CHARACTERIZATION OF TOXIC METAL EXPOSURE FROM ELECTRONIC CIGARETTE USE

by
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ABSTRACT

E-cigarettes are battery-powered devices that heat a liquid solution (e-liquid) to generate aerosols. Their use has grown since 2006, with over 8.1 million US adults (3.2%) currently using e-cigarettes in 2018. Current FDA regulation on e-cigarettes is limited to ensuring consumer safety. Of particular concern is their exposure pathway to metals, as the e-liquid is heated and aerosolized by a coil commonly made up of metals, such as Ni and Cr, which are recognized inhalation carcinogens.

The goals of this dissertation were first to determine the range of metal concentrations found in e-liquids and e-cigarette aerosol samples through a systematic review; second, to describe daily exclusive e-cigarette users by collecting demographic information, use behaviors and device characteristics; third, to evaluate whether e-cigarette use is associated with metal exposure, specifically Ni, Cr, Pb, and Mn, as determined by non-invasive biomarkers (urine, saliva, exhaled breath condensate (EBC)).

<u>In the systematic review</u>, metal concentrations showed substantial heterogeneity, although notably higher in e-liquids in contact with the coil, and higher in the aerosol. With the exception of Cd, metal biomarker levels were similar or higher compared to conventional cigarette users' levels. <u>In the analysis of daily exclusive e-cigarette users</u>, most were men (64%), white (82.7%), former smokers (89%), and vaped an average of 365 puffs/day. E-cigarette use was primarily reported as an aid to quit smoking, and less than half planned to quit vaping. More intense and frequent use was found among men and individuals with lower education levels. <u>In the biomarker analysis</u>, e-cigarette users had higher Ni EBC, Pb saliva and Mn EBC, compared to non-users. Metal aerosol concentrations were positively associated with corresponding Cr urine and Ni saliva.

Certain device characteristics and behaviors of increased use were also associated with higher metal biomarker levels (Cr, Ni, Mn saliva and EBC; Ni urine).

Overall, e-cigarette use may contribute to toxic metal exposure. These findings may inform the FDA for product review and regulation, specifically implementing metal standards in e-cigarette emissions, adequate labeling of device components, and best practice for use so as to inform users and prevent unwanted metal exposure.

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PREFACE

This dissertation is the culmination of the research work conducted together with my advisors, co-authors, committee members, and collaborators throughout my doctoral studies in the Department of Environmental Health and Engineering of the Johns Hopkins Bloomberg School of Public Health. It is organized as a series of manuscripts, beginning with Chapter 1, which contains background information on the topic, the motivations behind the main objective, and the specific aims of this dissertation. A review of each of the analyses conducted, which is formatted into chapters, follows. The second chapter is a systematic review of published studies on metal concentrations of e-liquid, e-cigarette aerosols, and biomarkers of e-cigarette users across e-cigarette device systems. The third chapter compares participant characteristics and self-reported health symptoms among daily exclusive e-cigarette users and non-users in Maryland, USA, as well as the association between e-cigarette device characteristics and vaping frequency with e-cigarette user demographics. The fourth chapter, a transitional chapter, briefly links chapters three and five. The fifth chapter compares metal biomarker concentrations of Maryland e-cigarette users to non-users, and investigates the association of e-cigarette use behaviors and metal concentrations in the aerosol with metal biomarker concentrations of e-cigarette users. This dissertation ends with an overall summary of the research findings, a discussion of the strengths and limitations of the analyses as well as its research and policy implications, proposed next steps, and final conclusions.

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ABBREVIATIONS

Electronic cigarette (E-cigarette)
Electronic nicotine delivery system (ENDS)
Electronic cigarette liquid (E-liquid)
Reusable modifiable electronic cigarette (MODs)
Electronic cigarette pod systems (PODs)
US Food and Drug Administration (FDA)
Centers for Disease Control (CDC)
National Health and Nutrition Examination Survey (NHANES)
National Health Interview Survey (NHIS)
Population Assessment of Tobacco and Health study (PATH)
Agency for Toxic Substances and Disease Registry (ATSDR)
Truth Longitudinal Cohort (TLC)
Minimum risk level (MRL)
US Environmental Protection Agency (EPA)
National Ambient Air Quality Standards (NAAQS)
Interquartile range (IQR)
Geometric mean (GM)
Geometric mean ratio (GMR)
Confidence interval (CI)
Standard deviation (SD)
Particulate matter (PM)
Total particulate mater (TPM)
Ultrafine particles (UFP)
Inductively coupled plasma mass spectrometry (ICP-MS)
Inductively coupled plasma optical emission spectrometry (ICP-OES)
Atomic absorptiometry (AAS)
Exhaled breath condensate (EBC)

CHAPTER 1: INTRODUCTION

Overview

In this chapter, we review the following topics: (1) the different e-cigarette device systems and how they work, (2) the prevalence and demographics of e-cigarette users in the US, (3) the different e-cigarette heating coils and their potential to leach metals, and (4) specific toxic metals of concern. The specific aims of this dissertation follow after these topics.

E-cigarette devices and how they work

E-cigarettes are comprised of a battery, a cartridge containing the e-cigarette liquid (e-liquid), and an atomizer, which heats and aerosolizes the e-liquid (Figure 1). There are various types of e-cigarette devices and, at the time this dissertation was conducted, they were classified into closed and open systems [1] (Figure 2). The term "closed system" refers to devices where the user doesn't have access to the liquid cartridge. Closed system devices include first-generation devices often referred as 'cig-a-likes' as they resemble combustible tobacco cigarettes, and the vape pod systems often referred as PODs, which are the newest type of e-cigarette in the market. While PODs tend to be smaller than cig-a-likes, and shaped like a USB thumb drive, both types of devices have the same mechanics and are comprised of a disposable cartridge and low-capacity re-chargeable batteries. Open system devices include reusable modifiable devices (MODs) or "tank-style" devices, which are typically larger in size with a more powerful battery and adjustable voltage/wattage delivery, a larger re-fillable e-liquid reservoir (tank), and replaceable heating coils and wicks in the atomizer. All of these e-cigarette devices produce an aerosol by heating e-liquid (composed of propylene glycol, glycerin, nicotine, and other flavoring chemicals) with a metallic coil [2]. E-liquids used in open systems can be manipulated and mixed by the user. Since their introduction to the U.S market in 2006, little is known about e-cigarette's long-term health effects. Current FDA regulation on these devices is limited, specifically lacking in guidelines on quality control and labeling of device composition, ingredient listing, and manufacturing. Research pertaining to the individual characterization of e-cigarettes by type of device and by use behaviors is needed to better understand variability in generating different chemical constituents and identify potential quality control issues.

Prevalence and demographics of e-cigarette users in the US

Electronic cigarettes (e-cigarettes), since introduced in the US in 2006, have grown in popularity and in use [3, 4]. According to the Population Assessment of Tobacco and Health (PATH) study (Wave 1), a national longitudinal study of tobacco use, the prevalence of current e-cigarette use is 2.4% (5.5 million), of which 1.0% (2.3 million) used the product daily and 1.4% (3.2 million) used them some days [5]. Current e-cigarette use is more common among men, non-Hispanic whites, adults aged 18 to 24 years, those with some post-secondary education, and current smokers (dual use) [5, 6].

E-cigarette promotion is ubiquitously seen on the Internet, e-cigarette (vape) shops, and vaping events [7, 8]. At vaping expo or conventions, thousands of visitors attend and are encouraged to vape at the event, which may be held at indoor locations with poor ventilation [9]. It is here where attendees can sample the latest e-liquids, purchase new devices, and join or watch vape competitions where competitors generate the largest plume or do artistic tricks (Figure 3) [10, 11]. Social media has also served as a prominent source of exposure to e-cigarettes, particularly among youth, whose use has sharply increased since 2011 [12]. JUUL, one of the first major POD brands to rely heavily on social media to promote its products [13], has surged in popularity among youth and young adults and reached the point where the Surgeon General has issued its use as a growing epidemic [14, 15]. According to the Truth Longitudinal Cohort (TLC), current JUUL use is seen in a greater proportion of males, young adults aged 18-21 years, those who identify as lesbian, gay, or bisexual (LGB), current smokers, those living with someone who uses ENDS, and those who report living comfortably with regard to their financial situation [16].

E-cigarettes as a source of toxic metals

Research on the chemical components of e-cigarettes continues to grow. There is concern for metal exposure from the metallic coil, which heats and aerosolizes the e-liquid that is inhaled by the user. Heating coils are made of metal alloys, with the most common coils used listed below:

I. **NiChrome** wire is an alloy of nickel (Ni) and chromium (Cr) and is one of the most commonly used coils because of its ability to rapidly heat with minimal ramp-up time. It is the wire of choice for majority of the e-cigarette coils released from China and is typically used when creating large plumes of vapor, also known as cloud-chasing [17].

II. **Kanthal**, an alloy of iron, chromium, and aluminum, is also a popular wire because it is widely available, low cost, and can be bought in multiple gauges making it versatile. It is also recommended for users with a nickel allergy. Compared to Kanthal, NiChrome has a shorter lifespan because of its lower melting temperature and lower maximum operating temperature [17].

