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Original Investigation

Biomarkers of Exposure to Tobacco-Related Toxicants among Adult Nicotine Pouch Users

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Abstract

Introduction: Nicotine pouches (NPs) are an emerging nicotine delivery system. Understanding nicotine and toxicant exposure among NP users compared with users of other tobacco products and non-users is critical for informing public health strategies.
Methods: Data ($n = 4527$) were drawn from the Population Assessment of Tobacco and Health Study Wave 7 (2022–2023). Participants were classified into four mutually exclusive groups: non-tobacco users, exclusive NP users, exclusive e-cigarette users, and exclusive cigarette smokers. Geometric mean concentrations of biomarkers from urinary nicotine metabolites, minor tobacco alkaloids, and heavy metals were compared across groups using general linear model adjusted for demographics and current marijuana use.
Results: Despite having higher levels of nicotine metabolites than non-tobacco users (eg, cotinine, 2137.2 vs. 0.2 ng/mg creatinine, $p < .0001$), exclusive NP users showed no significant differences in levels of metals or minor tobacco alkaloids ($p > .05$). Exclusive NP users had comparable levels of nicotine metabolites to exclusive cigarette smokers ($p > .05$), but significantly higher concentrations of certain nicotine metabolites than exclusive e-cigarette users (eg, TNE-2: 27.3 vs. 7.0, $p = .02$). Meanwhile, exclusive NP users exhibited lower levels of anabasine (0.6 vs. 9.3 ng/mg creatinine, $p < .0001$), anatabine (0.4 vs. 14.7 ng/mg creatinine, $p < .0001$), and lead (0.2 vs. 0.4 ng/mg creatinine, $p = .003$) than exclusive cigarette smokers and lower levels of lead ($p = .02$) than exclusive e-cigarette users.
Conclusions: NP users have substantially elevated nicotine exposure without a corresponding rise in selected tobacco alkaloids or metals. Findings from objective biomarker measures could inform harm reduction strategies and shape regulatory policies concerning emerging nicotine products.
Implications: Nicotine pouches (NPs) are gaining popularity in the United States. The long-term health effects of nicotine pouch use remain unknown, and this national study offered early evidence into the scope of toxicant exposure associated with nicotine pouch use. Exclusive nicotine pouch users exhibited higher levels of nicotine metabolites but lower concentrations of anabasine and lead compared to cigarette smokers, indicating that NPs may serve as a potential harm-reduction strategy for combustible cigarette smokers. Findings from this study add to the current field of tobacco regulatory science and may inform future efforts to evaluate their effectiveness in smoking cessation or substitution.

Introduction

Nicotine pouches (NPs) have emerged as a novel nicotine delivery system, offering an alternative to traditional combustible cigarettes and electronic cigarettes (e-cigarettes).[1] These small, pre-portioned pouches contain nicotine but no tobacco leaf, and are placed between the gum and lip, releasing nicotine through absorption by the oral mucosa.[2-4] Overall sales of NPs have increased from 126.06 million units from August to December 2019 to 808.14 million units from January to March 2022.[5] Additionally, the concentrations of nicotine in these pouches can vary, with products containing higher concentrations gaining popularity, adding concern about dose-related exposure via NPs.[5] While marketed as a less harmful alternative to smoking, the potential health effects of NPs remain a subject of ongoing research.[6-8]

Biomarkers are measurable indicators of biological processes, conditions, or responses to exposures.[9,10] In the context of tobacco exposure, biomarkers can provide insights into the uptake and metabolism of harmful chemicals associated with nicotine products.[11-13] These biomarkers can include metabolites of specific toxicants found in tobacco products, such as nicotine metabolites, minor tobacco alkaloids, and heavy metals. In January 2025, the Food and Drug Administration authorized the marketing of 20 Zyn nicotine pouch products with different flavors and nicotine strengths (3 and 6 mg), citing the potential to provide a benefit to adults who smoke cigarettes.[14] Measuring biomarkers of exposure (BOE) allows researchers to evaluate the levels of toxicants and better understand the potential health risks linked to nicotine pouch use.

