacco user group looking at sex, age, race/ethnicity, and educational attainment. Age was categorized as 18 to 24 years, 25 to 34 years, 35 to 54 years, and 55 years or greater. Race/ethnicity was categorized as non-Hispanic White, non-Hispanic other race including multi-racial, and Hispanic. Educational attainment was categorized as less than high school graduate, General Educational Development (GED) certificate, high school graduate, some college or associate’s degree, and completed college or more. Differences between groups for these characteristics were assessed using Rao-Scott chi-squared tests.

Geometric mean concentrations were calculated for smokers by CPD category and tobacco user group for each biomarker. Results for a set of biomarkers selected for their importance as biomarkers of harmful or potentially harmful constituents (HPHCs) are presented in the text, and results for the remaining biomarkers of exposure analyzed in the PATH Study as well as biomarker levels by sex and race/ethnicity group are presented as supplementary material. In this study, all available urinary and serum biomarkers of exposure were analyzed

for selected PATH participants. Comparisons of biomarker levels between tobacco user groups for particular CPD categories were conducted as t-tests of logged biomarker concentrations, and overall biomarkers levels between groups were compared with and without adjustment for CPD. Tests of linear trend in logged biomarker concentrations by CPD within groups were conducted using linear regression analysis. Geometric mean ratios were calculated comparing biomarker levels for tobacco users by CPD category to never tobacco users with adjustment for age, sex, race/ethnicity, and educational attainment. Smoothed percentile curves of biomarker concentrations by CPD were estimated using quantile regression and plotted as natural cubic spline curves with four degrees of freedom.

Estimates were considered potentially unreliable if the unweighted sample size of a non-proportion estimate or the denominator of a proportion was less than 50, the relative standard error of an estimate was greater than 30%, or an estimate was calculated from a sample in which more than 40% of the biomarker values were below the LOD. Estimates were suppressed if the sample size was less than three respondents or if the estimate would allow for calculation of an estimate with sample size less than three. All analyses were conducted using SAS version 9.4 (SAS Institute, Cary, NC), and all figures were constructed using R version 3.0.2 (R Core Team, Vienna, Austria). Restricted-use PATH biomarker data²¹ were analyzed using biomarker sample weights with balanced repeated replicate methods to account for the PATH complex survey design. Confidence intervals for proportions were calculated using the Wilson method.²²

Results

Table 1 presents the distribution of smokers in each tobacco user group by demographic characteristics and CPD category. Dual cigarette and e-cigarette users were more likely to be female ($p = 0.0094$) and dual cigarette and smokeless tobacco users were more likely to be male ($p < 0.0001$) than exclusive cigarette smokers. Dual cigarette and smokeless tobacco users were more likely to be younger than 35 years old than either exclusive cigarette smokers ($p < 0.0001$) or dual cigarette and e-cigarette users ($p < 0.0001$). Exclusive cigarette smokers were less likely to be white ($p < 0.0001$ and $p = 0.0003$ compared to dual e-cigarette and dual smokeless tobacco users, respectively) and more likely to be other race ($p < 0.0001$ and $p = 0.0022$) than dual cigarette and e-cigarette users and dual cigarette and smokeless tobacco users. More than a third of dual cigarette and smokeless tobacco users had less than a high school diploma or had a GED, making them more likely than dual cigarette and e-cigarette users ($p = 0.0002$) to be in these education categories. Over half of dual cigarette and e-cigarette users had at least some college education, more than exclusive cigarette users ($p < 0.0001$) and dual cigarette and smokeless tobacco users ($p = 0.0001$). The distributions of exclusive cigarette and dual cigarette and e-cigarette users by CPD category were similar, although dual cigarette and smokeless tobacco users were more likely than exclusive cigarette smokers to report smoking at least 20 cpd ($p = 0.042$) and had higher median CPD than the other two user groups.

Table 2 shows biomarker concentrations by CPD category for 10 biomarkers representative of different classes of HPHCs. Overall, dual cigarette and e-cigarette users had higher levels of TNE-2 ($p = 0.0036$), NNAL ($p = 0.0323$), 1-HOP ($p = 0.008$), HPMA ($p = 0.0445$), and

MHB3 ($p = 0.0164$) than exclusive cigarette smokers, and dual cigarette and smokeless tobacco users had higher levels of NNAL ($p < 0.0001$) and NNN ($p = 0.0236$) than exclusive cigarette smokers. In general, there was a clear dose-response relationship for each biomarker, although there was some evidence of concentrations leveling off at higher CPD levels for some biomarkers. For exclusive cigarette smokers and dual cigarette and e-cigarette users, the p -values for trend by CPD were <0.001 for lead and < 0.0001 for each of the other biomarkers. Sample size was more limited for dual cigarette and smokeless tobacco users, but p -values for trend by CPD were <0.05 for NNAL, NNN, HPMA, CYMA, MHB3, and cadmium. Estimates for the full set of biomarkers are presented as Supplementary Tables 1, and tables showing overall biomarker levels for the tobacco users groups by sex and race/ethnicity group are presented as Supplementary Tables 2.

