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A biomonitoring assessment of secondhand exposures to electronic cigarette emissions

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ABSTRACT

Background: Electronic cigarette (e-cigarette) conventions regularly bring together thousands of users around the world. In these environments, secondhand exposures to high concentrations of e-cigarette emissions are prevalent. Some biomarkers for tobacco smoke exposure may be used to characterize secondhand e-cigarette exposures in such an environment.

Methods: Participants who did not use any tobacco product attended four separate e-cigarette events for approximately six hours. Urine and saliva samples were collected from participants prior to the event, immediately after the event, 4-h after the event, and the next morning (first void). Urine samples from 34 participants were analyzed for cotinine, trans-3′-hydroxycotinine, *S*-(3-hydroxypropyl)-*N*-acetylcysteine (3-HPMA), *S*-carboxyethyl-*N*-acetylcysteine (CEMA), select tobacco-specific nitrosamines (TSNAs), and 8-isoprostane. Saliva samples were analyzed for cotinine and trans-3′-hydroxycotinine.

Results: Data from 28 of 34 participants were used in the data analysis. Creatinine-adjusted urinary cotinine concentrations increased up to 13-fold and peaked 4-h after completed exposure (range of adjusted geometric means [AGMs] = 0.352–2.31 μg/g creatinine). Salivary cotinine concentrations were also the highest 4-h after completed exposure (range of AGMs = 0.0373–0.167 ng/mL). Salivary cotinine and creatinine-corrected concentrations of urinary cotinine, trans-3′-hydroxycotinine, CEMA, and 3-HPMA varied significantly across sampling times. Urinary and salivary cotinine, urinary trans-3′-hydroxycotinine, and urinary 3-HPMA concentrations also varied significantly across events.

Conclusion: Secondhand e-cigarette exposures lasting six hours resulted in significant changes in exposure biomarker concentrations of both nicotine and acrolein but did not change exposure to tobacco-specific nitrosamines. Additional research is needed to understand the relationship between biomarker concentrations and environmental concentrations of toxicants in e-cigarette emissions.

1. Introduction

Tobacco products contribute to the death of nearly a half a million Americans every year (CDC, 2017a). Tobacco is used primarily because of nicotine addiction (U.S. Department of Health Human Services, 1988). To provide a less toxic smoking experience, devices have emerged that deliver nicotine without the high concentrations of many harmful chemicals in tobacco smoke. One such device is the electronic cigarette. Electronic cigarettes (e-cigarettes) aerosolize a liquid containing nicotine without producing tobacco combustion products (AIHA, 2014). E-cigarettes have rapidly grown in popularity and are now the most commonly used nicotine delivery products among youth (U.S. Department of Health and Human Services, 2016).

Because e-cigarettes are often excluded from indoor smoke-free laws (Tobacco Control Legal Consortium, 2015; U.S. Department of Health and Human Services, 2016) many users begin using them in places

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Table 1
E-Cigarette event characteristics.

VARIABLES	EVENT 1	EVENT 2	EVENT 3	EVENT 4
Location	Daytona Beach, Florida	Athens, Georgia	Chattanooga, Tennessee	Atlanta, Georgia
Venue type	Convention Center	Concert Venue	Convention Center	Exhibition/Tradeshow
Date	April 2016	September 2016	October 2016	March 2017
Estimate number of attendees	1000	300	150	1500
Number of Study Participants	10	9	11	4
Exposure Duration (mins)	(341–351)	(350)	(340)	(360–363)

where smoking is banned (Marynak et al., 2014). As of June 30, 2018, approximately 20% of US states, the District of Columbia, and Puerto Rico banned e-cigarette use in bars, restaurants, and private worksites (CDC, 2017c). In comparison, nearly 60% of states and Puerto Rico and the District of Columbia ban traditional cigarettes in bars, restaurants, and private worksites (CDC, 2017b).

Often studies characterize passive e-cigarette emission exposures in a controlled environment, but few characterize exposures in a real-use or public setting. Studies in controlled environments are often short in duration and cannot account for the variety of e-cigarette devices, liquids, and user behaviors that influence exposure (Melstrom et al., 2017; U.S. Department of Health and Human Services, 2016; Wang et al., 2017). Some studies use a regulatory commercial smoking machine to mimic the first-hand exposure of an e-cigarette device. These studies fail to account for the lung absorption of e-cigarette emissions that occurs when a human participant operates the device (Schripp et al., 2013). Understanding the secondhand exposures to toxicants in e-cigarette emissions under real-use conditions in natural settings is an important public health priority.

Because validated biomarkers specific to e-cigarette exposures have yet to be identified (Schick et al., 2017), we used conventional tobacco smoke exposure biomarkers to characterize e-cigarette emissions exposures. One of the most sensitive and specific tobacco exposure biomarkers is cotinine, the primary proximate metabolite of nicotine (Benowitz, 1999). Approximately 75% of absorbed nicotine is converted to cotinine, and approximately 60% of cotinine is further metabolized to trans-3′-hydroxycotinine (Hukkanen et al., 2005). The sum of these metabolites accounts for 60–80% of absorbed nicotine. Because nicotine is typically present in e-cigarette liquids, cotinine and trans-3′-hydroxycotinine are useful biomarkers for characterizing e-cigarette exposure (Schick et al., 2017).

