AN EXPOSURE ASSESSMENT OF ELECTRONIC CIGARETTES: USERS' NICOTINE DEPENDENCE AND NONUSERS' SECONDHAND EXPOSURES

by

JONA MARIE JOHNSON

(Under the Direction of Jia-Sheng Wang)

ABSTRACT

Background: Electronic cigarettes (e-cigarettes) are novel nicotine delivery devices. E-cigarette user nicotine dependence and secondhand e-cigarette exposure have not been well characterized. **Objectives:** 1) To characterize secondhand e-cigarette exposures during e-cigarette conventions to formaldehyde, acetaldehyde, acrolein, nicotine, and propylene glycol using environmental monitoring, 2) to characterize secondhand e-cigarette exposures to acrolein, nicotine, and tobacco-specific nitrosamines (TSNAs) during e-cigarette conventions using biomarkers of exposure, 3) to characterize secondhand e-cigarette exposures during e-cigarette conventions using 8-isoprostane, a biomarker of effect and, 4) to assess nicotine dependence of e-cigarette users attending an e-cigarette convention. **Methods:** Data was collected at five e-cigarette events throughout the Southeast from October 2015 to March 2017. During the first convention, e-cigarette users completed a survey that included questions from the Fagerstrom Test for Nicotine Dependence (FTND) and questions concerning behavioral and addiction characteristics. During the remaining four conventions (September 2016 to March 2017), a secondhand exposure assessment using biological and environmental monitoring was completed using non-smoking volunteers (n=34). Air pumps were placed inside backpacks worn by volunteers (n=22) and used to sample the air in e-cigarette environments. Urine and saliva samples were collected pre- and

post-exposure. Urine samples were analyzed for cotinine, trans-3'-hydroxycotinine, two acrolein metabolites, four tobacco-specific nitrosamines (TSNAs), and 8-isoprostane. Saliva samples were analyxed for cotinine and trans-3'-hydroxycotinine. **Results:** E-cigarette vapor contained elevated concentrations of propylene glycol (median=305.51 $\mu g/m^3$) and nicotine (median=1.10 $\mu g/m^3$). Airborne concentrations of formaldehyde, acetaldehyde, and acrolein during e-cigarette events were low. Concentrations of creatinine-corrected urinary cotinine, trans-3'-hydroxycotinine, *S*-(3-hydroxypropyl)-*N*-acetylcysteine (3-HPMA), *S*-carboxyethyl-*N*-acetylcysteine (CEMA), and salivary cotinine significantly varied across sampling times. Most users surveyed were classified as moderately dependent using the FTND (average FTND score = 5.0). Most (85.2%) e-cigarette users smoked tobacco cigarettes prior to beginning e-cigarette use. **Conclusion:** E-cigarette secondhand exposures in public settings with high-concentrations of e-cigarette vapor do not contain elevated concentrations of formaldehyde or acetaldehyde. Secondhand e-cigarette vapor does contain elevated concentrations of nicotine, propylene glycol, and possibly acrolein. E-cigarette use can result in average nicotine dependence higher than previously reported for e-cigarette users and tobacco smokers.

INDEX WORDS: Electronic Cigarettes, Secondhand Smoke, Secondhand Exposure Assessment, Biomonitoring, Air sampling, Nicotine Dependence

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DEDICATION

"Finish the Drill."

–The University of Georgia Athletic Department

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CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

INTRODUCTION

Tobacco use is the largest preventable cause of death and disease in the United States and kills nearly half a million Americans every year.[1] Tobacco products are highly addictive because they contain nicotine, a psychoactive drug that activates key receptors in the brain.[2] During smoking, users are also exposed to extremely harmful compounds present in tobacco.[3] In response, nicotine replacement products have been developed that deliver nicotine without exposing the user to the harmful toxicants in tobacco and tobacco smoke.[4] In 2004, a novel electronic nicotine delivery device, the electronic cigarette (e-cigarette), was introduced to the market as a smoking cessation device.[5] Data needed to evaluate the effectiveness of e-cigarettes as cessation devices and to characterize exposures, including secondhand exposures, and resulting adverse health consequences is limited.[6]

Secondhand exposures to e-cigarettes are not fully understood.[6] While e-cigarettes do not contain all combustible by-products associated with burning tobacco, e-cigarettes produce vapors that have been reported to contain chemicals of concern.[7-19] Results from available studies are inconsistent and realistic exposure scenarios are understudied.[6; 7]

In 2014, e-cigarettes surpassed traditional tobacco cigarettes to become the most commonly used tobacco product among youth.[6] Traditional tobacco cigarettes are still the most used tobacco product among adults (≥ 18 years old)[20], though adult e-cigarette use is rapidly increasing (2,167% from 2010-2013).[21] In 2014 U.S. Food and Drug Administration (FDA)

concluded there is no evidence e-cigarettes are successful smoking cessation tools.[22] Still, e-cigarettes are touted as safe alternatives to traditional cigarettes by highly respected organizations such as Public Health England and the Royal College of Physicians.[23]

The 2009 Family Smoking Prevention and Tobacco Control Act delegated authority over cigarettes, roll-your-own tobacco, and smokeless tobacco to the FDA.[23] E-cigarettes were not included in the legislation. On April 25, 2014, the FDA announced a proposal to expand FDA's authority to include e-cigarettes and other novel tobacco products.[22] In this proposal, FDA stated they would use their regulatory authority to 1) implement age restrictions for e-cigarette purchases, 2) require health warnings on e-cigarette products, 3) require product and business registration, 4) require submission of ingredients and reporting of potentially harmful ingredients, 5) prohibit vending machine sales, 6) prohibit use of modified risk descriptions on labels, 7) prohibit distribution of free e-cigarette liquid samples, 8) require premarket review of e-cigarette devices and liquids, and 9) enforce action against e-cigarette products that are misbranded or adulterated. On May 10, 2016, the FDA issued a final rule that extended the Agency's authority to cover e-cigarettes beginning August 8, 2016.[24] This ruling is currently being discussed in court.[6]

The FDA does not typically have the authority to regulate tobacco use in public places or indoors.[6; 25] This authority is delegated to states and local jurisdictions.[6] E-cigarettes do not emit smoke and are often excluded from state or local indoor smoke-free laws.[26] E-cigarette users have reported that they began using e-cigarettes because they use them in smoke-free places.[27] In 2012 when only three states had laws that banned e-cigarette use in restaurants, bars, and workplaces the Center for Disease Control and Prevention (CDC) estimated that 303 million people, including 70 million children, were at risk of secondhand e-cigarette vapor

exposures.[28]As of July 3, 2017, eight states, the District of Columbia, Puerto Rico, and 661 localities included e-cigarettes in their smoke-free indoor air laws.[29; 30] Though states and local jurisdictions are beginning to include e-cigarettes in their smoke free laws, there currently is vast opportunity for secondhand e-cigarette exposures in public spaces, homes, and workplaces.

Conventions designed to bring e-cigarette users together in a social setting are held nearly every month across the United States.[31] These gatherings can attract thousands of attendees. Secondhand exposures in this environment, likely the public setting with the highest concentration of e- cigarette vapor, have not been fully characterized.[32] The nicotine dependence of users that attend these events, perhaps representing the most active of e-cigarette users, is unknown. Therefore, this dissertation sought to characterize users' nicotine dependence and non-users' secondhand e-cigarette exposures using environmental and biological monitoring during e-cigarette conventions.

OUTLINE OF DISSERTATION

- **Chapter 1** provides an introduction, outline of the dissertation, and a review of the current and relevant literature.
- **Chapter 2** is a manuscript that presents environmental monitoring data collected during four e-cigarette conventions. This manuscript addresses the first objective of this research: to characterize secondhand e-cigarette exposures to formaldehyde, acetaldehyde, acrolein, nicotine, and propylene during e-cigarette conventions using air monitoring. This paper was submitted to *International Journal of Hygiene and Environmental Healt*h on 17 October, 2017.
- **Chapter 3** is a manuscript that presents biological monitoring data from non-smoking volunteers who attended at least one e-cigarette convention. This manuscript addresses the second and third objectives of this research: 2) to characterize secondhand e-cigarette exposures to acrolein, nicotine, and tobacco-specific nitrosamines (TSNAs) at e-cigarette conventions using biomarkers of exposure and 3) to characterize secondhand e-cigarette exposures at e-cigarette conventions using 8-isoprostane as a biomarker of effect. The paper will be submitted to *Environmental Health Perspectives.*
- **Chapter 4** is a manuscript that presents data characterizing nicotine dependence of users who attended an e-cigarette convention in Orlando, Florida in October 2015. This manuscript addresses the fourth objective of this research: to assess nicotine dependence of e-cigarette users attending an e-cigarette convention. The paper has been accepted and published by the *Journal of Community Health.*
- **Chapter 5** contains a summary and concluding remarks.

The environmental and biological monitoring manuscripts (Chapters 2 and 3) were pilot projects funded by the National Institute for Occupational Safety and Health (NIOSH) Education Research Center (ERC) Small Project/Pilot Grant through the University of Alabama at Birmingham (UAB) (Grant no: 2T420H008436). Air samples analysis in Chapter 2 was supported by the National Center for Environmental Health.

LITERATURE REVIEW

This literature review concisely summarizes 1) chemicals identified in e-cigarette vapor and 2) biomarkers of exposure to select e-cigarette vapor components, and 3) e-cigarette user nicotine dependence.

Chemicals Identified in E-cigarette Vapor

Studies analyzing chemical constituents of e-cigarette vapor are inconsistent. Most studies agree nicotine, particulate matter, propylene glycol, and/or glycerin are present in e-cigarette vapor. However, the available literature does not consistently report to what extent e-cigarette vapor contains volatile organic compounds (VOCs) (primarily formaldehyde, acetaldehyde, and acrolein), metals, or tobacco-specific nitrosamines (TSNAs).

There are many different e-cigarette device models, user behaviors, customizable parameters, and study designs that affect how the device is used and may explain the variety of secondhand exposure assessment results.[33; 34] For example, battery voltage and nicotine content have been reported to significantly affect VOC production.[11] The type of e-cigarette liquid used has also been shown to impact VOC vapor concentrations.[8] Many studies use a smoking machine

to produce e-cigarette vapor for secondhand exposure studies and are not truly representative of real-use conditions.[12]

Studies analyzing chemical constituents in e-cigarette vapor produced by smoking machines or human subjects are examined in this section. The chemicals most commonly reported in e-cigarette secondhand exposure studies are discussed. Air sampling information is also provided for the select chemicals measured as part of this dissertation.

Volatile Organic Compounds/Carbonyl Compounds

Volatile Organic Compounds (VOCs) are carbon-containing compounds that are easily volatilized at room temperature.[35; 36] VOCs are found ubiquitously in the environment.[35; 36] It is theorized that VOCs are formed in e-cigarette vapor when propylene glycol and/or glycerin passes over the heated wire in the device's atomizer during use.[37] The research in this dissertation focused on the three most commonly detected and discussed VOCs (formaldehyde, acetaldehyde, and acrolein) in the e-cigarette secondhand exposure literature.

Health Effects and Exposure Guidelines for Formaldehyde, Acetaldehyde, and Acrolein

The International Agency for Research on Cancer (IARC) classifies formaldehyde as a Group 1 carcinogen (carcinogenic to humans).[38] Formaldehyde is very reactive and, at elevated exposure concentrations, can irritate the biological tissues that it contacts.[39] Sufficient exposure can result in lung, nose, and throat irritation and/or worsening of asthmatic symptoms.[39] The Occupational Health and Safety Administration (OHSA) Permissible Exposure Limit (PEL) for an 8-hour formaldehyde exposure over an eight hour work shift (known was the 8-hour time-weighted average [8-hour TWA]) is 0.75 part-per-million (ppm) (0.92 mg/m^3).[40] The National

Institute for Occupational Safety and Health (NIOSH) Recommended Exposure Limit (REL) (8-hour TWA) for formaldehyde is 0.016 ppm (19.65 μg/m^3).[41] The Agency for Toxic Substances and Disease Registry (ATSDR) establishes chronic, intermediate, and acute minimal risk levels (MRLs) for community exposures.[42] The ATSDR acute, intermediate, and chronic MRLs for formaldehyde inhalation exposure are 0.04 ppm (49.13 μg/m^3), 0.03 ppm (36.85 μg/m^3) and 0.008 ppm (9.83 μg/m^3), respectively.[43] Exposures at or below these recommended exposure limits are not expected to result in adverse health effects for the average person.[42]

IARC classifies acetaldehyde as a Group 2B carcinogen (possible carcinogenic to humans).[44] Acetaldehyde can be irritating to the eyes, skin, and respiratory tract.[45] Erythema, coughing, pulmonary edema, and necrosis if exposures levels are sufficient.[45] The OSHA PEL (8-hour TWA) for acetaldehyde is 200 ppm (360 mg/m^3).[40] NIOSH has not established a REL specific to acetaldehyde.[46] The American Council of Governmental Industrial Hygienists (ACGIH) recommends acetaldehyde exposures no not exceed 25 ppm (45.04 mg/m^3).[47] The ATSDR has not established a MRL.[43]

IARC decided the carcinogenicity of acrolein in humans is not classifiable.[48] Acrolein can irritate the nasal cavity and lining of the lungs, which is consistent with the health effects reported by e-cigarette users.[49] Additionally, a rat study demonstrated that synergistic effects occur when rats inhale a mixture of acetaldehyde, formaldehyde, and acrolein.[50] OSHA, NIOSH, and ACGIH all set 8-hour TWAs of 0.1 ppm (230 μg/m^3) for acrolein exposures.[51] NIOSH recommends exposures of 15 minutes or less not exceed 0.3 ppm (690 μg/m^3).[46] The ATSDR MRL for acute and intermediate exposures is 0.003 ppm (6.88 μg/m^3) and 0.00004 ppm (0.09 μg/m^3), respectively.[43]

Formaldehyde in E-cigarette Vapor

Goniewicz et al. (2014) analyzed vapors generated by different brands and types of electronic cigarettes using a smoking machine.[10] The results from the e-cigarette vaping session were compared with vapor analysis of a Nicorette inhaler. Formaldehyde was detected in concentrations of 2.0-56.1 µg/150 puffs. Formaldehyde was also detected at an average concentration of 2.0 µg/150 puffs in the Nicorette inhaler, but it was not detected in the blank samples. The authors concluded that formaldehyde concentrations in exhaled e-cigarette vapor may be similar to that of exhaled vapor from traditional cigarettes.

A follow-up study by Kosmider et al. (2014) researched how the nicotine concentration in the vaping liquid and the e-cigarette devices' battery voltage affected levels of carbonyls produced.[11] Second generation e-cigarettes allow the user to adjust the voltage of the batteries, increasing the amount of nicotine delivered to the user. The authors used the same method and a similar smoking machine regimen as Goniewicz et al. (2014) but used 2 types of new e-cigarettes: 1) an e-cigarette with a 3.4 volt (V) battery and 2) an e-cigarette with a 3.2-4.8 V battery. One difference between this study and the Goniewicz et al. (2014) study is that the authors only sampled 15 puffs of e-cigarette vapor instead of 150 puffs. Overall, the authors found that increasing the battery voltage increased the levels of carbonyls in e-cigarette vapor. Specifically, formaldehyde concentrations ranged from below the limit of detection to (Mean ± SD) 59 ± 6 ng/15 puffs. Operating an e-cigarette device using a 4.8 V battery produced formaldehyde concentrations similar to those in traditional cigarettes. Increasing the battery voltage from 3.2-4.8 V increased carbonyl concentrations 4-200 times, indicating that second generation devices do produce more carbonyl compounds. It is important to highlight that the

Kosmider et al. (2014) study results were from only 15 puffs, the reported equivalent of one tobacco cigarette.

Geiss et al. (2015) characterized mainstream and passive vapor from popular brands of e-cigarettes.[8] In the study, second generation e-cigarettes were vaped using a smoking machine in a $30m^3$ emission chamber. Carbonyl concentrations could not be detected. However, Geiss et al. (2015) also collected vapor from the smoking machine in a 2L Tedlar ® bag and analyzed the chemical constituents. Formaldehyde concentrations ranged from 196-227 $\mu g/m^3$. The authors extrapolated this to mean that a concentration in a $60m^3$ room could be between 0.004 and 0.005 $\mu g/m^3$. Uchiyama et al. (2013) also used a smoking machine with an absorbent cartridge the authors developed (silica gel cartridge with hydroquinone and 2,4-DNPH) to create an e-cigarette vaping environment.[14] The authors reported a mean and maximum formaldehyde concentration of 61 mg/m^3 and 260 mg/m^3, respectively.

Schober et al. (2014) conducted a chamber study using human vapers to assess the impact of e-cigarette vapor on indoor air quality.[13] In the study, 6 vaping sessions were spread across 7 days (1 control day) and included 3 vapers/session. Formaldehyde was detected in the chamber at concentrations of 24-55 $\mu g/m^3$. In a similar study, Schripp et al. (2013) had one e-cigarette user use an e-cigarette in a chamber for approximately fifteen minutes.[12] Formaldehyde concentrations ranged from 8-16 $\mu g/m^3$. In a study by McAuley et al. (2012), e-cigarette vapor produced by a smoking machine was analyzed immediately as it left the e-cigarette.[52] Formaldehyde concentrations ranged from <35.6 $\mu g/m^3$ to 1064.8 $\mu g/m^3$. Compared to traditional cigarettes analyzed in the study, the authors reported these concentrations were low.

Acetaldehyde in E-cigarette Vapor

Goniewicz et al. (2014) measured concentrations of acetaldehyde in a study assessing the various vapors generated by different brands and types of e-cigarettes using a Palaczbot smoking machine.[10] The authors reported concentrations of acetaldehyde ranged from 1.1-13.6 µg/150 puffs. Acetaldehyde was measured in a nicotine inhaler blank at an average concentration of 0.6 µg/150 puffs. A follow-up study by Kosmider et al. (2014) assessed how the nicotine concentration in the vaping liquid and the e-cigarette devices' battery voltage affected levels of carbonyls.[11] Acetaldehyde concentrations ranged from below the limit of detection to (Mean ± SD) 104 ± 74 ng/15 puffs.

Geiss et al. (2015) measured acetaldehyde concentrations by characterizing mainstream and passive vapor from popular brands of e-cigarettes.[8] In the chamber study, using the same sampling regimen and equipment as for formaldehyde, acetaldehyde was not detected. However, high levels of acetaldehyde (81-399 µg/m^3) were detected when exhaled e-cigarette vapor was trapped in 2 L Tedlar sampling® bag. The authors found that using a "velvet" vaping liquid (a mixture of glycerol and water) and a 2nd generation e-cigarette caused concentrations of acetaldehyde in e-cigarette vapor to increase by 5-fold. In the Schober et al. (2014) chamber study, acetaldehyde was measured in concentrations ranging from 19-162 µg/m^3.[13] Uchiyama et al. (2013) reported a mean and maximum acetaldehyde concentration of 48 mg/m^3 and 210 mg/m^3, respectively.[14]

McAuley et al. (2012) used a smoking machine to produce 50 puffs of e-cigarette vapor. The vapor was analyzed immediately as it left the e-cigarette.[52] Acetaldehyde concentrations reportedly ranged from <77.6 µg/m^3 to 1317.4 µg/m^3. Long et al. (2014) had human subjects use an e-cigarette and produce ~99 puffs of e-cigarette vapor. [53] Exhaled breath and chamber air

were analyzed. Formaldehyde, acetaldehyde, and acrolein concentrations were all undetectable or comparable to control concentrations.

Acrolein in E-cigarette Vapor

Goniewicz et al. (2014) measured concentrations of acrolein produced in e-cigarette vapor using a smoking machine.[10] The authors reported concentrations of acrolein ranged from 0.7-41.9 µg/150 puffs. EPA did not design method TO-11A to measure acrolein and this method may not be appropriate.[54] In a follow-up study by Kosmider et al. (2014), acrolein was not detected in any exhaled vapor sample even when battery voltages were increased to 4.8 V.[11] The authors indicated that the small sampling volume (15 puffs) may have been the cause as their previous study assessing chemicals in 150 puffs did identify acrolein at quantifiable concentrations in the vapor. Uchiyama et al. (2013) reported a mean and maximum acrolein concentration of 27 mg/m^3 and 73 mg/m^3, respectively.[14]

Geiss et al. (2015) found that, depending on the type of vaping liquids (i.e. mixture of glycerol and water v. propylene glycol, glycerol, and water) and e-cigarette used (i.e. first or second generation), concentrations of acrolein in e-cigarette vapor can increase by 27-fold.[8] The authors reported that concentrations of acrolein may be underreported in this study because it forms multiple formation peaks and is hard to analyze. In the chamber component of the study, acrolein was not detected. In the section of the study capturing exhaled e-cigarette vapor in a 2L Tedlar® sampling bag, acrolein was detected in concentrations ranging from 5-135 µg/m^3.

An additional study by Schober et al. (2014) did not detect airborne concentrations of acrolein in their chamber study using NIOSH Method 2018. It is important to note that NIOSH does not recommend this method be used to measure acrolein because of poor recovery

percentages.[55] However, Schober et al. (2014) did identify a mecapturic acid metabolite indicated to be a product of the pyrolosis of acrolein in the urine of e-cigarette users who vaped liquids containing nicotine.[13] In a study by McAuley et al. (2012), 50 puffs of e-cigarette vapor were produced by an e-cigarette machine and immediately analyzed as it left the e-cigarette.[52] Acrolein concentrations could not be detected using EPA TO-11A (<58.7 μg/m^3).

Nicotine

Nicotine is a naturally occurring chemical found in tobacco plants.[56] For nicotine-containing e-cigarette liquids, nicotine is extracted from tobacco to be added to e-cigarette liquids.[57] Exposure to nicotine can result from inhalation, ingestion, dermal absorption, and mucous membrane absorption.[56] Health effects can include neurological effects such as tremors, increased heart rate, twitching, seizures, multi-system organ depression, vomiting, and death.[56] Disruption of the nicotinic acetylcholine receptors by nicotine in the fetal and adolescent brain can disrupt normal neurodevelopment.[58]

Average airborne nicotine concentration in homes with at least one tobacco cigarette smoker ranges from 2-14 μg/m^3.[59] Airborne nicotine concentrations in various types of rooms with tobacco smokers have been reported to range from 0.3 to >500 μg/m^3.[60]

Recommended levels for airborne exposure to nicotine help characterize secondhand e-cigarette exposures to nicotine. These recommendations are not available for the general population, but they are available for workers. ACGIH, OSHA, and NIOSH all recommend nicotine workplace exposures be no more than 0.5 mg/m^3 averaged over an eight hour work shift.[61] NIOSH determined that a airborne nicotine dose of 5 mg/m^3 is likely to cause death or serious adverse health effects in workers.[62]

Nicotine in E-cigarette Vapor

Czogala et al. (2014) conducted a chamber study to investigate potential secondhand exposures from e-cigarettes and reported significantly increased concentrations of airborne nicotine (range: 0.82-6.23 μg/m^3) from e-cigarette vapor compared to control samples.[16] E-cigarette vapor produced by a smoking machine for 3 seconds and trapped in a 10-L glass chamber had nicotine concentrations of 4.0-7.0 μg/m^3. Geiss et al. (2015) used a smoking machine chamber study to demonstrate that, on average, an e-cigarette delivers the same concentration of airborne nicotine as a traditional cigarette.[8] Maximum airborne nicotine concentrations were measured to be ~0.6 μg/m^3.