A key question surrounding NPs is how the exposure to harmful chemicals compares to that of other nicotine products like e-cigarettes and conventional cigarettes. Combustible cigarettes contain a complex mixture of thousands of chemicals, many of which are known carcinogens and toxins.[15-17] E-cigarettes, while generally considered to have a reduced harm profile compared to cigarettes, still expose users to various potentially harmful chemicals, albeit at lower levels.[18,19] A meta-analysis study found that oral NPs typically deliver fewer harmful and potentially harmful constituents (HPHCs)

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at lower levels than cigarettes and smokeless tobacco. However, there is a dearth of research regarding the measurement of health effects for NP users from the national probability sample in the real-world setting.[20]

This study analyzed the Population Assessment of Tobacco and Health Study (PATH) Wave 7 Adult interview files and biospecimen data to compare key BOE to tobacco-related toxicants across four classes of HPHCs—nicotine metabolites, minor tobacco alkaloids, and heavy metals. The study examined levels among exclusive nicotine pouch users relative to non-current tobacco users, exclusive e-cigarette users, and cigarette smokers. Given the high nicotine concentration in NPs and the absence of many toxicants found in traditional tobacco products,[21-24] we hypothesize that NP users may show elevated nicotine exposure (eg, cotinine) but reduced levels of certain other tobacco-related toxicants (eg, anabasine, lead).

Design and Methods

Data

The PATH Study is a longitudinal cohort study of tobacco use among a nationally representative sample of U.S. civilian, non-institutionalized individuals,[25] and it uses a four-stage, stratified probability sampling design that intentionally oversampled adult tobacco users, young adults, and African Americans. Wave 7 was conducted from January 2022 to April 2023 with a longitudinal follow-up of participants recruited in Wave 1, with additional participants replenished in Wave 4. The weighted adult interview response rate in Wave 7 was 54.3% for the Wave 1 cohort and 55.9% for the Wave 4 cohort. The PATH data collection was conducted by Westat and approved by Westat's Institutional Review Board.

At Wave 7, 30 801 participants completed the interview survey, and 6067 provided sufficient urine samples for analysis, representing diverse tobacco use and non-use groups. Details of biospecimen collection and laboratory procedures were provided in the biospecimen urine collection procedures.[26] Respondents reported their use of all nicotine-containing products within a 3-day period of biospecimen collection, and nicotine exposure questions were incorporated into the adult interview.[26] The PATH biomarker and adult survey data at Wave 7 were linked through their unique and de-identified personal ID.

Measures

Biomarkers of Exposure

We selected several biomarkers that are available at W7 PATH study and are relevant to the health effects of tobacco use from three groups of HPHC classes: (1) nicotine metabolites (eg, nicotine equivalents [TNE-2], cotinine, trans-3′-Hydroxycotinine), (2) minor tobacco alkaloids (anabasine, anatabine), (3) metals (cadmium, lead, and uranium). Biomarker concentrations below the limit of detection (LOD, Appendix Table 1) were imputed using a common substitution formula (the LOD divided by the square root of 2).[27]

Nicotine Pouch and Other Tobacco Use

Participants were asked whether they had ever used NPs or used NPs in the past 30 days. Those who reported using NPs in the past 30 days were classified as current NP users. To be consistent with the definition of NP use, participants who smoked combustible cigarettes in the past 30 days were classified as past 30-day (current) cigarette smokers. Similarly, those who reported using electronic nicotine products in the past 30 days were classified as past 30-day (current) e-cigarette users. Based on self-reported nicotine pouch, cigarette smoking, and exclusive e-cigarette use status, participants were classified into four mutually exclusive groups: non-current tobacco users, exclusive current NP users, exclusive current e-cigarette users, and exclusive cigarette smokers. Dual users were excluded to avoid confounding effects on biomarker measures.

Individuals who reported use of other tobacco products (eg, cigars, pipe, hookah, smokeless tobacco, bidi, kretek) in the past 30 days were classified as *other current tobacco users*, which are excluded from the analytical sample to remove the confounding effects of other tobacco use on biomarker concentration levels.[28]

Demographic and other correlates include age (continuous), sex (male, female), race/ethnicity (non-Hispanic [N.H.] White, NH-non-White [including Black, Hispanic, and other race]), education (less than a college degree, college graduates), annual household income (<$100 000 or missing, $100 000+), and past 12-month marijuana use (yes/no).

Statistical Methods

Sample characteristics of study participants were reported, overall, and by tobacco use status. Rao-Scott χ^2 test for categorical variables and general linear model for continuous variables were conducted to detect significant group differences.