Tobacco-specific nitrosamines (TSNAs) are a class of compounds only found in tobacco products (Schick et al., 2017). Several TSNAs have been detected in e-cigarette emissions (Goniewicz et al., 2014). A metabolite of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK), 4-(methylnitrosamino)-1-(3-pyridyl) butanol (NNAL) is often used as a tobacco exposure biomarker because it is stable, abundant in urine of smokers and tobacco users, and indicative of cancer risk (Schick et al., 2017). Acrolein is a potent irritant formed when glycerin and propylene glycol in e-cigarette liquids are heated inside an e-cigarette and oxidized to a variety of carbonyl compounds (IARC, 1993; Ohta et al., 2011; Sleiman et al., 2016). Although acrolein exposures are not specific to tobacco products, acrolein metabolites (i.e. *S*-(3-hydroxypropyl)-*N*-acetylcysteine [3-HPMA], *S*-carboxyethyl-*N*-acetylcysteine [CEMA]), may help assess the extent of tobacco or e-cigarette exposure.

Environmental toxicants, such as tobacco smoke, are known to generate reactive oxygen species in humans (CDC, 2010). A non-enzymatic peroxidation product of arachidonic acid, 8-isoprostane is a known biomarker for estimating oxidative stress (Kadiiska et al., 2005). Like acrolein, 8-isoprostane is not specific to tobacco products but can also aid in understanding exposures from tobacco or e-cigarette products.

The purpose of this study was to conduct a secondhand exposure assessment using biomonitoring to characterize passive e-cigarette exposures in a real-use setting with a high concentration of e-cigarette emissions. E-cigarette conventions are large social e-cigarette events described previously (Johnson et al., 2018a; Williams, 2015). E-cigarette conventions attract hundreds to thousands of e-cigarette users who gather in a relatively small space (i.e. convention hall). This environment provides a unique opportunity to conduct a secondhand exposure assessment representative of high exposures in public settings and exposures that last for approximately the length of a work shift.

2. METHODS

2.1. Study locations

This study was conducted at four e-cigarette events in the Southeastern United States between April 2016 and March 2017 described previously (Johnson et al., 2018b). Event 1 was held in a large convention center in Daytona Beach, Florida in April 2016. Event 2 was held in a small concert venue in Athens, GA in September 2016. Event 3 was held in a large convention center in Chattanooga, Tennessee in October 2016. Event 4 was held in a tradeshow venue in Atlanta, Georgia in March 2017. Events 1 and 4 attracted ≥1000 attendees. Events 2 and 3 attracted smaller crowds. Event and venue summaries are presented in Table 1.

2.2. Study participants

Study participants were recruited from University of Georgia (UGA) students and staff or friends and family members of the researchers. All participants gave written informed consent and completed a screening questionnaire to determine their eligibility. Participants received a $25 gift card, lodging (if necessary), and per diem for each event they completed. In order to participate, participants had to be healthy and at least 18 years old. Females could not be pregnant or breastfeeding. Additionally, participants could not be current e-cigarette, tobacco, nicotine replacement therapy, or smokeless tobacco users or live with anyone who uses these products. Thirty-four volunteers participated in this study. This total participant count includes 26 unique participants and 5 participants who attended two or more events. Participants ranged from 19 to 30 years old (Females = 19–28 years old; Males = 19–30 years old). Most participants were female (n = 23, 68%). The UGA Institution Review Board reviewed and approved this study.

2.3. Event visits

Prior to entering the venue, participants completed an entry survey that asked about confounding exposures they may have received in the past 6 days (i.e. exposure to secondhand smoke or e-cigarette emissions, wood smoke, and charcoal). The survey also asked the participant to list the food and drinks they had consumed in the past 24 h. Inside the venue, participants participated in the event as members of the public. Participants attended the events with a researcher for approximately six hours (Table 1). All participants remained inside the venue for the duration of sampling. A researcher had to exit the venue for less than 30 min during Event 1. No confounding exposures were noted during this time. Participants were instructed not to use an e-cigarette, nicotine

replacement-therapy, or other tobacco product while attending the event. An exit survey verified the participants had not used any nicotine product and asked about any adverse health effects experienced. The exit survey also asked participants what food and drink they consumed during the event inside the venue.

2.4. Biological sample collection

Urine and saliva samples were collected from each participant before entering the venue ("pre-exposure"), immediately prior to or just after they exited the venue ("immediate post-exposure"), 4-h after exiting the venue ("4-h post-exposure"), and first thing in the morning the day after the event ("first-void").

All urine and saliva samples were collected in urine collection cups and Salivettes®, respectively. Participants were instructed not to touch the inside of the collection cups or the Salivette to prevent contamination. Each urine cup and Salivette was labeled with a unique barcode to identify the participant, event, biological medium, and sampling time. Sampling supplies were provided to the participants to take home for samples not collected when the researchers were present (i.e. select 4-h post-exposure samples and first-void samples).