III. Other coils available in the market are **pure titanium (Ti), pure nickel, and stainless steel** [17]. Titanium coils raise a few concerns – it can heat to the point of ignition, thus making it a safety hazard, and at close to working temperatures it can form titanium dioxide powder, a 2B carcinogen, which is possibly carcinogenic to humans. Stainless steel is an alloy of chromium, nickel, and carbon.

All these types of coils have the potential to leach metals onto the e-liquid. Moreover, because most coils are likely made of complex alloys, users may be exposed to other metals beyond the ones mentioned in the label.

Apart from the coil components, other metals, such as tin, have been detected in joints of e-cigarette devices [18] as well as lead and arsenic in certain e-liquid solutions [19, 20]. A growing number of studies have found toxic metals, such as lead, nickel, and chromium in the e-liquid and in the aerosol [18, 21-24]. From one preliminary study where e-cigarette samples were collected from personal devices of daily users, levels of lead and zinc increased by more than 2000% in the aerosol compared to that found in the original liquid dispenser; levels of nickel, chromium, and tin increased more than 600% [20]. Moreover, the metal mass concentrations (mg/m^3) in collected aerosol samples were found to exceed current health-based standards by 50% or more. While some of the metals detected in the aerosol (zinc, manganese, copper) are essential elements when ingested, these metals are considered toxic when inhaled [25]. This is concerning given that many active smokers switch to using e-cigarettes in the belief that these devices are safe [26-30].

Specific metals of concern

Nickel (Ni) is a metal of particular concern since some e-cigarette heating coils are made of alloys containing nickel. The respiratory health effects from inhalation of nickel are well known [31]. Human studies have reported an increased risk of lung and nasal cancers from inhalation of nickel refinery dusts [32]. From one preliminary study, almost 60% of e-cigarette aerosol samples exceeded the Agency for Toxic Substances and Disease Registry's (ATSDR) chronic minimum risk level (MRL) of 0.0002 mg/m^3 (Figure 4) for Nickel.

Chromium (Cr) is also a key component to most e-cigarette heating coils, such as NiChrome, Kanthal and stainless steel. Hexavalent chromium (Cr VI) is a known potent inhalation carcinogen [33]. The US Environmental Protection Agency (EPA) has stated that "the classification of chromium (VI) as a known human carcinogen raises concern for the carcinogenic potential of chromium (III)" [34], particularly due to the possible oxidation of Cr (III) to Cr (VI) within the oxygen-rich environment of the lungs. Approximately 10% of the aerosol samples analyzed in the preliminary study exceeded the lowest-observed-adverse-effect level (LOAEL) of 0.002 mg/m^3 established by the EPA for the Cr (VI) compounds [34].

Although an essential nutrient when ingested, **manganese** (Mn) has been linked to irreversible Parkinson-like disease known as manganism through inhalation exposure [35]. Mn levels measured in the preliminary study are potentially part of the coil from Cr and stainless steel alloys. Although none of the aerosol samples exceeded the LOAEL of 0.05 mg/m^3, 78% of the samples were above EPA's Reference Concentration (RfC) of $5x10^{-6}$ mg/ m^3.

While there has been a reduction in population exposures because of broad public health interventions, continued research on **lead** (Pb) demonstrates significant increases in risk of adverse health outcomes [36-39]. While it is not disclosed as a component in the coil or other parts of e-cigarette devices, lead was detected in 95% of aerosol samples of our preliminary study. Fifty percent of these samples exceeded the EPA's National Ambient Air Quality Standards (NAAQS) of 0.00015 mg/m^3. It is suspected that these lead containing samples are a result of soldering materials that are in contact with the e-liquid.

Hypotheses and specific aims

Few studies have evaluated the components of e-cigarettes and whether the e-liquid composition changes once in contact with the device. Of particular concern is their potential as an exposure pathway to metals, as the e-liquid is heated and aerosolized by a coil commonly made up of metals, such as nickel (Ni) and chromium (Cr), which are recognized inhalation carcinogens [2, 40-43]. A small but growing body of evidence shows that e-cigarette aerosols contain relatively high levels of toxic metals [20, 23, 24, 30, 44, 45]. Our preliminary studies indicate marked increases in metal concentrations in the generated aerosol compared to the e-liquid from the refilling dispenser, demonstrating that metals are transferred from the device to the aerosol [46].

Informed by the evidence base, I **hypothesized** that

(1) Metal concentrations measured in the e-liquid and aerosols show large variation, but are relatively higher among samples that are in contact with the heating coil of the device,

(2) More intense use behaviors and device characteristics are seen in participants with certain socio-demographic characteristics,

(3) Compared to non-users, e-cigarette users report more respiratory and cardiovascular health symptoms, and

(4) Certain e-cigarette device characteristics and use patterns determine levels of metal exposure, and metal exposure among e-cigarette users is higher than that of non-users and non-smokers.

The **main objective** of this project was to evaluate the association of e-cigarette use behaviors and device characteristics with metal exposure and compare the levels of exposure to those of non-users.

The **specific aims** were the following:

1. **Determine the range of metal concentrations in e-liquids (bottles, cartridges), e-cigarette aerosols, and biomarkers of e-cigarette users.** A systematic review of published studies was conducted to determine the range of metal concentrations that have been reported in e-liquids (bottle, cartridges, other), e-cigarette aerosols, and biomarkers of e-cigarette users across the different e-cigarette device systems. Differences in metal level generation according to sample (e-liquid/aerosol), source of sample (dispenser bottle/cartridge/open wick/tank), and device type (open/closed system device) were reported.

2. Evaluate the demographic characteristics, perceptions of e-cigarette safety, and self-reported health status among e-cigarette users in Maryland. Questionnaire data on demographic characteristics, e-cigarette device characteristics, use behaviors (preferred nicotine concentration, volume of e-liquid consumed/week, frequency of coil change), self-reported health status were collected from daily exclusive e-cigarette users and non-users. Participant characteristics and self-reported health symptoms were compared between users and non-users. The association of e-cigarette device characteristics, vaping frequency, and e-liquid nicotine concentrations with e-cigarette user demographics was assessed.

3. **Investigate the contribution of e-cigarette use patterns that are associated with increased metal exposure among users in Maryland.** In addition to questionnaire data, biomarkers (urine, saliva, and exhaled breath) were collected from both Maryland e-cigarette users and non-users. The association of e-cigarette use patterns and metal concentrations in the aerosol with metal biomarker concentrations were assessed; metal biomarker levels of e-cigarette users were compared to those of non-users.

To achieve these aims, 100 e-cigarette users (includes previous smokers who have quit for at least 6 months) and 50 non-users/non-smokers were recruited. Samples of their e-liquid and e-cigarette aerosols were collected from their personal devices. Non-users/non-smokers were among friends or colleagues of e-cigarette users or of similar sociodemographic background in order to maximize comparability in socio-demographic and lifestyle factors, which can be determinants of metal exposure. For both groups, urine, saliva, and exhaled breath were collected. With the US Food and Drug Administration (FDA) recently extending their authority of tobacco products to cover e-cigarettes and with the FDA Center for Tobacco Products calling for research on e-cigarette toxicity [47], this dissertation, which evaluated the potential leaching of toxic metals from e-cigarette components to the aerosol and, ultimately, the user, may inform the FDA for product review and policy-level interventions. This work may also inform the Centers for Disease Control and Prevention (CDC)'s NHANES collection efforts to include more detailed questions pertaining to e-cigarette use behaviors and device characteristics.

FIGURES

Figure 1. Parts of an Electronic cigarette (e-cigarette)

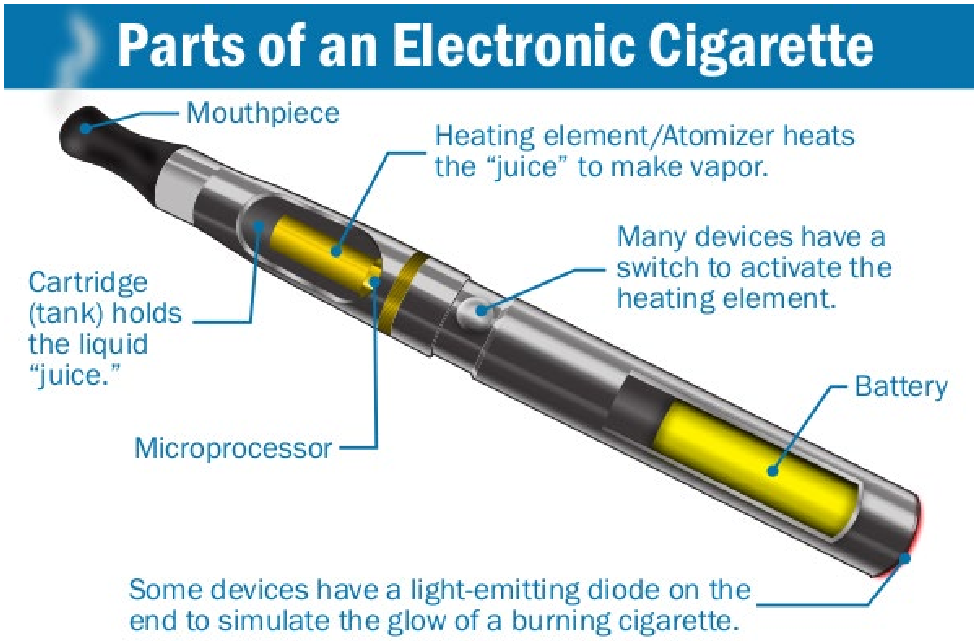


Image source: https://www.usfa.fema.gov/downloads/pdf/publications/electronic_cigarettes.pdf

Figure 2. The different e-cigarette device systems. Closed-system devices comprise rechargeable and disposable e-cigarettes. Open-system devices include tanks and Mods.

