Biomarkers of Exposure to Nicotine and Selected Toxicants in Individuals Who Use Alternative Tobacco Products Sold in Japan and Canada from 2018 to 2019

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ABSTRACT

Background: Comparisons of nicotine and toxicant exposures between people who use different alternative tobacco products remain underexplored.

Methods: This cross-sectional, multicountry study analyzed urinary metabolites of nicotine, tobacco-specific nitrosamines [4-methylnitrosamino-1-3-pyridyl-1-butanone (NNK)], and volatile organic compounds (acrolein, acrylamide, and acrylonitrile) among established users ($n = 550$) in Japan and Canada. Participants exclusively or concurrently used nicotine vaping products (NVP; Canada only), heated tobacco products (HTP; Japan only), and combustible cigarettes (CC; Japan and Canada) or abstained (Japan and Canada).

Results: All product groups showed substantial nicotine exposure. Both HTPs and NVPs exposed exclusive users to lower toxicant levels than exclusive CC use. Canadian participants who exclusively used NVPs exhibited lower NNK and acrolein exposure but higher acrylamide exposure than Japanese participants who exclusively used HTPs. Concurrent use of CCs alongside alternative products exposed users to higher toxicant levels compared with exclusive use of either alternative product.

Conclusions: Exclusive use of alternative tobacco products results in significant nicotine exposure but substantially lower toxicant exposure compared with exclusive CC use. People who use HTPs in Japan may experience higher exposure to nicotine and certain toxicants (NNK and acrolein) than people who use NVPs in Canada. Concurrent use results suggest that partially substituting CCs with alternative products may reduce toxicant exposure but to a lesser extent than completely transitioning to alternative products.

Impact: Exposure patterns between two popular alternative tobacco products differ. The overall toxicant exposure from these products is lower than from CCs, providing critical data for regulatory decisions and public health considerations.

Introduction

Nicotine vaping products (NVP; often termed "e-cigarettes") and heated tobacco products (HTP) are two prominent alternative tobacco products that emerged in the tobacco marketplace in the past decade (1–3). NVPs and HTPs deliver nicotine as an aerosol, although the aerosol is generated using two different approaches. NVP devices aerosolize a liquid solution with nicotine and flavorings without tobacco leaf, whereas HTP devices heat manufactured sticks/capsules containing tobacco leaf. For both NVPs and HTPs, the resulting aerosol contains nicotine but lower levels of toxicants than those found in combustible cigarettes (CC; refs. 4–6). When comparing emissions from NVPs and HTPs, a single study found that NVPs emitted lower amounts of tobacco-specific nitrosamines (TSNA) than HTPs (7), and a health risk modeling study suggested that HTPs may land somewhere between NVPs and CCs regarding cancer potency (8). However, whether nicotine and toxicant exposures differ between people who use NVPs and people who use HTPs under generalizable (i.e., "real-world") conditions is unresolved.

Comparing alternative tobacco products' health risks with CCs' health risks is essential in estimating the population-wide effects of introducing new products and replacing CCs with those alternatives. This is best evidenced by the risk/use equilibrium (9): for a product that is 90% less harmful than CCs, it would take 10 times the number of people who use alternative tobacco products to reach an equivalent health burden to that of CC smoking (e.g., 1,000 people who use alternative tobacco products for every 100 people who smoke CCs). For a product that is 50% less dangerous than CCs, it would take twice as many people who use it (e.g., 200 people who use it for every 100 people who smoke CCs). Along with comparisons with CCs, understanding the differences between alternative tobacco products is also valuable. Because NVPs and HTPs are relatively new products used mainly by people ≤40 years old, predictions about health outcomes that are more common in older people are challenging. In the short term, assessing biomarkers of exposure can establish potentially meaningful differences between anticipated health risks in individuals who use NVPs, HTPs, and CCs (10, 11).

In randomized trials in which participants were switched from CCs to NVPs, NVP use has consistently been shown to yield similar

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nicotine intake and decreased exposure to toxic smoke constituents 4 to 12 weeks after switching (12–14). Several cross-sectional studies that have compared biomarkers of exposure in people who exclusively use NVPs with those who use CCs have found lower concentrations of biomarkers of exposure to nicotine and toxicants in NVP users (15–19). Manufacturer-supported forced-switching studies using HTPs have reported no change in exposure to nicotine but decreased toxicant biomarkers in people who had used CCs and switched to an HTP (20–23). A single cross-sectional study from South Korea found that people who only used HTPs presented similar nicotine and toxicant biomarker levels compared with those who only used CCs, except for NNAL (4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol; a biomarker of exposure to the tobacco-specific nitrosamine NNK, a potent lung carcinogen) and CEMA (N-Acetyl-S-(2-carboxyethyl)-L-cysteine; a biomarker of exposure to acrolein, a respiratory irritant and suspected human carcinogen) which were lower in people who used HTPs (24). Although the studies consistently showed substantial (NVPs) and moderate (HTPs) reductions in toxicant exposure compared with CCs, the direct comparison of exposure in individuals who use NVPs and HTPs (including those using alternative products concurrently with CCs) has not been extensively studied.

The present study aimed to address the following gaps in the scientific literature regarding biomarkers of exposure in people who use NVPs, HTPs, or CCs:

1. Examine relative differences in nicotine and toxicant exposures from using NVPs compared with using HTPs; and
2. Establish and compare nicotine and toxicant exposures for different concurrent product use patterns (NVP + CC vs. HTP + CC use).

To accomplish these objectives, we conducted a multicountry study in Japan and Canada that measured biomarkers of exposure to nicotine and selected inhalation toxicants in adults who used NVPs (Canada only), HTPs (Japan only), and CCs (Japan and Canada).