Sample collection locations and storage methods are described in the supplementary material (Appendix A, Table 1). When participants collected samples after the events on their own (i.e. 4-hours post exposure and first void samples), they were instructed to place the samples in their freezer immediately or place them on wet ice until they could place them in their freezer. Most participants delivered these samples to a researcher the following morning. For the few remaining samples, the researcher traveled to an agreed upon location or drove to the participant's residence to collect the remaining samples the day after the event. One Event 3 participant lived a significant distance from UGA, and this participant's sample was kept in a freezer and delivered to a researcher on wet ice one week later. All samples were transported on wet ice to the U.S. Centers for Disease Control and Prevention (CDC) within a few weeks of collection.

The Division of Laboratory Sciences, National Center for Environmental Health, U.S. CDC analyzed urine samples for cotinine, trans-3′-hydroxycotinine, NNAL, N′-nitrosonornicotine (NNN), N′-nitrosoanabasine (NAB) and N′-nitrosoanatabine (NAT), 8-isoprostane, 3-HPMA, and CEMA. Saliva samples were analyzed for cotinine and trans-3′-hydroxycotinine.

2.5. Biological sample analysis

2.5.1. Cotinine and trans-3′-Hydroxycotinine analyses

2.5.1.1. Salivary measurements. Salivary cotinine and trans-3′-hydroxycotinine were measured by isotope dilution high performance liquid chromatography/atmospheric pressure chemical ionization tandem mass spectrometry (HPLC-APCI-MS/MS) using a modified version of a published procedure (Bernert et al., 2000). The limits of detection were 0.015 ng/mL for both analytes.

2.5.1.2. Urinary measurements. Urinary "total" (free plus conjugated glucuronide forms) cotinine and trans-3′-hydroxycotinine were measured by isotope dilution high performance liquid chromatography/atmospheric pressure chemical ionization tandem mass spectrometry (HPLC-APCI-MS/MS) using a modified version of a published procedure (Bernert et al., 2005). The limits of detection were 0.030 ng/mL for both analytes.

2.5.2. Volatile organic compound metabolites in urine (VOCM)

Urinary VOC metabolite (VOCM) concentrations were measured using ultrahigh performance liquid chromatography coupled with electrospray ionization tandem mass spectrometry (UPLC-ESI-MS/MS) according to a published procedure (Alwis et al., 2012). The limits of detection for CEMA and 3-HPMA were 6.96 ng/mL and 1.3 ng/mL, respectively.

2.5.3. Urinary tobacco specific nitrosamines (TSNAs)

Urinary "total" (free plus conjugated glucuronide forms) NNAL, NNN, NAB, and NAT were measured by isotope dilution high performance liquid chromatography/atmospheric pressure chemical ionization tandem mass spectrometry (HPLC-MS/MS) using a modified version of a published procedure (Xia et al., 2014). The limit of detection for urinary TSNAs ranged from 0.0006 to 0.0042 ng/mL, depending on the analyte.

2.5.4. 8-Isoprostane

Urinary "total" (free plus conjugated glucuronide forms) 8-iso-prostane (iPF2α-III) (8-iso-15(S)-Prostaglandin F2α) (8-epi PGF2α) (15-F2t-isoprostane) (9α,11α,15S-trihydroxy-(8β)-prosta-5Z,13E-dien-1-oic acid) was measured by isotope dilution ultrahigh-performance liquid chromatography/electrospray ionization tandem mass spectrometry (UHPLC–MS/MS) following urine digestion using β-glucuronidase. The limit of detection for urinary 8-isoprostane was 8.8 pg/mL.

2.5.5. Creatinine

Creatinine in urine was measured by a commercial automated, colorimetric enzymatic (creatinase) method implemented on a Roche/Hitachi Cobas 6000 Analyzer.

2.6. Data analysis

Concentrations below the limit of detection (LOD) were substituted with an imputed value ($LOD/\sqrt{2}$) (Hornung and Reed, 1990). All urinary endpoints were corrected for creatinine. Data were not normally distributed and so were log-transformed for analysis. Adjusted geometric means (AGM) and 95% confidence intervals (95% CI) of biomarker concentrations were calculated for the four sampling times and sampling events (Table 2). The median and range of select biomarker concentrations across events and sampling times are presented in Figs. 1–4.

A linear mixed effects model was used to analyze the log-transformed data across the four sampling times and four events. Sample time, event, and time by event interactions were treated as fixed effects. Participants were treated as random effects. A p-value ≤ 0.05 was considered statistically significant. Results are presented in Table 3. The adjusted mean ratio (i.e. the difference) between participants' log-transformed maximum biomarker concentrations and their log-transformed baseline biomarker concentrations for each endpoint and location are presented in Table 4.

3. Results

To ensure only participants with minimal to no recent secondhand tobacco exposures were included, participants with a salivary cotinine concentration > 0.1 ng/mL at the pre-exposure sampling time were excluded from the analysis (n = 6 total [Event 1 = 1; Event 3 = 5]). Samples from 28 of the 34 participants were analyzed. Two participants did not collect 4-h post exposure saliva samples for Event 1. A substance interfered with 8-isoprostane analysis in two Event 1 samples. Insufficient quantities of urine prevented the analysis of TSNAs in one Event 1 and one Event 2 sample. These data were treated as missing data in the analysis. Approximately 3 people smoking cigarettes passed by participants as they walked towards the Event 2 venue. One smoker was present as they exited the venue. Participants walked quickly past the smokers and avoided inhaling any secondhand smoke as much as possible.