Geometric mean and 95% confidence intervals of creatinine-corrected biomarker concentration levels were reported across tobacco use groups (ie, non-current tobacco users, exclusive NP users, exclusive cigarette smokers, and exclusive e-cigarette users) and geometric mean ratio (fold changes) between groups were calculated. General linear models that adjusted for age, income, and past-year marijuana use were performed to compare BOE differences between exclusive NP users and non-tobacco users, yielding adjusted results. Additional multivariable models were conducted to compare BOE differences of exclusive NP users with cigarette smokers and e-cigarette users.

All analyses were performed using the Wave 7 person-level urinary specimen sampling weight and 100 replicate weights. Variances were estimated through balanced repeated replication with a Fay coefficient of 0.3 for population-level inference.[29,30] Urinary biomarkers were normalized by urinary creatinine concentrations to account for variations in urine dilution. To address the skewness in the distribution, BOE data were transformed using the natural logarithm. Statistical analyses were performed using SAS 9.4 (Cary, NC). A p-value <0.05 was considered statistically significant.

Results

Figure 1 illustrates the analytic sample flowchart, which shows the linkage of adult interview data ($n = 30801$) and adult urinary biomarker data ($n = 6067$) in the PATH Wave 7, and participant selection criteria. The combined biomarker-adult interview data included 6067 participants. Individuals who reported using nicotine replacement therapies in the past 3 days or had creatinine values outside the normal range of (10–370 mg/dL) ($n = 141$) were excluded from this analysis.

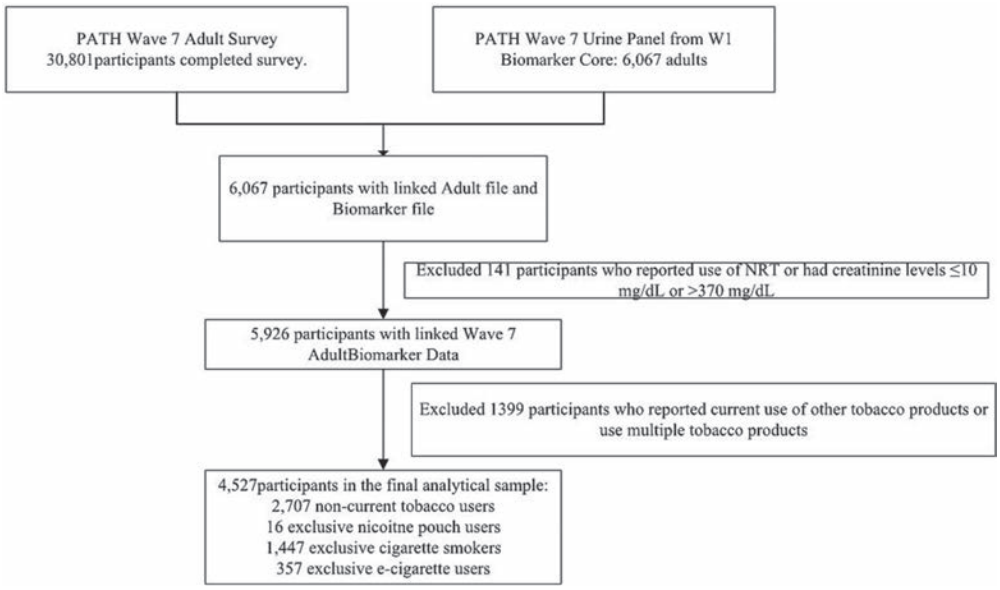

Figure 1. Flow chart and selection criteria for the analytical sample.

After excluding those who reported current use of other tobacco products other than NP, cigarettes, or e-cigarettes ($n = 1399$), the final analytical sample consisted of 4527 participants, including 2707 non-current tobacco users, 16 exclusive NP users, 1447 exclusive cigarette smokers, and 357 exclusive e-cigarette users.