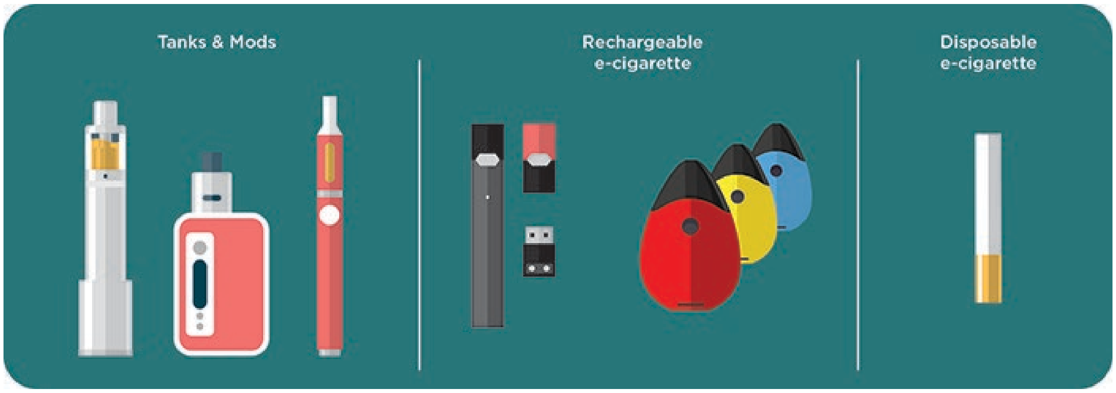
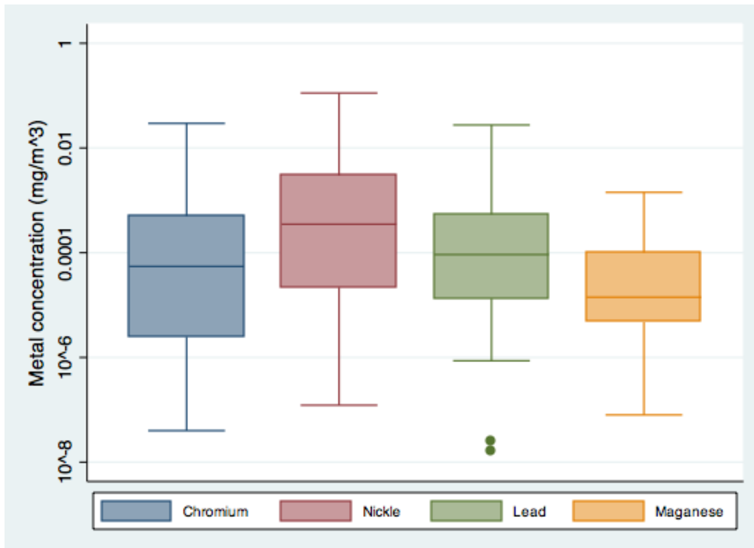


Image source: https://www.cdc.gov/tobacco/basic_information/e-cigarettes/about-e-cigarettes.html

Figure 3. Vaping competition in Baltimore, MD, April 2016. Jarmul et al. *Am J Public Health* 2016.

Figure 4. Metal concentrations in e-cigarette aerosol from preliminary study. *Olmedo et al Env Health Perspectives, 2018.* Samples were taken from 2nd or 3rd generation devices.



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CHAPTER 2

Metals in Electronic Cigarettes: A Systematic Review

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ABSTRACT

Background: Electronic cigarettes (E-cigarettes) are rapidly growing in popularity among youth. An increasing number of studies have found toxic metals in e-cigarette emissions. The objective of this study is to provide a systematic review of published studies on metal concentrations in e-liquid, e-cigarette aerosols, and biomarkers of e-cigarette users across e-cigarette device systems.

Methods: We searched PubMed/TOXLINE, Embase, and Web of Science and identified 24 studies on metals in e-liquid, e-cigarette aerosols, and biomarkers of e-cigarette users. For metal concentrations in e-liquid and aerosol samples, we collected or derived the mean and standard deviation. Metal concentrations in e-liquids and aerosols were converted and reported in μg/kg and ng/puff for easy comparison.

Results: Twelve studies reported metal concentrations in e-liquids (bottles, cartridges, other), twelve studies reported metal concentrations in e-cigarette aerosols (from *cig-a-likes* and open system devices), and four studies reported metal concentrations in biomarkers of e-cigarette users. Metal concentrations showed substantial heterogeneity depending on sample type, source of e-liquid, and device type. Metals in biomarkers of e-cigarette users were similar or higher compared to conventional cigarette users for most metals, and higher compared to cigar users.

Conclusion: Metal concentrations in e-liquid and aerosols varied largely. Metal concentrations in e-liquid from cartridges or tank/open wicks were higher than those from dispenser or bottle, possibly due to coil contact. Metal concentrations in the aerosol were generally higher than in the e-liquid samples. Biomarker studies indicate that e-cigarettes are a potential source of exposure to metals with the exception of cadmium.

INTRODUCTION

E-cigarettes are battery-operated devices that generate aerosols with or without nicotine by heating a liquid solution (e-liquid) with a metallic coil [1, 2]. The number of current e-cigarette users among US middle and high school students has increased from 2.1 million in 2017 to 3.6 million in 2018 [3]. The appealing flavors and perception of safety contribute to their popularity [4-6]. E-cigarettes, however, are not toxic-free. Numerous studies have measured elevated levels of toxic organic and inorganic chemicals in e-cigarettes[7-20].

The presence of metals and metalloids (e.g., arsenic, chromium, lead, nickel) in e-cigarette aerosol is a major concern given their serious health effects including cancer, cardiovascular disease, renal damage, and neurotoxicity [9, 21-24]. Metal exposure may originate from the coil [4, 25] but also from soldered joints and other parts of the device [26]. Commonly used coils are made of alloys (e.g., Kanthal (iron, chromium, and aluminum), and Nichrome (nickel and chromium)) or high purity metal (e.g., nickel or titanium) [4, 25]. Tin and other metals are used in solder joints [27].

The contribution of e-cigarettes to metal exposure is not fully understood, particularly because of the rapidly changing nature of devices and e-liquids. E-cigarette devices are classified into closed and open systems [28]. Closed system devices (e.g. first generation *cig-a-likes* and the recent *PODs* such as JUUL) are non-refillable, use low-voltage batteries, and are commonly used by youth and new e-cigarette users [29, 30]. Most studies on metals in closed system devices used *cig-a-likes*, except a recent study using JUUL products [9]. Open system devices (e.g. e-pen models and tank-like systems) are refillable, have adjustable power (modifiable e-cigarettes (mods)), and are commonly used by former smokers [31]. While a relatively large number of studies have measured metals in e-cigarettes, the individual studies are characterized by a small number of e-liquids, device types, and sample sizes. The objective of this systematic review is to determine the range of metal concentrations in e-liquids (bottle, cartridges, other), e-cigarette aerosols, and biomarkers of e-cigarette users across e-cigarette device systems to better understand the metals and metal levels e-cigarette users are exposed to, and the potential implications on health outcomes.

METHODS

Data source and search strategy

We searched PubMed/TOXLINE, Embase, and Web of Science through July 19, 2018 using keywords and Mesh terms listed in Supplementary file 1. Two research groups conducted the initial search independently, and both searches were combined removing duplicates before manuscript screening (Figure 1). Three authors conducted manuscript screening (DZ, AA1 and AA2), followed by full text reviews conducted individually by two authors (DZ and AA1). Conflicts regarding manuscripts to include and data abstraction were resolved through review of the original manuscripts and consensus among four other authors (AA2, ANA, AR, MH). We included studies published between January 2008 and December 2018. To be included, studies must have quantified metal levels in e-cigarette liquids, e-cigarette aerosols, and/or biomarkers from e-cigarette users. E-liquid was classified as coming from the bottle dispenser (with no contact from the coil and used with open system devices), from cartridges or *PODs* (in which the e-liquid is in contact with the coil and used in closed system devices), and from other sources (open wick and tanks, where the e-liquid is in contact with the coil and the samples were often collected after vaping the device). Studies measuring metals only in indoor air (reflecting secondhand exposure to e-cigarette aerosol) were excluded from this review [32-36]. We placed no restriction on the type or generation of e-cigarette device and/or e-liquid, the method of sample collection, or the method of metal analysis. Secondary data and reviews were excluded.