A total of 103 urine and 101 saliva samples were collected across the four events and used in this data analysis. A total of 34 saliva and 36 urine samples were analyzed and used in this data analysis for Event 1, 27 saliva and urine samples for Event 2, 24 saliva and urine samples for

Table 2
Adjusted geometric means and confidence intervals of biomarker concentrations[a].

Sample Time	Event	Value	Urinary Cotinine (μg/g)	Salivary Cotinine (ng/mL)	Urinary Trans-3′-Hydroxycotinine (μg/g)	3-HPMA (μg/g)	CEMA (μg/g)	8-Isoprostane (ng/g)
Pre-Exposure	1	Geometric Mean (GM)	0.106	0.0204	0.134	275	71.8	341
		95% CI of GM	0.0751–0.150	0.0150–0.0277	0.0775–0.231	183–413	52.2–98.8	261–446
	2	Geometric Mean	0.159	0.0172	0.168	186	61.5	346
		95% CI of GM	0.113–0.225	0.0127–0.0233	0.0974–0.290	124–279	44.8–84.5	265–452
	3	Geometric Mean	0.120	0.0185	0.147	196	64.7	346
		95% CI of GM	0.0805–0.179	0.0129–0.0266	0.0821–0.264	123–311	44.9–93.1	252–473
	4	Geometric Mean	0.191	0.0139	0.223	324	100	302
		95% CI of GM	0.118–0.307	0.00895–0.0216	0.116–0.429	187–560	64.8–155	207–442
Immediate Post-Exposure	1	Geometric Mean	0.379	0.0838	0.354	625	95.6	446
		95% CI of GM	0.268–0.536	0.0617–0.114	0.205–0.612	415–940	69.6–131	336–590
	2	Geometric Mean	0.738	0.113	0.434	199	97.0	260
		95% CI of GM	0.523–1.04	0.083–0.154	0.251–0.748	132–299	70.6–133	199–340
	3	Geometric Mean	0.282	0.0325	0.249	252	82.7	300
		95% CI of GM	0.189–0.420	0.0226–0.0467	0.139–0.447	159–401	57.4–119	219–411
	4	Geometric Mean	1.08	0.169	0.848	424	113	381
		95% CI of GM	0.673–1.75	0.109–0.262	0.441–1.63	245–735	73.1–175	260–557
4-Hours Post-Exposure	1	Geometric Mean	0.814	0.0836	0.731	734	116	373
		95% CI of GM	0.575–1.15	0.0594–0.1178	0.423–1.26	488–1100	84.1–159	286–487
	2	Geometric Mean	N.A.[b]	N.A.	N.A.	N.A.	N.A.	N.A.
		95% CI of GM	…	…	…	…	…	…
	3	Geometric Mean	0.352	0.0373	0.349	455	110	314
		95% CI of GM	0.236–0.524	0.0259–0.0537	0.194–0.626	286–723	76.5–159	229–430
	4	Geometric Mean	2.31	0.167	2.22	808	107	414
		95% CI of GM	1.43–3.72	0.107–0.259	1.15–4.26	466–1400	69.4–166	283–605
First Void	1	Geometric Mean	0.801	0.0649	0.884	839	169	297
		95% CI of GM	0.567–1.13	0.0478–0.0882	0.512–1.53	558–1260	123–233	225–392
	2	Geometric Mean	1.09	0.111	0.957	163	90.0	323
		95% CI of GM	0.768–1.53	0.0821–0.151	0.555–1.65	109–245	66.2–125	248–422
	3	Geometric Mean	0.312	0.0280	0.406	262	96.3	369
		95% CI of GM	0.209–0.465	0.0194–0.0402	0.226–0.728	165–417	66.9–139	269–505
	4	Geometric Mean	2.21	0.148	2.67	407	97.8	377
		95% CI of GM	1.37–3.56	0.0951–0.230	1.39–5.13	235–706	63.3–151	258–551

[a] All urinary endpoints were corrected for creatinine.
[b] 4h post-exposure samples were not collected after Event 2.

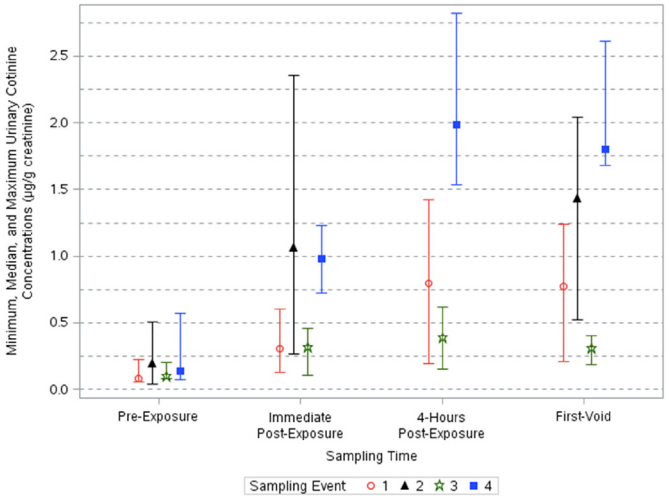

Fig. 1. Minimum, median, and maximum creatinine-corrected urinary cotinine concentrations across sampling times and events.