Materials and Methods

Data source

Data are from a supplemental study to the International Tobacco Control Policy Research Project, which has ongoing longitudinal studies in Japan, Canada, and several other countries. The supplemental grant supported a cross-sectional, multicountry study among individuals who used NVPs, HTPs, and CCs. The study collected survey data on tobacco product use and urine samples to measure biomarkers of exposure. The technical report (https://itcproject.org/methods/technical-reports/itc-japan-canada-heated-tobacco-products-survey-technical-report-may-2019/) provides a detailed description of the study methodology.

Multicountry participant recruitment

Study participants were recruited from Japan, the world's leading HTP market (2), and Canada, where the HTP brand *IQOS* has been sold since 2016 (25) and where NVP use is relatively widespread. Six groups of nicotine use were sampled across the two countries:

1. No use: those who indicated no nicotine use in the past 12 months
2. Exclusive NVP use: those who indicated they used NVPs daily for the past 3+ months (and neither HTPs nor CCs in the past 3 months)
3. Exclusive HTP use: those who indicated they used HTPs daily for the past 3+ months (and neither NVPs nor CCs in the past 3 months)
4. Dual NVP + CC use: those who indicated they used both NVPs and CCs daily for the past 3+ months (and no HTP use in the past 3 months)
5. Dual HTP + CC use: those who indicated they used both HTPs and CCs daily for the past 3+ months (and no NVP use in the past 3 months)
6. Exclusive CC use: those who indicated they smoked CCs daily for the past 3+ months (and neither NVPs nor HTPs in the past 3 months).

Previous research suggests that medium to large reductions (Cohen's $f = 0.25$; a medium effect size) in exposure to 3-hydroxypropylmercapturic acid (metabolite of acrolein) occurred after 90 days of switching to *IQOS* use, approximately half of that typically observed following CC smoking cessation (23). Power calculations indicated that a total of 360 participants (60 per group) would be sufficient for the present study to detect differences across groups with >80% statistical power at an α of 5%.

Survey fieldwork and biomarker ascertainment occurred from September 12 2018, to October 30, 2018, in Canada and from November 14, 2018, to January 31, 2019, in Japan. A popular online survey platform (https://member.insight.rakuten.us/) purposively utilized demographic information about existing panelists to recruit participants from each country. Panelists were further excluded from the study for (i) being younger than 20 years old; (ii) using "other" tobacco products in the last 3 months (e.g., pipes, cigars, cigarillos, hookah, snus, chewing tobacco, or dissolvable tablets); (iii) using nicotine replacement therapy in the last 3 months; (iv) using recreational drugs in the last 3 months; (v) being diagnosed with diagnosis/treatment of kidney disease, cancer, drug/alcohol dependency, or psychiatric conditions (other than anxiety or depression) in the last 12 months; or (vi) being pregnant or breastfeeding.

Under ideal conditions, similar numbers of Japanese and Canadian participants would have been enrolled in each product use group. This proved challenging in practice due to country-specific differences in the tobacco marketplace. It was recognized during study development that people who use NVPs could only be enrolled in Canada, as Japan's regulatory framework significantly reduced NVP availability. HTPs, by contrast, are exceedingly popular in Japan, and the decision was made *a priori* to oversample exclusive HTP use in Japan, including users of various HTP brands. Given *IQOS*'s availability in Canada since 2016, it was anticipated that enrolling people who use HTPs exclusively was feasible at a 1:2 ratio (Canada:Japan), and enrolling people who use HTPs concurrently alongside CCs would be achievable at a 1:1 ratio. In practice, just $n = 7$ people who used HTPs exclusively or concurrently with CCs were successfully enrolled in Canada, $n = 3$ of which were ultimately excluded from analyses due to their urinary creatinine levels (per below-described exclusion criteria), leaving $n = 4$ people who used HTPs from Canada, all of whom concurrently used CCs.

In totality, 603 ($n = 354$ Japanese participants and $n = 249$ Canadian participants) eligible panelists completed questionnaires on sociodemographic and product use information and self-collected spot urine samples. The final analytic sample excluded $n = 30$ for having urinary creatinine values outside an established reference range (19) and another $n = 23$ for having detectable levels of tobacco-specific biomarkers but self-reporting exclusive NVP use ($n = 18$) or no product use [$n = 5$; accomplished by applying ROC methods developed and described in Goniewicz and colleagues (26)]. This left an analytic sample of $\boldsymbol{n = 550}$

respondents (n = 140 no use; n = 26 exclusive NVP use; n = 128 exclusive HTP use; n = 53 NVP + CC dual use; n = 73 HTP + CC dual use; and n = 130 exclusive CC use; **Fig. 1**). These are comparable with sample sizes in a 90-day follow-up study (23).

Biospecimen collection

After providing sociodemographic and nicotine consumption information via web survey, participants were mailed a specimen kit with instructions for self-collecting spot urine samples. For quality control purposes, two samples per participant were requested. After collecting their samples, participants repackaged the collection kits into temperature-controlled biospecimen transport bags and shipped them to country-specific research sites. Samples were then transferred to −20°C freezers until shipment in dry ice to Roswell Park Comprehensive Cancer Center (Roswell Park) for biomarker analysis. The study was conducted in accordance with recognized ethical guidelines and approved by the Research Ethics Board at the University of Waterloo, Canada (REB#31499 and REB#31040), and the Roswell Park Institutional Review Board. Written informed consent was obtained from all participants before partaking, and all participants were compensated for their time.