Figure 1 presents biomarker concentrations expressed as smoothed percentile curves by CPD for exclusive cigarette smokers for four representative biomarkers – TNE-2, NNAL, 1-HOP, and CYMA. The general pattern for each biomarker is similar, with concentrations steadily increasing from 0 to 10 CPD and the rate of increase slowing down at higher CPD levels. The curve for 1-HOP is much flatter than those for TNE-2, NNAL, and CYMA. Figures 2 and 3 present similar plots for dual cigarette and e-cigarette users and dual cigarette and smokeless tobacco users (there were no dual cigarette and smokeless tobacco users with CPD greater than 40).

Additional tables presenting results for the full set of biomarkers available in the PATH Study are available as supplementary material.

Discussion

This analysis has examined the relationship between concentrations of biomarkers of exposure associated with tobacco use and cigarettes smoked per day among daily cigarette smokers. Biomarker concentrations generally increased with CPD, although the particular nature of the dose-response relationship often varied by biomarker and was not necessarily linear in form, particularly at higher CPD levels, such as those at and above 20 CPD. Dual cigarette and e-cigarette users had higher overall mean levels of TNE-2, NNAL, 1-HOP, HPMA, and MHB3 than exclusive cigarette smokers, and dual cigarette and smokeless tobacco users had higher levels of NNAL and NNN.

These results are generally consistent with previous findings, although this analysis has presented exposure data by CPD for a much larger set of biomarkers from the large, nationally representative prospective PATH cohort study. The shapes of the dose-response curves for NNAL and 1-HOP in exclusive cigarette smokers were generally consistent with those reported previously by Joseph et al.¹³ and Rostron,¹⁴ and the curve for TNE-2 was similar to the one observed previously for cotinine by Caraballo et al.¹⁵ Similar patterns by CPD were observed here for other biomarkers such as CYMA as well as for dual users of cigarettes and e-cigarettes or smokeless tobacco. The higher levels of TSNA's such as NNAL and NNN among dual cigarette and smokeless tobacco users compared to exclusive cigarette smokers seen in this study are consistent with previous research that has found that smokeless tobacco users have TSNA levels that often exceed those of cigarette smokers.^{23,24}

Dual users of cigarettes and e-cigarettes also had elevated levels of several biomarkers compared to exclusive cigarette smokers. Some of these results can be difficult to interpret, given that it is not entirely clear if these differences are due to higher cigarette consumption and exposure not necessarily captured by self-reported CPD or additional exposure due to e-cigarette use. Even so, these results are consistent with previous estimates from more limited data that found evidence that these dual users can have higher levels of total nicotine equivalents than exclusive cigarette smokers.¹¹ Our study also found increased NNAL levels among dual cigarette and e-cigarette levels, although previous research has found that exclusive e-cigarette users tend to have low levels of TSNA exposure.¹¹

This analysis also provides information and data that may prove useful in the assessment of potential reduced exposure tobacco products. Concentrations for a wide range of biomarkers of various types have been presented for multiple CPD levels. These values provide a benchmark for comparison with levels produced by new and modified products. The estimates presented here also demonstrate that even relatively low levels of tobacco use, as measured by CPD, still result in exposure to harmful constituents at levels that exceed and are often several times higher than exposure for never tobacco users.

There are certain limitations to this analysis. CPD was self-reported by participants, and it has been observed that respondents often report cigarette consumption using round numbers, in particular 20, which is the number of cigarettes commonly in a pack.²⁵ Care should be taken in interpreting biomarker estimates at high CPD levels, such as those above 40, given the relatively small number of smokers reporting such values. Sample size for some CPD categories was limited, particularly for dual cigarette and smokeless tobacco users. The analysis was restricted to daily cigarette smokers to ensure consistency in exposure and thus did not include some day smokers. The study also involved numerous statistical comparisons, and statistically significant associations may not represent causal relationships.

As noted, interpretation of results for dual users can be challenging, given that frequency and quantity of use of tobacco products can differ substantially among these users. As such, overall results may not represent the exposure patterns of particular users. To address this issue, we conducted a sensitivity analysis among dual cigarette and e-cigarette users looking specifically at daily and some day e-cigarette users. There was some suggestion among these users that daily e-cigarette users had higher levels of some biomarkers such as TNE-2 compared to some day e-cigarette users, although sample size was limited in terms of reaching conclusions.