The search strategy retrieved a total of 614 individual studies (Figure 1), including 3 studies identified through hand search [37-39]. After abstract and full text review, a total of 24 individual studies met the inclusion criteria. Among those 24 studies, 12 reported data on metals in e-liquid (9 from bottle, 4 from cartridges (3 from *cig-a-likes* and 1 from *POD)*, 1 from an open wick, 1 from both bottles and cartridges, and 1 from the tank after heating); 12 reported data on metals in e-cigarette aerosol (8 from closed system devices (all *cig-a-likes*), 3 from open system devices, and 1 from both closed and open system devices); and 4 reported data on metals in biomarkers of e-cigarette users.

Data abstraction and summary data

For each study, the following data were collected: first author, year of publication, source of e-cigarettes/e-liquids (e.g. online, local outlet, manufacturer), device/e-liquid brand, device type (open system device, closed system device (distinguishing between *cig-a-likes* and *PODs*)), e-liquid container (bottle, cartridge, open wick, tank), e-liquid flavor, nicotine content, puffing protocol, type of coil (Nichrome, Kanthal, other, not reported), whether the study accounted for background concentration or not (considered not done if not mentioned), sample size, analytical methods for metal determination, and summary metal concentrations. If the information was not available in the published manuscript, we contacted the study authors. In e-liquid and aerosol samples, we collected or derived the mean and standard deviation (SD). If metal concentrations were below the limit of detection (LOD), we replaced them by the LOD/√2. For biomarker samples, we collected the median and interquartile range (IQR) or the geometric mean (GM) and 95% CI. The number of metals analyzed across studies was diverse and some metals were only analyzed in one or two studies. In the tables for e-liquid and aerosol samples (shown separately), we prioritized metals that were analyzed in at least three studies: Aluminum (Al), antimony (Sb), arsenic (As), cadmium (Cd), cobalt (Co), chromium (Cr), copper (Cu), iron (Fe), lead (Pb), manganese (Mn), nickel (Ni), selenium (Se), tin (Sn), and zinc (Zn). In the results section, we reported the range of mean metal levels for e-liquid samples grouped based on the source of the e-liquid (bottle, cartridge, other) and for aerosol samples grouped based on device type (closed system and open system devices). The median of the mean in each of those groups was reported if there were 3 studies or more. For biomarker studies, both the summary statistics and the results of adjustment models are reported in the tables and result section.

For manuscripts reporting data with and without background correction [15], we reported values that accounted for background metal levels (subtraction of metal levels assessed in blanks or controls to account for interference or external contamination). For nicotine content, if only the fraction volume of nicotine (%) was provided, nicotine density of 1.01 g/ml [40] was used to convert this number into a mass concentration (mg/ml). For the study that reported two masses ($PM_{0.1}$ and $PM_{0.1\text{-}2.5}$), we kept only the $PM_{0.1}$, as it is likely inhaled deep into the lungs, and metals were not detected in the $PM_{0.1\text{-}2.5}$ [20]. Some studies reported data in a figure but not quantitatively [14, 27]. For those studies, we used an automated program to infer the underlying mean (SD) values

(Origin 9.0, OriginLab Corporation, MA, US). Other studies did not report the mean (SD) but reported metal levels for individual samples [8, 41]. For these studies, we calculated the mean and SD. For studies reporting the mean (SD) for multiple groups (e.g. by nicotine concentrations, by different flavors) [10, 12, 18, 23, 42], we calculated the weighted mean and total SD to facilitate the summary and comparison across studies and device types after confirming there were no major differences across flavors and nicotine levels. The total SD was estimated as it accounts for the SD within the groups as well as among the groups [43]. For some studies, the published data was insufficient to estimate the SD and only means are reported with no estimation of variability around those point estimates [8, 9, 15, 17, 19, 20, 26, 44]. The study by Olmedo et al. (2018) did not report means (SDs) in the original publication but we calculated them directly from the original data.

Most studies of e-liquids reported metals in µg/kg. For easy comparison, for studies reporting e-liquid metal concentrations in µg/L [8, 11, 15, 20, 23, 42] and ppb [9, 17, 41], the concentrations were converted to µg/kg, assuming the e-liquid density is 1.16 g ml^{-1} [45]. Most studies of aerosols reported metals in ng/puff. For studies reporting aerosol metal concentrations in different units [8, 10, 14, 15, 19, 20, 25-27], those were converted to ng/puff. The study by Mikheev et al. (2016) reported aerosol metal concentrations in ng/mg of total particulate matter (TPM), we used the average mass of TPM/puff of 2 mg to convert ng/mg TPM to ng/puff. The study by Olmedo et al. (2018) reported aerosol metal concentrations in µg/kg. First, we converted µg/kg to mg/m^3 using the equation described in the original paper, and then converted mg/m^3 to ng/puff using the conversion factor of 6.67×10^{-5} m^3/puff. The study by Zhao et al. (2018) reported aerosol metal concentrations (ng/ml) in particulate matter with aerodynamic diameter of ≤0.1 µm ($PM_{0.1}$). To facilitate the comparison with the other studies, we estimated the mass of metal per puff from the total aerosol mass (measured in $PM_{0.1}$) collected in 10 minutes divided by the number of puffs (17.65 puffs based on an inter-puff time of 30 s and puff duration of 4s) and multiplied by the metal mass fraction.

RESULTS

Metal concentrations in e-liquids

Twelve studies published between 2015 and 2018 met the inclusion criteria for analysis of metals in e-liquids (Table 1). E-liquid for metal analysis were collected from the bottle (no contact with the heating coil) in 9 studies, from the cartridge (*cig-a-likes*) in 3 studies, from the *POD* (JUUL) in one study, from the open wick (open system device) in one study, from both bottle and cartridge reported together in one study, and from the tank after heating the aerosol (open system device) in one study (the sum of these types of samples is higher than 12 as some studies collected multiple types of samples). E-cigarettes were obtained from the manufacturer, local or online stores, or e-cigarette users. The studies assessed between 1 and 10 e-liquid brands, and between 1 and 9 flavors. The reported nicotine concentrations ranged from 0 to 24 mg/ml. The number of different e-liquid samples ranged from 1 to 56, and the total number of samples ranged from 3 to 132. Eight studies utilized inductively coupled plasma mass spectrometry (ICP-MS) to quantify metals in e-liquids; others used atomic absorptiometry (AAS) [9], total reflection X-ray fluorescence [12], and molecular fluorescence [23, 42]. Three studies used a mixture solution of propylene glycol and glycerol as blank e-liquid to assess matrix effects [15, 25, 41]. Other studies did not report metal background correction.

Among nine studies reporting metal concentrations (μg/kg) in e-liquid from bottles (Table 1; Figure 2), the mean ranged from 6.6 to 15.0 (median 10.6) in four studies that reported Al; from undetectable to 3.6 (median 0.9) in six studies that reported As; from undetectable to 12.6 (median 0.2) in seven studies that reported Cd; from 0.1 to 0.2 (median 0.2) in three studies that reported Co; from 1.6 to 8.4 (median 5.4) in four studies that reported Cr; from undetectable to 20.0 (median 12.6) in five studies that reported Cu; from 3.5 to 65.2 in two studies that reported Fe; from 0.1 to 6.2 (median 1.7) in four studies that reported Mn; from undetectable to 28.9 (median 9.8) in seven studies that reported Ni; from undetectable to 10.5 (median 0.9) in eight studies that reported Pb; from 0.9 to 6.2 (median 1.2) in three studies that reported Sb; and from undetectable to 130 (median 81.5) in four studies that reported Zn.

Among three studies reporting metal concentrations (μg/kg) in e-liquids from cartridges (Table 1), one reported As (mean was undetectable), two reported Cd (mean ranged from undetectable to 176), and two reported Pb (mean ranged from undetectable (both in a cartridge from a cig-a-like and from a *POD*) to 1694), one reported Cr, Mn, and Ni from 5 brands (mean ranged from 46.4 to 1815 (median 199) for Cr, 24.7 to 5943 (median 172) for Mn, and from 50.5 to 19436 (median 398) for Ni).

One study reported a mean Zn concentration of 220 μg/kg in e-liquid from both bottles and cartridges [20]. One study reported Pb in e-liquid from an open wick (mean 202 μg/kg), which is in contact with the coil [9]. One study reported metals in e-liquid left from the tank after heating (means were 101 for Al, 4.2 for As, 0.4 for Cd, 10.8 for Co, 214 for Cr, 1990 for Cu, 1880 for Fe, 124 for Mn, 2510 for Ni, 517 for Pb, 3.6 for Sb, and 3250 for Zn [25]).