Appendix Table 2 shows the sample characteristics, overall, and by tobacco use groups. Overall, the mean(standard error) age of study participants was 54.0(0.2), including 54.3% females, 33.0% non-White minorities, 40.3% college graduates, and 32.3% with an annual household income of $100 000 or above. Moreover, 16.0% of study participants reported past-year marijuana use. Non-tobacco users had the highest mean age (54.9 years), whereas exclusive NP users had the lowest (38.2 years, $p < .0001$). Education and annual household income varied significantly between groups, with cigarette smokers having higher rates of less-than-college education and lower household income than other groups. A higher proportion of exclusive NP users (50.2%) had an annual household income above $100 000 compared to other groups. Marijuana use was most prevalent among exclusive e-cigarette smokers (41.7%), followed by exclusive cigarette smokers (32.0%), exclusive NP users (26.0%), and non-users (12.7%).

Table 1 presents BOE comparisons between non-current tobacco users and exclusive NP users. All urinary nicotine metabolites were significantly higher in exclusive NP users compared to non-tobacco users. For TNE-2, the geometric mean was 27.3 nmol/mg creatinine in exclusive NP users compared to 0.004 in non-tobacco users (GMR: 7797.0, $p < .0001$). Exclusive NP users had significantly higher mean concentration levels of Cotinine (2137.2 vs. 0.2 ng/mg creatinine; GMR: 10200.6, $p < .0001$) and Trans-3′-hydroxycotinine (2841.9 vs. 0.4 ng/mg creatinine; GMR: 6893.3, $p < .0001$) than non-users. In contrast, the mean concentrations of heavy metals, including cadmium, lead, and uranium, did not differ significantly between exclusive NP users and non-tobacco users (all $p > .05$).

Table 2 presents comparisons of BOEs among exclusive NP users, cigarette smokers, and e-cigarette users. Exclusive NP users exhibited similar mean concentration levels of most nicotine metabolites with exclusive cigarette smokers, but significantly higher levels of TNE-2, cotinine, and trans-3′-hydroxycotinine compared to exclusive e-cigarette users, with GMRs(95%CI) of 3.9(1.1–13.5), 4.7(1.4–16.1), and 5.7(1.4–23.4), respectively. In contrast, exclusive NP users had similar levels of minor tobacco alkaloids to exclusive e-cigarette users, but significantly lower levels of anabasine (0.6 vs. 9.3 ng/mg creatinine, GMR = 0.1, $p < .0001$) and anatabine (0.4 vs. 14.7 ng/mg creatinine, GMR: 0.03, $p < .0001$) than exclusive cigarette smokers.

In terms of heavy metals, exclusive NP users had significantly lower lead levels compared to cigarette smokers (0.2 vs. 0.4 ng/mg creatinine, GMR: 0.5, $p = .003$) and e-cigarette users (0.2 vs. 0.3 ng/mg creatinine; GMR: 0.7, $p = .02$).

Discussion

Biomarkers offer a crucial window into the potential health risks associated with nicotine product use.[12] By quantifying and comparing urinary levels of nicotine metabolites and toxic heavy metals between exclusive NP users, cigarette users, e-cigarette users, and non-tobacco users, this national study provided early evidence of the extent of toxicant exposure from these products. This comparative analysis is important for informing public health policies, developing effective harm reduction strategies, and conducting comprehensive risk assessments related to tobacco product use. A key limitation of this study is the small sample size of exclusive NP users ($n = 16$), which substantially limits statistical power for some comparisons, generalizability of the findings, and requires us to combine some groups or suppress some estimates in the sample characteristics. It is worth noting that small sample sizes are common in certain tobacco biomarker studies, particularly those involving specialized populations—for example, smokers who transitioned to e-cigarette use in the study by

Table 1. Comparisons of Biomarkers of Exposure to Toxicants Between Exclusive NP Users and Non-Tobacco Users[a]

	Non-tobacco users		Exclusive NP users			
	n[b]	Geometric mean (95% CI)	*n*[b]	Geometric mean (95% CI)	GMR (NP vs. Non Users)[c]	*p*-Value[d]
Urinary nicotine metabolites (ng/mg creatinine)						
TNE-2 (nmol/mg creatinine)	2606	0.004 (0.003–0.004)	16	27.3 (8.5–87.7)	7797.0 (2425.8–25061.8)	<.0001
Cotinine (COTT)	2625	0.2 (0.2–0.2)	16	2137.2 (674.7–6770.5)	10200.6 (3209.0–32425.5)	<.0001
Trans-3′-hydroxycotinine (HCTT)	2686	0.4 (0.4–0.5)	16	2841.9 (872.8–9253.3)	6893.3 (2119.8–22413.1)	<.0001
Heavy metals (ng/mg creatinine)						
Cadmium (UCD)	2704	0.2 (0.2–0.2)	16	0.1 (0.1–0.2)	0.7 (0.4–1.2)	1.00
Lead (UPB)	2704	0.3 (0.3–0.3)	16	0.2 (0.1–0.3)	0.6 (0.4–0.9)	.13
Uranium (UUR)	2704	0.01 (0.01–0.01)	16	0.01 (0–0.01)	1.1 (0.7–1.7)	.53