Ballbe et al. (2014) measured airborne nicotine concentrations in homes of e-cigarette users.[63] Control homes and e-cigarette users' homes airborne nicotine concentrations (Geometric Mean [GM] ± Geometric Standard Deviation [GSD]) were 0.02 μg/m^3 ± 3.51 μg/m^3 and 0.13 μg/m^3 ± 2.4 μg/m^3, respectively. Melstrom et al. (2017) reported nicotine concentrations produced by three e-cigarettes users over two hours in an office chamber-like environment ranged from 0.4-2.0 μg/m^3.[34] Schober et al. (2014) measured airborne nicotine concentrations while e-cigarette users operated an e-cigarette for two hours inside a chamber.[13] Nicotine concentrations ranged from <0.04-4.6 μg/m^3.

McAuley et al. (2012) used a smoking machine to trap e-cigarette vapor from 50 puffs on filters.[52] Nicotine concentrations on the filters reportedly ranged from <1.7 mg/m^3 to 6.8 mg/m^3. Similarly, a study by Pellegrino et al. (2012) reported nicotine concentrations of e-cigarette steam (e.g. immediately as the vapor leaves the e-cigarette) from 16 puffs produced by a smoking machine ranged from <0.01-6.21 mg/m^3.[17]

Particulate Matter

Particulate matter (PM) is the name for mixtures of airborne solid particles and liquid droplets.[64] Particulate matter in outdoor air pollution is classified as a Group 1 carcinogen (carcinogenic to humans) by IARC.[65] Particulate matter is likely formed from supersaturated propylene glycol in e-cigarette vapor.[66] The size of particulate matter can vary. Mixtures of particles and droplets that are ≤10 μm in diameter are called PM_{10}.[64] PM_{10} can be inhaled and deposited in the airways but typically is filtered by the nose and upperairway.[67] Mixtures with diameters ≤2.5 μm are called $PM_{2.5}$.[64] Mixtures that are ≤0.1 μm in diameter are called $PM_{0.1}$ or ultrafine particles (UFP).[68] Because these mixtures are so small, they can reach the aveloar region of the lung and cross the respiratory barriers to be introduced to other organs.[69]

Particles <0.5 μm in diameter are indicated to be the most harmful to human health.[70] The same study reported that adverse health effects were negatively correlated with particle size. Adverse health effects resulting for particulate matter can range from asthma exacerbation to premature death.[64]

Regulations are available that address particulate matter in the ambient environment and the workplace. The U.S. Environmental Protection Agency (EPA) identified particle pollution (particulate matter), including $PM_{2.5}$ and PM_{10}, as one of six criteria pollutants to be regulated by the National Ambient Air Quality Standards.[71] In this regulation, $PM_{2.5}$ and PM_{10} are not to exceed 35 $\mu g/m^3$ and 150 $\mu g/m^3$, respectively, over a 24-hour period averaged over three years. Though regulations address ambient particulate matter pollution, they provide a range of exposures considered to be acceptable for the general population's health. The California Division of Occupational Safety and Health (Cal/OSHA) and ACGIH recommend that worker exposures to total particulate matter not exceed an average of 10 mg/m^3 over an eight hour work

shift.[72] OSHA requires worker exposures do not exceed 15 mg/m^3 over the same time period.[72] Currently, there are no air quality guidelines specific to UFPs.[68] Particulate matter was not measured in this study and air sampling methods are not discussed.

Particulate Matter in E-cigarette Vapor

One study that measured $PM_{2.5}$ concentrations at a small e-cigarette convention reported median concentrations were 311.68-818.88 µg/m^3 throughout the two-day event.[73] Czogala et al. (2014) conducted a chamber study to investigate potential secondhand exposures from e-cigarettes and found significantly increased concentrations of $PM_{2.5.}$[16] In this study, concentrations of $PM_{2.5}$ were significantly higher (63.3-272.2 µg/m^3) when the vapor was produced by a human subject than the $PM_{2.5}$ concentrations in e-cigarette vapor produced by a smoking machine (15.0-80.0 µg/m^3). In a study that also used e-cigarette users to produce e-cigarette vapor (opposed to using a smoking machine), median $PM_{1.0}$, $PM_{2.5}$, and PM_{10} concentrations across seven sampling days were 13.0-421.0 µg/m^3, 18-561 µg/m^3, and 40-604 µg/m^3, respectively.[66]

A study that imitated an office environment using three e-cigarette users reported $PM_{2.5}$ concentrations ranged from 0.002 mg/m^3 to 19.972 mg/m^3 (medians=0.035-0.515 mg/m^3).[34] A similar study by Ruprecht et al. (2014) measured particulate matter produced by a human subject using an e-cigarette in an office environment.[74] In this study, $PM_{1.0}$, $PM_{2.5}$, $PM_{7.0}$, and PM_{10} concentrations after ~2-3 hours of e-cigarette use reached (Mean ± SD) 3.5 µg/m^3 ± 7.3 µg/m^3, 7.2 µg/m^3 ± 9.6 µg/m^3, 8.7 µg/m^3 ± 9.9 µg/m^3, and 9.9 µg/m^3 ± 10.3 µg/m^3 above background concentrations, respectively. The authors reported particulate matter concentrations after e-cigarette use were hundreds of times lower than those present after traditional cigarette use. A

study by the same research group reported that particulate matter concentrations after e-cigarette use for 14 minutes (1 user) were similar to outdoor particulate matter concentrations.[75]

A study by Pellegrino et al. (2012) used a smoking machine to produce e-cigarette vapor and reported that $PM_{1.0}$, $PM_{2.5}$, $PM_{7.0}$, and PM_{10} concentrations after 1.5-3 minutes of e-cigarette use by a smoking machine ranged from 0-14 $\mu g/m^3$, 3-43 $\mu g/m^3$, 8-40 $\mu g/m^3$, and 10-52 $\mu g/m^3$, respectively.[17] In a study that used a smoking machine to produce 50 puffs of e-cigarette vapor captured in a sampling bag, particle counts were about ten times lower than those produced by traditional cigarettes.[52]

Propylene Glycol and Glycerin

Propylene glycol is a liquid used often as a solvent and an additive in consumer products because it absorbs water and maintains moisture.[76] The Food and Drug Administration (FDA) classifies propylene glycol as Generally Regarded as Safe (GRAS)[77] and allows the chemical to be added to foods, cosmetics, and medicines.[76] Occupational exposure guidelines are not available for propylene glycol.[40; 46] The ATSDR's intermediate inhalation MRL is 9.00 ppb (28.01 $\mu g/m^3$).[76]

An exposure assessment of human subject volunteers demonstrated that exposures lasting just over a minute to an average concentration of 309 mg/m^3 propylene glycol resulted in ocular and throat irritation and slightly reduced lung capacity.[78] Though both propylene glycol and glycerin are used as e-cigarette liquid solvents, the environmental monitoring in this dissertation's research focused on propylene glycol.

Propylene Glycol and Glycerin in E-cigarette Vapor

McAuley et al. (2012) used a smoking machine to trap e-cigarette vapor from 50 puffs on filters.[52] Propylene Glycol concentrations reportedly ranged from 0.196 mg/m^3 to 120.00 mg/m^3. Schripp et al. (2013) measured propylene glycol in e-cigarette vapor produced by a human subject after 15 minutes of e-cigarette use.[12] Concentrations were not detectable (<1.0 μg/m^3) in the chamber study. The authors also used a smoking machine to produce vapor for 3 seconds that was captured in a 10-Liter glass chamber for analysis. Propylene glycol concentrations ranged from 53-175 mg/m^3. Schober et al. (2014) measured the amount of propylene glycol in e-cigarette vapor produced by human users during two hours of use in a chamber.[13] Concentrations ranged from 110-395 μg/m^3. A study by Pellegrino et al. (2012) measured the concentrations of propylene glycol and glycerin present in e-cigarette vapor immediately as it left the e-cigarette.[17] A smoking machine was used to generate the vapor. In this study, propylene glycol and glycerin concentrations ranged from 1650-1660 mg/m^3 and 580-610 mg/m^3, respectively.[17] Geiss et al. (2014) used a smoking machine in a chamber to measure propylene glycol after 65-70 minutes of e-cigarette use. Propylene glycol concentrations ranged from <1.0-2,000 μg/m^3. In the same study, e-cigarette vapor was also captured on a filter pad immediately was it left the e-cigarette device. Propylene glycol concentrations ranged from <2.2 -12.9 mg- ± 0.8 mg/filter.

Tobacco-Specific Nitrosamines (TSNAs)

Tobacco-specific nitrosamines (TSNAs) are a class of compounds that are unique to tobacco.[79] TSNAs are formed by nitrosation (i.e. addition of a "R-NO" functional group) of alkaloids (e.g. nicotine) when tobacco is processed, aged, and cured.[79-81] TSNAs are also formed during the smoking process and endogenously inside the body.[82] The presence of these

compounds in tobacco varies, primarily due to the manufacturing process and aging.[82] There are four TSNAs that are most commonly researched.[83] These four TSNAs are 1) 4-(methylnitrosamine)-1-(3-pyridyl)-1-butanone (NNK), 2) *N*-nitrosoanabasine (NAB), 3) *N*'-nitrosoanatabine (NAT), and 4) *N'*-nitrosonornicotine (NNN). 4-(methylnitrosamino)-1-(3-pyridyl) butan-1-ol (NNAL) is a major metabolite of NNK that researchers also often study.[82]

NNK and NNN are formed by the oxidation and nitrosation of nicotine mainly in tobacco.[79; 82] These two TSNAs are classified as Group 1 (carcinogenic to humans) carcinogens by the World Health Organization.[81] The Food and Drug Administration (FDA) identifies these compounds as two of the harmful and potentially harmful constituents (HPHCs) in tobacco products and tobacco smoke.[84]

NAT and NAB are formed by the nitrosation of anatabine and anabasine.[82] The World Health Organization classified these two TSNAs as Group 3 (not classifiable as to their carcinogenicity to humans) carcinogens.[85] There is limited evidence NAB is carcinogenic based on animal studies.[82] TSNAs were only measured in biological samples in this dissertation. Air sampling methods are therefore not discussed.

TSNAs in E-cigarette Vapor

Studies measuring TSNAs in e-cigarette vapor are limited. McAuley et al. (2012) could not detect NAB, NAT, and NNK in 50 puffs of e-cigarette vapor produced by an e-cigarette and immediately captured on filters (detection limit = <10 $\mu g/m^3$).[52] Concentrations of NNN reportedly ranged from <10 $\mu g/m^3$ to 18 $\mu g/m^3$. A second study assessed various vapors generated by different brands and types of e-cigarettes using a smoking machine.[10] NNN and

NNK were detected at concentrations of 0.8-4.3 ng/150 puffs and 1.1-8.3 ng/150 puffs, respectively.

Additional Chemicals Identified in E-cigarette Vapor in Secondhand Exposures Studies

Studies have inconsistently reported the presence of additional VOCs in e-cigarette vapor.[8; 10-14; 52] Metals (cadmium, nickel, and lead) were found in trace amounts in e-cigarette vapor from e-cigarettes smoked by a smoking machine.[10] Concentrations did not differ significantly (with the exception of cadmium in one sample) from control samples. Schober et al. (2014) detected aluminum in e-cigarette vapor, though no explanation was given as to why it might be present.[13] Saffari et al. (2014) measured the metals present in e-cigarette vapor produced by a human subject e-cigarette user stationed an office environment.[75] The authors reported that though overall metal concentrations were hundreds of times lower than those in traditional cigarette smoke, nickel and silver concentrations were comparable in e-cigarette aerosol compared to traditional cigarette smoke. The metals may be released from the e-cigarette device itself when it is heated to extremely high temperatures in a lab or by a smoking machine, potentially not representative of a real-life vaping environment.[15; 75; 79]

One study reported Polycyclic Aromatic Hydrocarbons (PAHs) were present in e-cigarette vapor.[66] PAHs are a class of toxic chemicals formed by incomplete combustion.[86] Given that e-cigarettes do not use combustion processes, it is likely that detectable PAH concentrations are from other sources.[87]

Air Sampling Methods

Formaldehyde and Acetaldehyde Air Sampling Methods

The 1999 EPA Compendium Method TO-11A is an accepted standard for sampling formaldehyde in ambient air.[88] This method is also appropriate to use when measuring ambient acetaldehyde concentrations, though not appropriate for measuring acrolein concentrations.[54; 89] This method can be used for up to 24 hours for aldehyde concentrations in the ppb range or for short durations when concentrations are in the ppm range.[88]

This method requires the use of a silica gel sorbent cartridge coated with 2,4-dinitrophenylhydrazine (DNPH).[88] EPA reports in the methods that silica gel sorbent materials have been proven to most accurately collect ambient organic chemicals with the least number of interferences compared with other sorbent materials available on the market (i.e. glass filters, XAD-2, C18, etc.).

Acrolein Air Sampling Methods

Studies have used method TO-11A to measure acrolein, though the EPA does not recommend using EPA-TO11A for this chemical because it reacts so easily and results in underestimation of acrolein concentrations.[54] Geiss et al. (2014) used NIOSH Method 2018 to measure acrolein, though NIOSH recommends against using this method because of low recovery rates.[55] Therefore OSHA Method 52 is the most appropriate method. OSHA 52 has a maximum volume of 48L at a recommended flow rate of 0.01 L/min.[90] The method's reliable quantification limit is 6.1 $\mu g/m^3$.

Nicotine Air Sampling Methods

Multiple methods are available to measure nicotine in ambient air. NIOSH Method 2551 is able to measure nicotine samples as low as 0.050 µg/sample, which is well below what is needed to measure nicotine concentrations as indicated by available studies.[91] NIOSH Method 2551 has a maximum volume of 600 L at a flow rate between 0.1-1 L/min. The method's limit of detection is 0.013 µg/sample with a working range between 0.05 and 2 µg/sample.

Propylene Glycol Air Sampling Methods

Both OSHA and NIOSH have active air sampling methods to measure concentrations of airborne propylene glycol. The OSHA method is only partially validated.[92] NIOSH developed Method 5523 for glycols.[93] This method can collect up to 800 µg/sample on one XAD-7 OVS tube. The recommended flow rate is 0.5-2 L/min for a minimum of 15 µg/sample.

Biomarkers of Exposure and Effect

The United States National Research Council (NRC) defines a biological marker (biomarker) as "an indicator signaling events in biological systems or samples".[94] The NRC identifies and defines three types of biomarkers: biomarkers of exposure, effect, and susceptibility. The NRC defines a biomarker of exposure as a biomarkers that identifies the presence of a foreign body in a system, an interaction between a system and a foreign body, or other exposure event.[94] The NRC explains that biomarkers of exposure should 1) vary consistently and quantitatively with exposures and 2) be specific to the exposure. The NRC reports that biomarkers of effect measure changes in components of a biological system, the

function of a biological system, or changes in the biological system that are indicative of impairment or disease.

Biomarkers of susceptibility identify systems that have reduced capacity to respond to a foreign body.[94] A recent paper by Schick et al. (2017) states that a good biomarker 1) has a clear dose-response relationship with exposure, 2) is detectable over a wide range of concentrations, 3) is easily detected in biological media and 4) is stable upon storage.[79] The NRC recommends all biomarkers be validated to ensure a correlation exists between exposure and the biological change investigated.

Validated biomarkers specific to e-cigarette exposures are not yet identified and.[79] E-cigarette research is new and has borrowed its selection of biomarkers from the field of traditional tobacco smoke exposure. [79] This dissertation research focused on select e-cigarette biomarkers of exposures and effect, which are briefly discussed in this section.

Biomarkers of Exposure

Nicotine, nicotine metabolites and tobacco-specific nitrosamine metabolites are biomarkers that are specific to tobacco and nicotine exposure.[79] Other biomarkers of exposure, like metabolites of VOCs, are less specific and can be a result of endogenous processes or additional exposure sources.[79] The research in this dissertation used urinary and salivary cotinine and urinary *trans*-3'-hydroxycotinine to characterize secondhand nicotine exposures. Metabolites of four tobacco-specific nitrosamines were used to characterize secondhand exposures to tobacco components. An isoprostane compound was used to characterize oxidative stress that occurred as a result of e-cigarette secondhand exposures.

Nicotine, Cotinine and Trans-3'-hydroxycotinine

In the body, nicotine is converted to six main metabolites: nicotine, cotinine, *trans*-3'-hydroxycotinine, cotinine-N-glucuronide, nicotine-N-glucuronide, and *trans*-3'-hydroxycotinine-O-glucuronide.[79] The most sensitive and specific biomarker available for traditional tobacco cigarettes is the metabolite of nicotine, cotinine.[95; 96] Approximately 75% of nicotine is converted to cotinine in the human body by liver enzyme CYP2A6.[97] The time it takes to convert nicotine to cotinine varies among individuals.[97; 98] Cotinine is favored as a biomarker over nicotine because cotinine has a longer half-life in biological media (~2 hours vs. 16-18 hours).[79; 99] Analysis of multiple nicotine metabolites is needed to provide a true characterization of nicotine exposure.[79]

Available studies show that nicotine absorbed in the body by both users and bystanders is similar to that of traditional cigarette use.[18; 100] Goniewicz et al. (2017) collected urine from volunteers who switched from traditional cigarettes to e-cigarettes for two weeks.[101] Of the 17 biomarkers of exposure analyzed, 12 significantly decreased. With one exception, no nicotine metabolite was significantly different between the two sampling periods. After two weeks, the concentration (Mean ± SD) in urine was 4686 μg/g creatinine ± 4409 μg/g creatinine for 3-hydroxycotinine and 1927 μg/g creatinine ± 1728 μg/g creatinine for cotinine. Schober et al. (2014) measured urine concentrations of nicotine metabolites in the urine of volunteers who used an e-cigarette use for two hours.[13] Nicotine and cotinine significantly increased to approximately 100-300 μg/g creatinine and 240-360 μg/g creatinine, respectively. Ballbe et al. (2014) measured salivary and urinary cotinine for five non-smoking volunteers who lived in homes with e-cigarette users.[63] Salivary cotinine concentrations were (GM± GSD) 0.19 ng/mL ± 2.17 ng/mL.

Urinary cotinine concentrations were (GM± GSD) 1.75 ng/mg creatinine ± 2.67 ng/mg creatinine.

Shahab et al. (2017) measured salivary and urinary cotinine among various groups of tobacco users (i.e. tobacco cigarette users, e-cigarette users, nicotine replacement therapy users, and dual users).[102] There was no significant difference in salivary or urinary nicotine and nicotine metabolites between the groups. Among e-cigarette only users, the average (GM [95% CI]) reported concentrations were 179.6 ng/mL (118.1,273.0) for salivary cotinine, 7.5 nmol/mg creatinine (4.5,12.4) for urinary cotinine, and 11.4 nmol/mg creatinine (6.5,19.9) for urinary trans-3'-hydroxycotinine.

The presence and quantity of nicotine metabolites in a person's urine can be used to determine smoking status.[103] For example, a sample of 466 urine samples from adolescent patients at a hospital indicated urinary cotinine at concentrations of <0.05 ng/mL, 0.05-0.25 ng/mL, 0.25-30 ng/mL, and 30 ng/mL were indicative of no exposure to tobacco smoke, light secondhand or thirdhand smoke exposure, secondhand smoke exposure or light smoking activity, and active smokers, respectively.[104] Using a questionnaire, Campo et al. (2016) determined 30 μg/L and 1.78 μg/L were appropriate urinary cotinine cutoff points for active and secondhand tobacco smoke exposure, respectively.[105] Goniewicz et al. (2011) measured the urinary cotinine concentrations in 373 active and 228 passive smokers.[106] The authors reported the optimal urinary cotinine cutoff point to distinguish primary versus secondhand tobacco exposure was 31.5 ng/mL.

Tobacco-Specific Nitrosamines (TSNAs)

TSNAs are described in detail in the "TSNAs in E-cigarette Vapor" section of this literature review. Because TSNAs are found nowhere else except tobacco, they are useful biomarkers for measuring tobacco exposure.[79] Urine is the preferred media for TSNA biomarker analysis.[79] Very few studies have analyzed TSNAs in the urine of either e-cigarette users or people exposures to secondhand e-cigarette vapor.

Pure nicotine, like that often advertised for use in e-cigarettes, should not contain TSNAs.[79] However, low levels of TSNAs have been detected in e-cigarette liquids.[10; 107]

NNAL is the most stable and common TSNA metabolite analyzed in biological media.[79] It is stable upon storage, one of the most abundant TSNAs in urine, and is a carcinogen.[79] NNAL has a estimated half-life of 10-18 days.[108] Among traditional tobacco cigarette smokers, the geometric mean (95% CI) for total NNAL in urine was 216 pg/mg creatinine (182, 257) in the 2011-2012 National Health and Nutrition Examination Survey (NHANES).[79; 109] In the same study, the geometric mean (95% CI) was 1.19 pg/mg creatinine (1.09, 1.29) among nonsmokers.

Limited data is available regarding TSNAs in the urine of those exposed to e-cigarette vapor. In a Goniewicz et al. (2017) longitudinal study, NNAL urine concentrations in smokers who switched to e-cigarettes significantly decreased to (Mean ± SD) 80 ± 69 ng/g creatinine.[101] Shahab et al. (2017) measured TSNAs in various groups of tobacco users, including e-cigarette-only users.[102] The reported average concentrations for e-cigarette users were (GM [95% CI]) 1.47 pg/mg creatinine (1.02,2.12) for urinary NNAL, 1.07 pg/mg creatinine (0.79,1.47) for NAB, and 1.79 1.07 pg/mg creatinine (1.21,2.76) for NAT.

Goniewicz et al. (2011) measured the urinary NNAL concentrations in 373 active and 228 passive cigarette smokers.[106] The authors determined a urinary NNAL cutoff point of 47.3

pg/mL could accurately separate those exposed to secondhand smoke and those that were active smokers. Bernert et. Al (2012) used the 2007-2008 National Health and Nutrition Examination Survey to determine the geometric mean, 75th percentile, and 95th percentile of Total NNAL in the urine of nonsmokers was <0.6 pg/mL, 2.7 pg/mL, and 24.4 pg/mL, respectively.[110] The 50th percentile of Total NNAL in the urine of smokers in the same study was 329 pg/mL.