Metals in aerosols of e-cigarette

Twelve studies published between 2013 and 2018 met the inclusion criteria for metals in e-cigarette aerosols generated by closed system devices (all *cig-a-likes*) (n=8), open system devices (n=3), and both closed and open system devices (n=1) (Table 2). E-cigarettes were obtained from the manufacturer, local or online stores, or e-cigarette users. The studies assessed between 1 and 11 e-liquid brands, and between 1 and 7 flavors. Nicotine concentrations ranged from 0 to 45 mg/ml. The puffing protocols to collect the aerosols were widely different, although seven studies used 4-second puffs. The total number of puffs ranged from 4 to 150. Background metal concentrations were used to correct aerosol metal levels in all studies except in Lerner et al. (2015). Ten studies utilized ICP-MS or inductively coupled plasma optical emission spectrometry (ICP-OES) and two studies used AAS [44, 46] to quantify metal concentrations. The number of different devices evaluated ranged from 1 to 56, and the total number of aerosol samples ranged from 3 to 108.

For studies reporting metal concentrations (ng/puff) in aerosols from *cig-a-likes* (n=8)(Figure 3), the mean ranged from 1.3 to 39.4 in two studies that reported Al; from undetectable to 0.6 (median 0.1) in five studies that reported As; from undetectable to 0.6 (median 0.6) in three studies that reported Cd; from undetectable to 4.0 (median 0.65) in six studies that reported Cr; from undetectable to 117 (median 8.0) in seven studies that reported Cu; from 0.8 to 52 (median 4.2) in three studies that reported Fe; from undetectable to 0.2 (median 0.2) in three studies that reported Mn; from undetectable to 2.0 (median 0.5) in seven studies that reported Ni; from undetectable to 1.7 (median 0.8) in four studies that reported Pb; from 0.3 to 0.7 in two studies that reported Sb; from undetectable to 5.3 (median 0.99) in three studies that reported Se; from undetectable to 88.6 (median 1.9) in six studies that reported Sn; from undetectable to 12.3 (median 4.8) in six studies that reported zinc.

For studies reporting metal concentrations (ng/puff) from aerosols of open devices (n=3), the mean ranged from 0.02 to 290.4 in two studies reporting Al; from undetectable to 0.13 (median 0.13) in three studies that reported As; from undetectable to 0.1 (median 0.0001) in three studies that reported Cd; from 0.07 to 7 in two studies that reported Cr; from undetectable to 0.05 in two studies that reported Cu; from 0.07 to 0.39 in two studies that reported Fe; from undetectable to 0.01 in two studies that reported Mn; from 0.32 to 14.5 in two studies that reported Ni; from undetectable to 2.7 (median 0.08) in three studies that reported Pb; from 0.002 to 0.7 in two studies reported Sb; from 0.54 to 61.9 in two studies that reported Zn, and it was 0.02 on one study that reported Sn.

For studies reporting metal concentrations from aerosols of both *cig-a-likes* and open system devices together (n=1), the mean Al, Cu, Fe, Mn, Ni, Pb, and Zn concentrations were 0.98, 0.98, 0.44, 0.01, 0.05, 0.21, and 0.65 ng/puff, respectively.

Metals in biomarkers of e-cigarette users

Four studies reported metal concentrations in biomarkers of e-cigarette users [7, 37-39] (Table 3). Aherrera et al. (2017) recruited 64 daily e-cigarette users from Maryland, USA (5 users of *cig-a-like* devices and 59 users of *MOD* devices). Badea et al. (2018) recruited 34 e-cigarette users (device type not reported) as well as 58 non-smokers and 58 conventional cigarette smokers from Brasov, Romania. Goniewicz et al. (2018) used data of 5105 US adults (247 e-cigarette users, 2411 cigarette smokers, 792 dual users, and 1655 never tobacco users) from the Population Assessment of Tobacco and Health Study in the US (PATH 2013-2014). Jain (2018) used data from cigars, cigarettes, and e-cigarettes users from the 2013-2014 National Health and Nutrition Examination Survey (NHANES) in the US (23 e-cigarette users, 417 conventional cigarette users, and 43 cigars users). All studies used ICP-MS. The number of e-cigarette users across the studies ranged from 23 to 247.

Among studies reporting metal concentrations in urine (n=3) [7, 38, 39], most metals (As, Ba, Be, Cd, Cr, Mn, Mo, Ni, Pb, Sb, Sn and W) were only reported in one study. The GM in urine of e-cigarette users were 0.3 μg/L [39](and 0.58 μg/g creatinine [38]) in two studies that reported Co; 114 μg/L [39] and 119 μg/g creatinine [38] in two studies that reported Sr; 0.1 μg/L [39] and 0.17 μg/g creatinine [38] in two studies that reported Tl; and undetectable [39] and 0.007 μg/g creatinine [38] in two studies that reported U. In adjusted models in NHANES, urinary Ba, Co, Mo, Sb, Sn, Tl levels were higher in e-cigarette users compared to cigar users but similar compared to cigarette smokers; urinary Sr levels, however, were higher in e-cigarette users compared to both cigar and cigarette smokers [39]. In the PATH study, urinary Be, Co, Mn, Pb, Sr, Tl, and U were similar in e-cigarette users compared to conventional cigarette smokers, while urinary Cd concentrations of e-cigarette users were significantly lower than cigarette smokers [38]. Neither PATH nor NHANES have measured nickel or chromium.

Among studies reporting metal concentrations in serum (n=2) [37, 39], most metals (Ag, As, Ba, Be, Cd, Co, Fe, Hg, Mn, Mo, Ni, Pb, Pd, Sb, Sn, Sr, Th, Tl, U and V) were only reported in one study. Two studies reported Cu in serum of e-cigarette users (median 892 μg/L [37] and GM 106 μg/L [39]); two studies reported Se (median 88.0

μg/L [37] and GM 131 μg/L [39]); and two studies reported Zn (median 871 μg/L [37] and GM 60.9 μg/L [39]). In NHANES, serum Cu and Se were higher in e-cigarette users compared to both cigar and cigarette users in adjusted models, even though the results were not statistically significant [39]. In e-cigarette users from Romania, Ag, Se, and V were higher among e-cigarette users compared to non-users and cigarette smokers [37].

One study reported Cr and Ni in urine, saliva and exhaled breath condensate (EBC) (μg/L) of e-cigarette users [7]. This is the only study correlating measures of metals reported in the aerosol of the e-cigarette devices used by the users with metal levels in urine, saliva and EBC. Compared to the lowest tertile, participants in the two highest tertiles of aerosol Ni showed 16% and 72% higher urinary Ni (p-trend 0.03), and 202% and 321% higher saliva Ni (p-trend 0.01) while no association was found with EBC (adjusted for sociodemographics). For aerosol Cr, the corresponding comparison showed 98% and 193% higher saliva Cr (p-trend 0.02) with no association with EBC. In NHANES, e-cigarette users had significantly higher blood Mn levels compared to cigar users in adjusted models (p-trend 0.02) [39].

DISCUSSION

Numerous metals/metalloids – Al, Sb, As, Cd, Co, Cr, Cu, Fe, Pb, Mn, Ni, Se, Sn, and Zn—are present in e-cigarette samples in the studies reviewed. For most metals, levels were heterogeneous according to sample (e-liquid/aerosol), source of the sample (dispenser bottle/cartridge/open wick/tank), and device type (open/closed system device). Metal levels in e-liquid samples not in contact with the heating coil (dispenser bottle) were generally lower than most e-liquid samples collected from cartridges or from open wicks/tanks, which have already been in contact with the coil. Studies on aerosol samples, which are particularly important as these samples reflect metal concentrations inhaled by the user, show elevated metal levels in samples from both open system devices as compared to *cig-a-likes* (the only closed system device available for aerosol samples). Biomarker studies support that e-cigarettes are a major source of metals as most metal biomarker levels, with the exception of Cd, are similar or even higher in e-cigarette users compared to conventional cigarette users, and higher compared to cigar users. The direct comparison of metal aerosol levels to biomarker levels [7] also provides direct support that the metals in the aerosol are inhaled and absorbed by the e-cigarette user.

Metals such as Al, Fe, Ni, and Zn were consistently found in studies looking at e-liquids and aerosols, while Cr, Cu, and Pb were more consistently found in aerosols. Notably, Cd levels were low and even undetectable in both e-liquid and aerosol samples in several studies. Only four studies compared metal levels measured in the e-liquid and the corresponding aerosol from the same device [8, 15, 20, 25]. These studies are particularly important as they allow us to compare changes in metal levels before and after the e-liquid is in contact with the device, which can contribute to identifying the source and processes that determine metal contamination in e-cigarettes. With the exception of Beauval et al. (2017), where metal levels in the aerosol were comparable to those of the e-liquid, studies found markedly higher levels in the aerosol than in the e-liquid. Zhao et al. (2018) only detected Zn in the e-liquid formulation but found Al, Cr, Cu, Fe, Mn, Ni, Pb and Zn in aerosols. Similarly, Palazzolo et al. (2017) found higher Al, As, Ni and Zn in aerosols compared to the liquid before aerosolization. Olmedo et al. (2018) reported markedly higher metal concentrations in the aerosol, with Pb and Zn aerosol levels 25 times higher, and Cr, Ni, and Sn levels 6 times higher than levels in the dispenser samples. Even higher metal concentrations were found in the remaining e-liquid from the tank after vaping, with Cr, Cu, Ni, Pb, and Zn aerosol levels being more than 35 times higher than levels in the dispenser.