Analysis of biomarkers of exposure to nicotine and tobacco-related toxicants

This study assessed biomarkers of exposure to nicotine and four selected toxicants with known or hypothesized tobacco-related etiologies of respiratory diseases. These included metabolites of TSNA NNK and three volatile organic compounds (VOC): acrolein, acrylamide, and acrylonitrile. Nicotine biomarkers included two primary nicotine metabolites: cotinine and 3-hydroxycotinine; thus, nicotine exposure was assessed as a molar sum of those two metabolites (total nicotine equivalence; TNE-2). Analytic details and summaries of the clinical relevance of each biomarker are provided in **Table 1**. Established laboratory assays were used to determine concentrations of metabolites in urine samples. The method developed by Liang (35) was used to measure nicotine metabolites, Jacob and colleagues's (32) method was used for the NNK metabolite (NNAL), and Alwis and colleagues's (33) method was used to quantify VOC metabolites. All laboratory assays were conducted at Roswell Park. Roswell Park laboratory technicians randomly selected one of each participant's two collected urine samples for biomarker analysis.

Data analysis

Initial analyses described sociodemographic characteristics and product use patterns (**Table 2**). The primary analysis utilized multiple linear regression analysis to compare geometric mean concentrations of exposure biomarkers across product use groups. All multivariable regression models were controlled for age, sex, education status, and urinary creatinine level. We conducted a *post hoc* analysis stratifying on self-reported NVP puffs per day to better understand associations with NVP use by accounting for intensity of exposure. Finally, a country-stratified analysis was conducted in participants who exclusively used CCs to scrutinize any within-product differences in toxicant exposure between Japanese and Canadian participants.

Data for all measured biomarkers were right-skewed. Regression models used log-transformed outcome variables, and geometric means are reported in the text. Arithmetic means can be viewed in **Fig. 2**. All means reported were corrected for urinary creatinine levels to adjust for differences in hydration status at sample collection. Thus, all biomarker results below are presented as molar number (TNE-2) or mass (NNAL and all VOC metabolites) of a biomarker per mg of urinary creatinine. Individual biomarker concentrations below the limit of quantitation (LOQ) for a given assay were imputed using established methodology (LOQ/$\sqrt{2}$; ref.

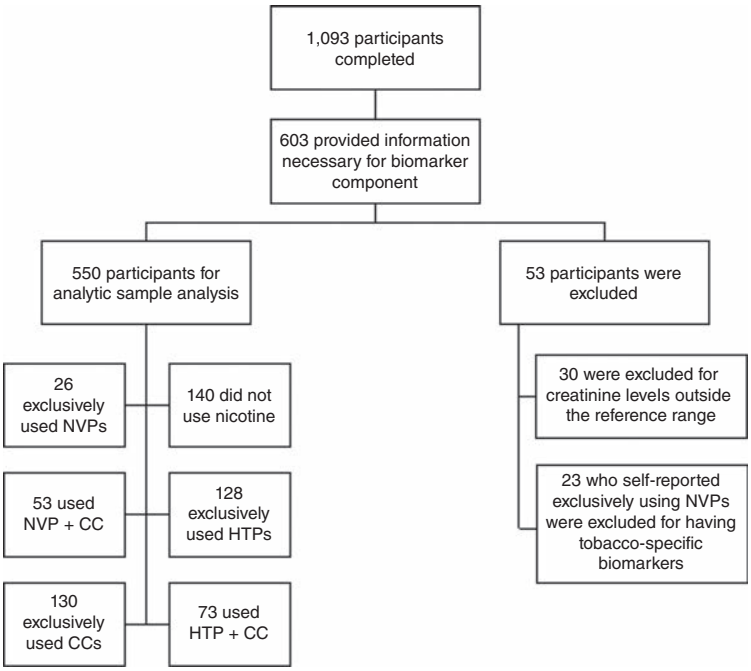

Figure 1.
Analytic sample flow diagram. CONSORT flow diagram of participant inclusion.

Table 1. Analytical limits of detection, biological half-lives, and assay methods for nicotine and toxicants assessed in International Tobacco Control Japan–Canada Heated Tobacco Project.

Urinary biomarker	Chemical exposure	Associated health concerns	Biological half-lives	Limit(s) of detection	Laboratory assay methods
Nicotine[a]					
Trans-3′-hydroxycotinine *TNE-2*[a]	Nicotine	• U.S. Centers for Disease Control: "Most of the dangers of smoking are due to the hundreds of toxic chemicals in the cigarette smoke, not the nicotine" (27). • Impairs fetal brain and lung development (28). • Alters cerebral cortex and hippocampus development during adolescence (28). • Causal factor in nicotine dependence, addiction, and withdrawal disorders (28).	6–7 hours	0.25–0.5 ng/mL	Previously validated high-performance liquid chromatography coupled with tandem mass spectrometric methods (29) were utilized to quantify nicotine metabolites
Cotinine *TNE-2*[a]	Nicotine		16–18 hours	1–5 ng/mL	
TSNAs					
4-Methylnitrosamino)-4-(3-pyridyl)-1-butanol *NNAL*	NNK[b]	• One of 16 compounds in cigarette smoke that have been classified as "carcinogenic to humans" by the International Agency for Research on Cancer (30).	10.3 days	1–3 pg/mL	Previously validated high-performance liquid chromatography coupled with tandem mass spectrometric methods (31, 32) were utilized to quantify TSNAs
VOCs					
N-acetyl-S-(2-carbamoyl-2-hydroxyethyl)-L-cysteine	Acrylamide		19–25 hours	10–30 ng/mL	Previously validated ultrahigh-performance liquid chromatography coupled with electrospray ionization tandem mass spectrometry (33) were utilized to quantify VOCs
N-acetyl-S-(2-cyanoethyl)-L-cysteine	Acrylonitrile	• One of 16 compounds in cigarette smoke that have been classified as "carcinogenic to humans" by the International Agency for Research on Cancer (30).	8 hours	10 ng/mL	
N-acetyl-S-(3-hydroxypropyl)-L-cysteine	Acrolein	• Acrolein exposure is estimated to be "leading cause of noncancer respiratory health effects in the United States" (34). • May contribute to lung carcinogenesis by damaging DNA and inhibiting DNA repair (34).	N/A	100 ng/mL	