CEMA and 3-HPMA

Acrolein is a chemical compound found in many environments.[111] It is found in the physical environment, in foods and drinks, and is formed through biological processes *in vivo*.[112] Exposure to acrolein can be assessed by measuring two of its metabolites, N-Acetyl-S-(3-hydroxypropyl)-L-cysteine (3-HPMA) and N-Acetyl-S-(2-carboxyethyl)-L-cysteine *(*CEMA), in biological media.[113]

Acrolein is theorized to form in conjunction with other VOCs when propylene glycol is heated and oxidized inside an e-cigarette.[14] Very few studies have measured CEMA and 3-HPMA in the urine of those exposed to e-cigarette vapor. Goniewicz et al. (2017) measured several VOC metabolites in the urine of smokers who switched to e-cigarettes for two weeks.[101] All metabolites significantly decreased by the end of the study. At the end of the two weeks, the average (SD) concentration of 3-HPMA in urine was 410 μg/g creatinine (465). Shahab et al. (2017) reported CEMA and 3-HPMA average concentrations (GM [95% CI]) were 54.6 ng/mg creatinine (41.7, 71.4) for CEMA, and 175.3 ng/mg creatinine (124, 247.8) for 3-HPMA among former smokers who used only e-cigarettes for at least six months at the time of the study.[102] Schober et al. (2014) reported urinary 3-HPMA was approximately 300-500 μg/g creatinine in volunteers who used an e-cigarette for two hours in a chamber study.[13]

Biomarkers of Effect

Tobacco smoke and other environmental exposures can produce reactive oxygen species (ROS).[114; 115] When the ROS in the body outnumbers the body's antioxidant defenses, oxidative stress occurs.[115] Oxidative stress is strongly associated with many acute and chronic diseases such as lung disease and cardiovascular disease.[115] Oxidative stress can activate pro-inflammatory factors that play a role in the pathogenesis of cancer and chronic obstructive pulmonary disease (COPD).[116]

One study demonstrated that e-cigarette vapor exposure causes oxidative stress.[117] In this study, human bronchial epithelial cells were exposed to e-cigarette liquid with and without nicotine, propylene glycol vapor, glycerol vapor, and tobacco smoke. Hydrogen peroxide was measured as an oxidative stress biomarker. Cells exposed to e-cigarette vapor had significantly lower viability and higher oxidative stress compared with control cells. The oxidative stress results were more pronounced in cells exposed to nicotine-containing liquids in one of the cell lines. Cells exposed to propylene glycol had reduced viability and increased oxidative stress levels compared with control cells. Cells exposed to only glycerol had significantly reduced viability compared to control cells and cells exposed to e-cigarette vapor. The magnitude of these effects were 4.5-8 times more pronounced in cells exposed to tobacco cigarette smoke.

Studies indicate exposure to e-cigarette use causes an inflammatory response in the user.[118] Inflammatory responses in non-users exposed to secondhand e-cigarette vapor have not been report. Fractional exhaled nitric oxide (FeNO) can be used as a marker of airway inflammation.[119; 120] Two studies measured FeNO from e-cigarette users after e-cigarette use.[13; 121] Both studies reported users' FeNO were altered by e-cigarette use, though one study reported an increase and the other a decrease.

Several lab studies have demonstrated e-cigarette vapor exposures causes inflammatory effects. For example, one study measured inflammatory responses in *in vitro* human airway epithelial cells and in C57BL/6J mice.[122] In the human cell line, e-cigarette vapor exposures increased secretion of inflammatory cytokines. *In vitro* lung cell fibroblasts appeared stressed and underwent morphological changes in response to e-cigarette vapor exposure. Pro-inflammatory cytokines increased and glutathione decreased in mice exposed to e-cigarette vapor.

8-isoprostane

8-isoprostane is a member of the F_2-isoprostane class and is one of the most specific and sensitive biomarkers for oxidative stress.[114; 115] 8-isoprostane is formed through the peroxidation of arachidonic acid.[115] 8-isoprostane has been vetted as a oxidative stress marker in traditional tobacco smokers.[123] Concentrations of 8-isoprostane in normal human urine samples typically range from 500-4,000 pg/mg creatinine.[124] 8-isoprostane can be found in all normal tissues and biological fluids and can be obtained through non-invasive methods (i.e. urine).[114; 125] To date, no study has measured 8-isoprostane in the urine of users or those exposed to e-cigarette vapor. Because it is one of the most sensitive biomarkers of oxidative stress available and it can easily be obtained from human subjects, 8-isoprostane was used as a biomarker of effect in this research.

E-cigarette User Nicotine Dependence

Addicted users of nicotine-containing products are largely addicted because of nicotine.[2] Inhaled nicotine is delivered to the lungs and travels to the user's brain where it binds to

nicotinic cholinergic receptors, resulting in the release of neurotransmitters.[126] The nicotine-induced release of neurotransmitters conditions dependence.[126] Psychosocial components are also known to contribute to nicotine dependence.[127]

In addition to nicotine, smokers are also exposed to thousands of chemicals, including at least 70 carcinogens, present in cigarette smoke.[128] In efforts to reduce the harms associated with cigarette use, devices that satisfy the nicotine craving of the user without exposing the user to tobacco smoke have emerged.[129] One such device, the e-cigarette, is also touted as a smoking cessation device.[129]

Users often begin using e-cigarettes to stop using traditional cigarettes.[27; 130] A person's future cessation of tobacco products can be predicted by his or her current nicotine dependence level.[131] There are many survey tools available to measure a users' nicotine dependence.[132; 133] One of the most popular is the Fagerstrom Test for Nicotine Dependence (FTND).[134]

The FTND has significantly predicted smoking cessation in multiple studies.[131; 133] The FTND characterizes a user's nicotine dependence by asking six questions pertaining to cigarette use and addiction characteristics.[134] These questions address (1) the time from waking to first cigarette use, (2) difficulty of refraining from cigarette use in public where use is forbidden, (3) whether the cigarette user would hate to give up the first cigarette in the morning vs. a cigarette at another time of day, (4) the number of cigarettes smoked per day, (5) whether the user uses cigarettes more frequently in the morning, and (6) if the user uses cigarettes even if he/she is sick in bed most of the day. Scores range from 0-10, representing low to high dependence.

Studies have shown tobacco biomarkers are significantly related to FTND scores. For example, studies show a user's FTND scores are significantly related to his/her exhaled carbon monoxide.[135; 136] Research shows that FTND scores are also significantly related to users' urinary

and salivary cotinine.[137-139] Recent research has indicated FTND scores are strongly associated with genetic polymorphisms and receptors related to nicotine dependence.[140-145]

The FTND has been adapted to measure nicotine dependence in e-cigarette users.[146] In the Etter et al. (2015) study, the average FTND score for e-cigarette users responding to a survey on an online "quit smoking" website ranged from 2.5-3.9. The author acknowledged the survey respondents were not intensive e-cigarette users. The average FTND scores for tobacco cigarette smokers in the United States range reportedly from 4.3-4.6.[147]

Data consistently indicate that e-cigarette users are less dependent on their devices than smokers are on their cigarettes.[148] Most e-cigarette users also feel that they are less dependent on e-cigarettes than they were on cigarettes.[146; 149-151] One survey asked users to respond to nicotine dependence survey questions as they would have previously when they were smokers and again as current e-cigarette users.[149] Results indicated users were significantly less dependent on nicotine using e-cigarettes than they had been on traditional cigarettes.

Various characteristics of e-cigarette devices are associated with higher nicotine dependence levels. For example, users of advanced e-cigarette devices tend to have higher levels of nicotine dependence.[149] Advanced e-cigarette devices allow the user to modify the battery voltage and length of time the coil is heated.[6] These parameters can be used to increase the amount of nicotine delivered to the user[152], which may lead to the increased dependence levels observed.

Summary

In summary, e-cigarettes are rapidly growing in popularity and are often excluded from indoor air bans and restrictions.[6; 25] E-cigarette addiction can be measured using the vetted

Fagerstrom Test for Nicotine Dependence tool.[153] E-cigarettes contain chemicals, though the current literature does not agree on which chemicals and at what concentrations these chemicals are present in e-cigarette vapor. [7-19] Exposures to many of the chemicals (i.e. formaldehyde, acetaldehyde, $PM_{2.5}$, acrolein, nicotine, and propylene glycol) identified in e-cigarette vapor are of public health concern.[38; 39; 48; 65; 76; 111] Health guidance values from NIOSH, OSHA, and ATSDR exist for many of the chemicals and can help characterize the severity of secondhand exposures to e-cigarette vapor.[40; 43; 46] Select chemicals can be identified using standard air sampling methods and biological monitoring. Using these tools, secondhand exposures to e-cigarettes can be characterized under real-use conditions to better characterize secondhand e-cigarette exposures.

CHAPTER 2

AIR MONITORING AT LARGE PUBLIC ELECTRONIC CIGARETTE EVENTS[1]

[1]Johnson, Jona M., Naeher, Luke P., Yu, Xiaozhong, Rathbun, Stephen L., Muilenburg, Jessica L., Wang, Jia-Sheng. Submitted to *International Journal of Hygiene and Environmental Health*, 10/17/2017.

ABSTRACT

Background: Electronic cigarette (e-cigarette) conventions bring thousands of e-cigarette users together socially regularly across the world. E-cigarette secondhand exposures to chemicals in this environment, likely the public setting with the highest concentration of e-cigarette vapor, have not been characterized.

Methods: Air sampling for formaldehyde, acetaldehyde, acrolein, nicotine, and propylene glycol was conducted at three e-cigarette conventions and one smaller event from April 2016 to March 2017 in three states in the Southeastern United States. Volunteers attended the events as members of the public and wore backpacks containing air sampling pumps. Control sampling was conducted when venues were crowded for non-e-cigarette events. Additional control sampling was conducted in two venues when the venues were empty.

Results: Formaldehyde and acetaldehyde concentrations during e-cigarette events were comparable to background concentrations. The median formaldehyde concentrations during events, crowded control events, and empty control events were 11.60, 10.45, and 12.50 $\mu g/m^3$, respectively. The median acetaldehyde concentrations during events, crowded control events, and empty control events were 9.70, 15.50, and 3.50 $\mu g/m^3$, respectively. Propylene glycol and nicotine were not detected during control sampling. The median nicotine concentration during e-cigarette events was 1.10 $\mu g/m^3$. The median propylene glycol concentration during e-cigarette events was 305.51 $\mu g/m^3$.

Conclusion: Results are similar to e-cigarette secondhand exposure studies that used human subjects to operate the e-cigarettes. Secondhand exposures to e-cigarettes did not contain high concentrations of formaldehyde or acetaldehyde. Additional research is needed to characterize exposures via inhalation to propylene glycol at concentrations measured in this study.

INTRODUCTION

Electronic cigarettes (e-cigarettes) are electronic devices that deliver nicotine to a user in a manner similar to traditional cigarettes, but e-cigarettes do not burn tobacco.[154] E-cigarettes are rapidly increasing in popularity and are currently the most commonly used tobacco product among American youth.[6] E-cigarettes are often excluded from smoke free laws and policies [26], and secondhand exposures are not fully understood. In order to provide information to public health policy and practice, it is important to better characterize human secondhand exposures to e-cigarettes.

Research regarding secondhand e-cigarette exposures is inconsistent.[7] Most studies agree nicotine, propylene glycol, and/or glycerin are present in e-cigarette vapor. However, the available literature does not consistently report to what extent e-cigarettes produce volatile organic compounds (VOCs) (i.e. formaldehyde, acetaldehyde, and acrolein). If present in e-cigarette vapor, these chemicals could cause adverse health effects in those exposed to secondhand e-cigarette vapor. Formaldehyde and acetaldehyde are Group 1 (carcinogenic to humans) and Group 2B (possibly carcinogenic to humans) carcinogens, respectively.[38; 44] Acrolein is a potent irritant.[111]

Secondhand exposures to VOCs, nicotine, and propylene glycol from e-cigarettes have been studied on a small scale (i.e. 1-10 e-cigarette users in a chamber or chamber-like environment)[12; 13; 16; 34; 53], in a home[63], and using a smoking machine.[8; 10-12; 14; 16; 17; 52] Research on e-cigarette exposures to these chemicals under real-use conditions in a public environment is not available.

This study aimed to characterize secondhand e-cigarette exposures in public e-cigarette conventions and events. E-cigarette conventions have been described previously as social events

designed to bring together users, manufacturers, and sellers of e-cigarettes and e-cigarette accessories.[32; 155] Hundreds to thousands of attendees come to the events and use e-cigarette devices. The air in the event venues is often thick with e-cigarette vapor. Environmental samples of nicotine, propylene glycol, formaldehyde, acetaldehyde, and acrolein were collected at three e-cigarette conventions and a fourth similar but smaller event in three states across the Southeastern United States. Exposure sampling was conducted for a length of time representative of a work shift to simulate occupational exposures because concession workers at e-cigarette conventions and employees at e-cigarette stores/shops may have high exposures. Control sampling was also conducted on days with no e-cigarette exposures.

METHODS

Study Locations

The study was conducted at four separate e-cigarette events that occurred in Daytona Beach, Florida (Event 1), Athens, Georgia (Event 2), Chattanooga, Tennessee (Event 3), and Atlanta, Georgia (Event 4). Data collection occurred from April 2016 to March 2017. Event 1, 3, and 4 were e-cigarette conventions held in large, open venues. Event 1 and 4 had an estimated 1,000-1,500 attendees. Event 2 was a social gathering of an estimated 300 e-cigarette users in a concert venue. Event 3 was smaller and had an estimated 150 attendees. These events were chosen because they were within driving distance of The University of Georgia (UGA) in Athens, GA and researchers expected the events to draw a large crowd of e-cigarette users based on the events' social media presence.

Participant recruitment and selection

Study subjects were recruited from UGA students and staff or friends and family members of the researchers. All subjects gave written informed consent and completed a screening questionnaire to determine their eligibility. The UGA Institution Review Board reviewed and approved this study. Participating subjects could elect to wear backpacks containing 2-4 active air sampling pumps while attending the e-cigarette event.

Sampling Methods

Air sampling was conducted at e-cigarette events using active air sampling pumps placed in backpacks worn by 21 volunteers across four events (one volunteer wore 2 backpacks). Volunteers were asked in an exit survey how they spent their time at the convention. Among volunteers that wore backpacks contain air sampling pumps, most (n=12, 57%) sat in a designated seated area near the vendors for at least 75% of the time. Some volunteers took their backpack off but kept it near them when seated. A minority of volunteers (n=6, 29%) visited vendor booths or stood in open vaping sections for at least 75% of the time. Three additional volunteers reportedly split the time equally between the two activities. Regardless, all activities were inside of the same venue in close proximity to each other. Event characteristics, including the number of backpacks worn at each event, are provided in Table 2.1.

Sampling was conducted using 3 types of calibrated air sampling pumps (AirChek XR5000 & 2000, SKC Inc., Eighty Four, PA, USA; Escort Elf, Zefon International, Inc., Ocala, FL, USA). Tygon tubing connected the pumps inside the backpack to sorbent tubes affixed to backpack straps. Sorbent tubes were placed in or near the breathing zone of volunteers. All pumps were pre- and post- calibrated within 12 hours of the event in the same city or

neighboring city of the event. Event 1 pumps were turned on as soon as researchers entered the venue. This was logistically difficult, so Event 2-4 pumps were programmed with a delayed start (20-55 minutes after entrance time depending on the venue design) to allow for the time spent waiting in queues to enter the event venue. Escort Elf pumps could not be programmed and were manually operated for each event. On average, researchers sampled for approximately 5.3 hours at each event (Table 2.1). The first event was open to the public for six hours. Sampling at later events was conducted for similar lengths of time for comparison.

Acrolein

Acrolein sampling was conducted at 100 mL/min in accordance with the Occupational Safety and Health Administration (OSHA) Method 52 using XAD-2 (2-hydroxymethyl Piperdine) sorbent tubes (SKC 226-118).[90]

Nicotine

Nicotine sampling was conducted at 1000 mL/min in accordance with the National Institute for Occupational Safety and Health (NIOSH) Method 2551 using XAD-4 sorbent tubes (SKC 226-93).[91]

Formaldehyde and Acetaldehyde

Formaldehyde and acetaldehyde sampling was conducted in accordance with EPA Compendium Method TO-11A[88] using Sep-Pak DNPH-silica plus short cartridges (Waters Corporation, WAT037500). Sampling was conducted at Event 1 at a flow rate of 800 mL/min for 120 minutes before sorbent tubes were replaced. Data from this event revealed larger air volumes

could be collected without saturating a sorbent tube. Therefore, sampling was conducted at a flow rate of 800 mL/min on one sorbent tube per pump during remaining events. Sep-Pak Ozone Scrubbers (Waters Corporation, WAT054420) were included on the sampling chain after Event 1. These were added to prevent ozone concentrations from affecting formaldehyde concentrations.

Propylene Glycol

Propylene glycol sampling was conducted in accordance with the NIOSH Method 5223 on XAD-7 OVS sorbent tubes (SKC 226-57).[93] This method recommends a maximum volume of 60 L. Event 1 samples were collected at 1000 mL/min for a total volume of 60 L before sorbent tubes were replaced. Event 1 data indicated larger volumes could be sampled without saturating the sorbent tube. Event 2 and 3 samples were collected at a flow rate of 800 mL/min using one sorbent tube per pump. Propylene glycol samples were collected at Event 4 at a flow rate of 1000 mL/min. A second day sampling of the event was conducted at 800 mL/min.

Control Sampling

For each event, control sampling was conducted in the venue to establish typical background concentrations of the chemicals. Sampling was conducted both when the venue was empty ("empty control sampling") and when a crowded, non-e-cigarette event occurred in the venue ("crowded control sampling").

Control sampling was conducted prior to the event in venues for Events 1 & 4 and after the event in venues for Events 2 & 3. Two venues (Event 1 & 3) were open to the public when the venue was empty. Pumps were placed in backpacks and were stationary during empty

control sampling. Researchers were not allowed in the exact room where the convention was held when the rooms were empty but were allowed in neighboring corridors for times and durations reflective of the event (Table 2.1). Empty control sampling was not feasible for Events 2 and 4.

Crowded control sampling for Events 1, 3, and 4 was conducted on the same day of the week for similar durations (± 45 minutes) at similar times of the day as the e-cigarette event. Control sampling was conducted during two crowded concerts lasting three hours on a weeknight in the Event 2 venue.

Air Sample Analyses

Samples and field blanks were shipped overnight on ice (~5°C) within one week to Bureau Veritas Laboratory in Novi, Michigan for analysis. Nicotine samples were analyzed in accordance with NIOSH Method 2551 using gas chromatography with a nitrogen phosphorus detector (reporting limit [RL]: 0.1μg/sample). In accordance with EPA Compendium Method TO-11A, formaldehyde and acetaldehyde samples were analyzed using high performance liquid chromatography (RLs: acetaldehyde, 0.5 μg/sample; formaldehyde, 0.1 μg/sample). Propylene glycol samples were analyzed in accordance with NIOSH Method 5523 using gas chromatography with a flame ionization detector (RL: 20 μg/sample). Acrolein samples were analyzed in accordance with OSHA Method 52 using gas chromatography and a nitrogen phosphorus detector (RL: 2.0 μg/sample).

Data analysis

Geometric means, medians, and ranges across events and control days were calculated for formaldehyde, acetaldehyde, nicotine, acrolein, and propylene glycol. OSHA recommends air pump pre- and post-calibrations have no more than 5% discrepancy.[156] This study includes formaldehyde/acetaldehyde data from 4 samples that exceed this range because data points from these pumps were the same or very similar to the data from pumps within recommended ranges. Exceptions are noted in Table 2.2. Data below the Bureau Veritas Laboratory RL is indicated by "<" followed by the RL concentration based on sample volume collected. One-half of the RL concentration for a chemical was used to calculate a geometric mean and median when the chemical was detected below the RL. In the dotplot in Figure 2.1, undetectable concentrations were assigned a value of "0". All acrolein samples (n=13, 6 at e-cigarette events) were below the RL (2.0 μg/sample) and are not presented here. SAS® University Edition.[157] and Microsoft Excel 2011 were used to make Tables 1& 2. SAS® University Edition was used to make Figure 2.1.

RESULTS

Venue Characteristics

Secondhand e-cigarette exposures at four large e-cigarette events (three e-cigarette conventions, one e-cigarette fundraiser concert) were studied in three Southeastern States in the United State from April 2106 to March 2017. Event attendance varied from approximately 150-1500 attendees (including vendors). Most attendees and vendors used an e-cigarette during events.

Venues for Events 1 & 3 were modern buildings with high ceilings typical of a large convention center (~30-45 feet) and a noticeable air draft that indicated a highly functioning

heating, ventilation, and air conditioning (HVAC) system. The Event 4 venue was a tradeshow with lower ceilings (~13 feet). The Event 2 venue was a small concert hall with 2 levels of balconies and a high ceiling (~35 feet). All venues had doors open during the events. Information about the event venues is provided in Table 2.2.

Exposure Sampling

In total, samples collected included 19 for nicotine, 13 for formaldehyde, 13 for acetaldehyde, 6 for acrolein, and 13 for propylene glycol. Acrolein was not detected on any control or event sampling sorbent tube and this chemical is excluded from the analysis. Samples were excluded due to random pump failure, > 5% pre/post pumps calibration agreement (unless otherwise noted), and tubing malfunctions inside the backpack (n=32). Results (geometric mean, median, range, and sample size) by chemical across sampling days, events, and overall are provided in Table 2.2. A visual representation of the data is presented in Figure 2.1.

Formaldehyde

Overall, formaldehyde concentrations at e-cigarette events were low and comparable to background concentrations. The median (25th, 75th, range) concentration of formaldehyde across all venues was 11.60 (9.67, 29.00, 6.48-59.00) $\mu g/m^3$ during e-cigarette events and 12.50 (10.95, 16.50, 9.90-20.00) $\mu g/m^3$ during empty control sampling, and 10.45 (9.55, 17.00, 9.00-45.00) $\mu g/m^3$ during crowded control sampling.

Formaldehyde concentrations in Event 1 measured during both control sampling days were higher than those measured at the e-cigarette event. Formaldehyde e-cigarette concentrations in Event 2 were similar to control concentrations. Event 3 formaldehyde

concentrations were highest during empty control sampling, though the lower end of the range of control concentrations did include concentrations measured at the e-cigarette event. Event 4 formaldehyde concentrations measured during the e-cigarette event were higher than those measured during control sampling, though event concentrations did overlap with crowded control concentrations.

Acetaldehyde

Acetaldehyde concentrations tended to be higher during crowded control sampling than during empty control sampling or e-cigarette events. The median (25th, 75th, range) concentration was 9.70 (6.96,14.00, 5.30-18.00) $\mu g/m^3$ during e-cigarette events, 15.50 (7.95, 24.00, 4.30-29.00) $\mu g/m^3$ during crowded control sampling, and 3.50 (1.45, 4.15, <2.70-4.20) $\mu g/m^3$ during sampling when the venue was empty.

Acetaldehyde concentrations were higher during crowded control sampling than concentrations measured at e-cigarette events for Event 1 and 2. Only during Event 3 were acetaldehyde concentrations higher during the e-cigarette event than during crowded control sampling. Event 4 acetaldehyde concentrations measured during the crowded control sampling day were slightly higher than those collected at the e-cigarette event, though concentrations overlapped.

Empty control sampling concentrations are only available for two events. Event 1 and Event 3 empty control concentrations are both lower than the crowded control and e-cigarette event concentrations for these venues.

Propylene Glycol

The median (25th, 75th, range) propylene glycol concentration across all e-cigarette events was 305.51 (230.00, 410.00, 210.00-490.00) $\mu g/m^3$. Propylene glycol was not detected during control sampling at any venue.

There was appreciable variation in the Event 1 propylene glycol concentrations [individual samples ≤ LOD-440.00 $\mu g/m^3$]. Weighted averages calculated for the whole sampling time (Time-Weighted Average ["TWA"]) were ≤ LOD-366.50 $\mu g/m^3$. Five samples that comprised one TWA were below the limit of detection. The volunteer wearing the backpack containing the pump for these 5 samples was exposed to e-cigarette vapor for the entire event.

Nicotine

The median (25th, 75th, range) nicotine concentration across all e-cigarette events was 1.10 (<0.37, 1.80, <0.36-2.20) $\mu g/m^3$. Nicotine was not detected during control sampling at any venue. Nicotine concentrations were below the limit of detection for all Event 3 samples (n=5). Event 3 had the smallest crowd and the least amount of visible e-cigarette vapor.

