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Research Article

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Associations of Cigarettes Smoked Per Day with Biomarkers of Exposure Among U.S. 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 more than 2,700 adult daily cigarette smokers in Wave 1 of the Population Assessment of Tobacco and Health 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 Total Nicotine Equivalents-2 ($P = 0.0036$) than exclusive cigarette smokers, and dual cigarette and smokeless tobacco users had higher levels of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol ($P < 0.0001$) and N′-nitrosonornicotine ($P = 0.0236$) 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.

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 Peto (3) and Law and colleagues (4) observed linear relationships between reported CPD and age-specific lung cancer risks. Kawachi and colleagues (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 with 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 current time, given that it has been suggested that some new tobacco products may provide reduced exposure to these constituents compared with 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 with 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 with tobacco-specific nitrosamines (TSNA) and volatile organic compounds (VOC) as exclusive cigarette smokers (11). More recent

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Note: Supplementary data for this article are available at Cancer Epidemiology, Biomarkers & Prevention Online (http://cebp.aacrjournals.org/).

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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; ref. 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 and colleagues (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. Rostron (14) analyzed NNAL by CPD and race/ethnicity for over 1,500 daily smokers using nationally representative data from the U.S. 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 and colleagues (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 2,700 U.S. 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. This 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 United States conducted by the NIH and the FDA (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) and 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, 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 noncancer 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 two 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 intraday 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 Materials and Methods.

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 3 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 CPD. 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 0–60 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 18–24 years, 25–34 years, 35–54 years, and 55 years or greater. Race/ethnicity was categorized as non-Hispanic White, non-Hispanic other

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Table 1. Percentage distribution of demographic characteristics and CPD for PATH Wave 1 adult daily cigarette smokers selected for biomarker analysis (%, 95% confidence interval)

	Exclusive cigarette smokers ($n = 1,952$)	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)[a]
Female	54.7 (51.3–58.2)	62.8 (58–67.4)	6.6 (3.3–12.9)[a]
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)[a]
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)[a]
Hispanic	9.3 (7.3–11.8)	7.1 (5.4–9.5)	4.6 (2.0–10.2)[a]
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)[a]
CPD			
0–4	5.7 (4.1–7.9)	2.8 (1.8–4.3)	4.7 (2.1–10.4)[a]
5–9	16.4 (13.7–19.7)	11.9 (9.3–15.2)	7.2 (3.7–13.6)[a]
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)[a]
20–24	28.1 (24.9–31.5)	31.6 (27.4–36.1)	36.0 (27.7–45.3)
25–60	12.8 (10.5–15.4)	10.4 (8.0–13.5)	15.5 (10.0–23.5)
Median CPD (25%, 75%)	14.5 (9.2–19.6)	14.7 (9.6–19.5)	18.1 (9.6–19.6)

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 3 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 PATH urinary biomarker weights.
[a]Relative SE > 30%.

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 χ^2 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 (HPHC) 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 Materials and Methods. 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 nonproportion estimate or the denominator of a proportion was less than 50, the relative SE 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), and all figures were constructed using R version 3.0.2 (R Core Team). Restricted-use PATH biomarker data (21) 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 with dual

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Table 2. Biomarker concentrations by CPD category and tobacco user group among PATH Wave 1 adult daily cigarette smokers selected for biomarker analysis (geometric mean, 95% confidence interval)