[a]Along with evaluating the two primary nicotine metabolites listed, TNE-2 was computed and included in statistical analyses as a reflection of systemic nicotine levels for participants. TNE-2 was calculated as the molar sum of cotinine and trans-3′-hydroxycotinine. Because it is a summary variable, TNE-2 does not have listings for limits of detection.
[b]Full compound name: 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone.

36), and biomarkers with 50% or more values below LOQ were not analyzed. All analyses were performed using R (version 4.2.0) and R Studio (version 2022.02.3) software (R Foundation for Statistical Computing). All significance tests were two-tailed, and the Bonferroni method accounted for multiple comparisons reported in **Fig. 2** and **Table 3**. P values < 0.05 were considered statistically significant. The data that support the findings of this study are available from the corresponding author upon reasonable request.

Results

Sample characteristics

Table 2 displays sociodemographic characteristics and product use patterns for 550 participants with valid results of urine sample analysis. The median age was 42.0 years or older for all product use groups aside from exclusive NVP use (34.5 ± 8.7 years) and dual NVP + CC use (37.0 ± 9.0 years) in Canada. The median number of CCs smoked per day was 12.5 ± 7.2 for the exclusive CC use group, whereas the dual HTP + CC use group in Japan reported medians of 10.0 ± 5.9 CCs per day and 10.0 ± 5.8 HTP inserts (e.g., one individual *IQOS* HEET/HeatStick) per day. Comparatively, the median number of HTP inserts per day was higher in people who exclusively used HTPs (15.0 ± 8.7, $P < 0.05$). Most people who exclusively used HTPs in Japan (84.6%) and all people (100%) who exclusively used NVPs in Canada had previously smoked CCs.

Nicotine and toxicant exposure in individuals who only used NVPs, HTPs, and CCs compared with nonusers

Figure 2 and **Table 3** show the distributions of biomarker concentrations for no use, exclusive NVP use in Canada, exclusive HTP use in Japan, and exclusive CC use in Japan and Canada (see Supplementary Table S1 for detailed regression results). As

Table 2. Descriptive characteristics of the analytic sample (n = 550).

Characteristic	No use (*N* = 140)	Exclusive NVP use (*N* = 26)	Exclusive HTP use (*N* = 128)	NVP + CC dual use (*N* = 53)	HTP + CC dual use (*N* = 73)	Exclusive CC use (*N* = 130)
Country of residence, *n* (%)						
Canada	70 (50.0%)	26 (100.0%)	0 (0.0%)	53 (100.0%)	4 (5.5%)	65 (50.0%)
Japan	70 (50.0%)	0 (0.0%)	128 (100.0%)	0 (0.0%)	69 (94.5%)	65 (50.0%)
Biological sex, *n* (%)						
Male	69 (49.3%)	12 (46.2%)	83 (64.8%)	36 (67.9%)	59 (80.8%)	75 (57.7%)
Female	71 (50.7%)	14 (53.8%)	45 (35.2%)	17 (32.1%)	14 (19.2%)	55 (42.3%)
Age, median ± SD	42.0 ± 10.6	34.5 ± 8.7	43.0 ± 9.3	37.0 ± 9.0	45.0 ± 9.6	45.0 ± 11.3
Age, *n* (%)						
20–24 years	13 (9.3%)	6 (23.1%)	8 (6.2%)	6 (11.3%)	3 (4.1%)	7 (5.4%)
25–39 years	48 (34.3%)	11 (42.3%)	35 (27.3%)	26 (49.1%)	13 (17.8%)	36 (27.7%)
40–54 years	68 (48.6%)	9 (34.6%)	81 (63.3%)	20 (37.7%)	50 (68.5%)	71 (54.6%)
55+ years	11 (7.9%)	0 (0.0%)	4 (3.1%)	1 (1.9%)	7 (9.6%)	16 (12.3%)
Education status, *n* (%)						
Low	22 (15.7%)	1 (3.8%)	48 (37.8%)	9 (17.0%)	27 (37.0%)	38 (29.2%)
Moderate	35 (25.0%)	19 (73.1%)	34 (26.8%)	26 (49.1%)	10 (13.7%)	39 (30.0%)
High	83 (59.3%)	6 (23.1%)	45 (35.4%)	18 (34.0%)	36 (49.3%)	53 (40.8%)
Income status, *n* (%)						
Low	16 (11.6%)	4 (15.4%)	29 (24.8%)	1 (1.9%)	6 (9.0%)	17 (13.7%)
Moderate	31 (22.5%)	6 (23.1%)	29 (24.8%)	21 (40.4%)	16 (23.9%)	34 (27.4%)
High	91 (65.9%)	16 (61.5%)	59 (50.4%)	30 (57.7%)	45 (67.2%)	73 (58.9%)
Marital status, *n* (%)						
Married	85 (60.7%)	13 (50.0%)	84 (65.6%)	27 (52.9%)	47 (64.4%)	77 (59.2%)
Unmarried	55 (39.3%)	13 (50.0%)	44 (34.4%)	24 (47.1%)	26 (35.6%)	53 (40.8%)
CCs per day, median ± SD	NA	NA	NA	8.0 ± 7.7	10.0 ± 5.8	12.5 ± 7.2
NVP puffs per day, median ± SD	NA	35.0 ± 79.0	NA	20.0 ± 31.4	NA	NA
HTP inserts per day, median ± SD	NA	NA	15.0 ± 8.7	NA	10.0 ± 5.9	NA
"Ever smoked a CC?" *n* (%)	75 (53.6%)	22 (84.6%)	128 (100.0%)	53 (100%)	73 (100%)	130 (100%)