DISCUSSION

A recent review concluded that e-cigarette vapor may contain chemicals at concentrations that could impact the health of those exposed.[7] Our study characterized secondhand e-cigarette exposures to formaldehyde, acetaldehyde, nicotine, acrolein, and propylene glycol using active air sampling methods in a natural e-cigarette environment with high levels of e-cigarette vapor. Results in our study indicate formaldehyde and acetaldehyde concentrations during e-cigarette events are comparable to concentrations present when the venue is empty and during non-e-

cigarette events in the venue. Low concentrations of nicotine and high concentrations of propylene glycol were present only during the e-cigarette events.

Acrolein was not detected during any control or event sampling period. This could be because the method was a low-flow method that allowed for only ~36 L of air to be sampled during the events. Other studies used additional methods in chamber environments and were also unable to detect acrolein in e-cigarette vapor.[8; 11; 13]

It is proposed that e-cigarette devices oxidize propylene glycol and glycerol to produce formaldehyde, acetaldehyde, acrolein, glyoxal and methylgloxal during use.[37] Results of this study do not provide convincing evidence that e-cigarette vapor produced by human subjects in a natural environment contains elevated levels of formaldehyde or acetaldehyde. The likely explanation for this discrepancy is that samples collected at the source of the e-cigarette, such as those in the Ohta et al. (2011) study and other studies reporting high concentrations of VOCs in e-cigarette vapor, are not representative of natural secondhand exposures and fail to account for absorption in the lung of the user and airborne aging of chemicals.[12]

This study shows that acetaldehyde concentrations were generally the highest during crowded control sampling days. Crowded control sampling events typically had attendance similar to, if not larger than, the e-cigarette events. Humans reportedly exhale concentrations of acetaldehyde even higher than concentrations measured in this study[158], which could explain the observation.

This study was designed, in part, to characterize occupational exposures. Formaldehyde concentrations detected in this study were well below the United States Occupational Safety and Health Administration's (OSHA) regulatory occupational Permissible Exposure Limit (PEL) 8-hour TWA of 0.75 ppm (0.92 mg/m^3).[40] However, samples collected during both control

sampling (Event 3 & 4) and an e-cigarette event (Event 4) were above the NIOSH Recommended Exposure Limit (REL) 8-hour TWA of 0.016 ppm (19.65 μg/m^3).[41] The United States Environmental Protection Agency (USEPA) has not established a Reference Concentration (RfC) for formaldehyde.[159] Reference Concentrations are estimates of the concentrations of chemicals to which members of the general population can be exposed continuously over a lifetime via inhalation without the exposures resulting in adverse health effects.[160]

Acetaldehyde concentrations collected during both control sampling days and e-cigarette events are below the OSHA 8-hour TWA PEL (200 ppm, 360 mg/m^3).[40] NIOSH has not established a REL for Acetaldehyde. The US EPA established an RfC of 9.0 μg/m^3 for acetaldehyde.[161] This RfC was exceeded during both e-cigarette events and crowded control events.

Nicotine concentrations detected in this study are comparable to those reported in the literature (range = <0.01-7.00 μg/m^3).[8; 12; 13; 16; 34; 63] All nicotine concentrations reported in this study are below the occupational OSHA PEL and NIOSH REL (0.5 mg/m^3, 8-hour TWA).[40; 162] For comparison, Ballbe et al. (2014) reported airborne nicotine inside the homes of e-cigarette users was (GM ± Geometric standard deviation [GSD]) 0.13 ± 2.4 μg/m^3.[63] In the same study, airborne nicotine inside the homes of tobacco smokers was reportedly (GM ± GSD) 0.74 ± 4.05 μg/m^3.

Propylene glycol concentrations found in this study are similar to the results of studies that used human subject e-cigarette operators. For example, propylene glycol concentrations reportedly ranged from 110.0-395.0 μg/m^3 in a chamber study using human subjects to operate the e-cigarettes.[13] Propylene glycol was not detected on five sorbent tubes during Event 1 of this

study. It could be that the tubes were exchanged before an acceptable mass could accumulate (every 60 minutes). This is likely, given vapor was visibly present the entire event. Schripp et al. (2013) also observed visible vapor in the chamber study but was unable to detect propylene glycol after a short sampling period.

Occupational exposure guidelines are not available for propylene glycol. The Agency for Toxic Substances and Disease Registry (ATSDR) established a propylene glycol Minimal Risk Level (MRL), which is a level at which continuous exposure is not likely to cause harm.[76] Most concentrations measured at e-cigarette events were above ATSDR intermediate MRL (established for >14-364 days of exposure) for inhalation exposure of 9.0 ppb (28.01 $\mu g/m^3$). Though exposures to e-cigarette vapors do not likely last 24-hours for this period of time, the MRL does provide a number for comparison. The U.S. EPA has not established a RfC for propylene glycol.[163] Given that e-cigarettes are emerging and that exposures measured in this study are much higher than the intermediate MRL, further research is needed to determine if a new health guidance value is warranted.

Five chemicals were sampled and measured in this study, though other chemicals (i.e. metals and additional VOCs) have been detected in e-cigarette vapor.[8; 10-12; 16; 52; 75] Particulate matter in the form of supersaturated propylene glycol has also been reported to be elevated in e-cigarette environments.[12] A recent study at a small e-cigarette convention reports particulate matter concentrations were elevated and ranged from 31.68-818.88 $\mu g/m^3$.[73] Future studies in this environment should sample for a wider range of chemicals to more comprehensively characterize secondhand e-cigarette exposures and include biological monitoring of non-e-cigarette users.

This study used standard sampling methods that are most appropriate for the chemicals of concern. However, as Kosmider et al. (2014) highlighted, standard air sampling methods are designed to capture gas-phase particles. The authors stated that chemicals present in the particle phase many not have been captured in the measurements. If so, this study may have underestimated the concentrations of chemicals present in e-cigarette environments.

Limitations of this study included a small sample size at four Southeastern e-cigarette events. Control concentrations were not collected on the day of the event. One must assume background concentrations were the same on both control and event days. No control over the e-cigarette environment was included. Recent literature indicates other products like cannabis oil may be aerosolized using e-cigarette devices, which were not captured in this study.[164]

CONCLUSION

This study is the first to sample for formaldehyde, acetaldehyde, nicotine, acrolein and propylene glycol in a public e-cigarette environment. The e-cigarette environment chosen likely had the highest concentration of e-cigarette vapor present in a public venue. A major strength of this study design is that it did not rely on assumptions regarding device type or user topography. Formaldehyde and acetaldehyde concentrations during e-cigarette events were comparable with control concentrations for each venue. Results show low levels of nicotine present. Propylene glycol concentrations were elevated. More research is needed to determine possible adverse health effects of the concentrations of propylene glycol found here. This study highlights the need for more studies with e-cigarette users rather than smoking machines to characterize e-cigarette exposures.

Funding Source

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Table 2.1. Sampling Characteristics

	Event 1	Event 2	Event 3	Event 4
E-Cigarette Event Details				
Location	Daytona Beach, Florida	Athens, Georgia	Chattanooga, Tennessee	Atlanta, Georgia
Date	April 2016	September 2016	October 2016	March,2017
Estimate number of attendees	1000	300	150	1500
Number of backpacks with air sampling pumps	5	7	6	4
Venue Characteristics				
Venue type	Convention	Concert	Convention	Exhibition/Tradeshow
Site (ft^2)	42,146	5100	36,000	205,000
Estimated Ceiling Height (ft)	45	35	30	13
Event Exposure Sampling[A]				
Sampling Day	Saturday	Friday	Sunday	Saturday and Sunday[B]
Sampling Hours	12:39-18:20	18:20-00:04	13:00-17:40	12:30-18:00 (Saturday) 11:30- 1700 (Sunday)
Sampling duration (mean ± SD) (minutes)	313 ± 37	337 ±9	279 ± 1	329 ± 2 (Saturday) 324 ±16 (Sunday)
Empty Venue Control Sampling				
Sampling Month	April		December	
Sampling Day	Friday	N.A.[C]	Monday	N.A[C]
Sampling Hours	10:55-16:58		13:00-1730	
Sampling duration (mean ± SD) (minutes)	297 ± 58		262 ± 15	
Crowded Venue Control Sampling				
Estimate number of attendees	1000	800	500	1000
Sampling Month	April	October & February[D]	December	March
Sampling Day	Saturday	Tuesday and Wednesday	Sunday[E]	Saturday
Sampling Hours	12:32-18:33	19:13-22:50 (Tuesday) 19:30-22:30 (Wednesday)	12:00-17:00	12:00-17:40
Sampling duration (mean ± SD) (minutes)	355 ± 3	208 ± 11 (Tuesday) 179 ±1 (Wednesday)	295 ± 8	333 ±16

[A]Samplling times only reflect samples included in analysis.
[B]Sampling on Sunday was only for nicotine and propylene glycol
[C]Not Available (N.A). --venue was not open to the public when venue was empty
[D]Initial control sampling was on a Wednesday night. Formaldehyde and acetaldehyde control concentrations were abnormal. Researchers repeated sampling for these two chemicals on a Tuesday night.
[E]Sampling was conducted on the previous Saturday, but due to logistical difficulties, the length of the sampling was not sufficiently representative of the event. Therefore, sampling was repeated on Sunday.

Table 2.2. Sampling Results

CHEMICAL	VALUE	LOCATION											
		EVENT 1 (FLORIDA)				EVENT 2 (GEORGIA)[A]		EVENT 3 (TENNESSEE)			EVENT 4 (GEORGIA)		OVERALL (events only)[F]
		Empty (control)	Crowd (control)	EVENT (individual samples)	EVENT (TWAs)	Crowd (control)[B]	EVENT	Empty (control)	Crowd (control)	EVENT[C,D]	Crowd (control)	EVENT[E]	
Nicotine	Geometric Mean (µg/m³)			**0.81**	**0.81**		**1.36**					**1.83**	**0.79**
	Median (µg/m³)			**0.85**	**0.85**		**1.45**					**1.90**	**1.10**
	Range (µg/m³)	<0.29	<0.28	**0.65-0.95**	**0.65-0.95**	<0.56	**1.10-1.50**	<0.37	<34.0-<36.0	**<0.36-<0.37**	<0.29-<0.34	**1.30-2.20**	**<0.36-2.20**
	Sample Size	1	3	**4**	**4**	1	**4**	2	2	**5**	2	**6**	**19**
Formaldehyde	Geometric Mean (µg/m³)	10.90	10.95	**7.46**	**7.72**	10.45	**10.44**	16.12	9.05	**12.64**	31.46	**40.83**	**15.25**
	Median (µg/m³)	10.95	11.00	**7.80**	**8.06**	10.46	**10.28**	16.50	9.05	**12.65**	33.50	**42.50**	**11.60**
	Range (µg/m³)	9.90-12.00	10.00-12.00	**4.50-9.60**	**6.48-8.80**	10.12-10.79	**9.68-11.60**	13.00-20.00	9.00-9.10	**12.10-13.20**	22.00-45.00	**29.00-59.00**	**6.48-59.00**
	Sample Size	2	2	**9**	**3**	2	**4**	2	2	**2**	2	**4**	**13**
Acetaldehyde	Geometric Mean (µg/m³)	1.98	11.96	**6.61**	**7.10**	29.00	**9.77**	4.15	4.59	**5.45**	18.49	**15.92**	**9.64**
	Median (µg/m³)	2.13	12.00	**7.90**	**6.96**	29.00	**9.85**	4.15	4.60	**5.45**	18.50	**16.00**	**9.70**
	Range (µg/m³)	<2.70-2.90	11.00-13.00	**<7.20-8.80**	**6.67-7.73**	29.00-29.00	**9.40-10.00**	4.10-4.20	4.30-4.90	**5.30-5.60**	18.00-19.00	**14.00-18.00**	**5.30-18.00**
	Sample Size	2	2	**9**	**3**	2	**4**	2	2	**2**	2	**4**	**13**
Propylene Glycol	Geometric Mean (µg/m³)			**251.49**	**264.71**		**233.67**			**226.32**		**422.48**	**299.79**
	Median (µg/m³)			**272.50**	**305.51**		**235.00**			**230**		**410.00**	**305.51**
	Range (µg/m³)			**<310.00-440.00**	**<331.30-366.50**	<140.00	**210.00-260.00**	<91.00-<92.00	<84.00-<85.00	**210.00-240.00**	<73.00	**380.00-490.00**	**210.00-490.00**
	Sample Size	0	0	**14**	**3**	2	**2**	2	2	**3**	2	**5**	**13**

[A] Most Event 2 calibrations were performed on the same sorbent tubes used to sample at the event. Based on control concentrations from all other control sampling, this would have only impacted formaldehyde and acetaldehyde concentrations. To address possible formaldehyde and acetaldehyde contamination, an exercise tested how much contamination was added to the tubes when they under went pre/post calibration and traveled to and from the event location. Based on this exercise, an average 0.09µg formaldehyde/sample (~ 2-times the typical background mass on formaldehyde field blanks) was added. This is within the acceptable background concentrations for DNPH sorbent tubes (<0.15 µg). Acetaldehyde was not detected. Control and event results for formaldehyde in Table 2.2 have been blank corrected based on this exercise. This error resulted in only minor adjustments to the reported concentrations.

[B] Formaldehyde concentration of 10.79 µg/m³ and acetaldehyde concentration of 29.00 µg/m³ collected on pump with 5.46% pre/post calibration agreement

[C] Formaldehyde concentration of 12.1µg/m³ and acetaldehyde concentration of 5.6 µg/m³ collected on pump with 5.3% pre/post calibration agreement

[D] Two formaldehyde and acetaldehyde tubes were not labeled & were matched volume sample:concentration by researchers

[E] Formaldehyde concentration of 29.0 µg/m³ and acetaldehyde concentration of 15.0 µg/m³ collected on pump with 14.99% pre/post calibration agreement; formaldehyde concentration of 56 µg/m³ and acetaldehyde concentration of 17 µg/m³ collected on pump with 13.67% pre/post calibration.

[F] Event 1 TWAs uses for these calculations

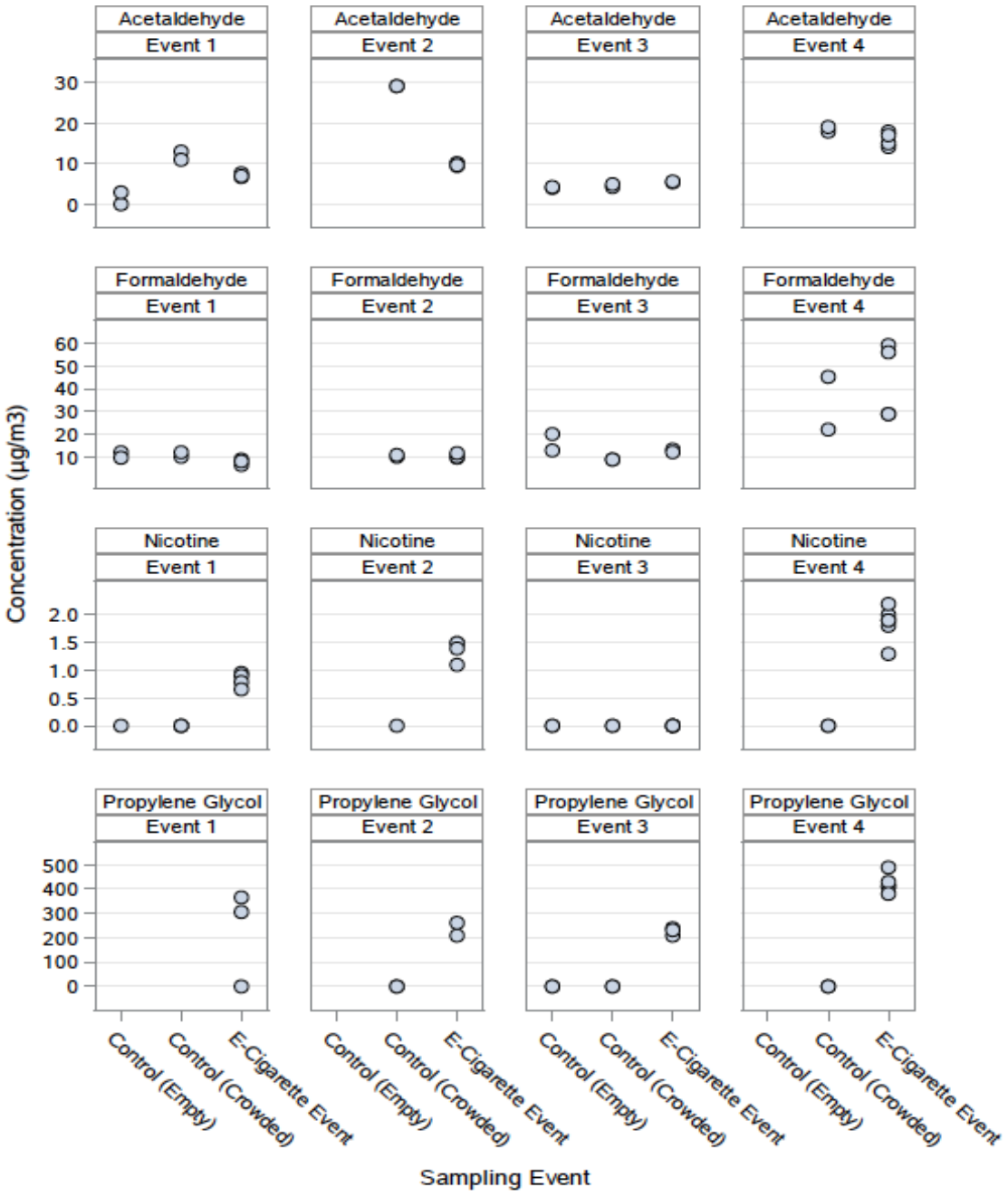

Figure 2.1. Chemical Concentrations Across Sampling Event

CHAPTER 3
A BIOMONITORING ASSESSMENT OF SECONDHAND EXPOSURES TO ELECTRONIC CIGARETTE VAPORS[2]

[2] Johnson, Jona M., Naeher, Luke P., Yu, Xiaozhong, Sosnoff, Connie, Wang, Lanqing, Rathbun, Stephen L., De Jesus, Victor, Xia, Baoyun, Holder, Cory, Muilenburg, Jessica L., Wang, Jia-Sheng. To be submitted to the *Environmental Health Perspectives.*

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 vapor are prevalent. Biomarkers for tobacco smoke exposure can be used to characterize secondhand e-cigarette exposures in such an environment.

Methods: Volunteers who did not use any tobacco product attended four separate e-cigarette events for approximately six hours. Urine and saliva samples were collected from volunteers prior to the event, immediately after the event, 4-hours after the event, and the next morning (first void). Urine samples from 34 volunteers 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 volunteers were used in the data analysis. Creatinine-adjusted urinary cotinine concentrations increased up to 13-fold and peaked 4-hours after completed exposure (range of adjusted geometric means [AGMs] = 0.35-2.31 ng/mg creatinine). Salivary cotinine concentrations were also the highest 4-hours after completed exposure (range of AGMs = 0.04-0.17 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 significantly varied across events.

Conclusion: Secondhand e-cigarette exposures lasting six hours resulted in significant changes in metabolite concentrations of both nicotine and acrolein but did not result in remarkable exposures to tobacco-specific nitrosamines. Additional research is needed to understand the

relationship between biomarker concentrations and environmental concentrations of toxicants in e-cigarette vapor.

Keywords: Electronic Cigarettes, Biological Monitoring, Secondhand Exposures, Nicotine, Cotinine

INTRODUCTION

Tobacco products contribute to the death of nearly a half a million Americans every year.[1] Users use tobacco because of nicotine addiction.[2] To provide a less toxic smoking experience, devices have emerged that deliver nicotine without the high concentrations of many harmful chemicals in tobacco products. One such device is the electronic cigarette. Electronic cigarettes (e-cigarettes) aerosolize a liquid containing nicotine without producing tobacco combustion products.[154] E-cigarettes have rapidly grown in popularity and are now the most commonly used nicotine delivery products among youth.[6]

Because e-cigarettes are often excluded from indoor smoke-free laws[6; 26] many users begin using them in places where smoking is banned.[28] As of June 30, 2017, less than 20% of states in the United States banned e-cigarette use in bars, restaurants, and private worksites.[29] Nearly 60% of states and Puerto Rico and the District of Columbia ban traditional cigarettes in bars, restaurants, and private worksites.[165]

Often studies characterize passive e-cigarette vapor 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.[6; 33; 34] 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 reported lung absorption of e-cigarette vapor that occurs when a human subject operates the device.[12] Understanding the secondhand exposures to toxicants in e-cigarette vapor 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,[79] we used conventional tobacco smoke exposure biomarkers to characterize e-cigarette vapor exposures. One of the most sensitive and specific tobacco exposure biomarkers is cotinine, the primary proximate metabolite of nicotine.[95] Approximately 75% of absorbed nicotine is converted to cotinine, and approximately 60% of cotinine is further metabolized to trans-3'-hydroxycotinine.[97] Together these metabolites account for 60 to 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.[79]

Tobacco-specific nitrosamines (TSNAs) are a class of compounds only found in tobacco products.[79] Several TSNAs have been detected in e-cigarette vapor.[10] 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, and indicative of cancer risk.[79] Acrolein is a potent irritant[48] formed when glycerin and propylene glycol in e-cigarette liquids are heated inside an e-cigarette and oxidized to a variety of carbonyl compounds.[37; 166] 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]), can help assess the extent of tobacco or e-cigarette exposure.[79; 113]

Environmental toxicants, such as tobacco smoke, are known to generate reactive oxygen species in humans.[3] A non-enzymatic peroxidation product of arachidonic acid, 8-isoprostane, is

a known biomarker for estimating oxidative stress.[167] 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 vapor. E-cigarette conventions are large social e-cigarette events described previously.[32; 155] 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.

METHODS

Study Locations

This study was conducted at four e-cigarette events through the Southeastern United States between April 2016 and March 2017. The events were chosen because of close proximity to The University of Georgia (UGA) in Athens, Georgia. Event 1 was held in a large convention center in Daytona Beach, Florida in April 2016. Event 2 was held in a small concert hall in Athens, GA in September 2016. Event 3 was held in a large convention hall in Chattanooga, Tennessee in October 2016. Event 4 was held in a tradeshow hall in Atlanta, Georgia in March 2017. Events 1 and 4 attracted ≥1,000 attendees. Events 2 and 3 attracted smaller crowds. Event and venue characteristics are presented in Table 3.1.

Study Volunteers

Study volunteers were recruited from UGA students and staff or friends and family members of the researchers. All volunteers gave written informed consent and completed a screening questionnaire to determine their eligibility. In order to participate, volunteers had to be healthy and at least 18 years old. Females could not be pregnant or breastfeeding. Additionally, volunteers could not be current e-cigarette, tobacco, nicotine replacement therapy, or smokeless tobacco users or live with anyone who uses these products. Two subjects in Event 1 indicated they had been exposed to cigarette smoke briefly within 6 days of the event. The extent and effect of these exposures was not known and the subjects were allowed to participate. Thirty-four volunteers participated in this study. This total includes repeated participation by 5 participants who attended two or more events. Participants ranged from 19-30 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.