Future analyses of these data will benefit from the longitudinal nature of the PATH Study and its extensive data collection and analysis. Biospecimens, specifically urine, have continued to be collected from participants in subsequent waves of the survey. When available, the resulting biomarker data will provide an excellent opportunity to examine how individuals are exposed to varying levels of constituents as they increase, decrease, or maintain the amount of a tobacco product that they consume. The data will also provide insight into the effects on exposure of initiating or quitting use of particular tobacco products as well as switching between different types of products, including to potentially reduced exposure products.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.
Biomarker concentrations by cigarettes per day for PATH Wave 1 adult exclusive daily cigarette smokers selected for biomarker analysis

Figure 2.

Biomarker concentrations by cigarettes per day for PATH Wave 1 adult daily cigarette smokers who use e-cigarettes every day or some days and were selected for biomarker analysis

Figure 3.

Biomarker concentrations by cigarettes per day for PATH Wave 1 adult daily cigarette smokers who use smokeless tobacco every day or some days and were selected for biomarker analysis

Table 1.

Percentage distribution of demographic characteristics and cigarettes smoked per day for PATH Wave 1 adult daily cigarette smokers selected for biomarker analysis (% , 95% Confidence Interval)

	Exclusive cigarette smokers (n = 1952)	Dual cigarette and e-cigarette users (n = 648)	Dual cigarette and smokeless tobacco users (n = 110)
Sex			
Male	45.3 (41.8, 48.7)	37.2 (32.6, 42)	93.4 (87.1, 96.7) *
Female	54.7 (51.3, 58.2)	62.8 (58, 67.4)	6.6 (3.3, 12.9) *
Age group (years)			
18-24	8.1 (6.4, 10.2)	6.9 (5.2, 9.1)	13.0 (7.3, 22.3)
25-34	21.0 (18.2, 24)	23.6 (20.3, 27.2)	38.9 (30.2, 48.3)
35-54	42.3 (38.6, 46.2)	45.3 (41.3, 49.4)	37.6 (28.4, 47.8)
55+	28.6 (25.1, 32.4)	24.2 (20.2, 28.6)	10.5 (4.7, 22.0) *
Race/ethnicity			
White, Non-Hispanic	72.9 (68.9, 76.6)	83.7 (80.6, 86.4)	90.5 (82.9, 94.9)
Other or Multi-Race, Non-Hispanic	17.8 (14.6, 21.4)	9.0 (7.1, 11.5)	4.9 (2.0, 11.5) *
Hispanic	9.3 (7.3, 11.8)	7.1 (5.4, 9.5)	4.6 (2.0, 10.2) *
Education			
Less than high school diploma	16.6 (14.0, 19.7)	11.4 (9.2, 14.2)	13.6 (8.4, 21.3)
GED	11.6 (9.3, 14.4)	8.7 (6.8, 11.1)	20.1 (13.7, 28.6)
High school diploma	33.3 (29.9, 36.9)	23.8 (20.2, 28.0)	33.8 (24.1, 45.0)
Some college/associate's degree	31.7 (28.5, 35.0)	41.2 (36.1, 46.5)	29.2 (20.5, 39.6)
Completed college or more	6.8 (5.6, 8.3)	14.8 (11.3, 19.1)	3.3 (1.2, 8.6) *
Cigarettes smoked per day			
0-4	5.7 (4.1, 7.9)	2.8 (1.8, 4.3)	4.7 (2.1, 10.4) *
5-9	16.4 (13.7, 19.7)	11.9 (9.3, 15.2)	7.2 (3.7, 13.6) *
10-14	21.9 (18.7, 25.5)	24.9 (21.4, 28.7)	25.0 (17.6, 34.2)
15-19	15.1 (12.0, 18.8)	18.4 (15.3, 22.0)	11.5 (5.9, 21.3) *
20-24	28.1 (24.9, 31.5)	31.6 (27.4, 36.1)	36.0 (27.7, 45.3)

	Exclusive cigarette smokers (n = 1952)	Dual cigarette and e-cigarette users (n = 648)	Dual cigarette and smokeless tobacco users (n = 110)
25-60	12.8 (10.5, 15.4) 14.5 (9.2, 19.6)	10.4 (8.0, 13.5) 14.7 (9.6, 19.5)	15.5 (10.0, 23.5) 18.1 (9.6, 19.6)
Median cigarettes smoked per day (25%, 75%)			

Note: Adults included in the analysis provided urine samples with creatinine levels between 10 and 370 mg/dL and reported currently smoking cigarettes every day. Individuals were excluded from the analysis if they currently used e-cigarettes, filtered cigars, cigarillos, traditional cigars, pipes, hookah, smokeless tobacco, snus, or dissolvables every day or some days or had used nicotine replacement therapy products in the past three days with the exception of dual cigarette and e-cigarette users who also currently used e-cigarettes every day or some days and dual cigarette and smokeless tobacco users who also currently used smokeless tobacco or snus every day or some days. All estimates were weighted by the PAXI urinary biomarker weights.