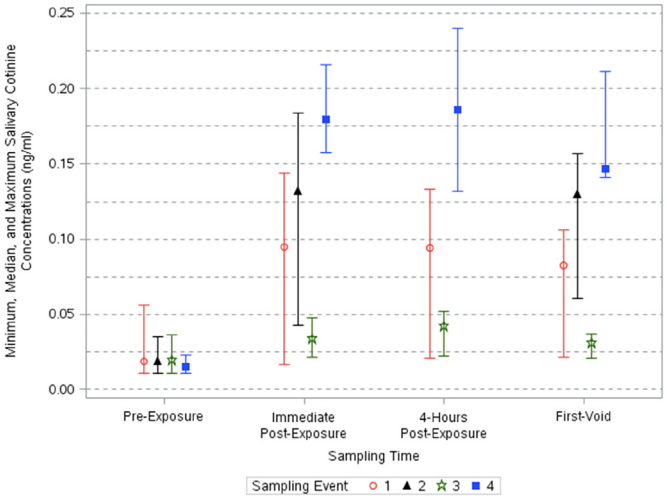

Fig. 2. Minimum, median, and maximum salivary cotinine concentrations across sampling times and events.

Event 3, and 16 saliva and urine samples for Event 4.

Samples were collected prior to exposure, immediately after exposure, 4-h after exposure, and first thing the following morning. Three participants in Event 1 forgot to collect first void samples, but did collect early morning samples. These are considered as first-void samples in this analysis. Samples were not collected 4-h after exposure for Event 2 because the sampling event ended at midnight.

Among the 28 participants used in the statistical analysis, most participants (n = 19, 68%) reporting sitting in a designated eating area inside the event venue 75% of their time. The remaining participants reported spending at least 75% of their time walking around and visiting vendors (n = 2, 7%), standing in e-cigarette use sections (n = 4,

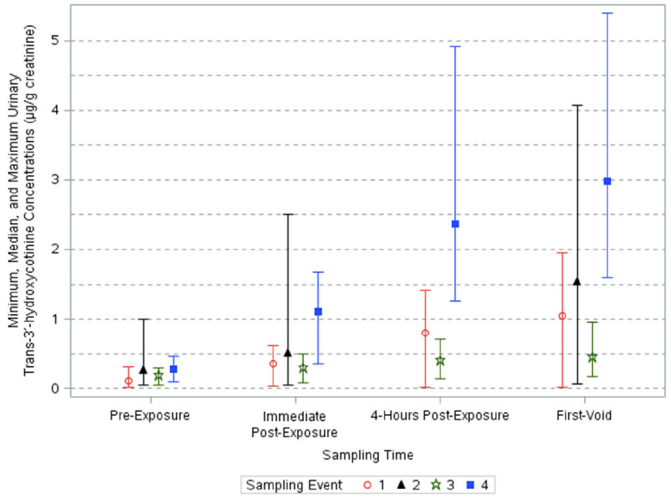

Fig. 3. Minimum, median, and maximum creatinine-corrected urinary trans-3′-hydroxycotinine concentrations across sampling times and events.

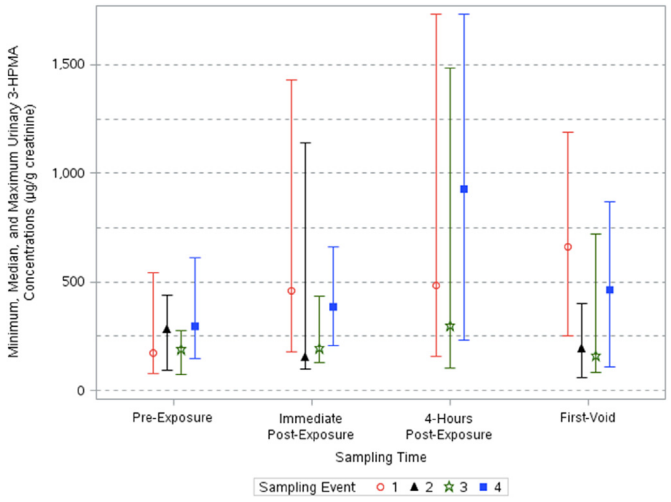

Fig. 4. Minimum, median, and maximum creatinine-corrected urinary 3-HPMA concentrations across sampling times and events.

14%), or split their time equally between sitting in the designated area and visiting vendors (n = 3, 11%). All participants verified they had not used a tobacco or e-cigarette product or touched e-cigarette liquid during the e-cigarette event.