	Exclusive cigarette smokers		Dual cigarette and e-cigarette users		Dual cigarette and smokeless tobacco users	
Biomarker CPD	***n***	**GM (95% CI)**	***n***	**GM (95% CI)**	***n***	**GM (95% CI)**
TNE-2 (μmol/g creatinine)						
Never tobacco users	1,633	0.01 (0.01–0.01)	1,633	0.01 (0.01–0.01)	1,633	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)[a]
5–9	305	26.43 (23.23–30.07)	83	35.62 (30.23–41.97)[b]	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	1,951	46.42 (43.22–49.85)	648	55.43 (51.52–59.63)[b,c]	110	51.20 (42.72–61.36)
P_{trend}		<0.0001		<0.0001		0.0557
NNAL (ng/g creatinine)						
Never tobacco users	1,653	0.92 (0.82–1.04)[a,d]	1,653	0.92 (0.82–1.04)[a,d]	1,653	0.92 (0.82–1.04)[a,d]
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)[b]
10–14	474	295.76 (267.15–327.43)	172	276.26 (239.83–318.24)	27	399.45 (311.63–512.03)[b]
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	1,951	298.01 (275.37–322.50)	646	345.24 (318.60–374.11)[b,c]	110	453.88 (383.26–537.51)[b,c]
P_{trend}		<0.0001		<0.0001		0.0131
NNN (ng/g creatinine)						
Never tobacco users	1,647	1.92 (1.81–2.04)[d]	1,647	1.92 (1.81–2.04)[d]	1,647	1.92 (1.81–2.04)[d]
0–4 CPD	108	4.58 (3.36–6.26)	24	3.20 (2.04–5.02)[d]	6	3.81 (1.25–11.66)[a,d]
5–9	292	8.43 (7.07–10.07)	81	9.21 (6.41–13.23)	10	12.40 (5.24–29.31)
10–14	459	14.84 (12.95–17.01)	167	10.98 (9.00–13.39)[b]	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	1,862	14.89 (13.81–16.06)	631	14.05 (12.66–15.59)[c]	109	18.60 (14.57–23.74)[b]
P_{trend}		<0.0001		<0.0001		0.0026
1-Hydroxypyrene (ng/g)						
Never tobacco users	1,655	128.13 (120.68–136.04)	1,655	128.13 (120.68–136.04)	1,655	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)[b]	10	418.41 (250.83–697.98)[b]
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)[b]
20–24	560	370.95 (341.65–402.76)	197	441.09 (408.32–476.49)[b]	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	1,952	335.76 (318.11–354.40)	648	391.17 (370.99–412.44)[b,c]	110	332.00 (278.16–396.25)
P_{trend}		<0.0001		<0.0001		0.4101
2-Hydroxyfluorene (ng/g)						
Never tobacco users	1,655	167.20 (158.05–176.88)	1,655	167.2 (158.05–176.88)	1,655	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)[b]
5–9	305	834.00 (743.00–936.13)	83	877.41 (736.63–1,045.10)	10	1,011.39 (534.33–1,914.39)
10–14	474	1,134.92 (1,047.54–1,229.59)	172	1,113.77 (1,009.56–1,228.73)	27	1,252.91 (758.39–2,069.91)
15–19	276	1,377.47 (1,229.28–1,543.53)	112	1,409.11 (1,237.47–1,604.56)	12	1,145.81 (1,028.41–1,276.62)[b]
20–24	560	1,469.89 (1,359.39–1,589.38)	197	1,632.8 (1,481.08–1,800.06)	38	1,013.46 (825.23–1,244.63)[b]
25–60	226	2,008.64 (1,827.04–2,208.30)	60	2,104.13 (1,871.03–2,366.27)	17	1,386.77 (964.98–1,992.93)
All daily smokers	1,952	1,231.23 (1,173.36–1,291.97)	648	1,329.39 (1,249.40–1,414.50)	110	1,061.43 (925.93–1,216.76)[c]
P value for trend		<0.0001		<0.0001		0.0789
HPMA (μg/g)						
Never tobacco users	1,653	261.73 (247.18–277.13)	1,653	261.73 (247.18–277.13)	1,653	261.73 (247.18–277.13)
0–4 CPD	110	604.78 (509.87–717.36)	24	477.01 (354.06–642.66)[e]	6	246.89 (190.60–319.79)[b,e]
5–9	301	746.81 (620.88–898.29)	83	953.00 (790.29–1,149.22)	10	1,128.29 (715.74–1,778.64)[e]
10–14	466	1,357.84 (1,225.63–1,504.30)	172	1,231.16 (1,096.02–1,382.97)	25	1,093.16 (891.71–1,340.14)[e]
15–19	273	1,550.18 (1,383.86–1,736.49)	111	1,634.81 (1,366.15–1,956.29)	11	1,215.16 (1,047.12–1,410.17)[b,e]
20–24	553	1,727.8 (1,584.74–1,883.78)	196	2,096.29 (1,824.04–2,409.17) [b]	37	1,163.48 (921.17–1,469.53)[b,e]
25–60	222	2,566.52 (2,234.33–2,948.10)	60	2,880.50 (2,451.94–3,383.97)	17	1,809.66 (1,230.85–2,660.65)[e]
All daily smokers	1,925	1,388.98 (1,299.67–1,484.42)	646	1,582.81 (1,457.69–1,718.67)[b,c]	106	1,143.82 (970.45–1,348.17)[b,c]
P_{trend}		<0.0001		<0.0001		0.0007