expected, people who did not use nicotine displayed minimal exposure to nicotine. Biomarkers of exposure to nicotine (**Fig. 2A**) were significantly elevated in all exclusive product user groups compared with the reference group of nonusers.

Compared with individuals who did not use any products, all exclusive product use groups showed elevated levels of NNAL (**Fig. 2B**).

No statistically significant differences in biomarkers of exposure to acrylamide (**Fig. 2C**), acrylonitrile (**Fig. 2D**), or acrolein (**Fig. 2E**) were observed between exclusive NVP use in Canada and nonuse groups in Canada and Japan. No statistically significant differences in exposure to acrylamide or acrylonitrile were observed between exclusive HTP use in Japan and nonuse groups in Canada and Japan. However, the biomarker of exposure to acrolein was approximately 32% lower in nonusers. Individuals who exclusively use CCs in Canada and Japan showed significantly elevated levels of all biomarkers of exposure to VOCs. Geometric mean concentrations of the biomarker of exposure to acrylamide, acrylonitrile, and acrolein were approximately 100%, 38%, 90%, and 56% lower in people who did not use any tobacco products in Canada and Japan, respectively.

Nicotine and toxicant exposure in individuals who only used NVPs or HTPs compared with individuals who only smoked CCs

When comparing the exclusive use of NVPs in Canada and HTPs in Japan with the multicountry exclusive CC use group, geometric mean concentrations of TNE-2 were significantly lower among people who exclusively use NVPs. However, they were similar to people who exclusively use HTPs. In *post hoc* exploratory analyses, we found that people in Canada who exclusively used NVPs and self-reported 100 or more puffs per day had comparable nicotine exposure to the multicountry exclusive CC use group (Supplementary Table S2).

When comparing the exclusive use groups of NVPs in Canada and HTPs in Japan with the multicountry exclusive CC use group, the geometric mean concentrations of NNAL were almost 100% lower among people who exclusively used NVPs and approximately 81% lower among people who used HTPs exclusively.

Biomarkers of exposure to acrylamide were 20%, 82%, and 63% lower with exclusive NVP use in Canada compared with the multicountry exclusive CC use group. Biomarkers of exposure to acrylamide were 39%, 89%, and 23% lower in people who exclusively used HTPs in Japan than those who exclusively smoked CCs in Canada or Japan.

Nicotine and toxicant exposure in individuals who only used NVPs compared with individuals who only used HTPs

In comparisons of people who exclusively used NVPs in Canada and exclusively used HTPs in Japan, geometric mean concentrations of TNE-2 were approximately 88% lower in exclusive NVP users in Japan.