3.1. Nicotine metabolites

3.1.1. Cotinine

Urinary cotinine concentrations corrected for creatinine varied significantly across sampling times ($p < 0.0001$) and events ($p < 0.0001$) (Table 3). A significant interaction between location and sampling time was also found ($p < 0.05$). Urinary cotinine concentrations corrected for creatinine increased up to 13-fold after the events (Table 4). The adjusted geometric means of urinary cotinine concentrations across all events ranged from 0.106 to 0.191, 0.282–1.08, 0.352–2.31, and 0.312–2.21 µg/g creatinine for pre-exposure, immediate post-exposure, 4-h post-exposure, and first-void samples, respectively (Table 2). Urinary cotinine concentrations were all below 0.58 µg/g creatinine in the pre-exposure samples. After exposure, concentrations increased through post-exposure sampling times and then decreased slightly in the first-void samples (Fig. 1). The largest increases in creatinine-corrected cotinine were observed in Event 4 and Event 2, while Event 3 showed little variation in cotinine exposure

Table 3
Analysis of variance of biomarker concentrations across sampling events and times[a,b].

Biological Endpoint	Effect	F-Value	*p*-Value
Urinary Cotinine	Sampling Time	116.91	< 0.0001***
	Event	28.88	< 0.0001***
	Sampling Time*Event	3.60	0.0015*
Salivary Cotinine	Sampling Time	95.12	< 0.0001***
	Event	23.01	< 0.0001***
	Sampling Time*Event	6.66	< 0.0001***
Urinary Trans-3′-Hydroxycotinine	Sampling Time	87.31	< 0.0001***
	Event	17.28	< 0.0001***
	Sampling Time*Event	2.61	0.0149*
Urinary 3-HPMA	Sampling Time	11.45	< 0.0001***
	Event	11.99	< 0.0001***
	Sampling Time*Event	2.75	0.0106*
Urinary CEMA	Sampling Time	6.47	0.0006**
	Event	1.35	0.2647
	Sampling Time*Event	1.88	0.0780
Urinary 8-isoprostane	Sampling Time	0.10	0.9594
	Event	0.62	0.6019
	Sampling Time*Event	1.64	0.1300

*Significant at $p \leq 0.05$.
**Significant at $p \leq 0.001$.
***Significant at $p \leq 0.0001$.
[a] All urinary endpoints were adjusted for creatinine for this analysis.
[b] Sampling Events included Events 1, 2, 3, and 4. Sampling times included pre-exposure, immediate post-exposure, 4-h post-exposure (Events 1, 3, and 4 only), and the morning after the events.

across time. The highest concentrations were detected in samples collected 4-h post-exposure for Event 4 (AGM: 2.31 µg/g creatinine [95% CI: 1.43,3.72]).

Salivary cotinine concentrations varied significantly across sampling times ($p < 0.0001$) and events ($p < 0.0001$) (Table 3). A significant interaction between sampling time and event was also found ($p < 0.0001$). Salivary cotinine concentrations increased up to 12-fold after the events (Table 4). Concentrations were elevated through post-exposure sampling times and decreased slightly in first-void samples (Fig. 2). The highest concentrations were measured in Event 4 samples collected 4-h after exposure (AGM: 0.17 ng/mL [95% CI: 0.11, 0.26]). The geometric mean and 95% CIs for this sample time were the same as those calculated for immediate post-exposure samples, but concentrations collected 4-h post-exposure had a wider range of concentrations. The adjusted geometric means of salivary cotinine across all events ranged from 0.0139 to 0.0204, 0.0325–0.169, 0.0373–0.167, and 0.0280–0.148 ng/mL for pre-exposure, immediate post-exposure, 4-h post-exposure, and first-void samples (Table 2).

3.1.2. Trans-3′-Hydroxycotinine

Creatinine-corrected urinary trans-3′-hydroxycotinine concentrations varied significantly across sampling times ($p < 0.0001$) and sampling events ($p < 0.0001$) (Table 3). A significant interaction between sampling time and location was detected ($p < 0.05$). Urinary trans-3′-hydroxycotinine concentrations increased up to 8.8-fold after the events (Table 4). The adjusted geometric means of concentrations ranged from 0.134 to 0.223, 0.249–0.848, 0.349–2.22, and 0.406–2.67 µg/g creatinine pre-exposure, immediate post-exposure, 4-h post-exposure, and first void samples, respectively (Table 2).

Table 4
Adjusted mean ratio of participants' maximum over baseline biomarker concentrations by endpoint and location[a].

Biological Endpoint	Event	Adjusted Mean Ratio
Urinary Cotinine	1	8.14
	2	6.77
	3	2.67
	4	13.16
Salivary Cotinine	1	4.58
	2	7.07
	3	2.02
	4	12.68
Urinary Trans-3′-Hydroxycotinine	1	6.84
	2	5.68
	3	2.24
	4	8.79
Urinary 3-HPMA	1	3.82
	2	1.28
	3	2.18
	4	1.83
Urinary CEMA	1	2.40
	2	1.82
	3	1.92
	4	1.16
Urinary 8-Isoprostane	1	1.37
	2	0.95
	3	1.07
	4	1.48

[a] All urinary endpoint were corrected for creatinine.