Event visits

Prior to entering the venue, subjects completed an entry survey that asked about confounding exposures they may have received in the past 6 days (i.e. secondhand smoke or e-cigarette vapor exposure, wood smoke, and charcoal). The survey also asked the subject to list the food and drinks they had consumed in the past 24 hours. Inside the venue, subjects participated in the event as members of the public. Volunteers attended the events with researchers for approximately six hours (340-363 minutes) (Table 3.1). All subjects remained inside the venue for the duration of sampling. One researcher had to exit the venue for less than 30 minutes during Event 1. No confounding exposures were noted during this time. Subjects

were instructed not to use an e-cigarette, nicotine replacement-therapy, or other tobacco product while attending the event. An exit survey verified the subjects 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.

Biological Sample Collection

Each subject collected urine and saliva samples before entering the venue ("pre-exposure"), immediately as or just after they exited the venue ("immediate post-exposure"), 4-hours after exiting the venue ("4-hours post-exposure"), and first thing in the morning the day after the event ("first-void"). The timing of each sampling event is presented in Table 3.1.

All urine and saliva samples were collected in urine collection cups and Salivettes®, respectively. Each urine cup and Salivette was labeled with a unique barcode to identify the subject, event, biological medium, and sampling time. Sampling supplies were provided to the subjects to take home for samples not collected when the researchers were present (i.e. select 4-hours post-exposure samples and first-void samples).

Researchers chose not to collect pre-exposure samples inside the venue because many of the site restrooms were located in close proximity to or inside of the e-cigarette event, which could have resulted in subjects' secondhand e-cigarette exposures prior to the pre-exposure sample collection. Instead, Events 1, 3, and 4 pre-exposure samples were collected in hotel restrooms near the event venues. Once samples were collected, they were immediately stored on wet ice in a portable cooler and later frozen. For each sampling time and event, ice was refreshed every 8-10 hours in all coolers until researchers returned from the event and could access a freezer (~8-30 hours after event). Pre-exposure samples from Event 2 were collected

by volunteers in the UGA Environmental Health Science (EHS) building located 15 minutes away from the event. Samples taken from Event 2 were immediately stored in a -80° C freezer. After pre-exposure samples were collected, a researcher drove all subjects (Event 1 & Event 2) or caravanned with subjects (Event 3 & 4) to each event venue to limit the possibility of confounding exposures occurring prior to all events.

Samples collected immediately after exposure were collected by subjects in restrooms located in hallways near the event (Event 1 & 3), in a restroom in the EHS building (Event 2), or in a restroom inside the event immediately before exiting (Event 4). Event 1, 3, and 4 samples were placed on wet ice in a cooler within 10 minutes of collection and later frozen. Event 2 samples were collected and immediately stored at -80° C.

The location of Event 1 required participants to stay overnight after the event in the same city as the event. Event 1 samples collected 4-hours after exposures were stored and transported on wet ice in a cooler until they were frozen. Samples collected 4-hours post-exposure and first thing in the morning for Events 2-4 were collected when subjects were at their residences or other individual locations. Subjects exited Event 2 at midnight and 4-hour post-exposure samples were not collected. Subjects were instructed to collect samples and immediately place them in their residential freezers for overnight storage.

Subjects delivered 4-hours post-exposure and first-void samples to a researcher the day after the event. One Event 3 subject lived a significant distance from the venue. This subject's samples were frozen for one week in the subject's residential freezer before being retrieved by the researcher. All samples were transported on wet ice and stored in a freezer until they were transported to the U.S. Centers for Disease Control and Prevention (CDC) within one (Events 1,3,4) to three (Event 2) weeks after 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.

Biological Sample Analysis

Cotinine and trans-3'-Hydroxycotinine Analyses

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.[168] The limits of detection were 0.015 ng/mL for both analytes.

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.[169] The limits of detection were 0.030 ng/mL for both analytes.

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.[170] The limits of detection for CEMA and 3-HPMA were 6.96 ng/mL and 1.3 ng/mL, respectively.

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.[171] The limit of detection for urinary TSNAs ranged from 0.0006 to 0.0042 ng/mL, depending on the analyte.

8-Isoprostane

Urinary "total" (free plus conjugated glucuronide forms) 8-isoprostane (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.

Creatinine

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

Data Analysis

Concentrations below the limit of detection (LOD) were substituted with an imputed value (LOD/$\sqrt{2}$).[172] 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 3.2). The median and range of select biomarker concentrations across events and sampling times are presented in Figures 3.1-3.4.

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 interaction were treated as fixed effects. Subjects were treated as random effects. A p-value ≤ 0.05 was considered statistically significant**.** Results are presented in Table 3.3. The adjusted mean ratio (i.e. difference) between subjects' log-transformed maximum biomarker concentrations and their log-transformed baseline biomarker concentrations for each endpoint and location are presented in Table 3.4.

RESULTS

To ensure only volunteers with minimal to no recent secondhand tobacco exposures were included, subjects with a salivary cotinine concentration > 0.1 ng/mL at the pre-exposure sampling time were excluded from the analysis (n=6 [Event 1=1;Event 3=5]). Samples from 28

of the 34 participants were analyzed. Two participants did not collect 4-hour 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.

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

Samples were collected prior to exposure (n=28), immediately after exposure (n=28), 4-hours after exposure (n=17 saliva, 19 urine), and first thing the following morning (n=28). Three subjects in Event 1 forgot to collect first void samples, but did collect early morning samples once they remembered. These are considered as first-void samples in this analysis. Samples were not collected 4-hours after exposure for Event 2 because the sampling event ended at midnight.

Among the 28 subjects used in the statistical analysis, most subjects (n=19, 68%) sat in a designated sitting area inside the event venue for 75% of their time. The sitting areas were in close proximity to the vendors. The remaining subjects reported spending at least 75% of their time walking around and visiting vendors (n=2, 7%), standing in e-cigarette use sections (n=4, 14%), or split their time equally between sitting in the designated area and visiting vendors (n=3, 11%). All subjects verified they had not used a tobacco or e-cigarette product or touched e-cigarette juice during the e-cigarette event.

Nicotine Metabolites

Cotinine

Urinary cotinine concentrations corrected for creatinine varied significantly across sampling times ($p<0.0001$) and events ($p<0.0001$) (Table 3.3). A significant interaction effect between location and sampling time was found ($p<0.05$). Urinary cotinine concentrations increased up to 13.16-fold after the events (Table 3.4). The adjusted geometric means of urinary cotinine concentrations across all events ranged from 0.11-0.19, 0.28-1.08, 0.35-2.31, and 0.31-2.21 ng/mg creatinine for pre-exposure, immediate post-exposure, 4-hours post-exposure, and first-void samples, respectively (Table 3.2). Urinary cotinine concentrations were all below 0.58 ng/mg creatinine in the pre-exposure samples. After exposure, concentrations increased through post-exposure sampling times and then decreased slightly in the first-void samples (Figure 3.1). The largest increases in cotinine were observed in Event 4 and Event 2, while Event 3 showed little variation in cotinine exposure across time. The highest concentrations were detected in samples collected 4-hours post-exposure for Event 4 (AGM: 2.31 ng/mg creatinine [95% CI: 1.43,3.72]).

Salivary cotinine concentrations varied significantly across sampling times ($p<0.0001$) and events ($p<0.0001$) (Table 3.3). A significant interaction effect between sampling time and event was also found ($p<0.0001$). Salivary cotinine concentrations increased up to 12.68-fold after the events (Table 3.4). Concentrations were elevated through post-exposure sampling times and decreased slightly in first-void samples (Figure 3.2). The highest adjusted geometric mean of concentrations was calculated from Event 4 samples collected 4-hours 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-hours post-exposure had a wider range of concentrations. The adjusted geometric means of salivary cotinine across all events ranged from 0.01-0.02, 0.03-0.17, 0.04-0.17, and 0.03-0.15 ng/mL for pre-exposure, immediate post-exposure, 4-hours post-exposure, and first-void samples (Table 3.2).

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 3.3). A significant interaction between sampling time and location was detected ($p<0.05$). Urinary trans-3'-hydroxycotinine concentrations increased up to 8.79-fold after the events (Table 3.4). The adjusted geometric means of concentrations ranged from 0.13-0.22, 0.25-0.85, 0.35-2.22, and 0.41-2.67 ng/mg creatinine pre-exposure, immediate post-exposure, 4-hours post-exposure, and first void samples, respectively (Table 3.2). Concentrations increased at each successive post-exposure sampling time and peaked in first-void samples (Figure 3.3). Event 4 first-void concentrations were the highest among events and sampling times (AGM: 2.67 ng/mg 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.

Urinary Acrolein Metabolites

3-HPMA

Creatinine-corrected 3-HPMA urinary concentrations varied significantly across sampling times ($p<0.0001$) and events ($p<0.0001$) (Table 3.3). A significant interaction effect between sampling time and event existed ($p<0.05$). Urinary 3-PHMA concentrations increased up to 3.82-fold after the events (Table 3.4). The adjusted geometric means of pre-exposure, immediate post-exposure, 4-hour post exposure, and first-void adjusted concentrations ranged from 185.69-323.50, 199.08-624.72, 454.55-807.87, and 163.09-838.82 ng/mg creatinine, respectively (Table 3.2). Event 1, 3, and 4 concentrations of 3-HPMA peaked at different times after events but they all increased after exposure, unlike Event 2 concentrations which did not increase appreciably (Table 3.2, Figure 3.4).

CEMA

Creatinine-corrected CEMA concentrations varied significantly across sampling times ($p<0.01$) but not across sampling events. Urinary CEMA concentrations increased up to 2.40-fold after the events (Table 3.4). The adjusted geometric means of concentrations ranged from 61.48-100.13, 82.67-113.00, 107.19-115.64, and 90.95-169.30 ng/mg creatinine in pre-exposure, immediate post-exposure, 4-hour post-exposure, and first void samples, respectively (Table 3.2).

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). TSNAs were not included in the statistical analyses because of the low detection rates.

Urinary 8-Isoprostane

Creatinine-corrected 8-isoprostane concentrations did not vary significantly across sampling times or events (Table 3.3). The adjusted geometric mean of concentrations ranged from 302.41-345.92, 260.13-445.59, 314.00-413.56, ad 296.78-377.06 pg/mg creatinine in pre-exposure, immediate post-exposure, 4-hour post-exposure, and first void samples, respectively (Table 3.2).

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 Among all 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 the vapor.

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 vapor lasting approximately six hours resulted in significant changes 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 significantly varied 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. Biomarker concentrations post-exposure (sampling times 2, 3, and 4) are largely dependent on the extent of exposure inside the sampling event. Therefore, the concentrations measured for a given sampling time would largely be influenced by the conditions of the sampling event, resulting in a significant interaction effect.

The highest urinary cotinine concentrations were observed after Event 4. Urinary cotinine concentrations measured 4-hours after this event were (AGM [95% CI]) 2.31 ng/mg creatinine (1.44, 3.72). Ballbe et al. (2014) measured cotinine concentrations in urine from five volunteers passively exposed to e-cigarette vapor at least two hours a day by living in the homes of e-cigarette users.[63] Reported urinary cotinine concentrations (GM ± Geometric SD [GSD]: 1.75 ± 2.67) 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 ng/mg 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-hours after Event 4 (AGM: 0.17 ng/mL [95% CI: 0.11, 0.26]). 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.[173] In that study, volunteers 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 subjects exposed to sidestream smoke from approximately three tobacco cigarettes in a chamber study.[174]

Urinary acrolein metabolites increased after e-cigarette exposure. The CDC reports the average 3-HPMA and CEMA urinary concentrations among a representative sample of non-smokers in the U.S. population from 2005-2006 were (Median [25^{th}, 75^{th}]) 219 ng/mg creatinine (140, 353) and 78.8 ng/mg creatinine (51.8, 121), respectively.[175] Adjusted average concentrations of 3-HPMA in this study exceeded these national estimates by up to fourfold. Average concentrations of CEMA in this study were similar to or slightly higher than the national 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.[13] 3-HPMA is the major metabolite and CEMA is a minor metabolite of acrolein.[175] This could explain the discrepancy in patterns of change observed. There are many sources of acrolein exposures both in the environment and endogenously. The acrolein concentrations measured in this study are likely not completely a result of 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.[13; 121] 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).[78] 8-Isoprostane is recognized as the most specific and sensitive biomarker for oxidative stress.[114; 115] It is used as an oxidative stress marker in traditional tobacco smokers.[123] 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 vapor and biomarkers of e-cigarette exposure. Volunteers' consumption of food and drink was recorded but not incorporated into biological analysis. Future research should consider the contribution of food and drink to biomarker concentrations. Ventilation rates inside e-cigarette event venues should also be considered in future research.

CONCLUSION

This study is the first to characterize secondhand exposures to chemicals present in e-cigarette vapor in public settings. While the exposure duration was relatively short (~6 hours), volunteers' 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.[63] Secondhand e-cigarette vapor may be a source of acrolein exposures but is 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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Table 3.1. E-cigarette Event Characteristics

VARIABLES	EVENT 1	EVENT 2	EVENT 3	EVENT 4
E-CIGARETTE EVENT				
Location	Daytona Beach, Florida	Athens, Georgia	Chattanooga, Tennessee	Atlanta, Georgia
Date	April 2016	September 2016	October 2016	March 2017
Estimate number of attendees	1000	300	150	1500
Number of Study Volunteers	10	9	11	4
VENUE				
Venue type	Convention	Concert Hall	Convention	Exhibition/ Tradeshow
Site (ft^2)	42,146	5,100	36,000	205,000
Estimated Ceiling Height (ft)	45	35	30	13
Exposure time (Duration, min)	12:39-16:20/30 (341-351)	18:16-00:06 (350)	12:05-17:45 (340)	12:10-18:10/13 (360-363)
SAMPLE COLLECTION TIME				
Pre-Exposure	11:30	17:15	10:30	10:24
Immediately Post-Exposure	18:30	0:30	17:53	18:10
4h Post-Exposure	22:30	N.A.[B]	22:00	22:10-22:40
First-Void	First void[A]	First void	First void	First void

[A]Three subjects forgot to collect first-void samples. They collected second void samples within 1-2 hours of waking which are used for this analysis.
[B]Subjects exited Event 3 at 00:06. A 4-hour post-exposure sample was not collected because the sample collection would have been within 2-4 hours of the first-void sample.

Table 3.2. Adjusted Geometric Means and Confidence Intervals of Biomarker Concentrations[A]

Sample Time	Event	Value	Urinary Cotinine (ng/mg)	Salivary Cotinine (ng/mL)	Urinary Trans-3'-Hydroxycotinine (ng/mg)	3-HPMA (ng/mg)	CEMA (ng/mg)	8-Isoprostane (pg/mg)
Pre-Exposure	1	Geometric Mean (GM)	0.11	0.02	0.13	274.60	71.83	341.31
		95 % CI of GM	0.08-0.15	0.01-0.03	0.08-0.23	182.53-413.15	52.24-98.76	261.39-445.68
	2	Geometric Mean	0.16	0.02	0.17	185.69	61.48	345.92
		95 % CI of GM	0.11-0.22	0.01-0.02	0.10-0.29	123.56-279.08	44.75-84.46	264.97-451.60
	3	Geometric Mean	0.12	0.02	0.15	195.90	64.65	345.50
		95 % CI of GM	0.08-0.18	0.01-0.03	0.08-0.26	123.22-311.41	44.88-93.12	252.30-473.14
	4	Geometric Mean	0.19	0.01	0.22	323.50	100.13	302.41
		95 % CI of GM	0.12-0.31	0.01-0.02	0.12-0.43	186.79-560.26	64.81-154.72	206.77-442.31
Immediate Post-Exposure	1	Geometric Mean	0.38	0.08	0.35	624.72	95.63	445.59
		95 % CI of GM	0.27-0.54	0.06-0.11	0.21-0.61	415.26-939.92	69.55-131.49	336.37-590.22
	2	Geometric Mean	0.74	0.11	0.43	199.08	96.95	260.13
		95 % CI of GM	0.52-1.04	0.08-0.15	0.25-0.75	132.46-299.20	70.57-133.19	199.26-339.61
	3	Geometric Mean	0.28	0.03	0.25	252.17	82.67	300.16
		95 % CI of GM	0.19-0.42	0.02-0.05	0.14-0.45	158.62-400.86	57.40-119.08	219.18-411.04
	4	Geometric Mean	1.08	0.17	0.85	424.20	113.00	380.92
		95 % CI of GM	0.67-1.75	0.11-0.26	0.44-1.63	244.94-734.65	73.13-174.60	260.45-557.13
4-Hours Post-Exposure	1	Geometric Mean	0.81	0.08	0.73	733.85	115.64	372.82
		95 % CI of GM	0.58-1.15	0.06-0.12	0.42-1.26	487.75-1104.01	84.11-159.02	285.52-486.82
	2	Geometric Mean	N.A.[B]	N.A.	N.A.	N.A.	N.A.	N.A.
		95 % CI of GM	…	…	…	…	…	…
	3	Geometric Mean	0.35	0.04	0.35	454.55	110.18	314.00
		95 % CI of GM	0.24-0.52	0.03-0.05	0.19-0.63	285.92-722.56	76.49-158.71	229.29-429.96
	4	Geometric Mean	2.31	0.17	2.22	807.87	107.19	413.56
		95 % CI of GM	1.43-3.72	0.11-0.26	1.15-4.26	466.47-1399.12	69.37-165.62	282.76-604.86
First Void	1	Geometric Mean	0.80	0.06	0.88	838.82	169.30	296.78
		95 % CI of GM	0.57-1.13	0.05-0.09	0.51-1.53	557.58-1262.06	123.12-232.78	224.57-392.25
	2	Geometric Mean	1.09	0.11	0.96	163.09	90.95	323.31
		95 % CI of GM	0.77-1.53	0.08-0.15	0.55-1.65	108.51-245.11	66.20-124.96	247.65-422.08
	3	Geometric Mean	0.31	0.03	0.41	262.25	96.29	368.93
		95 % CI of GM	0.21-0.46	0.02-0.04	0.23-0.73	164.98-416.92	66.85-138.70	269.40-505.22
	4	Geometric Mean	2.21	0.15	2.67	407.48	97.82	377.06
		95 % CI of GM	1.37-3.56	0.10-0.23	1.39-5.13	235.29-705.71	63.31-151.14	257.80-551.48

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

Table 3.3. Analysis of Variance of Biomarker Concentrations Across Sampling Times and Events[A]

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

[A]All urinary endpoints were adjusted for creatinine for this analysis
*Significant at $p \leq .05$
**Significant at $p \leq .001$
***Significant at $p \leq .0001$

Table 3.4. Adjusted Mean Ratio of Subjects' 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

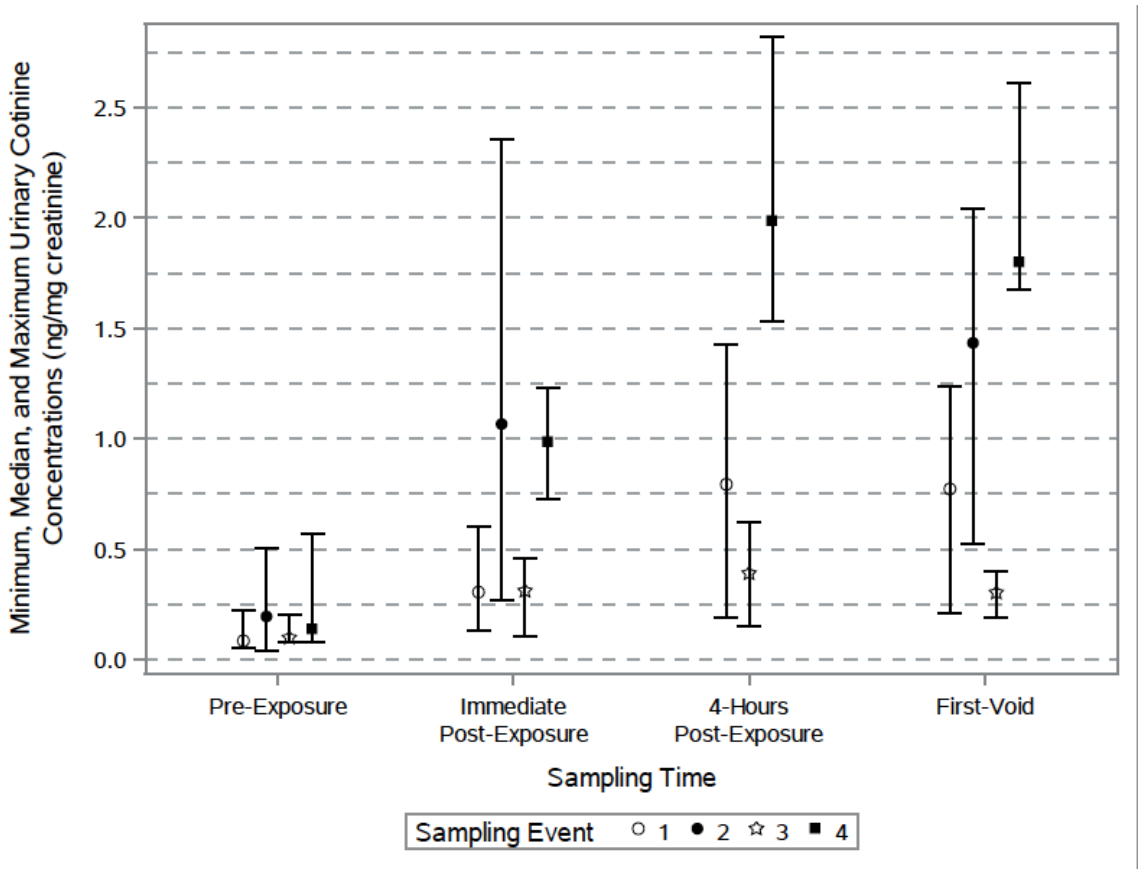

Figure 3.1 Creatinine-Corrected Urinary Cotinine Concentrations Across Sampling Times and Events