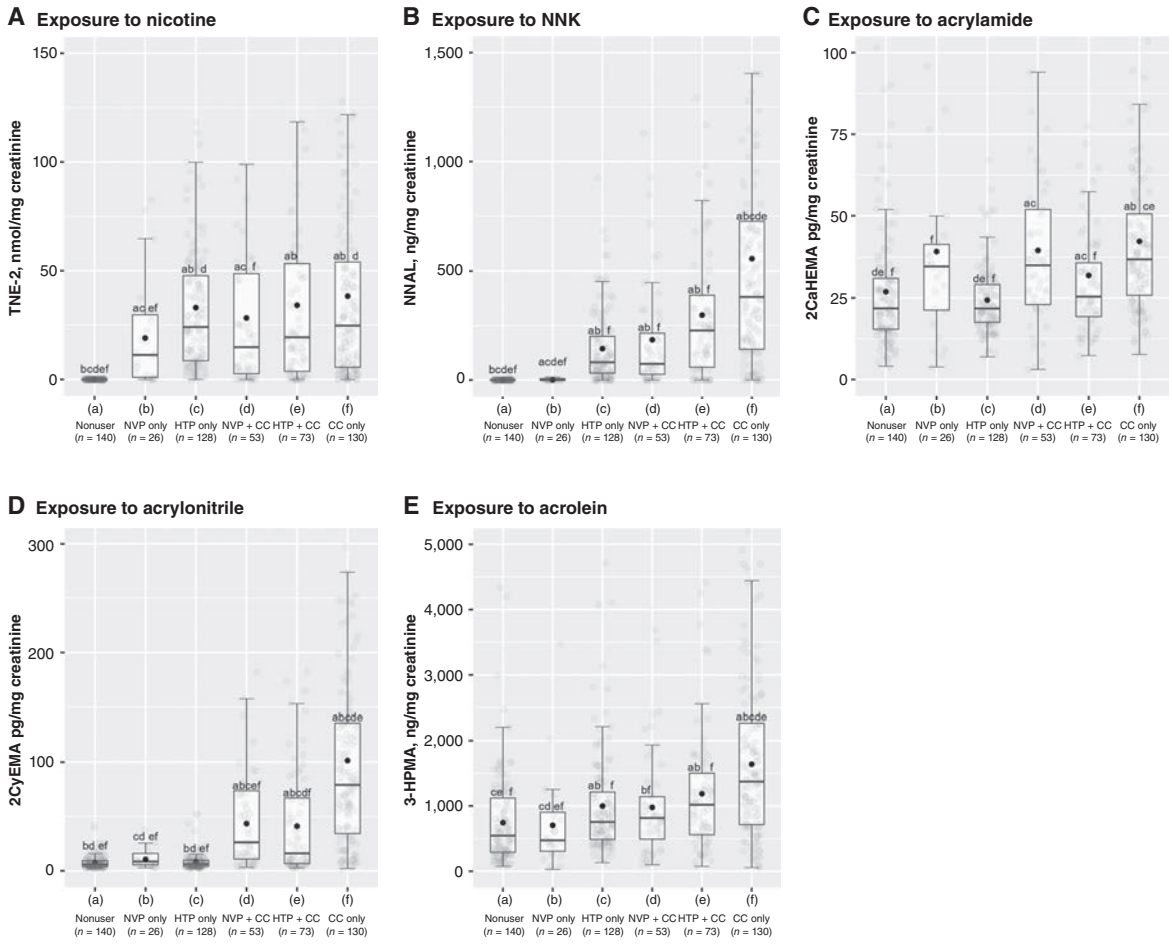

Figure 2.

Biomarkers of exposure among participants who use CCs, NVPs, and/or HTPs, International Tobacco Control Japan–Canada Heated Tobacco Project, 2018–2019 (n = 550). The distribution of biomarker exposure concentrations within each tobacco user group are summarized by box and whisker plots, with individual values plotted beneath as jitters. Data are presented untransformed, such that black dots represent arithmetic means. **A,** Displays exposure to nicotine, (**B**) displays exposure to NNK, (**C**) displays exposure to acrylamide, (**D**) displays exposure to acrylonitrile, and (**E**) displays exposure to acrolein. Superscript letters indicate Bonferroni-adjusted $P < 0.05$ in *post hoc* multiple comparisons between the user group represented by the box and whisker plot and another user group(s) notated by their group letter [a, no use (Canada and Japan); b, exclusive NVP use (Canada only); c, exclusive HTP use (Japan only); d, NVP + CC dual use (Canada only); e, HTP + CC dual use (Japan only); and f, exclusive CC use (Canada and Japan)]. 3-HPMA, 3-hydroxypropylmercapturic acid; 2CaHEMA, n-acetyl-S-(2-carbamoyl-2-hydroxyethyl)-L-cysteine; 2CyEMA, n-acetyl-S-(2-cyanoethyl)-L-cysteine.

Concentrations of NNAL were almost 100% lower among people who exclusively used NVPs in Canada, and concentrations of acrolein biomarkers were approximately 43% lower in exclusive NVP users in Canada than exclusive HTP users in Japan. No statistically significant differences in exposure to acrylonitrile or acrylamide were observed between exclusive NVP use in Canada and exclusive HTP use in Japan.

Nicotine and toxicant exposure in individuals who concurrently used NVPs or HTPs with CCs ("dual use") compared with individuals who only smoked CCs

Figure 2 shows the distributions of biomarker concentrations for dual HTP + CC use in Japan, dual NVP + CC use in Canada, and the multicountry exclusive CC use group (see Supplementary Table S1 for detailed regression results).

Geometric mean concentrations of TNE-2 were approximately 70% lower among people who used NVP + CC in Canada. No statistically significant differences in TNE-2 were observed between Japan's HTP + CC use and the multicountry exclusive CC use groups. No statistically significant differences in exposure to nicotine were observed between NVP + CC use in Canada versus HTP + CC use in Japan groups.

Concentrations of NNAL were approximately 87% lower among people who used NVP + CC in Canada and 77% lower among people who used HTP + CC in Japan compared with those who exclusively used CCs in Canada and Japan. No statistically significant differences

Table 3. Geometric means (± SD) of biomarkers of exposure according to nicotine use group.

User group	TNE-2	NNAL	2CaHEMA	2CyEMA	3-HPMA
(a) No use (Canada and Japan; $n = 140$)	0.01 ± 5.28^{bcdef}	0.03 ± 14.60^{bcdef}	22.39 ± 1.85^{def}	6.54 ± 2.11^{bdef}	541.34 ± 2.39^{cef}
(b) Exclusive NVP use (Canada only; $n = 26$)	2.07 ± 37.56^{acef}	0.15 ± 45.04^{acdef}	29.15 ± 2.26^{f}	11.53 ± 2.94^{cdef}	457.50 ± 2.76^{cdef}
(c) Exclusive HTP use (Japan only; $n = 128$)	17.56 ± 4.53^{abd}	32.57 ± 22.39^{abf}	22.44 ± 1.49^{def}	7.05 ± 2.18^{bdef}	806.24 ± 2.00^{abf}
(d) NVP + CC dual use (Canada only; $n = 53$)	3.83 ± 32.55^{acf}	22.84 ± 46.92^{abf}	31.75 ± 2.09^{ac}	29.07 ± 3.56^{abcef}	706.72 ± 2.36^{bf}
(e) HTP + CC dual use (Japan only; n = 73)	7.26 ± 19.55^{ab}	41.11 ± 51.08^{abf}	26.56 ± 1.76^{acf}	20.71 ± 3.62^{abcdf}	883.62 ± 2.26^{abf}
(f) Exclusive CC use (Canada and Japan; $n = 130$)	12.76 ± 10.02^{abd}	175.43 ± 16.15^{abcde}	36.61 ± 1.71^{abce}	63.31 ± 3.03^{abcde}	1,229.17 ± 2.58^{abcde}

Abbreviations: 3-HPMA, 3-hydroxypropylmercapturic acid; 2CaHEMA, n-acetyl-S-(2-carbamoyl-2-hydroxyethyl)-L-cysteine; 2CyEMA, n-acetyl-S-(2-cyanoethyl)-L-cysteine.