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Table 2. Biomarker concentrations by CPD category and tobacco user group among PATH Wave 1 adult daily cigarette smokers selected for biomarker analysis (geometric mean, 95% confidence interval) (Cont'd)

	Exclusive cigarette smokers		Dual cigarette and e-cigarette users		Dual cigarette and smokeless tobacco users	
Biomarker CPD	***n***	**GM (95% CI)**	***n***	**GM (95% CI)**	***n***	**GM (95% CI)**
CYMA (μg/g)						
Never tobacco users	1,653	1.27 (1.19–1.36)	1,653	1.27 (1.19–1.36)	1,653	1.27 (1.19–1.36)
0–4 CPD	111	46.98 (31.16–70.83)	24	48.31 (30.20–77.27)[e]	6	6.03 (1.32–27.65)[a,b,e]
5–9	305	103.06 (87.17–121.85)	83	115.83 (93.60–143.34)	10	94.98 (43.59–206.96)[e]
10–14	474	179.87 (160.60–201.46)	172	153.58 (133.50–176.68)	26	139.72 (114.48–170.52)[e]
15–19	276	213.27 (182.96–248.61)	112	219.57 (183.71–262.44)	12	162.34 (122.19–215.68)[e]
20–24	560	218.83 (201.97–237.11)	197	246.04 (215.03–281.52)	38	147.85 (109.78–199.13)[b,e]
25–60	225	306.41 (271.79–345.43)	60	359.21 (307.82–419.17)	17	224.59 (136.45–369.68)[e]
All daily smokers	1,951	176.38 (164.66–188.94)	648	194.79 (178.92–212.07)	109	130.94 (107.27–159.84)[b,c]
P_{trend}		<0.0001		<0.0001		0.0060
MHB3 (μg/g)						
Never tobacco users	1,653	4.44 (4.24–4.64)	1,653	4.44 (4.24–4.64)	1,653	4.44 (4.24–4.64)
0–4 CPD	111	13.52 (11.40–16.04)	24	13.89 (10.40–18.53)[e]	6	5.99 (4.14–8.69)[b,e]
5–9	305	21.26 (18.37–24.61)	83	25.23 (20.00–31.83)	10	33.72 (22.08–51.50)[e]
10–14	474	35.28 (31.23–39.85)	172	31.05 (27.09–35.59)	26	29.55 (23.36–37.38)[e]
15–19	276	37.61 (33.39–42.36)	112	43.02 (37.35–49.57)	12	31.12 (25.97–37.29)[e]
20–24	560	43.13 (39.90–46.62)	197	52.09 (44.71–60.67)[b]	38	28.37 (22.48–35.80)[b,e]
25–60	225	52.45 (47.96–57.35)	60	62.13 (53.80–71.75)	17	36.50 (23.95–55.63)[e]
All daily smokers	1,951	34.53 (32.64–36.53)	648	39.82 (36.59–43.34)[b,c]	109	28.34 (24.49–32.79)[b,c]
P_{trend}		<0.0001		<0.0001		0.0300
Cadmium (μg/g)						
Never tobacco users	1,652	0.15 (0.14–0.16)	1,652	0.15 (0.14–0.16)	1,652	0.15 (0.14–0.16)
0–4 CPD	111	0.23 (0.18–0.29)	24	0.13 (0.09–0.19)[b]	6	0.14 (0.05–0.38)[d]
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)[b]
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	1,944	0.31 (0.29–0.33)	647	0.30 (0.28–0.33)	110	0.17 (0.14–0.22)[b,c]
P_{trend}		<0.0001		<0.0001		0.0005
Lead (μg/g)						
Never tobacco users	1,653	0.35 (0.33–0.37)	1,653	0.35 (0.33–0.37)	1,653	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)[a]
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)[a]
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)[a]
All daily smokers	1,944	0.49 (0.47–0.52)	647	0.51 (0.48–0.54)	110	0.47 (0.41–0.54)
P_{trend}		0.0004		0.0005		0.1503