In comparison to conventional cigarettes, e-cigarette aerosols may result in less exposure to Cd but not to other toxic metals found in tobacco. In the United States, the highest metal concentrations in mainstream smoke of conventional cigarettes were for Cd (<5.0 - 80 ng/cigarette) followed by Pb (<5.0 – 23 ng/cigarette), while other metal levels were markedly lower (As, Co, Cr, Mn, Ni) or undetectable (Ni, Cr) [48]. In closed system devices (Blu *cig-a-likes*), Cu levels were 6.1 times higher than conventional smoke [46], and in open system devices, concentrations of Cr and Ni were higher in e-cigarettes while Pb and Zn were similar to those of conventional cigarette smoke [49].

Comparisons between e-cigarette users and cigarette smokers have also been drawn in biomarker studies and reveal comparability in metal biomarker levels. From two US nationally representative datasets, there were no statistically significant differences in urinary Ba, Be, Co, Mo, Mn, Sb, Sn, Tl levels between e-cigarette users and cigarette smokers [38, 39], except for urinary Sr levels, which were higher among e-cigarette users compared to cigarette smokers and cigar users [39], and urinary Cd levels, which were significantly lower in e-cigarette users [38]. Other types of metal biomarkers show higher levels among e-cigarette users as serum Ag, Se, and V levels were higher compared to cigarette smokers [37], and blood Mn were higher compared to cigar users [39]. Only one study compared aerosol metal levels to corresponding metal biomarker levels and found positive associations between Ni and Cr levels in the aerosol with urine Ni and saliva Cr levels, respectively [7], providing direct support that metals in the aerosol are absorbed by the e-cigarette user. E-cigarette use behaviors may influence metal exposure as e-cigarette users who changed their heating coil more frequently and consumed more e-liquid per week were associated with higher urinary Ni levels [7], and being a "daily" e-cigarette user versus a "some day" user had significantly higher urinary Pb and Sr levels [38].

As indicated, most e-liquids sampled from cartridges or from tanks/open wicks that were in contact with the coil had higher metal level concentrations compared to e-liquids sampled from the dispenser. Numerous studies have shown that e-liquids in contact with heating coils like Nichrome or Kanthal [15, 19, 20, 25-27] facilitate leaching of metals into the liquid present in the tank/cartomizer. Other device components may also transfer metals into the e-liquid as the presence of brass clamps and copper wires with silver coatings have been associated with higher Zn, Cu, Ag, and Al in the aerosol. Furthermore, the presence of solder joints of poor quality or with signs for fraying was associated with higher Sn levels [19, 26, 27], emphasizing that poor manufacturing techniques [50] have a notable contribution to potential metal impurities that may reach the user. The e-cigarette user's vaping regimen, which includes modifications in voltage, resistance, temperature, puff duration, may also play a role in the degradation of the heating coil and other metal elements, and in turn modify the aerosol composition and degree of metal exposure, although few studies have evaluated their contribution.

Inhaled metals are rapidly absorbed through the respiratory tract [51] and those that were detected in the studies on this review have been associated with serious adverse health effects. For instance, long-term inhalation of nickel hydroxide nanoparticles induced oxidative stress and inflammation in lung tissues in mice [52] and inhaled Ni exposure induced rhinitis and sinusitis in humans [51]. Ni and Cr (VI) are established inhalation carcinogens [53, 54] and have also been associated with decreased lung function, increased risk of asthma, bronchitis [51], and cardiovascular disease [55]. While total Cr is reported in these studies, there is concern for Cr (III)'s carcinogenic potential due to the possible oxidation of Cr (III) to Cr (VI) within the oxygen-rich environment of lungs [56]. Pb, which only requires low levels of exposure to result in health effects [57], is associated with increased risk for cardiovascular and kidney disease and is a major neurotoxicant particularly for children and the aging population [58, 59]. Mn, which is an essential nutrient through ingestion, has been linked to an irreversible Parkinson-like disease known as manganism if inhaled [60]. Cu is known to cause respiratory irritation, coughing, sneezing, chest pain, and runny nose [61]. In an *in vitro* study, exposure to Cu nanoparticles from e-cigarette aerosols increased mitochondrial oxidative stress and DNA fragmentation [62]. Exposure to Al at high levels can lead to impaired lung function and fibrosis as well as decreased performance in motor and cognitive function [63]. Fe can produce metal fume fever, siderosis, and fibrosis [64] while Zn can cause chest pain, dyspnea, metal fume fever and shortness of breath [65]. Lastly, arsenic is highly toxic to numerous organs and body systems, and exposure to inorganic As is associated with cancer and cardiovascular disease [22, 66]. The health effects of metals through inhalation have mostly been studied in occupational settings. While the exposure pattern in occupational settings might be different from chronic e-cigarette exposure, Olmedo et al. (2018) have reported that close to 50% or more of their aerosol samples from daily e-cigarette users from Maryland exceeded current health-based limit concentrations for Cr, Mn, Ni, and Pb.

This systematic review has several limitations. A major issue was the differing puffing protocols –from varied puff counts, seconds/puff, and the puff volume across all studies ranging from 13-70 ml. Some studies left out important aspects of their protocols such as puff flow rates, number of samples analyzed, and the limit of detection or quantification. There is a great need to standardize the reporting of vaping conditions in the study of e-cigarette contaminants. Other studies reported their findings using graphics (box blots, bar graphs, pie charts), which provided rough estimations as opposed to exact values. Some studies only reported means, which limited our analysis in the spread of data. Background correction after measuring blanks was sometimes missing or unclear, particularly in studies measuring metal concentrations in aerosols. We recommend reporting blank or control corrected metal levels. Particularly for the biomarker studies, some had a small sample size, lacked a control group, and based their analysis of e-cigarette use on one question, without sufficient information on the frequency of use or the type of device. Notwithstanding these limitations, this review has several strengths. This is the first review of its kind to analyze metal levels in e-liquids, cartomizers and tanks, aerosols and biomarkers in such detail and compare across studies standardizing units as much as possible. We strove to include all information presented to identify the metals of concern, the devices and sources of e-liquids that give off relatively higher metal levels, and the levels in comparison to conventional cigarettes. Lastly, this review has identified the need for standardization both in the conduction of the experiments, such as puffing protocols and accounting for background contamination, and in the reporting of the findings (units, measures of central tendency and variability) as this would aid in a more straightforward analysis in future e-cigarette studies.

CONCLUSIONS

Overall, the number of studies consistently support that e-cigarettes are a major concern for exposure to toxic metals. There is substantial heterogeneity across products and, in particular, e-liquids that are in contact with the heating coil. There is also evidence that aerosols have higher metal concentrations than those found in the e-liquids. These findings indicate that higher metal concentrations in aerosol samples are at least in part due to the metal components of the device. While the studies included in this review found lower Cd levels in biomarkers of e-cigarette users than in conventional cigarette and cigar users, other metal levels were similar or even higher in e-cigarette users. Manufacturing procedures could have a major contribution to potential metal impurities and could influence metal release during vaping. Regulation is needed to inform e-cigarette users on possible metal exposure through vaping as well as to prevent metal exposure during e-cigarette use.

TABLES

Table 1. Metal concentrations in e-liquid samples (µg/kg) used in e-cigarette devices. Studies reporting data for e-liquid samples collected from bottle dispensers are listed first followed by studies reporting metals in e-liquid samples from cartridges. A total of 12 studies were identified. The findings for Dunbar and Olmedo have been split for bottles and cartridge/tank separately to better compare results for samples with contact with the coil (cartridge, open wick, tank) with those without contact with the coil (bottle dispenser).