Superscript letters indicate Bonferroni-adjusted $P < 0.05$ in *post hoc* multiple comparisons between the user group represented by a geometric mean ± SD and another user group(s) notated by their group letter (a, no use; b, exclusive NVP use; c, exclusive HTP use; d, NVP + CC dual use; e, HTP + CC dual use; and f, exclusive CC use). TNE-2 is a biomarker of exposure (BoE) for nicotine; NNAL is a BoE for NNK; 2CaHEMA is a BoE for acrylamide; 2CyEMA is a BoE for acrylonitrile; and 3-hydroxypropylmercapturic acid is a BoE for acrolein.

in exposure to nicotine, NNK, acrylamide, or acrolein were observed between HTP + CC use in Japan and NVP + CC use in Canada.

Biomarkers of exposure to acrylonitrile and acrolein were approximately 54% and 43% lower in NVP + CC use in Canada than the multicountry exclusive CC use group, respectively. No statistically significant differences in exposure to acrylamide were observed between NVP + CC use in Canada and the multicountry exclusive CC use group. Biomarkers of exposure to acrylamide, acrylonitrile, and acrolein were approximately 27%, 67%, and 28% lower among people who used HTP + CC in Japan than those who exclusively used CCs in Canada and Japan, respectively. In comparisons of people who used HTP + CC in Japan with those who used NVP + CC in Canada, geometric mean concentrations of acrylonitrile biomarker were 29% lower with HTP + CC use. No statistically significant differences in exposure to acrylamide or acrolein were observed between HTP + CC use in Japan and NVP + CC use in Canada groups.

Discussion

Study participants who used tobacco had considerable nicotine exposure compared with those who did not use tobacco. Whereas the exclusive NVP use group in Canada exhibited lower nicotine exposure than the multicountry exclusive CC use group, the exclusive HTP use group in Japan did not. Furthermore, we found that people who exclusively used NVPs in Canada and HTPs in Japan showed substantially lower levels of exposure to toxicants compared with those who exclusively smoked CCs in Canada and Japan. A novel finding is that exclusive HTP use in Japan was associated with higher levels of NNK (by order of magnitude) and acrolein biomarkers than exclusive NVP use in Canada, consistent with prior emissions studies (7, 37–40). By contrast, exclusive NVP use in Canada had similar acrylamide exposure as the multicountry exclusive CC use group, whereas exclusive HTP use in Japan exhibited approximately half as much exposure. This aligns with results from the Korean THINK study (24), which also sampled people who exclusively used NVPs and HTPs for direct comparisons of toxicant exposure.

This study also contributes information about toxicant exposure from dual product use patterns. Both concurrent use groups (NVP + CC use in Canada and HTP + CC use in Japan) were more exposed to all four toxicants measured in this study than the exclusive use of HTPs in Japan or NVPs in Canada but less exposed to some toxicants than the multicountry exclusive CC use group. This differs from other biomarker studies that have observed higher toxicant exposures for dual NVP + CC use than exclusive CC use (18, 19). Real-world use patterns for NVPs and HTPs frequently include some concurrent CC smoking (41, 42), albeit at lower intensities than exclusive CC use. It remains essential to evaluate whether any harm reduction can be achieved from this partial substitution of CCs and moderate exposure reduction seen in concurrent users of alternative tobacco products and CCs.

Our findings align with prior independently funded NVP (6) and industry-funded HTP (5) biomarker studies. In addition, our NVP results are consistent with prior biomarker studies of people who used early-generation NVP devices (e.g., cig-a-likes; refs. 18, 19, 43). However, our study was conducted when most people who used NVPs were using modifiable tank-style NVP devices [sometimes referred to as third-generation devices (44)] with purportedly improved nicotine delivery (45, 46). Differences in TNE-2 levels across various user groups may reflect an actual difference in the ability to deliver nicotine from NVPs versus HTPs and CCs. Farsalinos and colleagues (47) previously observed lower nicotine yields from a third-generation NVP device than from HTP product *IQOS* when both devices were puffed for 2 seconds. However, no difference in nicotine yield was observed when both alternative products were puffed for 4 seconds. Furthermore, in our study, people who exclusively used NVPs and self-reported 100 or more puffs per day had comparable nicotine exposure with those who exclusively smoked CCs (Supplementary Table S2). Puffs per day are challenging for research participants to estimate (48). Even still, there seem to be subpatterns of daily NVP use (e.g., frequent vs. infrequent use) that result in differential exposure to nicotine (49).

Aside from nicotine, the biomarkers measured in our study were metabolites of known carcinogens and respiratory toxicants. NNK and all three VOCs we assessed were elevated in all product use groups compared with no use, indicating that people who use NVPs, HTPs, and CCs are all exposed to those toxicants more than people who abstain from all tobacco products altogether. However, exclusive HTP use in Japan and NVP use in Canada groups had less exposure to NNK and the VOCs measured in our study than the multicountry exclusive CC use group. How varying degrees of toxicant exposure are associated with future health effects remains unknown. There is also the possibility that emerging toxicants may be specific to NVPs or HTPs. As such, it should be emphasized that biomarker studies provide more substantial evidence about potential absolute risks of a product use compared with abstinence than

they do about the relative risks between product classes. An important finding from our study was that NVP use in Canada and HTP use in Japan expose people to different levels of toxicants. Whether those tobacco-related toxicants contribute differentially to the future risk of specific diseases is not precisely understood. The findings from this study suggest that HTPs are likely more hazardous than NVPs as they expose users to higher levels of those toxicants. However, caution is warranted until dose–response relationships between toxicant exposures and disease processes are better understood.