Abbreviations: CI, confidence interval; GM, geometric mean.
[a]Relative SE > 30%.
[b]*P* value for difference from exclusive cigarette smokers <0.05.
[c]*P* value for difference from exclusive cigarette smokers among all daily smokers <0.05, controlling for CPD.
[d]More than 40% of values were below the LOD.
[e]$n < 50$.

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

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Table 3. Adjusted geometric mean ratios by CPD 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	1,287.21 (761.10–2,177.00)	2,525.78 (1,541.02–4,139.83)	615.19 (74.46–5,082.36)
5–9	3,354.27 (2,689.61–4,183.18)	5,361.13 (4,229.35–6,795.78)	5,556.14 (2,788.91–11,069.07)
10–14	6,008.74 (5,003.96–7,215.28)	6,430.47 (5,001.24–8,268.14)	7,182.14 (5,011.02–10,293.95)
15–19	6,700.97 (5,225.61–8,592.86)	8,655.06 (6,667.36–11,235.34)	9,027.42 (5,915.15–13,777.22)
20–24	7,951.44 (6,233.11–10,143.47)	10,000.37 (7,619.72–13,124.82)	5,618.91 (3,851.28–8,197.84)
25–60	9,395.01 (7,173.43–12,304.61)	1,3067.02 (9,930.25–17,194.64)	7,988.60 (5,011.32–12,734.73)
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)
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)
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)

(Continued on the following page)

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Table 3. Adjusted geometric mean ratios by CPD category and tobacco user group (Cont'd)

Biomarker CPD	Exclusive cigarette smokers	Dual cigarette and e-cigarette users	Dual cigarette and smokeless tobacco users
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.