Creatinine-corrected concentrations increased at each successive post-exposure sampling time and peaked in first-void samples (Fig. 3). Event 4 first-void concentrations were the highest among events and sampling times (AGM: 2.67 μg/g creatinine [95% CI: 1.39, 5.13]).

Salivary trans-3′-hydroxycotinine concentrations were below the limit of detection for 66% of samples (n = 67). This was most pronounced in Event 3 samples where concentrations were < LOD for 92% of samples (n = 22). Salivary trans-3′-hydroxycotinine was not included in statistical analyses because of the low detection rates.

3.2. Urinary acrolein metabolites

3.2.1. 3-HPMA

Creatinine-corrected 3-HPMA urinary concentrations varied significantly across sampling times ($p < 0.0001$) and events ($p < 0.0001$) (Table 3). A significant interaction between sampling time and event existed ($p < 0.05$). Urinary 3-PHMA concentrations corrected for creatinine increased up to 3.8-fold after the events (Table 4). The adjusted geometric means of pre-exposure, immediate post-exposure, 4-h post exposure, and first-void adjusted concentrations ranged from 186 to 324, 199–625, 455–808, and 163–839 μg/g creatinine, respectively (Table 2). Event 1, 3, and 4 concentrations of creatinine-corrected 3-HPMA peaked at different times after events but they all increased after exposure, unlike Event 2 concentrations which did not increase appreciably (Table 2, Fig. 4).

3.2.2. CEMA

Creatinine-corrected CEMA concentrations varied significantly across sampling times ($p < 0.01$) but not across sampling events. Urinary CEMA concentrations increased up to 2.4-fold after the events (Table 4). The adjusted geometric means of concentrations ranged from 61.5 to 100, 82.7–113, 107–116, and 91.0–169 μg/g creatinine in pre-exposure, immediate post-exposure, 4-h post-exposure, and first void samples, respectively (Table 2).

3.3. Urinary tobacco-specific nitrosamines

Total NAB, NAT, and NNN concentrations were below the limit of detection in all samples for all sampling times and sampling events. NNAL was < LOD in 84% of samples (n = 85). Interestingly, 38% of detected NNAL concentrations were in pre-exposure samples (n = 6/16) collected from participants prior to Events 1 (n = 1), 2 (n = 3), and 3 (n = 2). TSNAs were not included in the statistical analyses because of the low detection rates.

3.4. Urinary 8-isoprostane

Creatinine-corrected 8-isoprostane concentrations did not vary significantly across sampling times or events (Table 3). The adjusted geometric mean of concentrations ranged from 302 to 346, 260–446, 314–414, 297–377 ng/g creatinine in pre-exposure, immediate post-exposure, 4-h post-exposure, and first void samples, respectively (Table 2).

3.5. Health effects

Participants completed an exit survey that asked about adverse health effects they experienced during the event. No adverse health effects were reported during Event 2. Among Event 1, 3, and 4 participants, 15% (n = 5) reported experiencing some type of adverse health effect (i.e. headache, dry mouth, cough, dry/burning eyes) during the e-cigarette convention that they attributed to secondhand exposures to e-cigarette emissions. One participant reported experiencing a headache but thought that it could be due to factors other than the event.

4. Discussion

This study characterized secondhand exposures to e-cigarettes by analyzing tobacco exposure biomarkers in urine and saliva of 28 non-users who attended at least one large e-cigarette event. Secondhand exposures to e-cigarette emissions lasting approximately six hours resulted in significant increases in salivary and urinary cotinine and urinary trans-3′-hydroxycotinine, 3-HPMA, and CEMA concentrations. Urinary and salivary cotinine and urinary trans-3′-hydroxycotinine and 3-HPMA concentrations varied significantly across sampling events. Significant interaction effects between sampling event and sampling time were found for urinary and salivary cotinine, urinary trans-3′-hydroxycotinine, and urinary 3-HPMA.

Significant interaction effects indicate the effect of sampling time is dependent on the sampling event. This can likely be explained by the differences in exposures inside each event venue determined by variables such as venue size, the number of active e-cigarette users, venue ventilation rates, mixing of outdoor air from opened doors, etc. Biomarker concentrations post-exposure are largely dependent on the extent of exposure inside the sampling event.

The highest urinary cotinine concentrations were observed after Event 4. Urinary cotinine concentrations measured 4-h after this event were (AGM [95% CI]) 2.31 μg/g creatinine (1.43, 3.72). Ballbe et al. (2014) measured cotinine concentrations in urine from five participants passively exposed to e-cigarette emissions at least two hours a day by living in the homes of e-cigarette users. Though there are differences between the Ballbe et al. (2014) and the present study such as e-cigarette user density, length of exposure, etc., the Ballbe et al. (2014) study offers valuable comparison data. Reported urinary cotinine concentrations (GM ± Geometric SD [GSD]: 1.75 ± 2.67 μg/g creatinine) were slightly lower than those found in this current study. For comparison, the reported urinary concentrations for twenty-five non-users living in homes with cigarette smokers were (GM ± GSD) 2.46 ± 2.67 μg/g creatinine in the Ballbe et al. study. It is not clear whether Ballbe et al. measured total cotinine or only the free form. The latter may account for the lower concentrations they found.