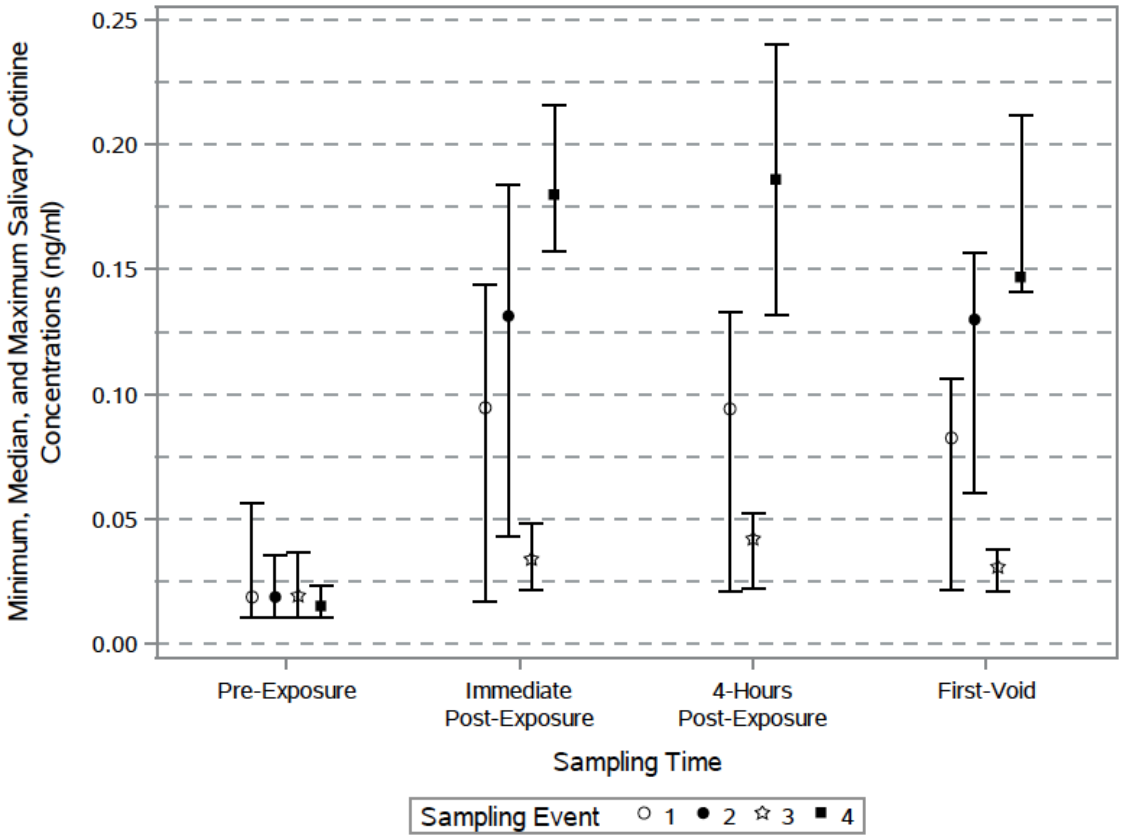

Figure 3.2 Salivary Cotinine Concentrations Across Sampling Times and Events

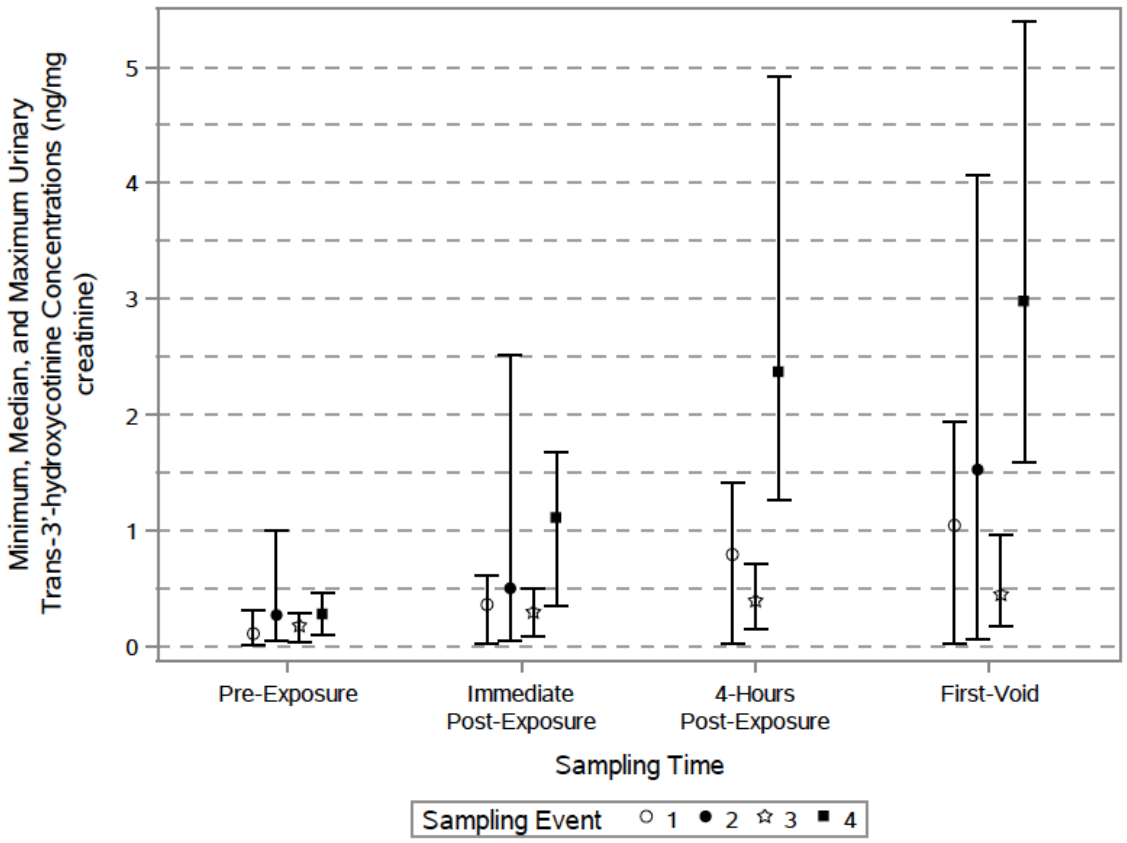

Figure 3.3 Creatinine-Corrected Urinary Trans-3'-hydroxycotinine Concentrations Across Sampling Times and Events

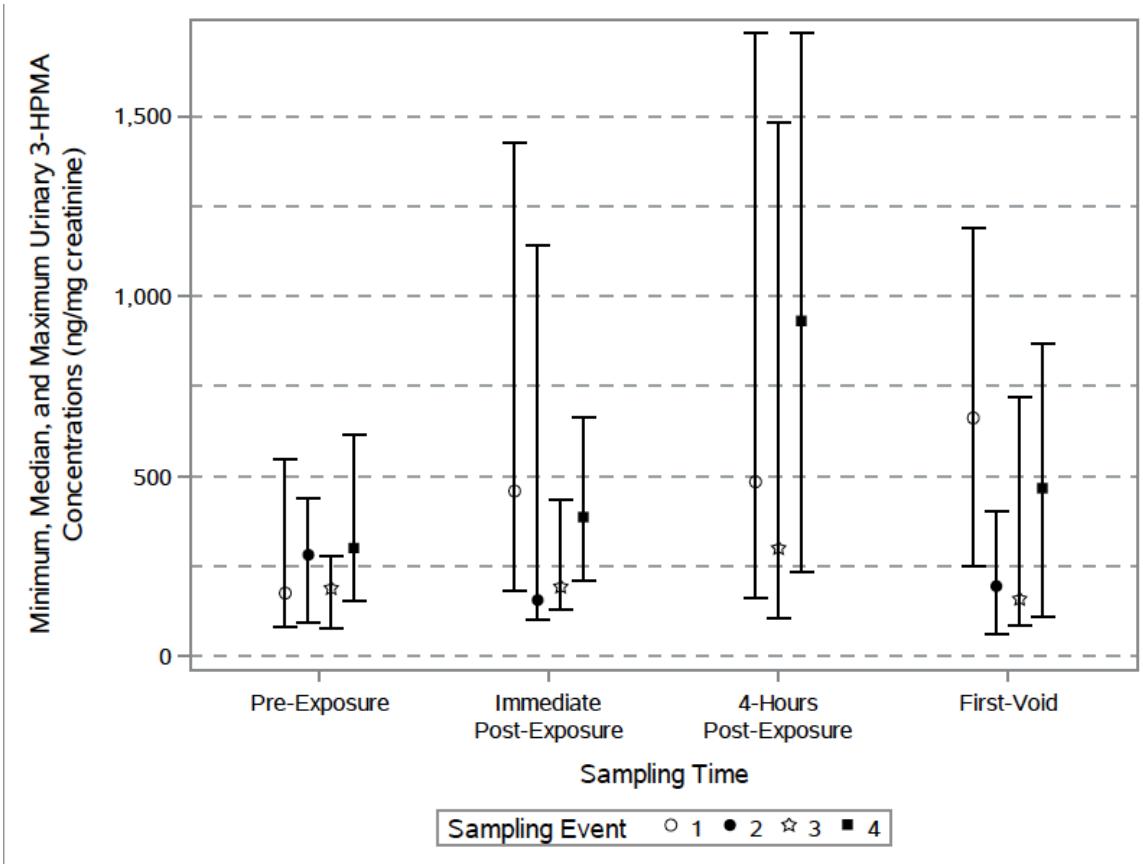

Figure 3.4 Creatinine-Corrected Urinary 3-HPMA Concentrations Across Sampling Times and Events

CHAPTER 4

ELEVATED NICOTINE DEPENDENCE SCORES AMONG ELECTRONIC CIGARETTE USERS AT AN ELECTRONIC CIGARETTE CONVENTION[3]

[3] Johnson, Jona M., Muilenburg, Jessica L., Rathbun, Stephen L., Yu, Xiaozhong, Naeher, Luke P., Wang, Jia-Sheng. 2017, *Journal of Community Health*, E-Published ahead of print. Reprinted here with permission of Springer.

ABSTRACT

Bakground: Electronic cigarette (e-cigarette) conventions are trade shows held across the globe to promote e-cigarette products and provide a venue for users to socialize. E-cigarette users that attend these events likely represent the most intensive e-cigarette user group. No study has characterized addiction and behavior characteristics in this population

Methods: We surveyed 131 e-cigarette users attending a large Southeastern e-cigarette convention in Fall 2015. All questions from the Fagerstrom Test for Nicotine Dependence (FTND), select questions from the Penn State Electronic Cigarette Dependence Index, and novel user behavior questions were included. In total, 25 questions were included in the survey. FTND scores were calculated for each respondent who answered all 6 FTND questions (n=117). Fisher's Exact Chi-square test was used to assess the relationship between addiction and behavior characteristics and FTND scores.

Results: Most respondents were classified as moderately dependent (score 5-7, 45.3% of respondents). Length of use, waking at night to use an e-cigarette, strength of cravings, strength of urges over the past week, and frequency of visiting e-cigarette blogs were significantly associated with FTND scores.

Conclusion: E-cigarettes users have average FTND scores higher than tobacco smokers. Scores were not significantly associated with prior tobacco cigarette use. Characteristics associated with tobacco smokers' nicotine addiction, such as waking at night to smoke and strength of cravings experienced, are relevant to e-cigarette users. E-cigarettes do not contain the magnitude of toxicants in tobacco cigarettes, but e-cigarettes may produce new chemical exposures evidenced by the adverse health effects reported by some respondents

INTRODUCTION

Electronic cigarettes ("e-cigarettes") are electronic devices that deliver nicotine and/or flavoring via a liquid, called the vaping fluid, to the user without using tobacco products.[176] In 2015 the US Centers for Disease Control and Prevention ("CDC") reported that e-cigarette use had tripled among middle and high school students in just one year.[177] The same study estimated 2.4 million youth were e-cigarette users in 2014, which surpassed the number of current youth users of any other tobacco product. From 2010-2013 ever-use of e-cigarettes by adults increased by 2,167%.[21] As evidenced by the quick uptake by consumers, e-cigarettes are a new phenomenon in the nicotine replacement industry. Despite the rising popularity of e-cigarettes, knowledge concerning e-cigarette efficacy and safety is notably deficient.

Former smokers often begin using e-cigarettes to quit smoking traditional cigarettes.[150] Future cessation can be predicted through a self-reported nicotine dependence questionnaire.[131] The Fagerstrom Test for Nicotine Dependence ("FTND") is a reliable tool used to estimate nicotine dependence.[134] The FTND estimates a respondent's nicotine dependence based on scores for six questions pertaining to cigarette use and addiction characteristics (Table 4.1). Scores range from 0-10, representing low to high dependence. Prevalence studies have shown FTND scores for traditional cigarette smokers in the United States range from 4.3-4.6.[147]

The FTND can be adapted to measure nicotine dependence in e-cigarette users.[146] Most e-cigarette users report that they are less dependent on e-cigarettes than they were on cigarettes.[146; 149; 150] One study reported the average FTND score for e-cigarette users responding to a survey on an online "quit smoking" website ranged from 2.5-3.9.[146] However, they also acknowledged that the survey respondents were not particularly intensive vapers.

One setting with a particularly intense population of e-cigarette users is an e-cigarette convention. E-cigarettes have grown to be so popular that conventions designed to bring e-cigarette users together in a social setting are held nearly every month across the United States.[31] The goal of the e-cigarette convention is to provide a large, social venue that promotes e-cigarette vendors and products.[32] These gatherings can range from meetings at a local e-cigarette shop and drawing a crowd of 100 attendees to regional convention attended by thousands of people. E-cigarette instructional seminars, social events, and competitions are also common. Research explaining why users attend these events is unavailable. One could hypothesize it is for the ease of access to hundreds of new e-cigarette products in a common place and to partake in social activities with many other e-cigarette users. Others may attend because they enjoy the thick, hazy environment, music, and e-cigarette competitions, and to support the e-cigarette industry.

The events have created a dedicated following of intense e-cigarette users. This subgroup of e-cigarette users is likely to have higher nicotine dependence scores compared with the general e-cigarette population because (1) These events often promote the newest e-cigarette devices designed to deliver more nicotine[11], and (2) attendees use their e-cigarette not only in response to nicotine dependence, but also frequently for social purposes and competitions. The increased use may result in more frequent nicotine delivery over a longer period of time, which likely results in higher dependence. The purpose of this study was to investigate nicotine dependence using a modified FTND test among e-cigarette users at a large Southeastern e-cigarette convention.

MATERIAL AND METHODS

Subjects

This study surveyed 131 adult attendees at the "Orlando Vape Convention" on October 17, 2015. An estimated 1,000 people attended to socialize and purchase e-cigarette liquids and supplies from approximately 40 vendors. An e-cigarette convention was chosen as the venue because it attracted a large number of intense e-cigarette users over a short amount of time. Only current e-cigarette users were eligible to complete the survey. The convention required all attendees to be at least 18 years old and provide proof of age. Therefore, only those ≥18 years old completed this study. All surveys were completed anonymously using pen and paper during the convention. Of those that completed the survey, 72% were male and 28% female. On average, males were 31 years old [range 18-68, standard deviation (SD)=11.1] and females were 35 years old [range 18-56, SD=11.5]. No personal identifying information was collected.

Procedures

The e-cigarette convention organizer granted permission to conduct the study on site. The University of Georgia Institutional Review Board for Human Subject Protection granted their approval of the project. Surveys were placed on a table manned by a researcher in the convention center entrance lobby. Subjects were able to approach the table and receive a paper survey to complete.

Instrument/Measures

The survey was largely a hybrid of two data collection instruments. All questions from the Fagerstrom Test for Nicotine Dependence were included as well as select questions from the

Penn State Electronic Cigarette Dependence Index.[134; 149] Additional questions were added to obtain information about e-cigarette use behavior, health effects, and secondhand exposure. In total 25 questions were included in the survey and discussed in this paper.

Table 4.1 demonstrates how each Fagerstrom Test for Nicotine Dependence question was scored. Question 4, "How many times a day do you usually use your e-cigarette?" was modified in accordance with the Penn State Electronic Cigarette Dependence Index. The answer choices are comparable to the same question in the Fagerstrom questionnaire. Question 4 was scored as follows: answers indicating 0-4 and 5-9 times a day were assigned a score of 0; answers indicating 10 to 14 and 15-19 times a day were assigned a score of 1; answers indicating 20-29 times were assigned a score of 2; answers indicating more than 30 times a day were assigned a score of 3.

Data Analyses

SAS® University Edition[157] and Microsoft Excel was used to create descriptive tables of addictive and behavioral characteristics of e-cigarette users, the distribution of Fagerstrom nicotine dependence rankings, and contingency tables to assess the relationship between addictive and behavioral characteristics and Fagerstrom dependence rankings. Only scores of subjects who completed all six FTND questions (n=117) are included in the FTND score distribution and in the contingency tables. Fisher's Exact Chi-square test was used to assess the relationship between addiction and behavioral characteristics and the Fagerstrom nicotine dependence rankings. A test with a p value ≤ 0.05 was considered statistically significant.

RESULTS

Fagerstrom Scores

Questions numbered one through six in Table 4.2 were scored according to the Fagerstrom Test for Nicotine Dependence methodology for the 117 subjects who completed all six FTND questions. The scores from each question were summed to establish an overall total score for nicotine dependence. The average FTND score was 5.0 (SD=2.32). Males' scores did not differ significantly from females' scores. Total scores were then categorized into one of four categories. These categories were "low dependence" (score=1-2, 17.1% of respondents), "low to moderate dependence" (score= 3-4, 22.2% of respondents), "moderate dependence" (score=5-7, 45.3%), and "high dependence" (score=8+, 15.4% of respondents).

Addiction Characteristics

Table 4.2 summarizes the distribution for addiction characteristics and individual FTND questions for all subjects. Nearly half of all subjects used an e-cigarette within 15 minutes of waking (25.2% within 5 minutes, 22.9% within 6-15 minutes of waking). Most respondents would rather give up any other e-cigarette session than the first in the morning (67.7%). One half of respondents used their e-cigarette for at least 10 minutes at a time 30 times a day (approximately 300 minutes or 5 hours a day) (50.0%).

Most e-cigarette users reported they did not awaken at night to use an e-cigarette (84.0%). Some respondents reported they did not wake at night but responded to the question asking how many nights per week they wake to use and e-cigarette (n=58). If their response was "no" to waking at night to use an e-cigarette and "0-1 nights/week", then it was assumed they did not typically waken at night to use an e-cigarette and they are not included in the analysis of the

number of nights per week a user wakes to use an e-cigarette. Two respondents reported not waking at night to use an e-cigarette, but then reported they wake 2-3 nights per week to use an e-cigarette. For the purposes of this analysis, neither response was adjusted because their responses were mutually exclusive and the correct answer could not be identified. Of those who did quantify how often they wake at night (n=23), the majority reported waking 2-3 nights per week to use an e-cigarette (39.1%).

Over half of respondents reported ever experiencing moderate to extremely strong cravings to use their e-cigarette (51.9%) and most (60.2%) experienced moderate to extremely strong urges to use their e-cigarette over the past week. Nearly one third of users reported feeling irritable or anxious if they could not use their e-cigarette (31.0% and 26.9%, respectively). Some respondents wrote "sometimes" on the survey for questions asking about irritability and anxiety. These responses were categorized as being "yes" because "sometimes" implies that they have experienced irritability or anxiety when they were unable to use an e-cigarette at some point in time.

Most reported use of vaping fluid that contained 1-12 mg/mL nicotine (71.5%), though 20.0% reported using a vaping liquid that did not contain nicotine. Another 4% of respondents used both nicotine and non-nicotine containing vaping fluids.

Behavioral Characteristics

Table 4.3 summarizes the distribution of behavior characteristics among all respondents. Notably, most reported using an e-cigarette for the past 1-5 years (54.6%), though over one third have started using e-cigarettes within the past year (40.0%). Most do not plan to quit using e-cigarettes in the future (66.9%). Some respondents selected both "yes" and "no" or wrote

"maybe" regarding their plans to quit e-cigarette use. We created the "maybe" response to reflect these answers (4.6%).

While a majority smoked tobacco cigarettes prior to using e-cigarettes (86.2%), a modest percentage did not use traditional cigarettes prior to e-cigarette use (13.9%). Most use only e-cigarettes currently and no longer use traditional cigarettes (90.1%).

Most reported that quitting or avoiding relapse of smoking tobacco cigarettes was a reason they started using e-cigarettes (72.1%). A smaller percentage began using e-cigarettes to reduce tobacco consumption with no intention of quitting smoking (11.6%). Informative answers categorized as "other" (20.9%) were gathered. For example, three respondents reported beginning e-cigarette use because they perceived it to be a safer alternative. Three respondents began using e-cigarettes to help them quit "dipping" tobacco or smoking water pipes (hookah). Other reported reasons for beginning e-cigarette use included to reduce stress, because of the flavored juices, to help with focus issues, for family and grandchildren, to prolong life, to host the event, enjoyment of the clouds e-cigarette vapors create, to reduce coughing, as a result of clinical diagnosis and doctor recommendation, to reduce food cravings, and because the perceived cost of e-cigarette use is lower than the cost of traditional cigarette use.

The most common type of e-cigarette device reportedly used was a second-generation device that allows the user to control the voltage (88.5%). The majority of respondents use their e-cigarette in the home (82.3%), cars (63.7%) and public places (45.2%).

Over half of respondents visit an e-cigarette blog at least once a week (51.2%). Most have not experienced any adverse health effects while vaping, though a small fraction reported symptoms such as throat and/or eye irritation, headaches, and nausea (n=20, 80.0 % of subjects who reported an adverse health effect). A small number identified other health effects they have

experienced since beginning the use of an e-cigarette that were not included in the list on the survey. These included a light buzz, dehydration, slight weight gain, and wheezing.

Some respondents used their response to the health effect question to report positive health gains they've experienced while using e-cigarettes. These included heightened lung capacity, better breathing, better overall health, and improved sense of taste. The positive health effects were not included in the Chi-squared analysis because the intent was to measure association between nicotine dependence and adverse health effects. However, it is worthwhile to acknowledge positive health impacts reported from e-cigarette use by former traditional cigarette smokers.

The majority of respondents have never had someone around them complain while they were using their e-cigarette (72.3%), but roughly one-fourth have someone complain of adverse health effects because of their vaping. Of those that have been using their e-cigarette and had someone around them complain, respondents reported the presence of vapor or smoke (identified in text for explanations for "other"), the smell (identified in text for explanations for "other"), and throat and/or eye irritation were the most common complaints (n=23, 74.2% of subjects who reported a complaint).

Association between Addiction and Behavior Characteristics and FTND Scores

Table 4.4 summarizes the relationships between select addiction and behavior characteristics and Fagerstrom categories for subjects that completed all six FTND questions (n=117). In an effort to keep the contingency table relatively small, some of the addiction and behavior characteristics were dichotomized. Fisher's Exact Chi-squared test revealed five characteristics significantly associated with the Fagerstrom score of a participant.

First, the length of e-cigarette use, when dichotomized to <1 year or >1 year of use, was significantly (p=0.0069) associated with Fagerstrom scores. More than half of respondents who have used e-cigarettes for >1 year were ranked as moderately or highly nicotine dependent (70.5%). Less than half (45.7%) who have used e-cigarettes <1 year were ranked as moderately to highly nicotine dependent.

Interestingly, a significant relationship between level of nicotine used and prior use exists (Chi-square=8.09, p=0.0100). Among those that were not traditional cigarette users prior to becoming e-cigarette users, 43.8% reported using e-cigarette fluid that does not contain nicotine. Only 14.1% of former smokers of traditional cigarettes reported using e-cigarette fluid with no nicotine.

Waking at night to use an e-cigarette was significantly associated with Fagerstrom scores (p=0.0082). Among those that reported waking at night to use an e-cigarette, 94.5% were ranked moderately or highly nicotine dependent. Among those that did not report awakening at night to vape, 54.5% were ranked in the top two tiers of dependence.

The strength of cravings and urges experienced by vapers, both at any previous time and over the past week, were also significantly associated with Fagerstrom scores (p=0.0075 and p=0.0005, respectively). Among those that reported very strong to extremely strong cravings to ever use an e-cigarette, 100% were classified as moderately to highly dependent. In contrast, 42.6% of those who reportedly have no to only slight cravings to vape were classified as moderately or highly dependent. Finally, those that visited e-cigarette blogs at least once a year were largely ranked as moderately to highly dependent (63.6%) compared with 43.8% of those that visit e-cigarette blogs no more than once a year (p=0.0235).

Variables that were not significantly associated with nicotine dependence scores included gender, nicotine level used, prior use of traditional cigarettes, dual use of e-cigarettes and traditional cigarettes, plans to quit e-cigarette use in the future, belief that one is less dependent on e-cigarettes compared with past traditional cigarette use, feeling anxious and/or irritable when unable to use an e-cigarette, experiencing adverse health effects since beginning e-cigarette use, and having someone nearby complain of health effects during e-cigarette use.