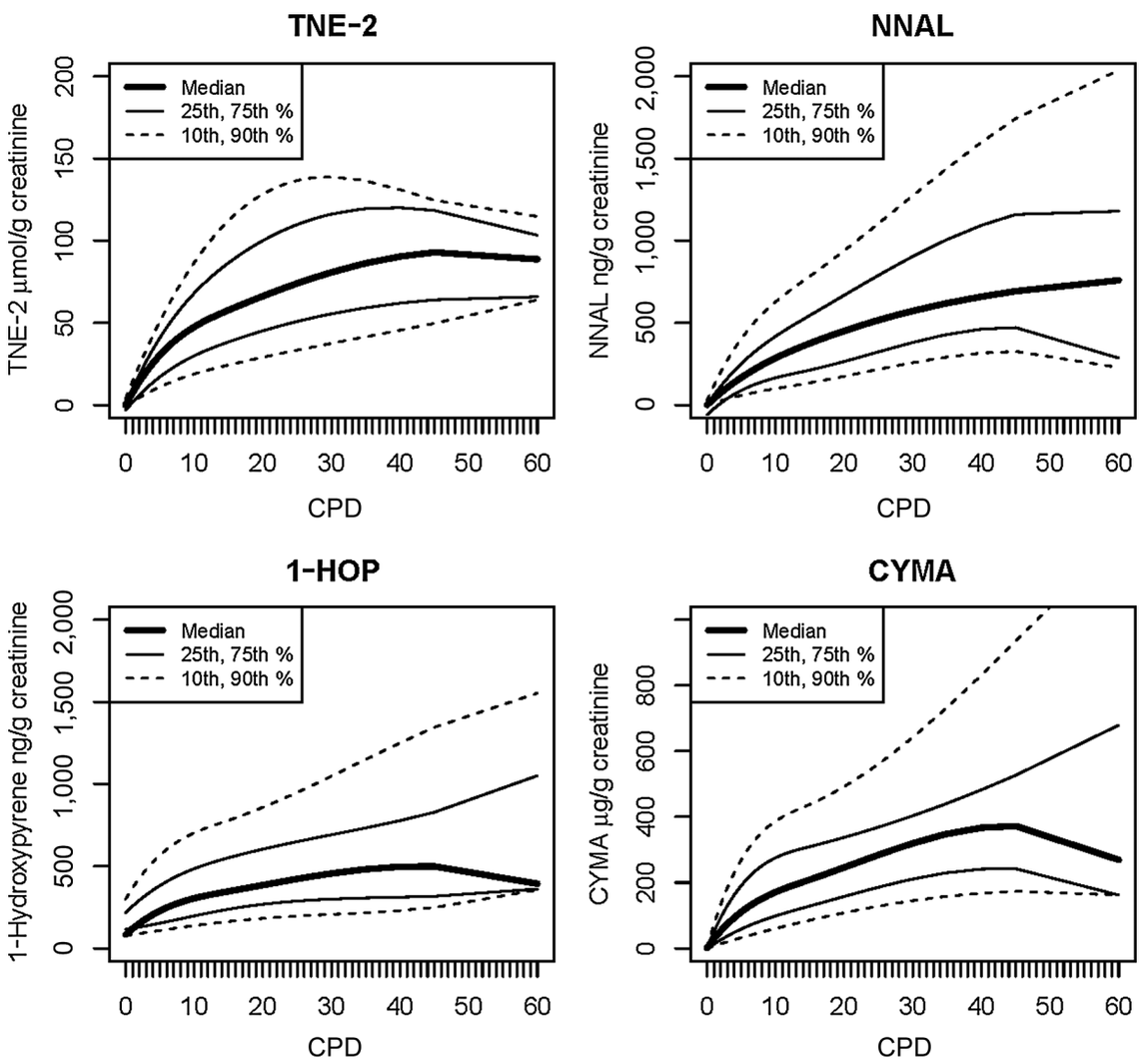

Figure 1.
Biomarker concentrations by CPD for PATH Wave 1 adult exclusive daily cigarette smokers selected for biomarker analysis.