Salivary cotinine concentrations in this study also reached the highest values at 4-h after Event 4 (AGM: 0.167 ng/mL [95% CI: 0.107, 0.259]). These concentrations are slightly lower than salivary cotinine concentrations reported for non-users living with e-cigarette users in the Ballbe et al. (2014) study (GM ± GSD: 0.19 ± 2.17 ng/mL). Ballbe et al. (2014) reported salivary cotinine concentrations of non-users who lived with tobacco cigarette smokers were twice as high (GM ± GSD: 0.38 ± 2.34 ng/mL). Salivary cotinine concentrations in the current study were similar to those reported in a study of secondhand tobacco exposures in a bar in Athens, GA (St Helen et al., 2012). In that study, participants stood or sat near tobacco smokers in a bar for three hours. After the three hours, mean salivary cotinine concentrations were (GM [95% CI]) 0.161 ng/mL (0.14, 0.18]). Results indicate that six hours of e-cigarette secondhand exposures can result in salivary cotinine concentrations similar to those reported for people living in homes with e-cigarette users or those exposed for a few hours to secondhand tobacco smoke in a bar. These concentrations are approximately twenty times lower than those reported for participants exposed to sidestream smoke from approximately three tobacco cigarettes in a chamber study (Avila-Tang et al., 2013).

Urinary acrolein metabolites increased after secondhand exposure to e-cigarette emissions. The CDC reports the average 3-HPMA and CEMA urinary concentrations among a representative sample of non-smokers in the U.S. population from 2005 to 2006 were (Median [25th, 75th]) 219 μg/g creatinine (140, 353) and 78.8 μg/g creatinine (51.8, 121), respectively (Alwis et al., 2015). Adjusted average concentrations of 3-HPMA in this study exceeded these estimates by up to four-fold. Average concentrations of CEMA in this study were similar to or slightly higher than the median reported by CDC. Similarly, Schober et al. (2014) reported the 3-HPMA was elevated among e-cigarette users, but they found no elevation in CEMA (Schober et al., 2014). 3-HPMA is the major metabolite and CEMA is a minor metabolite of acrolein (Alwis et al., 2015). This could explain the discrepancy in patterns of change observed. There are many sources of acrolein exposures both in the environment and endogenously, thus the acrolein concentrations measured in this study are only partially attributable to passive e-cigarette exposure.

E-cigarette use has been shown to result in inflammation in the user, but inflammation from secondhand e-cigarette exposures has not been reported. For example, two studies used the concentrations of fractional exhaled nitric oxide (FeNO) to measured bronchial inflammation in e-cigarette users (Schober et al., 2014; Vardavas et al., 2012). In both studies, the concentration of FeNO changed after primary e-cigarette use, though the responses were in opposite directions. Propylene glycol exposures have resulted in ocular and airway irritation, though the concentrations used to cause these health effects were much higher than those likely present at an e-cigarette event (GM = 309 mg/m^3) (Wieslander et al., 2001). 8-Isoprostane is recognized as the most specific and sensitive biomarker for oxidative stress (Czerska et al., 2016; Montuschi et al., 2007). Cigarette smoking is associated with increased urinary 8-isoprostane (Morrow and Roberts, 1997). However it was the only biomarker in this study that did not significantly change across sampling events or sampling times. Secondhand e-cigarette exposures in this study did not result in oxidative stress in those passively exposed, suggesting that an increase in oxidative stress may be a chronic effect biomarker of exposure or that the exposure levels in this study did not have an effect on oxidative stress.

Limitations of this study include a small sample size and a subset of only four e-cigarette events. This study only measured acute exposures. Chronic exposures may result in different outcomes. Future research should analyze the relationship between environmental components of e-cigarette emissions and biomarkers of e-cigarette exposure. Participants' consumption of food and drink was recorded but not incorporated into biological analysis. All participants consumed food or drinks inside the venue. Participants could have received third-hand e-cigarette exposures from e-cigarette emissions present on food or drink products, serving containers, or the participants' hands during consumption. Future research should consider the contribution of food and drink to biomarker concentrations, because chemicals of interest (i.e. acrolein) are inherently in many foods and drinks and third-hand exposures can contribute to overall e-cigarette exposures.

Ventilation rates inside e-cigarette event venues should also be considered in future research. Researchers did not observe any additional tobacco products being used inside the venues, but it is possible that attendees used other tobacco products during the events that could contribute to the concentrations of contaminants reported here.

5. Conclusion

This study is the first to characterize secondhand exposures to chemicals present in e-cigarette emissions in public settings. While the exposure duration was relatively short (~6 h), participants' salivary and urinary cotinine concentrations were comparable to those reported for non-users living with e-cigarette users or sitting near tobacco smokers in a bar (Ballbe et al., 2014). Secondhand e-cigarette emissions may be a source of acrolein exposures but are not a strong source of tobacco-specific nitrosamines. Secondhand e-cigarette exposures occurring for a short period of time do not result in measurable increases in an oxidative stress biomarker.

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Declarations of interest

None.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijheh.2019.04.013.

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