DISCUSSION

To our knowledge this is the first study that shows e-cigarette users can have higher average nicotine dependence levels than traditional cigarette users. Former studies of traditional cigarettes revealed FTND scores among users of traditional cigarettes in the United States ranged from 4.3-4.6.[147] Average nicotine-dependence scores for e-cigarette users have been reported to range from 2.5-3.9.[146] In the current study, the average FTND for e-cigarette users who completed all 6 FTND questions was 5.0. More than half of the e-cigarette users obtained a score $\geq$ 5.0 (60.7%). We acknowledge that we did have a small sample of respondents from one of the most, if not the most, intense e-cigarette user groups.

The length of e-cigarette use (<1 year vs. >1 year) and the level of nicotine used in e-cigarette liquid (none vs. any level of nicotine) were significantly associated with nicotine dependence scores. These results are supported by the Foulds et al. (2015) survey of 3,609 e-cigarette users. In that study, e-cigarette users who had used e-cigarettes for more time were more likely to have higher e-cigarette dependence index scores than those who had used e-cigarettes for a shorter period of time. Additionally, those who used e-cigarette fluid with no nicotine had lower scores than did those who used fluids that contained nicotine. One user in the

current study reported the intent to change nicotine concentration over time to end with an e-cigarette liquid that did not contain nicotine. If a user is able to effectively reduce nicotine intake over time, e-cigarettes may be a useful tobacco cessation tool. Additional research on the efficacy of this approach is needed.

Known indicators of nicotine dependence were significantly associated with FTND scores in our study. First, it has been reported that waking at night to use a traditional cigarette is a strong indicator of future cessation, and thus an indicator of nicotine dependence.[178] Our study shows the association between waking at night to use an e-cigarette and nicotine dependence is significant. Second, the strength of cravings ever experienced and those experienced over the past week were significantly associated with nicotine dependence scores. These measures have been reported to predict the difficulty of quitting traditional cigarettes, and thus nicotine dependence, with varying results.[179; 180] Our study is the second study to show these variables are significantly associated with nicotine dependence among e-cigarette users.[149]

Adverse health effects were weakly associated with nicotine dependence (p=0.0597). The types of adverse health effects most commonly reported in the current study were throat irritation and headaches (n=17 subjects, 68% of those who reported adverse health effects). These findings are supported by a internet survey of 81 e-cigarette users that found "dry mouth and throat" and "vertigo, headache or nausea" to be two of the most common undesirable effects experienced by e-cigarette users.[181]

This survey focused on short-term adverse health effects resulting from e-cigarette use. Because e-cigarettes were introduced to the consumer market only recently, studies examining the long-term health effects of e-cigarette use are unavailable.[6] Future studies examining the

long-term impacts of e-cigarette use are necessary to truly characterize adverse health effects resulting from e-cigarette use.

The reported reasons for beginning e-cigarette use are noteworthy. Nearly all "other" responses can be grouped into two categories: 1) The respondent perceived e-cigarettes to be a safer alternative to traditional cigarette use and 2) E-cigarettes were attractive because of the flavors, costs, and vapor clouds. Because we found a significant association between nicotine dependence and the frequency of visiting online e-cigarette blogs, we believe e-cigarette websites and blogs may be a good place for delivering health messaging to e-cigarette users.

The survey did not ask respondents why they were attending the event. It is assumed that users attended the event to visit with vendors, enjoy the samples available, purchase new e-cigarette products (i.e. liquids, devices, etc.) and socialize with other e-cigarette users. As Williams (2015) suggested, it could be that users who attend e-cigarette conventions somehow differ from those who do not.[32] Perhaps one difference is the nicotine dependence between the two user groups. Future research should investigate what impact e-cigarette convention attendance has on nicotine dependence and other e-cigarette use outcomes.

Limitations

This study is narrow in that it is representative of a small group of active users attending only one large e-cigarette convention. We did not ask respondents why they attended the e-cigarette convention. The small sample of respondents represents a highly active subgroup of e-cigarette users who may not represent the typical e-cigarette user. The modified FTND tool should be used among other intense e-cigarette user groups to validate results. Another limitation is that question four of the FTND asks users how many traditional cigarettes they smoke a day.

E-cigarettes are typically not disposable and are repeatedly used throughout the day. Thus, this question is difficult for e-cigarette users to answer.[149] Half of our respondents reported using their e-cigarette more than 30 times a day, so scoring is not problematic for at least 50% of our sample.

CONCLUSION

We used the Fagerstrom Test for Nicotine Dependence to characterize nicotine dependence in e-cigarette users. This study demonstrates that e-cigarette use can result in nicotine dependence equivalent to or higher than nicotine dependence resulting from tobacco cigarette use. E-cigarette users surveyed in this study have addiction behaviors similar to traditional cigarette users. We recognize our sample population was an intense user group. Notably, the environment in which we recruited is recreated for thousands of users at least monthly at e-cigarette gatherings around the world. Additional research is needed to determine the long-term efficacy of e-cigarettes as traditional cigarette cessation tools and to characterize exposures that result from e-cigarette use.

ACKNOWLEDGEMENTS

We would like to thank the e-cigarette convention organizers for allowing us to complete this study in their venue. We would also like to thank the survey respondents for their participation.

Table 4.1 Scoring for the Fagerstrom Test for Nicotine Dependence (FTND) Adapted for E-cigarette Use

Question	Answers	Points
1. How soon after waking do you use your e-cigarette?	Within 5 minutes	3
	6-15 minutes	2
	16-30 minutes	2
	31-60 minutes	1
	61-120 minutes	0
	More than 2 hours	0
2. Do you find it difficult to refrain from smoking in places where you are not supposed to (e.g. college campuses, church, library, movie theaters, etc.)?	Yes	1
	No	0
3. Which e-cigarette would you hate to give up?	The first in the morning	1
	Any other	0
4. How many times a day do you usually use you e-cigarette? Assume one "time" consists of around 15 puffs or lasts around 10 minutes.	0-4 times/day	0
	5-9 times/day	0
	10-14 times/day	1
	15-19 times/day	1
	20-29 times/day	2
	More than 30 times/day	3
5. Do you use your e-cigarette more frequently in the morning?	Yes	1
	No	0
6. Do you smoke even if you are sick in bed most of the day?	Yes	1
	No	0

Table 4.2 Addiction Characteristics (N=131)

Characteristic	%	(n)
1. How soon after waking do you use your e-cigarette? [A]		
Within 5 minutes	25.2	33
6-15 minute	22.9	30
16-30 minutes	19.1	25
31-60 minutes	13.7	18
61-120 minutes	11.5	15
More than 2 hours	7.6	10
2. Do you find it difficult to refrain from smoking in places where you are not supposed to (e.g. college campuses, church, library, movie theaters, etc.)? [A]		
No	83.1	108
Yes	16.9	22
3. Which e-cigarette would you hate to give up? [A]		
The first in the morning	32.3	40
Any other	67.7	84
4. How many times a day do you usually use you e-cigarette? Assume one "time" consists of around 15 puffs or lasts around 10 minutes. [A,B]		
0-4 times	6.9	9
5-9 times	10.0	13
10-14 times	13.9	18
15-19 times	8.5	11
20-29 times	10.8	14
More than 30 times	50.0	65
5. Do you use your e-cigarette more frequently in the morning? [A]		
No	73.2	93
Yes	26.8	34
6. Do you use your e-cigarette even if you are sick in bed most of the day? [A]		
No	53.5	68
Yes	46.5	59
7. Do you sometimes awaken at night to use your e-cigarette? [B, C]		
No	84.0	110
Yes	16.0	21
8. If yes [you do sometimes awake at night you use your e-cigarette], how many nights a week do you typically awaken to use to use your electronic cigarette (n=21)? [B, C]		
0-1 nights	30.4	7
2-3 nights	39.1	9
More than 4 nights	30.4	7
9. Do you ever have strong cravings to use your e-cigarette? [B, D]		
None/slight	48.1	63
Moderate/strong	46.6	61
Very strong/extremely strong	5.3	7
10. Over the past week, how strong have the urges to use an e-cigarette been? [B, E]		
None/slight	39.8	47
Moderate/strong	49.2	58
Very strong/extremely strong	11.0	13
11. When you haven't used an e-cigarette for a while, or when you tried to stop using your e-cig, did you feel more irritable because you couldn't use an e-cigarette? [B, D]		
No	69.0	89
Yes	31.0	40
12. Did you feel nervous, restless, or anxious because you couldn't use an e-cigarette? [B, D]		
No	73.1	95
Yes	26.9	35
13. What level of nicotine do you most often use in your vaping fluids?		
Vaping fluid that does not contain nicotine	20.0	26
1-12 mg/mL	71.5	93
>13 mg/mL	2.3	3
Both 1-12 mg/mL and >13 mg/mL	2.3	3
Both vaping fluid that does not contain nicotine and 1-12 mg/mL	3.9	5
14. Do you believe you are less nicotine dependent on e-cigarettes compared to when you smoked traditional cigarettes?		
No	10.2	13
Yes	89.8	114

[A] Question adapted or borrowed from the Fagerstrom Test for Nicotine Dependence (FTND)
[B] Question adapted or borrowed from the Penn State [Electronic] Cigarette Dependence Index developed by Foulds et al. (2015)[149]
[C] Originally adapted from Bover et al. (2008)[178] by Foulds et al. (2015)[149]
[D] Originally adapted from the "Hooked On Nicotine Checklist" by Foulds et al. (2015)[149]
[E] Originally adapted from Fidler et al. (2011)[180] by Foulds et al. (2015)[149]

Table 4.3 Behavioral Characteristics (N=131)

Characteristic	%	(n)
1. How long have you been using e-cigarettes?		
1-2 months	9.2	12
3 months – 1 year	30.8	40
1-5 years	54.6	71
>5 years	5.4	7
2. Do you plan to quit using e-cigarettes in the future?		
No	66.9	87
Yes	28.5	37
Maybe	4.6	6
3. Did you smoke traditional cigarettes prior to beginning the use of e-cigarettes?		
No	13.9	18
Yes	86.2	112
4. Do you currently smoke both traditional cigarettes and e-cigarettes?		
No	90.1	100
Yes	9.9	11
5. Why did you start using e-cigarettes? (Multiple answers selected)		
To quit smoking or avoid relapse of smoking	72.1	93
To reduce tobacco consumption with no intention of quitting smoking	11.6	15
Other	20.9	27
6. What type of e-cigarette do you use? (Multiple answers selected)		
A first generation e-cigarette that is the same size and shape as a regular cigarette	5.4	7
An e-cigarette that activates the heating coil at the press of a button and allows the voltage to be controlled by the user	88.5	115
Other	10.0	13
7. Where do you commonly smoke your e-cigarette? (Multiple answers selected)		
Home	82.3	102
In the car	63.7	79
Public places (campus, shops, library, movie theaters, etc.)	45.2	56
Other	1.5	2
8. How often do you visit vaping blogs, online forums, or vaping events?		
Never	7.8	10
Once a year	7	9
1-3 months/year	17.1	22
1-2 times/month	17.1	22
At least once a week	51.2	66
9. What, if any, health effects have you experienced while smoking an e-cigarette? (Multiple answers selected)		
None	80.5	103
Throat irritation	10.2	13
Skin irritation	0	0
Eye irritation	1.6	2
Headaches	3.9	5
Nausea	1.6	2
Other	3.9	5
10. How often does someone physically near you (i.e. standing by, or riding in the same car) complain of health effects (irritation, nausea, headache) because of your e-cigarette?		
Never	72.3	94
Sometimes	23.9	31
Often	2.3	3
Frequently	1.5	2
11. If people around you DO complain of health effects when you use your e-cigarette, what health effects do they complain of? (Multiple answers selected) (n=49)		
Skin irritation	2	1
Eye irritation	10.2	5
Headaches	8.2	4
Nausea	0	0
Throat irritation	12.2	6
None	36.7	18
Other	30.6	15

Table 4.4 Association Between Characteristics of E-cigarette Usage and Fagerstrom Scores (N=117)

Characteristic	Low Dependence (%) n=20	Low to moderate Dependence (%) n=26	Moderate Dependence (%) n=53	High Dependence (%) n=18	Chi-Square	p Value*
Gender					4.10	0.2424
Male	15.7	22.9	50.6	10.8		
Female	20.0	23.3	33.3	23.3		
Length of e-cigarette use					11.87	0.0069*
< 1 year	26.1	28.3	26.1	19.6		
> 1 year	11.3	18.3	57.8	12.7		
Prior Use of traditional cigarettes					2.55	0.4833
No	31.3	18.8	37.5	12.5		
Yes	15.0	23.0	46.0	16.0		
Uses both traditional cigarettes and e-cigarettes					0.80	0.9094
No	15.9	25.0	43.2	15.9		
Yes	18.2	18.2	54.6	9.1		
Nicotine level used					8.29	0.0539
Liquid with no nicotine	36.4	22.7	36.4	4.6		
Liquid with nicotine	12.8	22.3	46.8	18.1		
Plan to quit using e-cigs					7.39	0.2512
No	21.3	25.0	42.5	11.3		
Yes	9.1	18.2	48.5	24.2		
Maybe	0.0	0.0	66.7	33.3		
Believe less dependent					3.66	0.3137
No	16.7	8.3	41.7	33.3		
Yes	16.8	22.8	46.5	13.9		
Waking at night to vape					10.56	0.0082*
No	20.2	25.2	41.4	13.13		
Yes	0.0	5.6	66.7	27.8		
Strength of cravings to use e-cig					16.59	0.0075*
None/slight	25.9	31.5	35.2	7.4		
Moderate/strong	10.5	15.8	52.6	21.1		
Very strong/extremely strong	0.0	0.0	66.7	33.3		
Strength of urges to use e-cig over the past week					26.09	0.0005*
None/slight	24.4	36.6	34.2	4.9		
Moderate/strong	13.5	17.3	53.9	15.4		
Very strong/extremely strong	0.0	0.0	45.5	54.6		
Feeling anxious without e-cig					1.52	0.7086
No	17.7	24.7	42.4	15.3		
Yes	15.6	15.6	53.1	15.6		
Feeling irri without e-cig					2.95	0.3979
No	20.5	20.5	46.2	12.8		
Yes	10.3	25.6	45.6	20.5		
Visits e-cigarette blogs					8.99	0.0235*
No more than once a year	37.5	18.8	18.8	25.0		
At least once a year	13.1	23.2	49.5	14.1		
Has experienced (adverse) health effects while vaping					8.07	0.0597
No	12.1	25.3	47.3	15.4		
Yes	34.6	11.5	38.5	15.4		
Has had someone nearby complain of health effects from the e-cig					5.64	0.1531
Never	19.1	22.6	47.6	10.7		
Sometimes, Often, or Frequently	12.5	21.9	37.5	28.1		

* A test with a p value ≤ 0.05 was considered statistically significant.

CHAPTER 5

SUMMARY AND CONCLUSIONS

SUMMARY

Electronic cigarettes (e-cigarettes) are devices that deliver nicotine and/or flavoring via a liquid (called the vaping fluid) to the user without using tobacco.[154] E-cigarettes do not contain the combustible by-products associated with tobacco but still may expose bystanders to chemicals of concern.[6] E-cigarettes are often touted as smoking cessation devices, but there is limited evidence they are effective as such.[22] Knowledge of the nicotine dependence levels of e-cigarette users is limited.

E-cigarettes have grown to be so popular that large social e-cigarette gatherings occur regularly around the world.[32] These conventions attract hundreds to thousands of people who use e-cigarettes in a relatively small venue over the course of a few hours or intermittently over a few days. Secondhand exposures to chemicals in e-cigarette vapor in this environment or any other public setting have not been characterized.

The purpose of the work in this dissertation is to characterize e-cigarette secondhand exposures in a high-concentration e-cigarette environment and assess e-cigarette users' nicotine dependence. Passive e-cigarette exposures were characterized using environmental and biological monitoring. Nicotine dependence was assessed using a survey tool that included questions from the Fagerstrom Test for Nicotine Dependence.

The first manuscript in this dissertation presents data from a secondhand exposure assessment using environmental monitoring to characterize e-cigarette vapor exposures. Air sampling pumps were placed inside the backpacks of select volunteers (n=22) across four events.

Air samples of nicotine, formaldehyde, acetaldehyde, acrolein, and propylene glycol were collected using standard air sampling methods during e-cigarette events and during control days for comparison. Data indicated e-cigarette vapor did not contain elevated concentrations of select volatile organic compounds but did contain elevated concentrations of nicotine and propylene glycol. The median nicotine concentration across the four events was 1.10 $\mu g/m^3$ (range = <0.36-2.20 $\mu g/m^3$). The median propylene glycol concentration across the events was 305.51 $\mu g/m^3$ (range = 210.00-490.00 $\mu g/m^3$). Nicotine concentrations were well below available health guidance values.[40; 162] An applicable health guidance value has not been established for propylene glycol.

The second manuscript presents data from a secondhand exposure assessment using biological monitoring to characterize secondhand e-cigarette exposures. A total of 34 volunteers attended four e-cigarette events. Urine (n=103) and saliva (n=101) samples were collected from volunteers immediately before the event, immediately after the event, 4-hours after the event, and the next morning. Data from samples of 28 volunteers was used and analyzed using a mixed linear model. Concentrations of creatinine-corrected urinary cotinine ($p<0.0001$), trans-3'-hydroxycotinine ($p<0.0001$), 3-HPMA ($p<0.0001$), CEMA ($p<0.001$), and salivary cotinine ($p<0.0001$) varied significantly across sampling times. All biomarkers except urinary CEMA and 8-isoprostane also varied significantly across events. A significant interaction effect between sampling time and sampling location was detected for all biomarkers except CEMA and 8-isoprostane. Adjusted urinary-cotinine concentrations in this study 4-hours after e-cigarette secondhand exposures for one event (GM [95% CI]), (2.31 ng/mg creatinine [1.44, 3.72]) were similar to those reported for non-users living with e-cigarette users.[63] Salivary cotinine concentrations (GM [95% CI], (0.17 ng/mL [0.11,0.26]) 4-hours after exposure at the same event

were similar to those of non-users who lived in homes with e-cigarette users and non-users who visited a bar and were in close proximity to tobacco smokers.[173] Results indicated e-cigarette secondhand exposures are not strong sources of exposures to TSNAs. Although acrolein was not detected in any air sample during environmental monitoring, biological samples indicate e-cigarette vapor may contain acrolein. Acrolein is produced endogenously in the body and is present in foods and the environment, which may be a source of urinary acrolein observed.[175] Additional research is needed to clarify the presence of acrolein in e-cigarette vapor. Across events and sampling times, 8-isoprostane remained unchanged.

The third manuscript presents the data from the survey of e-cigarette users attending an e-cigarette convention (n=131). Based on responses to questions from the Fagerstrom Test for Nicotine Dependence, respondents were classified as having "low dependence" (score=1-2, 17.1%), "low to moderate dependence" (score= 3-4, 22.2%), "moderate dependence" (score=5-7, 45.3%), and "high dependence" (score=8+, 15.4%). The average score was 5.0. Results indicated e-cigarette users' nicotine dependence is higher than previously reported.[146] The results from this study also show e-cigarette users' average nicotine dependence can be higher than the average nicotine dependence of tobacco smokers.[147] Interestingly, over half of respondents reported they use an e-cigarette 10 times a day at least 30 minutes at a time (total time = 5 hours). Most smoked tobacco cigarettes prior to using e-cigarettes (86.2%). Almost all (90.1%) only use e-cigarettes and no longer use tobacco cigarettes. Most began using an e-cigarette to quit smoking or avoid relapse of smoking (72.1%). The relationship between FTND and behavioral and addiction characteristics was analyzed using the Fisher's Exact Chi-squared test. Many of the characteristics associated with tobacco smokers' nicotine dependence (i.e. waking at night to smoke [p=0.0082], experiencing strong cravings over the past week [p=0.0005] and ever

[p=0.0075], length of use [p=0.0069]) were significantly associated with nicotine dependence among e-cigarette users.

CONCLUSIONS

Results indicate e-cigarette secondhand exposures are sources of elevated nicotine and propylene glycol exposure but not formaldehyde, acetaldehyde, or tobacco-specific nitrosamines typically. E-cigarette vapor may be a source of secondhand acrolein exposure, though more research is needed.

The survey of e-cigarette respondents reported in manuscript three revealed many e-cigarette users begin using the devices because they want to cease tobacco cigarette use. Many of them reported switching to e-cigarettes completely and successfully. Though these results appear promising, it is important to remember the exposures, health consequences, and cessation outcomes resulting from chronic e-cigarette use are not fully understood.

Multiple sources, including the volunteers who participated in the e-cigarette events reported here and the survey respondents, report eye irritation, throat irritation, and headaches. The cause of these adverse health effects is unknown. It is possible that e-cigarettes result in novel exposures not measured in this work.

Currently, very few states and localities ban e-cigarettes in public places where tobacco smoking is banned.[26; 29] Approximately half of survey respondents reported using their e-cigarette in public places. Results from this body of work, specifically the types and magnitude of exposures that can result from e-cigarette secondhand exposures, can be used to better inform indoor e-cigarette use policies.

FUTURE RESEARCH

Though both environmental and biological monitoring were conducted at these events, the relationship between these two data sets was not analyzed in this dissertation. Future work should consider how the environmental components of e-cigarette vapor exposure correlate with biological endpoints in those exposed. Consumption of food and drink were not incorporated in this analysis. Some foods are known to contain some of the contaminants assessed in this study (i.e. acrolein[111]) and should be included in future work.

A minority of subjects in this study reported experiencing adverse health effects that they attributed to the e-cigarette event (15%, n=5). The health effects reported (i.e. headache, eye irritation, and throat irritation) were similar to those reported by survey respondents discussed in manuscript three and 81 e-cigarette users who responded to an internet survey.[27] Only urinary 8-isoprostane was analyzed as a marker of oxidative stress in this study. Results did not indicate secondhand e-cigarette exposures in this study resulted in an increase in 8-isoprostane. Future studies should consider additional markers of inflammation and oxidative stress. For example, two studies have demonstrated that fractional exhaled nitric oxide (FeNO) concentrations change after e-cigarette use.[13; 121]

E-cigarettes likely cause fewer toxicant exposures compared to traditional tobacco products.[6] However, chronic-use studies need to be completed to verify the findings from the acute-duration studies presented in this dissertation.

This study only sampled for a select number of chemicals previously reported in e-cigarette vapor. Future studies should sample for additional contaminants, such as particulate matter. Additionally, methods that allow for e-cigarette vapor to be captured in a way that allows the

vapor to be analyzed in a laboratory for a multitude of chemicals (i.e. samples collected in a canister) should be considered to expand the quantity of chemicals that can be assessed.

Lastly, this body of work was conducted at only one type of e-cigarette event and represents possibly the highest exposures a member of the public will likely experience. E-cigarette exposures can occur in many different types of public environments. Additional locations where e-cigarettes are used (i.e. restaurants, bars, cars, worksites, etc.) should be considered for future work to fully characterize e-cigarette secondhand exposures in public settings.

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APPENDICES

APPENDIX A: SECONDHAND EXPOSURE ASSESSMENT CONSENT FORM

UNIVERSITY OF GEORGIA
CONSENT FORM

Secondhand Exposure Assessment of E-Cigarette Vapor at an E-Cigarette Convention

Researcher's Statement

We are asking you to take part in a research study. Before you decide to participate in this study, it is important that you understand why the research is being done and what it will involve. This form is designed to give you the information about the study so you can decide whether to be in the study or not. Please take the time to read the following information carefully. Please ask the researcher if there is anything that is not clear or if you need more information. When all your questions have been answered, you can decide if you want to be in the study or not. This process is called "informed consent." A copy of this form will be given to you.