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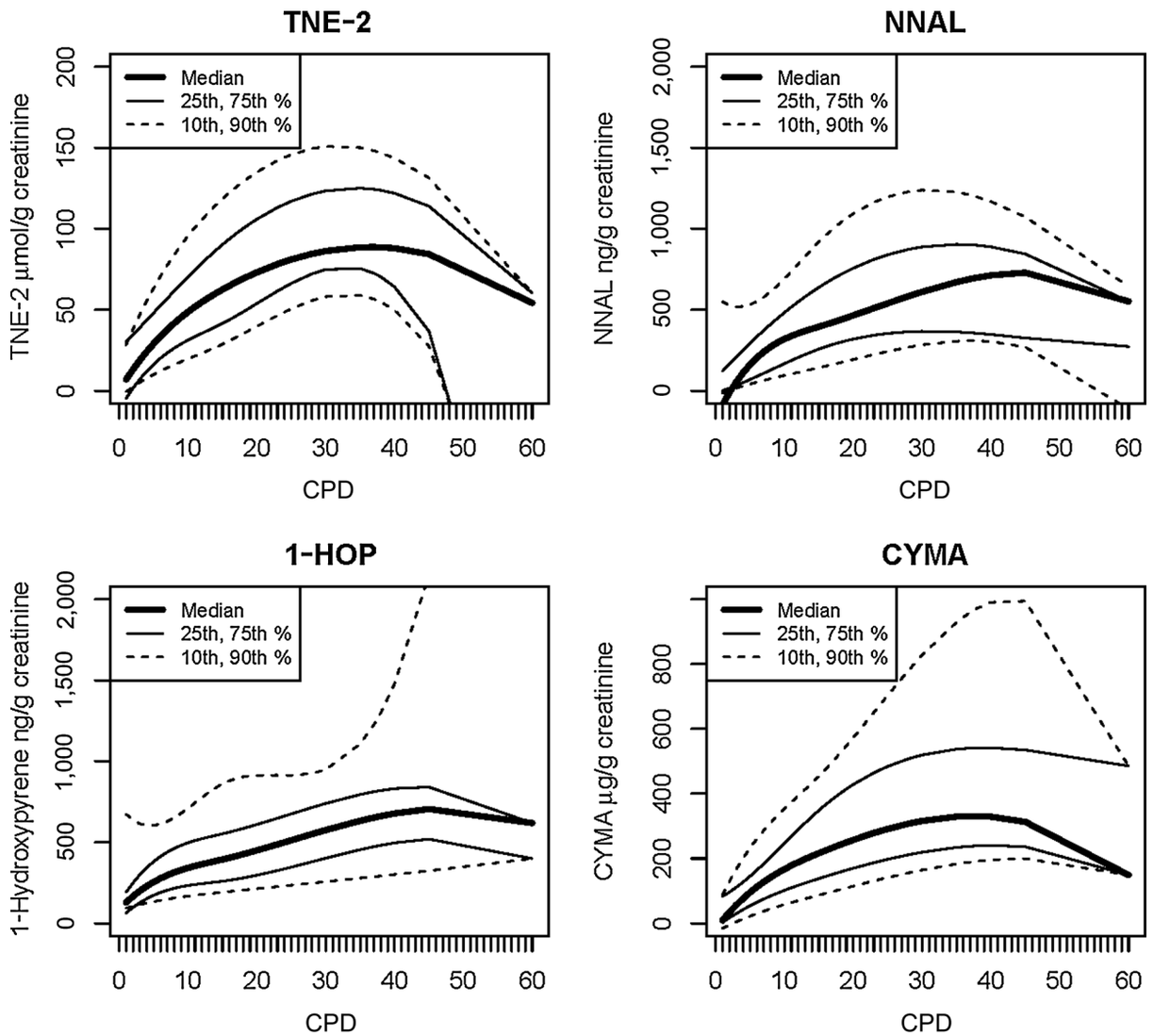

Figure 2.

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

the full set of biomarkers available in the PATH Study are presented as Supplementary Table S1, and tables showing overall biomarker levels for the tobacco users groups by sex and race/ethnicity group are presented as Supplementary Table S2. Table 3 gives adjusted geometric mean ratios by CPD and tobacco user group for the 10 biomarkers and shows a similar dose–response relationship.

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).