Abbreviation: GED - General Education Development.

* Relative standard error > 30%

Table 2.

Biomarker concentrations by cigarettes per day category and tobacco user group among PATH Wave 1 adult daily cigarette smokers selected for biomarker analysis (Geometric Mean, 95% Confidence Interval)

Biomarker CPD	Exclusive cigarette smokers		Dual cigarette and e-cigarette users		Dual cigarette and smokeless tobacco users	
	n	GM (95% CI)	n	GM (95% CI)	n	GM (95% CI)
TNE-2 (µmol/g creatinine)						
Never tobacco users	1633	0.01 (0.01, 0.01)	1633	0.01 (0.01, 0.01)	1633	0.01 (0.01, 0.01)
0-4 CPD	110	9.88 (5.82, 16.78)	24	16.91 (11.01, 25.96)	6	5.30 (0.59, 47.78) [#]
5-9	305	26.43 (23.23, 30.07)	83	35.62 (30.23, 41.97) [*]	10	48.47 (20.72, 113.38)
10-14	474	47.98 (44.14, 52.16)	172	46.46 (41.85, 51.57)	27	56.22 (46.37, 68.18)
15-19	276	53.56 (47.95, 59.83)	112	57.52 (50.12, 66.02)	12	69.89 (48.43, 100.88)
20-24	560	62.54 (57.12, 68.48)	197	70.23 (61.05, 80.80)	38	52.13 (43.67, 62.23)
25-60	226	78.11 (69.12, 88.26)	60	87.70 (77.53, 99.22)	17	68.40 (46.40, 100.82)
All daily smokers	1951	46.42 (43.22, 49.85)	648	55.43 (51.52, 59.63) ^{*/}	110	51.20 (42.72, 61.36)
<i>p</i> -value for trend		<0.0001		<0.0001		0.0557
NNAL (ng/g creatinine)						
Never tobacco users	1653	0.92 (0.82, 1.04) ^{##}	1653	0.92 (0.82, 1.04) ^{##}	1653	0.92 (0.82, 1.04) ^{##}
0-4 CPD	111	67.24 (45.76, 98.80)	24	75.73 (46.22, 124.07)	6	202.84 (65.72, 626.09)
5-9	304	155.26 (133.54, 180.50)	83	204.14 (160.74, 259.25)	10	481.67 (165.73, 1399.95) [*]
10-14	474	295.76 (267.15, 327.43)	172	276.26 (239.83, 318.24)	27	399.45 (311.63, 512.03) [*]
15-19	276	343.03 (309.39, 380.33)	111	397.63 (325.43, 485.84)	12	602.12 (321.04, 1129.29)
20-24	560	405.17 (372.37, 440.85)	196	435.39 (380.30, 498.47)	38	431.48 (330.57, 563.20)
25-60	226	582.55 (492.78, 688.68)	60	622.59 (534.86, 724.70)	17	631.52 (429.53, 928.49)
All daily smokers	1951	298.01 (275.37, 322.50)	646	345.24 (318.60, 374.11) ^{*/}	110	453.88 (383.26, 537.51) ^{*/}
<i>p</i> -value for trend		<0.0001		<0.0001		0.0131
NNN (ng/g creatinine)						
Never tobacco users	1647	1.92 (1.81, 2.04) ⁺	1647	1.92 (1.81, 2.04) ⁺	1647	1.92 (1.81, 2.04) ⁺
0-4 CPD	108	4.58 (3.36, 6.26)	24	3.20 (2.04, 5.02) ⁺	6	3.81 (1.25, 11.66) ^{##}
5-9	292	8.43 (7.07, 10.07)	81	9.21 (6.41, 13.23)	10	12.40 (5.24, 29.31)