First Author	Type of e-liquid	Source of e-liquid	E-liquid brand	E-liquid flavor (Nicotine mg/ml)	Analytical methods	N-e-liquid	N-samples	Background correction	Al[a]	As	Cd	Co	Cr	Cu	Fe	Mn	Ni	Pb	Sb	Zn
Talio, 2015	Bottle	Internet	NR	Tobacco, Cappuccino, Ice Min, Tobacco Whinston (0-18)	Molecular Fluorescence	4	16	NR										0.8 (0.9)		
Beauval, 2016	Bottle	Manufacturer	NHOSS	Cherry and others (0 or 16)	ICP-MS	54	54	Y	11.1 (2.8)	1.0 (0.6)	<0.3 (LOQ)	0.2 (0.1)	6.2 (1.1)	12.6 (2.2)		1.7 (1.4)	<13.8 (LOQ)	<0.9 (LOQ)	6.2 (27.1)	130 (47.8)
Beauval, 2017	Bottle	Manufacturer	NHOSS	Unflavored, Tobacco, Mint (0,16)	ICP-MS	6	6	NR	10.0 (1.5)	0.8 (0.4)	<0.3 (LOQ)	0.1 (0.08)	4.5 (1.1)	16.3 (6.7)		1.6 (0.9)	<13.8 (LOQ)	<0.9 (LOQ)	1.2 (0.1)	<172 (LOQ)
Palazzolo, 2017	Bottle	Local outlet	7's	Tobacco (24)	ICP-MS	1	4	Y	6.6 (0.4)	0.07 (0.03)	<0.009 (LOD)			<0.009 (LOD)	3.5 (0.2)	0.1 (0.005)	0.1 (0.006)	<0.009 (LOD)		0.4 (0.03)
Talio, 2017	Bottle	Online	NR	Tobacco, Cappuccino, Mint (0-18)	Molecular Fluorescence	4	16	NR			12.6 (4.0)						14.5 (6.8)			
Dunbar, 2018	Bottle	Canadian or US outlet	Multiple brands[b]	Multiple flavors[c] (6-24)	AAS	12	36	NR										<7.8 (LOQ)		
Kamilari, 2018	Bottle	Market (USA, France, Turkey, Greece)	NR	NR	Total Reflection X-Ray Fluorescence	22	132	NR		<1.0 (LOD)	<25.0 (LOD)		8.4 (55.5)	9.5 (47.3)			4.7 (27)	2.2 (10.3)		
Olmedo, 2018	Bottle	Daily e-cig users in MD	NR	NR	ICP-MS	56	56	Y	15.0 (11.7)	3.6 (10.3)	0.07 (0.000)	0.2 (0.3)	1.6 (2.2)	20.0 (38.1)	65.2 (102)	6.2 (20.7)	28.9 (43.8)	1.04 (1.94)	0.9 (3.7)	41.3 (137)
Song, 2018	Bottle	Local retails	NR	Tobacco	ICP-MS	3	NR	NR		1.9	0.7		NR	NR			3	10.5		NR
Flora, 2016	Cartridge	Indiana and Arizona outlet	MarkTen	Menthol and Classic (15)	ICP-MS	4	12	NR		<430 (LOQ)	<220 (LOQ)									
Hess, 2017	Cartridge	US outlet and online	Brand A	NR (1.6-1.8)	ICP-MS	10	20	NR			176 (273)		1815 (4489)			5943 (10492)	19436 (20984)	1694 (1247)		
			Brand B			10	20				1.0 (0.9)		678 (244)			576 (243)	11524 (3904)	50 (68.3)		
			Brand C			8	16				1.4 (1.1)		199 (61.6)			172 (29.2)	398 (114)	5.0 (1.5)		
			Brand D			10	20				0.8 (0.7)		65.4 (9.5)			35.7 (12.0)	50.5 (19.3)	4.2 (0.8)		
			Brand E			10	20				0.4 (0.3)		46.4 (6.0)			24.7 (8.4)	98 (42.4)	80.3 (69.2)		
Dunbar, 2018	Cartridge (*cig-a-like*)	Canadian outlet	DUNE	Strawberry (0)	AAS	1	3	NR										<7.8 (LOQ)		
	Cartridge (*POD*)	US outlet	JUUL	Crème Brulee, Fruit (0)	AAS	2	6	NR										<7.8 (LOQ)		
	Open wick	Canadian or US outlet	EZEE, DUNE, EVO	Fruitalicious, Mint, Grape, Menthol, Berry (0)	AAS	6	18	NR										202		
Zhao, 2018	Bottle, cartridge	Local retails and online	Blu, NJOY	Tobacco (10)	SF-ICPMS	NR	NR	NR												220
Olmedo, 2018	Tank	Daily e-cig users in MD	NR	NR	ICP-MS	49	49	Y	101 (176)	4.2 (11.6)	0.4 (1.0)	10.8 (17.1)	214 (346)	1990 (5550)	1880 (3860)	124 (247)	2510 (8160)	517 (1520)	3.6 (7.5)	3250 (9640)

Note: AAS: atomic absorption spectrometer, ICP-MS: inductively coupled plasma–mass spectrometry, LOD: limit of detection, LOQ: limit of quantification, MD: Maryland, NR: not reported.

[a] All metal concentrations were reported as mean (standard deviation). The study by Palazzolo et al. (2017) reported the mean (standard error) instead of the standard deviation. The studies by Flora et al. (2016) and Zhao et al. (2018) did not report the standard deviation or any other measure of variability. We calculate mean and SD for individual samples by Beauval et al. (2016) and Beauval et al. (2017). We calculate the weighted mean and total SD for multiple groups by Talio et al. (2015), Talio et al. (2017), and Kamilari et al. (2018). The study by Olmedo et al. (2018) did not report means (SDs) in the original publication but we calculated them directly from the original data.

[b] Multiple brands include Cosmic Fog, Cool Vape, Premium Labs', Blue V, Club, V, Vaper's Knoll, High Caliber, Good E-Juice, House of Vapor.

[c] Multiple flavors include Milk & Honey, Banana Mama, Strawberry, Watermelon, Cola, Menthol, Pina Colada, Bubble Gum, Vanilla

Table 2. Metal concentrations in aerosol samples (ng/puff) collected from e-cigarette devices. Studies reporting data for aerosol samples collected from cig-a-likes are listed first followed by studies reporting metals in aerosol samples from open devices.

First Author	Device type	Source of e-cigarette	Device brand	E-liquid Flavor (nicotine mg/ml)	Type of coil	Puffing protocol	Analytical methods	N-devices	N-samples	Background correction	Al[a]	As	Cd	Cr	Cu	Fe	Mn	Ni	Pb	Sb	Se	Sn	Zn
Williams, 2013	*Cig-a-likes*	Local outlet and online	NR	NR (0)	Nichrome	60 puffs, 4.3 s/puff	ICP-OES	1	3	Y	39.4			0.7	20.3	52	0.2	0.5	1.7			3.7	5.8
Goniewicz, 2014	*Cig-a-likes*	Online	Multiple brands[b]	Marlboro, regular, trendy, menthol, camel, tobacco (4-18)	NR	1.8 s/puff,10 s interval, puff volume of 70 ml, 150 puffs	ICP-MS	12	108	Y		<LOD (NR)	0.6 (0.6)	<LOD (NR)	<LOD (NR)		<LOD (NR)	1.2 (0.8)	0.6 (1.1)				<LOD (NR)
Tayyarah, 2014	*Cig-a-likes*	Manufacturers	Blu, SKYCIG	Tobacco, menthol, cherry (16, 18, 24)	NR	99 puffs, 55 ml/puff, 2 puffs/min	ICP-MS	5	19	Y		<1.4 (LOQ)	<1.4 (LOQ)	1.4			<1.3 (LOQ)	<1.4 (LOQ)	<1.4 (LOQ)		<1.4 (LOQ)	<1.4 (LOQ)	
Lerner, 2015	*Cig-a-likes*	E-cigarette users	Blu	Tobacco (16)	NR	4 puffs, 4s/puff	AAS	1	4	N					117 (83.6)								
Williams, 2015	*Cig-a-likes*	Local outlet and online	4 brands (name NR)	NR	Nichrome	60 puffs, 4.3 s/puff	ICP-OES	4	12	Y				0.6 (0.5)	8.9 (10.2)			2.0 (3.7)				88.6 (322)	3.8 (6.2)
Margham, 2016	*Cig-a-likes*	NR	Vype	Tobacco (18.6)	Nichrome	puff volumes of 55 cm³ puff duration 3s, twice/min	ICP-MS, AAS	1	5	Y		0.2 (NQ)	<0.2 (LOD)	0.4	1.9	4.2		0.6 (NQ)	<0.5 (LOD)		<0.08 (LOD)	<0.6 (LOD)	12.3
Mikheev, 2016	*Cig-a-likes*	NR	Blu	Tobacco, menthol, cherry, java jolt, peach, colada, vanilla (0, 12-16)	NR	17.5 ml/s, 75 puffs, 4.3 s/puff, 60 s interval	ICP-MS	1	42	Y		0.14 (0.02, 1.0)		4.0 (0.16, 9.2)	1.2 (0.08, 12.6)			0.3 (0.1, 13.5)		0.3 (0.06, 1.1)		0.1 (0.04, 180)	6.2 (3.2, 105.6)
Williams, 2017	*Cig-a-likes*	Local outlet and online	Multiple brands[c]	Multiple falvors[d](12.5-45)	Nichrome	4.3 s/puff every 5 mins, 60 puffs	ICP-OES	6	18	Y	13	0.6			8.0	0.8		0.4		0.7	5.3	2.9	3.6
Beauval, 2017	Open	Manufacturer	NHOSS	Unflavor, Tobacco, Mint (0,16)	NR	55 ml puff over 3 s, twelve/ minute	ICP-MS	1	18	Y		<0.7 (LOQ)	0.1	7					2.7	0.7			
Palazzolo, 2017	Open	Local outlet	Triple 3 eGo	Tobacco (24)	NR	400 ml/puff, volume:33.6 ml, 45 puffs, 5 s/puff, 10s interval	ICP-MS	1	8	Y	290	0.13	<LOD		<LOD	0.07	<LOD	14.5	<LOD				61.9
Olmedo, 2018	Open	Daily e-cigarette users in Maryland	NR	NR	Kanthal or others	puff volume of 66.67ml, 4s/puff, puff interval 30s,1 L/min	ICP-MS	56	56	Y	0.02 (0.05)	0.004 (0.01)	0.0001 (0.003)	0.07 (0.27)	0.05 (0.12)	0.39 (1.33)	0.01 (0.02)	0.32 (1.06)	0.08 (0.27)	0.002 (0.004)		0.02 (0.06)	0.54 (0.88)
Zhao, 2018	*Cig-a-likes*, Open	Local retails and online	Blu, NJOY	Tobacco (10)	NR	30 L/min for 10 min, 3.7 V, puff volume of 55ml, 4s/puff, puff interval 30s.	SF-ICP-MS	NR	NR	Y	0.98				0.98	0.44	0.01	0.05	0.21				0.65

Note: AAS: atomic absorption spectrometer, ICP-MS: inductively coupled plasma–mass spectrometry, LOD: limit of detection, LOQ: limit of quantification, NR: not reported, NQ: not quantifiable.