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,

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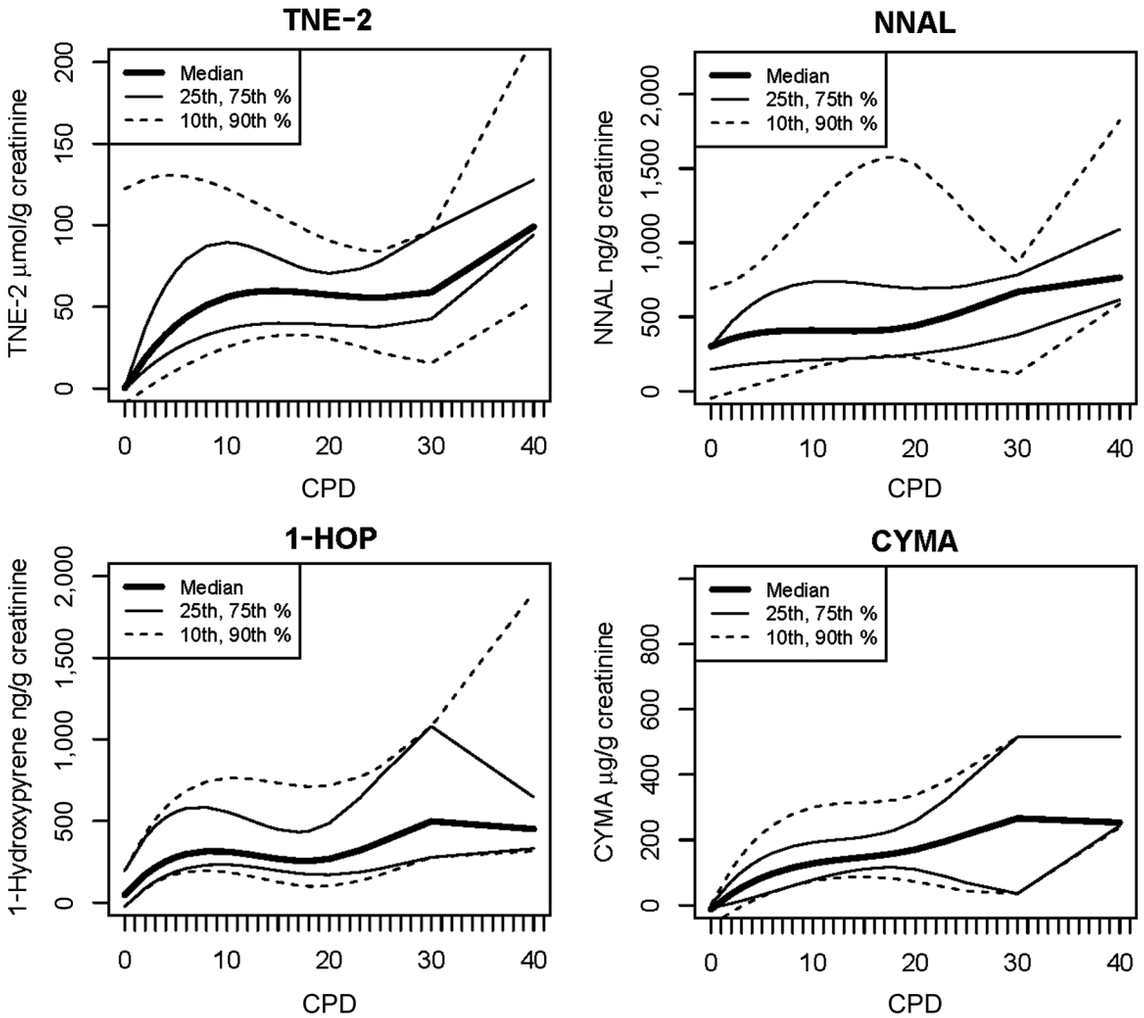

Figure 3.

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

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 and colleagues (13) and Rostron (14), and the curve for TNE-2 was similar to the one observed previously for cotinine by Caraballo and colleagues (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 TSNAs such as NNAL and NNN among dual cigarette and smokeless tobacco users compared with 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 with exclusive cigarette smokers. Some of these results can be difficult to interpret, given that it is not entirely clear whether 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.

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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 with 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.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Disclaimer

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