Biomarker CPD	Exclusive cigarette smokers			Dual cigarette and e-cigarette users			Dual cigarette and smokeless tobacco users		
	n	GM (95% CI)		n	GM (95% CI)		n	GM (95% CI)	
10-14	459	14.84 (12.95, 17.01)		167	10.98 (9.00, 13.39) *		26	17.24 (13.68, 21.74)	
15-19	266	18.75 (14.49, 24.26)		110	16.25 (13.46, 19.61)		12	26.22 (11.99, 57.36)	
20-24	523	18.58 (16.90, 20.41)		190	17.58 (14.14, 21.86)		38	21.46 (13.62, 33.83)	
25-60	214	24.23 (20.90, 28.10)		59	23.94 (20.14, 28.45)		17	22.70 (12.50, 41.22)	
All daily smokers	1862	14.89 (13.81, 16.06)		631	14.05 (12.66, 15.59) /		109	18.60 (14.57, 23.74) *	
p-value for trend		<0.0001			<0.0001			0.0026	
1-Hydroxypyrene (ng/g)									
Never tobacco users	1655	128.13 (120.68, 136.04)		1655	128.13 (120.68, 136.04)		1655	128.13 (120.68, 136.04)	
0-4 CPD	111	203.61 (171.86, 241.22)		24	193.08 (158.38, 235.39)		6	137.76 (93.07, 203.91)	
5-9	305	237.27 (215.70, 261.00)		83	335.98 (279.59, 403.74) *		10	418.41 (250.83, 697.98) *	
10-14	474	326.84 (297.15, 359.50)		172	332.93 (301.22, 367.98)		27	412.34 (227.26, 748.15)	
15-19	276	376.63 (316.09, 448.76)		112	396.3 (344.40, 456.04)		12	280.12 (231.86, 338.41) *	
20-24	560	370.95 (341.65, 402.76)		197	441.09 (408.32, 476.49) *		38	283.10 (209.87, 381.90)	
25-60	226	482.01 (424.41, 547.43)		60	559.97 (464.29, 675.38)		17	450.72 (275.64, 737.00)	
All daily smokers	1952	335.76 (318.11, 354.40)		648	391.17 (370.99, 412.44) */		110	332.00 (278.16, 396.25)	
p-value for trend		<0.0001			<0.0001			0.4101	
2-Hydroxyfluorene (ng/g)									
Never tobacco users	1655	167.20 (158.05, 176.88)		1655	167.2 (158.05, 176.88)		1655	167.2 (158.05, 176.88)	
0-4 CPD	111	536.81 (452.87, 636.31)		24	449.24 (329.64, 612.23)		6	231.41 (170.69, 313.74) *	
5-9	305	834.00 (743.00, 936.13)		83	877.41 (736.63, 1045.10)		10	1011.39 (534.33, 1914.39)	
10-14	474	1134.92 (1047.54, 1229.59)		172	1113.77 (1009.56, 1228.73)		27	1252.91 (758.39, 2069.91)	
15-19	276	1377.47 (1229.28, 1543.53)		112	1409.11 (1237.47, 1604.56)		12	1145.81 (1028.41, 1276.62) *	
20-24	560	1469.89 (1359.39, 1589.38)		197	1632.8 (1481.08, 1800.06)		38	1013.46 (825.23, 1244.63) *	
25-60	226	2008.64 (1827.04, 2208.30)		60	2104.13 (1871.03, 2366.27)		17	1386.77 (964.98, 1992.93)	
All daily smokers	1952	1231.23 (1173.36, 1291.97)		648	1329.39 (1249.40, 1414.50)		110	1061.43 (925.93, 1216.76) /	
p-value for trend		<0.0001			<0.0001			0.0789	
HPMA (µg/g)									

Biomarker CPD	Exclusive cigarette smokers			Dual cigarette and e-cigarette users			Dual cigarette and smokeless tobacco users		
	n	GM (95% CI)		n	GM (95% CI)		n	GM (95% CI)	
Never tobacco users	1653	261.73 (247.18, 277.13)		1653	261.73 (247.18, 277.13)		1653	261.73 (247.18, 277.13)	
0-4 CPD	110	604.78 (509.87, 717.36)		24	477.01 (354.06, 642.66) [^]		6	246.89 (190.60, 319.79) [^]	
5-9	301	746.81 (620.88, 898.29)		83	953.00 (790.29, 1149.22)		10	1128.29 (715.74, 1778.64) [^]	
10-14	466	1357.84 (1225.63, 1504.30)		172	1231.16 (1096.02, 1382.97)		25	1093.16 (891.71, 1340.14) [^]	
15-19	273	1550.18 (1383.86, 1736.49)		111	1634.81 (1366.15, 1956.29)		11	1215.16 (1047.12, 1410.17) [^]	
20-24	553	1727.8 (1584.74, 1883.78)		196	2096.29 (1824.04, 2409.17) [*]		37	1163.48 (921.17, 1469.53) [^]	
25-60	222	2566.52 (2234.33, 2948.10)		60	2880.50 (2451.94, 3383.97)		17	1809.66 (1230.85, 2660.65) [^]	
All daily smokers	1925	1388.98 (1299.67, 1484.42)		646	1582.81 (1457.69, 1718.67) ^{*/}		106	1143.82 (970.45, 1348.17) ^{*/}	
<i>p</i> -value for trend		<0.0001			<0.0001			0.0007	
CYMA (µg/g)									
Never tobacco users	1653	1.27 (1.19, 1.36)		1653	1.27 (1.19, 1.36)		1653	1.27 (1.19, 1.36)	
0-4 CPD	111	46.98 (31.16, 70.83)		24	48.31 (30.20, 77.27) [^]		6	6.03 (1.32, 27.65) ^{*/^}	
5-9	305	103.06 (87.17, 121.85)		83	115.83 (93.60, 143.34)		10	94.98 (43.59, 206.96) [^]	
10-14	474	179.87 (160.60, 201.46)		172	153.58 (133.50, 176.68)		26	139.72 (114.48, 170.52) [^]	
15-19	276	213.27 (182.96, 248.61)		112	219.57 (183.71, 262.44)		12	162.34 (122.19, 215.68) [^]	
20-24	560	218.83 (201.97, 237.11)		197	246.04 (215.03, 281.52)		38	147.85 (109.78, 199.13) ^{*/^}	
25-60	225	306.41 (271.79, 345.43)		60	359.21 (307.82, 419.17)		17	224.59 (136.45, 369.68) [^]	
All daily smokers	1951	176.38 (164.66, 188.94)		648	194.79 (178.92, 212.07)		109	130.94 (107.27, 159.84) ^{*/}	
<i>p</i> -value for trend		<0.0001			<0.0001			0.0060	
MHB3 (µg/g)									
Never tobacco users	1653	4.44 (4.24, 4.64)		1653	4.44 (4.24, 4.64)		1653	4.44 (4.24, 4.64)	
0-4 CPD	111	13.52 (11.40, 16.04)		24	13.89 (10.40, 18.53) [^]		6	5.99 (4.14, 8.69) ^{*/^}	
5-9	305	21.26 (18.37, 24.61)		83	25.23 (20.00, 31.83)		10	33.72 (22.08, 51.50) [^]	
10-14	474	35.28 (31.23, 39.85)		172	31.05 (27.09, 35.59)		26	29.55 (23.36, 37.38) [^]	
15-19	276	37.61 (33.39, 42.36)		112	43.02 (37.35, 49.57)		12	31.12 (25.97, 37.29) [^]	