Abbreviations: CI = confidence interval, GMR = Geometric mean ratio, NP = nicotine pouch, TNE = Total nicotine equivalents. [a]All analyses applied single-wave sample weight, 100 replicated weights, and the balanced repeated replication method with Fay's adjustment = 0.3 to account for the PATH study's complex design. [b]Among non-tobacco users, 242 observations had BOEs for Cotinine N-oxide (COXT), Nicotine (NICT), Nornicotine (NNCT), Nicotine 1′-oxide (NOXT), TNE-6, Anabasine (ANBT), and Anatabine (ANTT). Thus, these BOEs are not reported in this table. [c]Unadjusted ratio and 95% CI for geometric means of pairwise comparisons between groups were reported. [d]*p*-Values were from multivariable regressions adjusted by age, income, and current marijuana use.

Goniewicz et al.[31] ($n = 20$). The small sample size for exclusive NP users is expected, given the relatively low prevalence of its use, with about 0.4% of U.S. adults reporting current use of NP in 2022.[32] Some large differences in certain BOEs (eg, nicotine metabolites between exclusive NP use vs. non-tobacco use) are unlikely to be significantly affected by the small sample size, while some other comparisons (eg, nicotine metabolites between exclusive NP users and exclusive e-cigarette users) may suffer from limited statistical power. Some observed differences might be due to insufficient control for covariates. As such, the small number of NP users highlights the need for cautious interpretation, and future research with larger samples of nicotine pouch users is warranted to validate these findings.

This study found elevated levels of urinary nicotine metabolites, including TNE-2, cotinine, and trans-3′-hydroxycotinine, among exclusive NP users compared to non-users. It suggests that NP use leads to substantial nicotine absorption and exposure. Additionally, exclusive NP users exhibited significantly higher levels of nicotine metabolites (TNE-2, cotinine, and trans-3′-hydroxycotinine) than exclusive e-cigarette users and levels comparable to cigarette smokers. Nicotine in tobacco is highly addictive. Acute nicotine increases brain reward function through its effects on dopamine release.[33,34] On one hand, the high nicotine levels might be concerning for young people when their brains are still in development, since nicotine exposure during adolescence can negatively impact learning, memory, and attention.[35,36] On the other hand, high nicotine levels might assist current smokers in switching from combustible tobacco use, which may represent a harm reduction strategy. Understanding nicotine exposure of NP users can provide insights to current field of tobacco regulatory science and future efforts to evaluate NP's effectiveness in smoking abstinence.

The high nicotine levels in NP users might be due to the high nicotine concentrations in current nicotine pouch products. NP typically offers a range of nicotine concentrations ranging from 1.79 to 47.5 mg/pouch.[37] During 2019–2022, NPs with 6 mg were the most commonly sold while sales of products with 8 mg nicotine concentration level increased more than those with lower nicotine concentration.[5] Laboratory evaluations of nicotine pharmacokinetics indicate that NPs can deliver nicotine in quantities comparable to those absorbed by cigarette smokers.[4] Additionally, the cumulative nicotine release patterns exhibit a dose-dependent response across all nicotine concentrations.[3] Our prior study showed that 12.6% of U.S. adults who made recent quit attempts tried NPs,[32] and the nicotine uptake of NP use can be rapid since NPs are placed between a person's lip or cheek and gums and nicotine is absorbed into the bloodstream through mucous membranes in their mouth.[38] Frequency and intensity of tobacco product use can also influence the nicotine exposure as daily use typically exhibited higher levels of nicotine exposure than occasional users. Future randomized control trials should assess changes in BOE and health effects of transitions from combustible cigarette smoking to NP use and the effectiveness of NP use in smoking cessation.