The present study was limited by challenges with enrollment that resulted in lower sample sizes than estimated from *a priori* power calculations. A sizeable proportion of self-reported exclusive NVP users were excluded from the analytic sample for exhibiting exposure to tobacco-specific biomarkers, meaning they were likely to have misreported their CC smoking status. Additionally, the low prevalence of HTP use in Canada during study enrollment (50, 51) meant we could not recruit exclusive HTP users in Canada for this study. Even with a lower sample size than anticipated, we detected significant differences across our user groups where expected. Our results must be interpreted within the context of the multiplecountry study design. Geography- and ethnicity-related residual confounding could influence our study in a few manners. First, differences in toxicant levels between the same product categories in Canada and Japan could introduce variability unrelated to individual use patterns, as manufacturing standards and product formulations differ between these regions. Prior research has observed country-specific differences in toxicant emissions for CCs (52, 53), corresponding with data from country-stratified biomarker studies (54, 55). Our study's sensitivity analyses of exclusive CC smokers showed no differences in biomarker concentrations between Japanese and Canadian exclusive CC smokers except for NNAL, which was higher in Japanese participants (Supplementary Table S3). There may also be country-specific differences for other product types, but this remains poorly understood. In a recent product analysis study, average N-nitrosonornicotine (NNN) yields were four-fold higher in Canadian inserts than in Japanese HTP IQOS tobacco inserts (40); however, a similar disparity was not observed for NNK. Using or possessing nicotine-containing NVPs is illegal without a prescription in Japan (56), so this relatively small cross-sectional study was not designed to evaluate country-specific exposure differences for NVPs. Beyond physical characteristics of tobacco products in each country, ethnic differences in toxicant metabolism could influence our results. For example, it has been posited that ethnicity-specific genetic polymorphisms may partly explain differences in lung cancer rates of cigarette smokers from varying ethnic groups. Prior research from the United States–based multiethnic cohort study (57) has found that East Asian ancestry was associated with higher cotinine retention compared with Caucasians; however, no ethnic differences were observed in the metabolism of NNAL. A third possibility is that environmental exposures to toxicants differ between Canadian and Japanese participants of our study, particularly those biomarkers that are not specific to tobacco. Taken together, caution is warranted when interpreting comparisons of Japanese-only (e.g., exclusive HTP use) versus Canadian-only (e.g., exclusive NVP use) groups in our study.

In summary, the results from the present study indicate that using alternative products, such as NVPs or HTPs, may reduce toxicant exposure compared with CCs while providing similar nicotine doses. Therefore, if people who smoke CCs transition completely to alternative products, they may experience meaningful reductions in toxicant exposure. Consistent with prior product testing and emissions studies, our biomarker data also suggest that HTPs expose users in Japan to higher levels of nicotine and toxicants than NVPs in Canada. Concurrent use of alternative products with CCs results in overall higher exposure to tobacco-related respiratory toxicants than exclusive use of alternative products but moderately reduced exposure to some toxicants than exclusive CC use. These findings can contribute alongside other research domains to inform regulators, clinicians, and consumers of potential relative health risks associated with using various alternative tobacco products. Research within larger samples of exclusive use groups and with a larger panel of biomarkers (including biomarkers of potential harm) may further clarify differences in potentially differential health risks between NVPs and HTPs.

Authors' Disclosures

K.M. Cummings reports grants from the US NCI during the conduct of the study and reports receiving payment, in the past and currently, as an expert witness on behalf of plaintiffs suing cigarette manufacturers. R.J. O'Connor reports grants from the NIH during the conduct of the study and personal fees from BMJ Journals and the World Health Organization and grants from Roswell Park Alliance Foundation and Louis Sklarow Memorial Fund outside the submitted work. M.L. Goniewicz reports grants from the NCI during the conduct of the study and personal fees from Johnson & Johnson and grants from Pfizer outside the submitted work; in addition, has also consulted with the US FDA, the World Health Organization, and the Campaign for Tobacco-Free Kids on the toxicity of tobacco products and tobacco control policies; he is also a member of the International Association for the Study of Lung Cancer's Tobacco Control and Smoking Cessation Committee and American Association for Cancer Research Tobacco Product and Cancer Subcommittee. No disclosures were reported by the other authors.

Authors' Contributions

C.R. Miller: Conceptualization, software, formal analysis, investigation, visualization, methodology, writing–original draft, writing–review and editing. **L.M. Schneller-Najm:** Software, formal analysis, writing–review and editing. **N.J. Leigh:** Data curation, investigation, methodology, writing–review and editing, conduct laboratory assays. **T. Agar:** Data curation, project administration, writing–review and editing. **A.C.K. Quah:** Resources, project administration, writing–review and editing. **K.M. Cummings:** Conceptualization, resources, supervision, funding acquisition, investigation, project administration, writing–review and editing. **G.T. Fong:** Conceptualization, resources, data curation, supervision, funding acquisition, investigation, project administration, writing–review and editing. **R.J. O'Connor:** Conceptualization, resources, data curation, supervision, funding acquisition, investigation, project administration, writing–review and editing. **M.L. Goniewicz:** Conceptualization, resources, data curation, supervision, funding acquisition, investigation, project administration, writing–review and editing.

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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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