Biomarker CPD	Exclusive cigarette smokers		Dual cigarette and e-cigarette users		Dual cigarette and smokeless tobacco users	
	n	GM (95% CI)	n	GM (95% CI)	n	GM (95% CI)
20-24	560	43.13 (39.90, 46.62)	197	52.09 (44.71, 60.67) *	38	28.37 (22.48, 35.80) * [^]
25-60	225	52.45 (47.96, 57.35)	60	62.13 (53.80, 71.75)	17	36.50 (23.95, 55.63) [^]
All daily smokers	1951	34.53 (32.64, 36.53)	648	39.82 (36.59, 43.34) */	109	28.34 (24.49, 32.79) */
<i>p</i> -value for trend		<0.0001		<0.0001		0.0300
Cadmium (µg/g)						
Never tobacco users	1652	0.15 (0.14, 0.16)	1652	0.15 (0.14, 0.16)	1652	0.15 (0.14, 0.16)
0-4 CPD	111	0.23 (0.18, 0.29)	24	0.13 (0.09, 0.19) *	6	0.14 (0.05, 0.38) ⁺
5-9	303	0.20 (0.17, 0.23)	83	0.21 (0.17, 0.26)	10	0.13 (0.06, 0.29)
10-14	473	0.29 (0.26, 0.33)	172	0.24 (0.21, 0.28)	27	0.14 (0.11, 0.19)
15-19	275	0.29 (0.25, 0.34)	112	0.30 (0.23, 0.41)	12	0.20 (0.11, 0.38)
20-24	558	0.37 (0.33, 0.42)	196	0.37 (0.32, 0.43)	38	0.15 (0.11, 0.20) *
25-60	224	0.53 (0.46, 0.61)	60	0.50 (0.42, 0.59)	17	0.35 (0.21, 0.59)
All daily smokers	1944	0.31 (0.29, 0.33)	647	0.30 (0.28, 0.33)	110	0.17 (0.14, 0.22) */
<i>p</i> -value for trend		<0.0001		<0.0001		0.0005
Lead (µg/g)						
Never tobacco users	1653	0.35 (0.33, 0.37)	1653	0.35 (0.33, 0.37)	1653	0.35 (0.33, 0.37)
0-4 CPD	111	0.50 (0.40, 0.63)	24	0.39 (0.31, 0.50)	6	0.47 (0.30, 0.75) [#]
5-9	303	0.39 (0.34, 0.44)	83	0.46 (0.38, 0.56)	10	0.31 (0.23, 0.42)
10-14	473	0.47 (0.43, 0.51)	172	0.45 (0.41, 0.50)	27	0.43 (0.33, 0.56)
15-19	275	0.51 (0.47, 0.57)	112	0.50 (0.42, 0.59)	12	0.56 (0.32, 1.00) [#]
20-24	558	0.52 (0.49, 0.56)	196	0.56 (0.49, 0.62)	38	0.46 (0.37, 0.57)
25-60	224	0.62 (0.56, 0.69)	60	0.67 (0.55, 0.81)	17	0.64 (0.34, 1.20) [#]
All daily smokers	1944	0.49 (0.47, 0.52)	647	0.51 (0.48, 0.54)	110	0.47 (0.41, 0.54)
<i>p</i> -value for trend		0.0004		0.0005		0.1503