Second, tobacco smoke contains many alkaloids that are structurally similar to nicotine.[39] These minor alkaloids, such as anabasine and anatabine, are present in cigarettes and have been used as a marker to distinguish between cigarette smoking and e-cigarette use, as e-cigarette products typically contain lower levels of these alkaloids due to the purification of nicotine used in e-liquids.[40] This study also found that exclusive NP users had low levels of anabasine and anatabine, which are similar to exclusive e-cigarette users, and significantly lower than exclusive cigarette smokers. The low levels of minor alkaloids are due to that NPs do not include tobacco leaf, and some derive nicotine from tobacco plants, while others use lab-synthesized nicotine.[5,41] Therefore, the BOE of minor tobacco alkaloids can be used to distinguish between cigarette smokers and NP users as a marker in smoking cessation efforts.

Cigarette smoking contains thousands of chemicals including some heavy metals like lead and cadmium, that are highly toxic.[42] Certain metal elements have also been identified in e-cigarette aerosols, liquids, and human biospecimens, either as contaminants or byproducts of the heating process.[43-46] Since NPs do not contain tobacco, the presence of heavy metals in exclusive NP users is expected to be minimal, as

Table 2. BOE Comparisons Among Exclusive NP, Cigarette, and E-cigarette Users[a]

	Exclusive NP users		Exclusive cigarette smokers				Exclusive E-cigarette users			
	n	Geometric mean (95% CI)	*n*	Geometric mean (95% CI)	GMR (NP vs. cigarette)[b]	*p*-Value[c]	*n*	Geometric mean (95% CI)	GMR (NP vs. E-cigarette)[b]	*p*-Value[c]
Urinary nicotine metabolites (ng/mg creatinine)										
TNE-2 (nmol/mg creatinine)	16	27.3 (8.5–87.7)	1445	15.0 (8.9–25.1)	1.8 (0.6–6.0)	0.09	354	7 (4.5–10.9)	3.9 (1.1–13.5)	0.02
Cotinine (COTT)	16	2137.2 (674.7–6770.5)	1446	941.5 (552.3–1604.7)	2.3 (0.7–7.5)	0.06	357	455.6 (290–715.7)	4.7 (1.4–16.1)	0.01
Cotinine N-oxide (COXT)	15	315.0 (209.1–474.7)	1446	334.1 (303–368.5)	0.9 (0.6–1.4)	0.76	354	285.5 (234.4–347.6)	1.1 (0.7–1.8)	0.59
Trans-3′-hydroxycotinine (HCTT)	16	2841.9 (872.8–9253.3)	1358	1670.3 (1005.6–2774.3)	1.7 (0.5–5.6)	0.10	297	494.8 (229.4–1067.2)	5.7 (1.4–23.4)	0.01
Nicotine (NICT)	15	709.2 (250–2011.7)	1358	1249.1 (1105–1411.9)	0.6 (0.2–1.6)	0.39	297	900.9 (710.8–1141.7)	0.8 (0.3–2.4)	0.68
Nornicotine (NNCT)	15	26.5 (12.7–55.5)	1358	67 (60.2–74.6)	0.4 (0.2–0.8)	0.04	297	37.2 (31–44.6)	0.7 (0.3–1.6)	0.41
Nicotine 1′-oxide (NOXT)	15	305.8 (140.6–665.2)	1358	389.1 (343.2–441.1)	0.8 (0.4–1.7)	0.92	297	307.2 (246.9–382.1)	1.0 (0.4–2.3)	0.96
TNE-6 (nmol/mg creatinine)	15	56.7 (35.7–90.1)	1358	60.6 (55.3–66.5)	0.9 (0.6–1.5)	0.88	297	47.3 (38.8–57.8)	1.2 (0.7–2.0)	0.42
Minor tobacco alkaloids (ng/mg creatinine)										
Anabasine (ANBT)	15	0.6 (0.3–1)	1358	9.3 (8.4–10.4)	0.1 (0.04–0.11)	<.0001	297	0.9 (0.7–1.1)	0.7 (0.4–1.2)	0.27
Anatabine (ANTT)	15	0.4 (0.2–0.6)	1358	14.7 (12.9–16.8)	0.03 (0.02–0.04)	<.0001	297	0.6 (0.5–0.7)	0.6 (0.4–1.0)	0.14
Heavy metals (ng/mg creatinine)										
Cadmium (UCD)	16	0.1 (0.1–0.2)	1446	0.4 (0.3–0.4)	0.4 (0.2–0.7)	0.19	356	0.2 (0.1–0.2)	0.9 (0.5–1.5)	0.87
Lead (UPB)	16	0.2 (0.1–0.3)	1446	0.4 (0.4–0.4)	0.5 (0.3–0.7)	0.003	357	0.3 (0.2–0.3)	0.7 (0.4–1.0)	0.02
Uranium (UUR)	16	0.01 (0–0.01)	1446	0.01 (0.01–0.01)	0.8 (0.5–1.3)	0.74	357	0.01 (0.01–0.01)	1.0 (0.6–1.6)	0.79