Principal Investigator: Dr. Luke P. Naeher
Associate Professor
Environmental Health Science Department

Purpose of the Study

The goal of this study is to characterize exposures to e-cigarette vapor in a high-concentration, public e-cigarette environment. We are recruiting participants in this study who are 18 years and older, generally in good health, not smokers nor e-cigarette users who do not use any form of tobacco product or nicotine-replacement product, and are not pregnant.

Study Procedures

If you agree to participate, you will be asked to …

- Complete a screening survey that asks about your current and past smoking, e-cigarette, cigar/pipe, and chew/dip history. If you currently smoke cigarettes or cigars/pipes, vape e-cigarettes, chew/dip tobacco, or use a nicotine replacement product you will not be eligible for this study. Additionally, if you participate in these activities within 6 days before the convention, you will not be eligible to participate in the study.
- You may be withdrawn from further participation based on your screening survey responses
- Attend a 30-minute meeting within a month of the study to learn about day-of study logistics including travel plans, meal expenses, and reward gift cards
- Complete a day-of survey asking about your exposure to of cigarettes, e-cigarettes, and cigars/pipes, 6 days prior to the e-cigarette convention
- Attend an e-cigarette convention either in Fall 2016
- During the convention, we ask that you visit with vendors and act as a convention participant. We ask that you do not sit near open doors, as these may reduce the presence of e-cigarette vapor in the area. This may reduce your exposure so much that researchers cannot detect what they need to in your biological samples. Additionally, it may produce non-detectable levels of chemicals in air samples.
- You may be asked to wear a backpack containing sampling pumps during your time in the convention to allow us to measure airborne contaminants at the convention and correlate it with metabolites in your urine and saliva. If you do not wish to wear the

backpack with sampling pumps, you may decline. You decision to wear the sampling pumps or not will not affect your ability to participate in the study.

- Provide urine and saliva samples to researchers immediately before you enter the convention, immediately after leaving the convention, the evening after the convention, and the morning after the convention
- These samples will be used to analyze urinary and salivary nicotine, select VOC metabolites hypothesized to be in e-cigarette vapor, TSNAS that indicate tobacco exposure, and 8-isoprostane, a marker of DNA oxidative stress.
    - Time commitment: 18-hour time commitment on day 1 (collecting pre- and post-exposure urine samples, convention attendance).
    - 10-minute commitment on day 2 (collecting post-exposure samples)
    - If you participate in a convention far enough away to require overnight stay, your time commitment will be roughly 24 hours.

In summary: The estimated total time commitment for you as a participant in the study is approximately 18 hours for each convention that you attend. Additional travel time and over-night stay may be required depending on the location of the convention.

Risks and discomforts

- Some e-cigarette users have reported experiencing headaches, nausea, eye, nose and throat irritation when using e-cigarettes. It is possible that these symptoms can also be experienced if you are passively exposed to e-cigarette vapor. If you experience these symptoms, you are free to decide if you would like to remain in the convention or exit the convention until your symptoms resolve.
- The survey questionnaires will seek self-reported health-related information. However, information provided will be kept confidential. You can also skip any questions that you do not feel comfortable responding to. Furthermore, to maintain your confidentiality, no one except the researchers conducting this study will see any of your questionnaires. Your completed questionnaires should be immediately handed directly to the research professional who will review it. All partially or completed questionnaires will be locked in a secure location in a locked file box.
- There are no foreseeable risks for urine or saliva collection. They are safe and non-invasive procedures. You are encouraged to wash your hands before and after sampling collection to maintain sanitation.
- All urine samples will be collected in public restrooms. Males, especially, may feel uncomfortable collecting a urine sample in view of other people in the restroom. If this is the case for you as a male or a female, researchers will make all reasonable efforts to locate a private bathroom or a bathroom that has enclosed stalls. Restrooms at conventions centers the researchers have visited do have enclosed stalls, so it is highly likely that accommodations exist or can be arranged for you to feel most comfortable.
- You may be asked to wear a backpack containing sampling pumps during the convention. The backpack will not be heavy and will only weigh a few pounds. If at any time you are uncomfortable and want to take the backpack off, you will be allowed to do so. We do ask that you let the researchers know when you decide to no longer wear the backpack. If you do not wish to wear the sampling pumps in the backpack at all, you will not have to**.**

Not agreeing to wear the pack of sampling pumps in this study will not affect your participation in the study.

Please Select One and Initial:

- ( ) Initial:_____1. **I agree** to wear a backpack containing sampling pumps during the convention.

OR

( ) Initial:_____ 2. **I do not agree** to wear a backpack containing sampling pumps during the convention.

- There are no anticipated risks or discomforts from information that will be obtained from the analysis of your saliva or urine.

Chemicals Identified in E-cigarette Vapor:

Formaldehyde, acetaldehyde, acrolein, nicotine, and particulate matter (PM) are the main chemicals identified and attributed to vaping liquids that have known public health effects. Nicotine can have neurological affects that aide in addiction and have developmental affects in fetuses. PM is a known irritant and carcinogen. Formaldehyde is classified as a Group 1 (human carcinogen) and acetaldehyde is a Group 2B carcinogen (possible carcinogenic to humans) by the International Agency for Research on Cancer. Acrolein can irritate the nasal cavity and lining of the lungs, which is consistent with the health effects reported by e-cigarette users. Our preliminary data indicates that the concentrations of these chemicals, with the exception of particulate matter, present at e-cigarette conventions do not exceed human health guidance values; therefore, harmful exposures are unlikely to occur. Additionally, tobacco-specific nitrosamines (TSNAs) have been identified in e-cigarette vapor in at very low levels, though not all studies have been able to detect them. TSNAs are known carcinogens at concentrations associated with typical tobacco cigarettes.

Below is a picture of the type of environment this study in which this study will occur:

Benefits

- By participating in this study, you are allowing researchers to characterize exposures that result from e-cigarette use. The results of this study will help inform you of risks associated with e-cigarette use, both as a user and a bystander.

- Secondhand exposures to e-cigarettes are not well understood, though they frequently occur. By participating in this study, you are allowing us to characterize secondhand exposures that occur in public, dense, e-cigarette environments. This characterization will inform prudent public health practice and address knowledge gaps about exposure to e-cigarettes that have existed for some time.

Privacy/Confidentiality
You will be assigned a subject ID that will only be known by the investigators and yourself. All questionnaire forms you fill out will ask for your subject ID, not your name, in efforts to protect your privacy. All completed questionnaire forms and personal information will be kept confidential and locked in a file box in a locked room. Information that links your personal name to your assigned number will be promptly shredded and disposed after data collection is completed. Your personal identifiable information will be stored on the password-protected computer of the researcher and no one else will have access to your information. The project's research records may be reviewed by the departments at the University of Georgia responsible for regulatory and research oversight. Data obtained from your salivary and urinary samples will be kept in a locked cabinet that only the researchers have access to. Only your subject ID will be on the sample and the forms with your samples' data. Data from your samples will be kept for 5 years, unless you request otherwise on the form below.

Your saliva and urinary samples will not be used for any drug screening.

Researchers will not release identifiable results of the study to anyone other than individuals working on the project without your written consent unless required by law.

Taking part is voluntary
Your involvement in the study is voluntary, and you may choose not to participate or to stop at any time without penalty or loss of benefits to which you are otherwise entitled. If you decide to stop or withdraw from the study, the information/data collected from or about you up to the point of your withdrawal will be kept as part of the study and may continue to be analyzed. Your decision to participate or not participate will have no bearing on your grade or class standing.

Reward: As a reward for participating in this study, each volunteer will receive a $25 gift card for each e-cigarette convention he or she attends. The gift card will be awarded when the participant meets the researcher the day after the e-cigarette convention to deliver the last urine and saliva sample. Additionally, volunteers that participate in all 3 conventions will receive an extra $25 gift card for a total of $100.

The researchers of this study are required to document and track all payments made to study volunteers. To do so, we will have you sign on a log sheet to acknowledge that you received a gift card for your participation. This form will be stored in the office of the Environmental Health Science Department Administrative Associate. The log will be stored in a filing cabinet. The room will be locked and secured when the Administrative Associate is not present.

REIMBURSEMENT: You will be required to register in the University of Georgia Unified Vendor Database or complete a Non-Employee Payment Form in order to receive per diem reimbursement. Researchers will send you instructions through email on how to register once you are deemed eligible for the study.

If you are injured by this research The researchers will exercise all reasonable care to protect you from harm as a result of your participation. In the event that any research-related activities result in an injury, the sole responsibility of the researchers will be to arrange for your transportation to an appropriate health care facility. If you think that you have suffered a research-related injury, you should seek immediate medical attention and then contact Luke Naeher right away at 706-542-4104. In the event that you suffer a research-related injury, your medical expenses will be your responsibility or that of your third-party payer, although you are not precluded from seeking to collect compensation for injury related to malpractice, fault, or blame on the part of those involved in the research. Expenses from car accidents and injuries from car accidents will be your responsibility or that of your third-party payer. All subjects who drive to the e-cigarette convention must have up-to-date car insurance and car registration.

Consent to bank urine and saliva samples

This requests you to allow us to store a portion of your urine and saliva sample in the Centers for Disease Control and Prevention (CDC) Division of Laboratory Services Tobacco and Volatile Organics laboratory. Your sample data will be stored in a locked cabinet at the CDC. Researchers would like to store a portion of your samples and your sample data for 5 years. In time, additional analysis of your samples and sample data may address data gaps regarding secondhand exposure assessment. Researchers would like to keep your samples and sample data for a maximum of 5 years in the event that researchers identify analysis that would address existing data gaps surrounding secondhand exposure to e-cigarettes. If you agree to have and urine and saliva samples and sample data stored for up to 5 years, you can ask that the stored samples and/or sample data be destroyed at any time after by contacting Dr. Luke Naeher at 706-542-4104. Refusal to agree to this consent to bank would in no way prevent you from participating in the study.

Please Select Two and Initial:

( ) Initial:_____ 1. **I agree** to allow a portion of my saliva and urine sample from this study to be stored so that researchers may use it for future analysis supported by the findings in this study.

( ) Initial:_____ 2. **I agree** to allow my data from this study to be stored so that researchers may use it for future analysis supported by the findings in this study.

OR

( ) Initial:_____ 3. **I do not agree** to allow my data and a portion of my saliva and urine sample from this study to be stored for future testing. These samples should not be used for anything but this study, and the samples should be destroyed one year after this study is finished.

( ) Initial:_____ 4. **I do not agree** to allow my data from this study to be stored future analysis supported by the findings in this study. These data should not be used for anything but this study, and the samples should be destroyed one year after this study is finished.

If you have questions:
The main researcher conducting this study is Luke Naeher, a professor at the University of Georgia. Please ask any questions you have now. If you have questions later, you may contact Luke Naeher at lnaeher@uga.edu or at 706-542-4104. If you have any questions or concerns regarding your rights as a research participant in this study, you may contact the Institutional Review Board (IRB) Chairperson at 706-542-3199 or irb@uga.edu.

Research Subject's Consent to Participate in Research:
To voluntarily agree to take part in this study, you must sign on the line below. Your signature below indicates that you have read or had read to you this entire consent form, and have had all of your questions answered.

_____	_____	_____
Name of Researcher	Signature	Date
_____	_____	_____
Name of Participant	Signature	Date

Please sign both copies, keep one and return one to the researcher.

APPENDIX B: SECONDHAND EXPOSURE ASSESSMENT PRE-SCREENING QUESTIONNAIRE

Electronic Cigarette Secondhand Exposure Assessment Questionnaire

Subject ID: _____ **Date:** _____

BACKGROUND

AGE: _____years **SEX** (Circle one): MALE or FEMALE

Weight: _____lbs **HEIGHT**: _____Feet _____Inches

Are you pregnant? YES or NO

1. Do you have any diagnosed respiratory problems? YES or NO

2. Are you breastfeeding? YES or NO

3. Do you have a serious medical or psychiatric condition or other condition requiring regular medication use? YES or NO

4. Do you have a history of Chronic Obstructive Pulmonary Disease (COPD), stroke, and/or cancer within the past 5 years? YES or NO

5. Have you received chemotherapy treatment within the past month? YES or NO

6. Do you have severe cardiac disease? YES or NO

7. Do you have a history of asthma or severe allergic rhinitis? YES or NO

8. Are you allergic or hypersensitive to nicotine, nicotine components, propylene glycol or vegetable glycerin? YES or NO

9. Do you have a personal history of hypertension (Blood Pressure $\geq$ *150/95 at screening after 5 min rest)?* YES or NO

SMOKING STATUS

10. Have you smoked more than 100 cigarettes during your lifetime?	YES or NO
11. Do you currently smoke cigarettes?	YES or NO
12. If YES, how many cigarettes a day do you smoke?	_____
13. Have you ever used electronic cigarettes?	YES or NO
14. If YES how many sessions (~10 puffs/session) do you vape a day?	_____
15. Do you currently smoke pipes or cigars?	YES or NO
16. If YES, how many pipes or cigars do you smoke a day?	_____
17. Do you currently use smokeless tobacco products (i.e. chew/dip)?	YES or NO
18. If YES, how many times a day do you use smokeless tobacco?	_____
19. Does anyone smoke cigarettes within your home on a regular basis?	YES or NO
20. If YES, how many cigarettes does that person smoke a day?	_____
21. Does anyone use electronic cigarettes within your home regularly?	YES or NO
22. If YES, how many vaping sessions (~10 puffs) does the person vape a day?	_____
23. Do you currently use a nicotine-replacement therapy?	YES or NO

APPENDIX C: SECONDHAND EXPOSURE ASSESSMENT EVENT ENTRY SURVEY

Electronic Cigarette Secondhand Exposure Assessment Questionnaire

To be completed immediately before leaving for the convention

Subject ID: _____ Date: _____

1. Have you vaped an e-cigarette, smoked a cigarette, chewed or dipped tobacco, or used a nicotine replacement product in the **past 6 days**? YES or NO
2. Were you around anyone smoking cigarettes within the past 6 days? YES or NO
2. **If YES,** how many cigarettes did that person smoke around you in the past 6 days? _____
3. **If YES**, when were you last around someone smoking cigarettes (day and time)? _____
4. Did anyone use electronic cigarettes around you within the past 6 days? YES or NO
5. **If YES**, how many e-cig sessions (~10 puffs/session) did that person smoke around you in the past 6 days? _____
6. **If YES,** when were you last around someone using an e-cigarette (day and time)? _____
7. Were you around woodsmoke in the past 6 days? YES or NO
8. **If YES**, how many hours were you around woodsmoke in the past 6 days? _____
9. **If YES,** when were you last around woodsmoke (day and time)? _____
10. Were you around a charcoal or gas grill within the past 6 days? YES or NO
11. **If YES**, how many hours were you around a grill the past 6 days? _____
12. **If YES**, when were you last around a charcoal or gas grill (day and time)? _____

Please list the food and drinks you have consumed in the past 24 hours:

_____

_____

_____

_____

APPENDIX D: SECONDHAND EXPOSURE ASSESSMENT EVENT EXIT SURVEY

APPENDIX D: SECONDHAND EXPOSURE ASSESSMENT EVENT EXIT SURVEY

To be completed when upon your final exit from the e-cigarette convention

Time of **exit** : _____

1. Did you rotate around to different sections of the convention room? YES or NO

2. How long do you estimate you stayed in the main convention room _____

3. What was your **primary** (>75% of your time) activity during the e-cigarette convention
   a. Walking around visiting vendors
   b. Sitting in the seated area
   c. Standing in the open vaping section
   d. Other (please explain):
   _____
   _____

4. Did you experience any health effects while attending the convention? YES or NO

5. If YES, please explain:
   _____
   _____

4. Did you use an e-cigarette during the convention? YES or NO

6. If YES, how many sessions (10 puffs/session) did you vape during the convention? ____

7. Did you use nicotine-replacement therapy during the convention? YES or NO

8. If YES, what nicotine-replacement therapy did you use and how often?
   _____

8. Did you use any tobacco product during the e-cigarette convention? YES or NO

9. If YES, what tobacco product did you use during the e-cigarette convention and how often?_____

10. Did you consume any food or beverage during the convention? YES or NO

11. Did you touch any e-juice in the convention? YES or NO

12. If YES, please list the food and drinks you consumed **DURING THE CONVENTION**
    _____
    _____
    _____

APPENDIX E: E-CIGARETTE SURVEY CONSENT FORM

UNIVERSITY OF GEORGIA
CONSENT FORM
Electronic Cigarette Survey

Researcher's Statement
We are asking you to take part in a research study conducted by the Department of Environmental Health at the University of Georgia. Before you decide to participate in this study, it is important that you understand why the research is being done and what it will involve. Your involvement in this study is voluntary, and you may choose to not participate or stop at any time without penalty or loss of benefits to which you are otherwise entitled. The findings from this survey may provide information on characterization on e-cigarette use. If you have any questions regarding this research, you can ask me now. If you have any questions regarding you rights as a participant, you can contact the University of Georgia Institutional Review Board at 706-542-3199.

This form is designed to give you the information about the study so you can decide whether to be in the study or not. Please take the time to read the following information carefully. Please ask the researcher if there is anything that is not clear or if you need more information. When all your questions have been answered, you can decide if you want to be in the study or not. This process is called "informed consent."

Principal Investigator: Dr. Luke P. Naeher
Associate Professor
Environmental Health Science Department

Purpose of the Study
The goal of this study is address current knowledge gaps regarding electronic cigarette use.

Study Procedures
If you agree to participate, you will be asked to:

- Complete a survey asking you questions about your experience using, or not using, electronic cigarettes
- Time commitment: 5-minute time commitment

Risks and discomforts

- There are no expected risks or discomfort resulting from completing this study

Benefits

- By participating in this study, you are allowing researchers to characterize e-cigarette use patterns, possible health effects resulting from e-cigarette use, and addiction patterns of e-cigarette users. This characterization will inform prudent public health practice and address knowledge gaps about exposure to e-cigarettes that have existed for some time.

Privacy/Confidentiality
We are not collecting personal identifiable information. We will *not* ask for your name, address, or birthday. All surveys will be kept confidential and locked in a file cabinet in a locked room. Once the information from the survey is coded for statistical analyses, the surveys will be shredded. The project's research records may be reviewed by the departments at the University of Georgia responsible for regulatory and research oversight.

Taking part is voluntary
Your involvement in the study is voluntary, and you may choose not to participate or to stop at any time without penalty or loss of benefits to which you are otherwise entitled. If you decide to stop or withdraw from the study, the information/data collected from or about you up to the point of your withdrawal will be kept as part of the study and may continue to be analyzed.

If you are injured by this research The researchers will exercise all reasonable care to protect you from harm as a result of your participation. The researchers do not believe there is any risk of injury by completing this survey.

If you have questions
The main researcher conducting this study is Luke Naeher, a professor at the University of Georgia. Please ask any questions you have now. If you have questions later, you may contact Luke Naeher at lnaeher@uga.edu or at 706-542-4104. If you have any questions or concerns regarding your rights as a research participant in this study, you may contact the Institutional Review Board (IRB) Chairperson at 706.542.3199 or irb@uga.edu.

APPENDIX F: E-CIGARETTE SURVEY

ELECTRONIC CIGARETTE STUDY

The University of Georgia

AGE: _____ HEIGHT: **_____WEIGHT: _____SEX (circle one):** Male or Female

QUESTION	ANSWER
1. How many times per day do you usually use your e-cigarette? (assume one "time" consists of around 15 puffs or lasts around 10 minutes)	A. 0-4 times/day B. 5-9 times/day C. 10-14 times/day D. 15-19 times/day E. 20-29 times/day F. More than 30 times/day
2. How soon after waking up do you use your e-cigarette?	A. More than 2 hours B. 61-120 minutes C. 31-60 minutes D. 16-30 minutes E. 6-15 minutes A. Within 5 minutes
3. Do you sometimes awaken at night to use your e-cigarette?	A. YES B. NO
4. If yes, how many nights per week do you typically awaken to use your electronic cigarette?	A. 0-1 nights B. 2-3 nights C. More than 4 nights
5. Do you ever have strong cravings to use your e-cigarette?	A. None/slight B. Moderate/strong C. Very strong/extremely strong

6. Over the past week, how strong have the urges to use an e-cigarette been?	A. None/slight B. Moderate/strong C. Very strong/extremely strong
7. Do you find it difficult to refrain from using an e-cigarette in places where you are not supposed to ? E.g. college campus, shopping, church, library, movie theaters, etc.	A. YES B. NO
8. When you haven't used an e-cigarette for a while or when you tried to stop using your e-cig, did you feel more irritable because you couldn't use an e-cigarette?	A. YES B. NO
9. Did you feel nervous, restless, or anxious because you couldn't use an e-cigarette?	A. YES B. NO
10. Which e-cigarette would you hate to give up?	A. The first in the morning B. Any other
11. Do you use your e-cigarette more frequently in the morning?	A. YES B. NO
12. Do you use your e-cigarette even if you are sick in bed most of the day?	A. YES B. NO
13. How long have you been using e-cigs?	A. 1-2 months B. 3 months – 1 year C. 1-5 years D. >5 years
14. Did you smoke traditional cigarettes prior to beginning the use of e-cigarettes?	A. Yes B. No

15. Do you currently smoke both traditional cigarettes and e-cigarettes?	A. Yes B. No
16. Why did you start using e-cigarettes?	A. To quit smoking or avoid relapse of smoking B. To reduce tobacco consumption with no intention of quitting smoking C. Other, please explain: _____ _____ _____ _____ _____
17. Do you plan to quit using e-cigs in the future?	A. YES B. NO
18. What level of nicotine do you most often use in your vaping fluids?	A. Vaping fluid that does not contain nicotine B. 1-12 mg/nl C. >13 mg/nl D. both 1-12 mg/nl and >13 mg/nl
19. What type of e-cigarette do you use (select all that apply)?	A. A first-generation e-cigarette that is the same size and shape as a regular cigarette B. An e-cigarette that activates the heating coil at the press of a button and allows the voltage to be controlled by the user C. Other, please explain: _____ _____ _____ _____

20. Do you believe you are less nicotine dependent on e-cigarettes compared to when you smoked traditional cigarettes?	A. Yes B. No
21. Where do you commonly smoke your e-cigarette (select all that apply)	A. Home B. In the car C. Public places (campus, shops, library, movie theaters, etc.) D. Other, please explain
22. How often do you visit vaping blogs, online forums, or vaping events?	A. At least once a week B. 1-2 times/month C. 1-3 months/year D. Once a year E. Never
23. What, if any, health effects have you experienced while smoking an e-cigarette (select all that apply)?	A. Throat irritation B. Skin irritation C. Eye irritation D. Headaches E. Nausea F. Other, please explain: _____ _____ _____ G. None
24. How often does someone physically near you (i.e. standing by, or riding in the same car) complain of health effects (irritation, nausea, headache), because of your e-cigarette?	A. Never B. Sometimes C. Often D. Frequently

25. If people around you <u>DO</u> complain of health effects (irritation, nausea, headache) when you use your e-cigarette, what health effects do they complain of?	A. Throat irritation B. Skin irritation C. Eye irritation D. Headaches E. Nausea F. Other, please explain: _____ _____ _____ _____ G. None