Abbreviations: CI - Confidence Interval, CPD - cigarettes per day, GM - Geometric Mean

* - *p*-value for difference from exclusive cigarette smokers < 0.05

/ - *p*-value for difference from exclusive cigarette smokers among all daily smokers < 0.05, controlling for CPD

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- Relative standard error > 30%
+ - More than 40% of values were below the Limit of Detection
v - n < 50

Table 3.

Adjusted geometric mean ratios by cigarettes per day category and tobacco user group

Biomarker CPD	Exclusive cigarette smokers	Dual cigarette and e-cigarette users	Dual cigarette and smokeless tobacco users
TNE-2			
Never tobacco users	1	1	1
0-4 CPD	1287.21 (761.10, 2177.00)	2525.78 (1541.02, 4139.83)	615.19 (74.46, 5082.36)
5-9	3354.27 (2689.61, 4183.18)	5361.13 (4229.35, 6795.78)	5556.14 (2788.91, 11069.07)
10-14	6008.74 (5003.96, 7215.28)	6430.47 (5001.24, 8268.14)	7182.14 (5011.02, 10293.95)
15-19	6700.97 (5225.61, 8592.86)	8655.06 (6667.36, 11235.34)	9027.42 (5915.15, 13777.22)
20-24	7951.44 (6233.11, 10143.47)	10000.37 (7619.72, 13124.82)	5618.91 (3851.28, 8197.84)
25-60	9395.01 (7173.43, 12304.61)	13067.02 (9930.25, 17194.64)	7988.60 (5011.32, 12734.73)
NNAL			
Never tobacco users	1	1	1
0-4 CPD	62.36 (41.54, 93.60)	86.37 (51.12, 145.95)	173.81 (76.64, 394.16)
5-9	144.70 (117.05, 178.87)	202.88 (158.00, 260.51)	390.03 (177.54, 856.82)
10-14	253.63 (219.38, 293.22)	259.90 (214.29, 315.23)	352.47 (257.73, 482.04)
15-19	304.62 (246.04, 377.14)	384.59 (304.26, 486.12)	574.11 (316.61, 1041.06)
20-24	358.20 (296.53, 432.68)	392.18 (320.29, 480.2)	364.09 (266.51, 497.39)
25-60	460.14 (372.35, 568.63)	505.72 (401.22, 637.43)	508.55 (362.38, 713.68)
NNN			
Never tobacco users	1	1	1
0-4 CPD	2.40 (1.86, 3.10)	2.12 (1.33, 3.39)	1.87 (0.63, 5.56)
5-9	4.39 (3.55, 5.42)	4.30 (3.35, 5.53)	5.58 (3.68, 8.46)
10-14	6.76 (6.11, 7.47)	5.19 (4.35, 6.18)	8.11 (5.61, 11.73)
15-19	8.88 (7.33, 10.74)	7.13 (6.08, 8.36)	13.73 (6.04, 31.22)
20-24	8.73 (8.04, 9.49)	7.29 (6.28, 8.46)	10.71 (7.40, 15.51)
25-60	9.60 (8.33, 11.07)	7.51 (6.10, 9.24)	9.56 (6.29, 14.52)
1-Hydroxypyrene			
Never tobacco users	1	1	1
0-4 CPD	1.65 (1.42, 1.90)	1.59 (1.34, 1.89)	1.19 (0.68, 2.09)
5-9	1.78 (1.61, 1.97)	2.35 (1.98, 2.79)	2.90 (1.72, 4.91)