Abbreviations: CI = confidence interval, GMR = geometric mean ratio, NP = nicotine pouch, TNE = total nicotine equivalents. [a] All analyses applied single-wave sample weight, 100 replicated weights, and the balanced repeated replication method with Fay's adjustment = 0.3 to account for the PATH study's complex design. [b] Unadjusted ratio and 95% CI for geometric means of pairwise comparisons between groups were reported. [c] *p*-Values were from multivariable regressions adjusted by age, income, and current marijuana use.

shown in this study with similar mean concentration levels of heavy metals between exclusive NP users and non-tobacco users. This study also found that the mean concentration of lead among exclusive NP users was nearly half of exclusive cigarette smokers and 30% lower than that of exclusive e-cigarette users, suggesting reduced metal exposure associated with pouch use in comparison with other combustible or non-combustible tobacco use.

This study is subject to limitations. First, the tobacco use status is self-reported and thus may be subject to recall and social desirability biases. NPs are relatively new to the market and participants might misreport snus or similar smokeless tobacco products as NPs. Second, the cross-sectional study design precludes causal inference. Third, biomarker outcomes with long half-lives (eg, metals) may originate from previous combustible tobacco use, passive tobacco exposure, or other sources.[47] Additionally, several biomarkers (eg, NNAL, VOCs) are not available yet in PATH Wave 7 and future studies should conduct a comprehensive analysis of exposure to tobacco-related toxicants associated with NP use. Fourth, this study did not assess NP use frequency, which can influence the BOE levels. Fifth, this study used data from Wave 7 of the PATH study, which were collected between January 2022 and March 2023. The NP and e-cigarette market has rapidly evolved, and biomarker outcomes may differ based on the types of NP products, e-cigarette devices, and nicotine strengths used. Therefore, continued monitoring of BOE is essential to understand the health effects of evolving tobacco products. Finally, the smokeless tobacco product is a close analog to NP products, and the comparison of BOEs between these two products would provide valuable insights. However, the dataset only had 7 exclusive smokeless tobacco users, limiting both the ability to report detailed sample characteristics and the statistical power of comparisons. Future research should further explore BOE differences between users of NPs and smokeless tobacco products.

Conclusion

This analysis of urinary biomarkers demonstrates that individuals who exclusively use NP products exhibit significantly elevated levels of nicotine metabolites, comparable to those found in cigarette smokers. Moreover, exposure to minor tobacco alkaloids and heavy metals does not appear to be elevated in nicotine pouch users relative to non-users and lower than in cigarette smoking, suggesting a potential harm reduction. These findings highlight the need for comprehensive risk assessments and inform public health policies to address the public health implications of nicotine pouch use. Ultimately, this study provides critical insights into the toxicological profile of NPs, which can inform ongoing efforts to explore their effectiveness in smoking cessation or substitution.

Author Contributions

Hongying Dai (Conceptualization [lead], Data curation [equal], Formal analysis [lead], Funding acquisition [lead], Investigation [lead], Methodology [lead], Project administration [lead], Resources [equal], Software [equal], Supervision [lead], Validation [supporting], Visualization [equal], Writing—original draft [equal], Writing—review & editing [equal]) and Brian Young (Data curation [equal], Investigation [supporting], Methodology [supporting], Resources [equal], Validation [lead], Writing—original draft [equal], Writing—review & editing [equal])

Supplementary Material

Supplementary material is available at *Nicotine and Tobacco Research* online.

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Declaration of Interests

The authors have no conflicts of interest to disclose.

Data Availability

Data can be accessed at https://www.icpsr.umich.edu/web/NAHDAP/studies/36840/datadocumentation.

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