Biomarker CPD	Exclusive cigarette smokers	Dual cigarette and e-cigarette users	Dual cigarette and smokeless tobacco users
10-14	2.35 (2.15, 2.58)	2.45 (2.18, 2.75)	3.02 (1.52, 5.99)
15-19	2.75 (2.42, 3.13)	2.81 (2.48, 3.17)	2.31 (1.93, 2.78)
20-24	2.77 (2.49, 3.08)	3.04 (2.67, 3.47)	2.39 (1.78, 3.20)
25-60	3.36 (2.96, 3.82)	3.45 (2.87, 4.15)	3.44 (2.01, 5.89)
2-Hydroxyfluorene			
Never tobacco users	1	1	1
0-4 CPD	4.47 (3.53, 5.67)	4.35 (3.10, 6.11)	1.60 (0.96, 2.66)
5-9	7.01 (6.04, 8.13)	8.04 (6.59, 9.80)	7.63 (3.61, 16.13)
10-14	9.91 (8.83, 11.12)	9.70 (8.62, 10.93)	10.55 (6.19, 17.99)
15-19	12.26 (11.01, 13.66)	12.77 (11.19, 14.57)	9.01 (7.69, 10.54)
20-24	13.25 (11.98, 14.66)	13.75 (11.94, 15.83)	9.39 (7.06, 12.49)
25-60	16.25 (14.44, 18.29)	16.22 (13.82, 19.05)	13.10 (8.80, 19.50)
HPMA			
Never tobacco users	1	1	1
0-4 CPD	2.44 (1.98, 3.02)	2.00 (1.52, 2.62)	0.93 (0.69, 1.25)
5-9	2.97 (2.49, 3.56)	3.57 (3.04, 4.19)	4.56 (2.80, 7.45)
10-14	5.19 (4.72, 5.71)	4.91 (4.37, 5.52)	4.43 (3.48, 5.63)
15-19	6.03 (5.45, 6.68)	6.09 (5.19, 7.14)	4.83 (4.02, 5.81)
20-24	6.63 (6.07, 7.25)	7.69 (6.74, 8.76)	5.09 (3.82, 6.78)
25-60	9.31 (8.02, 10.81)	10.03 (8.38, 12.01)	6.82 (4.61, 10.10)
CYMA			
Never tobacco users	1	1	1
0-4 CPD	35.36 (22.87, 54.69)	43.12 (27.07, 68.69)	4.60 (0.94, 22.48)
5-9	81.13 (66.90, 98.40)	89.72 (72.40, 111.17)	75.58 (33.51, 170.45)
10-14	135.85 (117.94, 156.48)	124.87 (106.26, 146.75)	113.08 (82.05, 155.85)
15-19	163.56 (143.20, 186.83)	171.03 (142.22, 205.67)	132.38 (93.93, 186.58)
20-24	167.00 (147.39, 189.23)	184.57 (156.77, 217.30)	127.72 (85.13, 191.61)
25-60	220.77 (186.31, 261.60)	255.13 (212.77, 305.93)	172.90 (103.76, 288.11)
MHB3			
Never tobacco users	1	1	1
0-4 CPD	3.14 (2.54, 3.89)	3.51 (2.68, 4.59)	1.34 (0.80, 2.23)

Biomarker CPD	Exclusive cigarette smokers	Dual cigarette and e-cigarette users	Dual cigarette and smokeless tobacco users
5-9	5.04 (4.37, 5.82)	5.64 (4.50, 7.07)	8.44 (5.72, 12.44)
10-14	7.85 (6.99, 8.82)	7.38 (6.61, 8.25)	7.18 (5.43, 9.49)
15-19	8.50 (7.70, 9.38)	9.38 (8.18, 10.76)	7.44 (6.25, 8.86)
20-24	9.52 (8.79, 10.3)	11.03 (9.52, 12.77)	7.47 (5.70, 9.80)
25-60	10.93 (9.80, 12.2)	12.85 (11.25, 14.68)	8.33 (5.44, 12.74)
Cadmium			
Never tobacco users	1	1	1
0-4 CPD	1.52 (1.29, 1.78)	1.24 (0.93, 1.66)	1.31 (0.74, 2.33)
5-9	1.51 (1.35, 1.70)	1.61 (1.38, 1.88)	1.56 (0.88, 2.77)
10-14	1.97 (1.78, 2.18)	1.84 (1.59, 2.12)	1.71 (1.29, 2.26)
15-19	2.23 (1.95, 2.56)	2.25 (1.79, 2.83)	2.30 (1.40, 3.78)
20-24	2.73 (2.42, 3.07)	2.56 (2.32, 2.83)	1.92 (1.48, 2.48)
25-60	3.49 (3.05, 4.00)	3.28 (2.60, 4.15)	3.48 (2.14, 5.66)
Lead			
Never tobacco users	1	1	1
0-4 CPD	1.38 (1.13, 1.69)	1.47 (1.21, 1.80)	1.42 (0.77, 2.61)
5-9	1.22 (1.05, 1.42)	1.39 (1.15, 1.69)	1.09 (0.64, 1.85)
10-14	1.29 (1.18, 1.43)	1.34 (1.19, 1.52)	1.45 (1.07, 1.96)
15-19	1.48 (1.34, 1.63)	1.45 (1.27, 1.67)	1.98 (1.15, 3.42)
20-24	1.45 (1.33, 1.58)	1.49 (1.34, 1.64)	1.73 (1.35, 2.22)
25-60	1.54 (1.38, 1.73)	1.53 (1.27, 1.86)	1.91 (1.02, 3.56)

Note: Geometric mean ratios were adjusted for age, sex, race/ethnicity, and educational attainment.