[a] All metal concentrations were reported as mean (standard deviation). The studies by Williams et al. (2013), Margham et al. (2016), Williams et al. (2017), Palazzolo et al. (2017), and Zhao et al. (2018) did not report the standard deviation or any other measure of variability. The metal concentrations by Mikheev et al. (2016) and Williams et al. (2015) were derived using an automated program from the figure. The mean and SD were calculated for individual samples by Beauval et al. (2017). The study by Olmedo et al. (2018) did not report means (SDs) in the original publication but we calculated them directly from the original data. We calculate the weighted mean and total SD for multiple groups by Goniewicz et al. (2014) and Tayyarah et al. (2014).

[b] Multiple brands include Joye, Janty, DSE, Trendy, Nicore, Mild, Ecis, Dekang, Intellicig, Colinss, Premium.

[c] Multiple brands include Blucig, Mistic, NJOY King, Square 82, V2 Cig, Vype

[d] Multiple flavors include Tobacco, Menthol, Traditional, Original Red, Red, Classic Regular

Table 3. Metal concentrations in biomarker samples from e-cigarette users.

Author	Aherrera et al., 2017			Badea et al. 2018	Goniewicz et al. 2018	Jain et al., 2018		
Participants	64 participants from Baltimore, Maryland (5 *cig-a-likes*, 59 *MOD*)			150 Romanian participants (58 non-smokers, 58 conventional cigarette smokers, 34 e-cigarette users)	5105 US adults (247 e-cigarette users, 2411 cigarette smokers, 792 dual users, and 1655 never tobacco users) of the Population Assessment of Tobacco and Health Study (PATH 2013-2014)	US adult users from (cigars, cigarettes, and e-cigarettes users) the 2013-2014 National Health and Nutrition Examination Survey (NHANES)		
Analytical methods	ICP-MS			ICP-MS	ICP-MS	ICP-MS		
Biomarker	Urine	Urine-Creatinine	Saliva	Serum	Urine	Blood	Urine	Serum
Unit	μg/L	μg/g creatinine	μg/L	μg/L	μg/g creatinine		μg/L	
N	64	64	63	34	247	23	14	
Summary statistics	Median (IQR)			Median (IQR)	GM (95% CI)	GM (95% CI)		
Ag				0.2 (0.1, 0.5)				
As				0.2 (0.1, 0.3)			3.2 (0.8, 13.4)	
Ba				2.5 (1.9, 3.1)			0.9 (0.3, 2.5)	
Be				0.3 (0.3, 0.3)	0.01 (0.01, 0.01)			
Cd				0.03 (0.0, 0.0)	0.19 (0.17, 0.23)			
Co				0.3 (0.2, 0.4)	0.58 (0.52, 0.64)		0.3 (0.2, 0.6)	
Cr	0.5 (0.4, 0.8)	0.4 (0.3, 0.5)	1.5 (0.8, 2.9)					
Cu				892 (799, 958)				106 (70.7, 160)
Fe				1151 (888, 1515)				
Hg				0.5 (0.5, 0.5)				
Mn				0.8 (0.6, 1.0)	0.14 (0.12, 0.16)	10.3 (8.7, 12.2)		
Mo				0.6 (0.4, 0.9)			31.3 (14.6, 67.2)	
Ni	0.9 (0.6, 1.6)	0.7 (0.4, 1.4)	2.3 (1.0, 4.9)	7.0 (3.9, 10.0)				
Pb				2.2 (1.0, 3.5)	0.43 (0.38, 0.49)			
Pd				0.01 (0.0, 0.0)				
Sb				1.2 (1.1, 1.6)			0.04 (0.02, 0.08)	
Se				88.0 (79.6, 95.0)		186 (163, 211)		131 (108, 160)
Sn				5.4 (4.9, 6.6)			0.4 (0.04, 3.1)	
Sr				23.2 (20.0, 29.1)	119 (101, 140)		114 (38.5, 337)	
Th				0.01 (0.0, 0.1)				
Tl				0.03 (0.0, 0.0)	0.17 (0.15, 0.19)		0.1 (0.03, 0.3)	
U				0.01 (0.0, 0.0)	0.007 (0.006, 0.008)		0 (0, 0.02)	
V				0.3 (0.2, 0.3)				
W							0.02 (0, 0.1)	
Zn				871 (781, 1008)				60.9 (39.1, 95)

FIGURES

Figure 1. Summary of the search and screening process. Footnote: The number of studies below adds to 28 because some studies reported data both for e-liquid and aerosol metal concentrations.

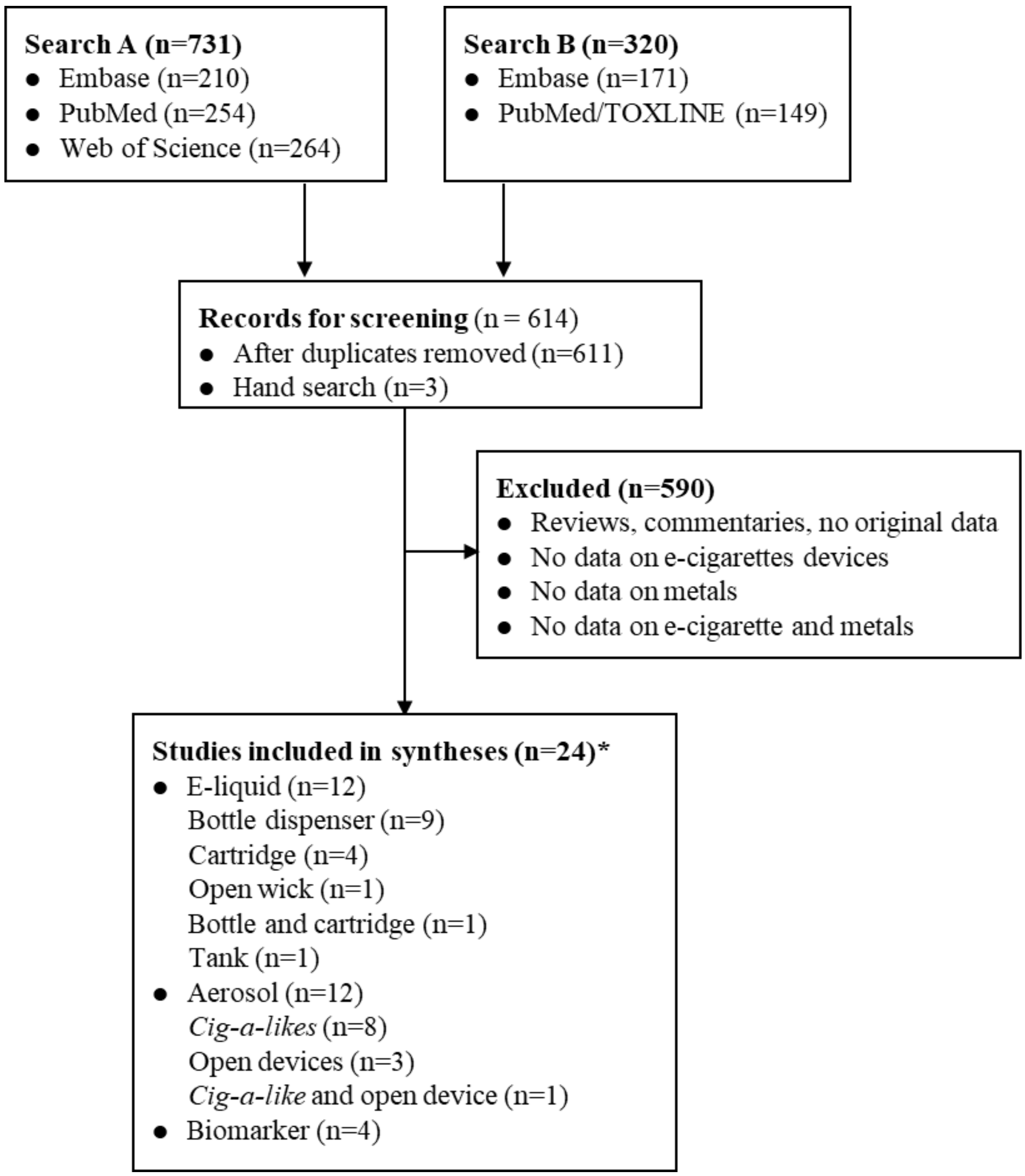
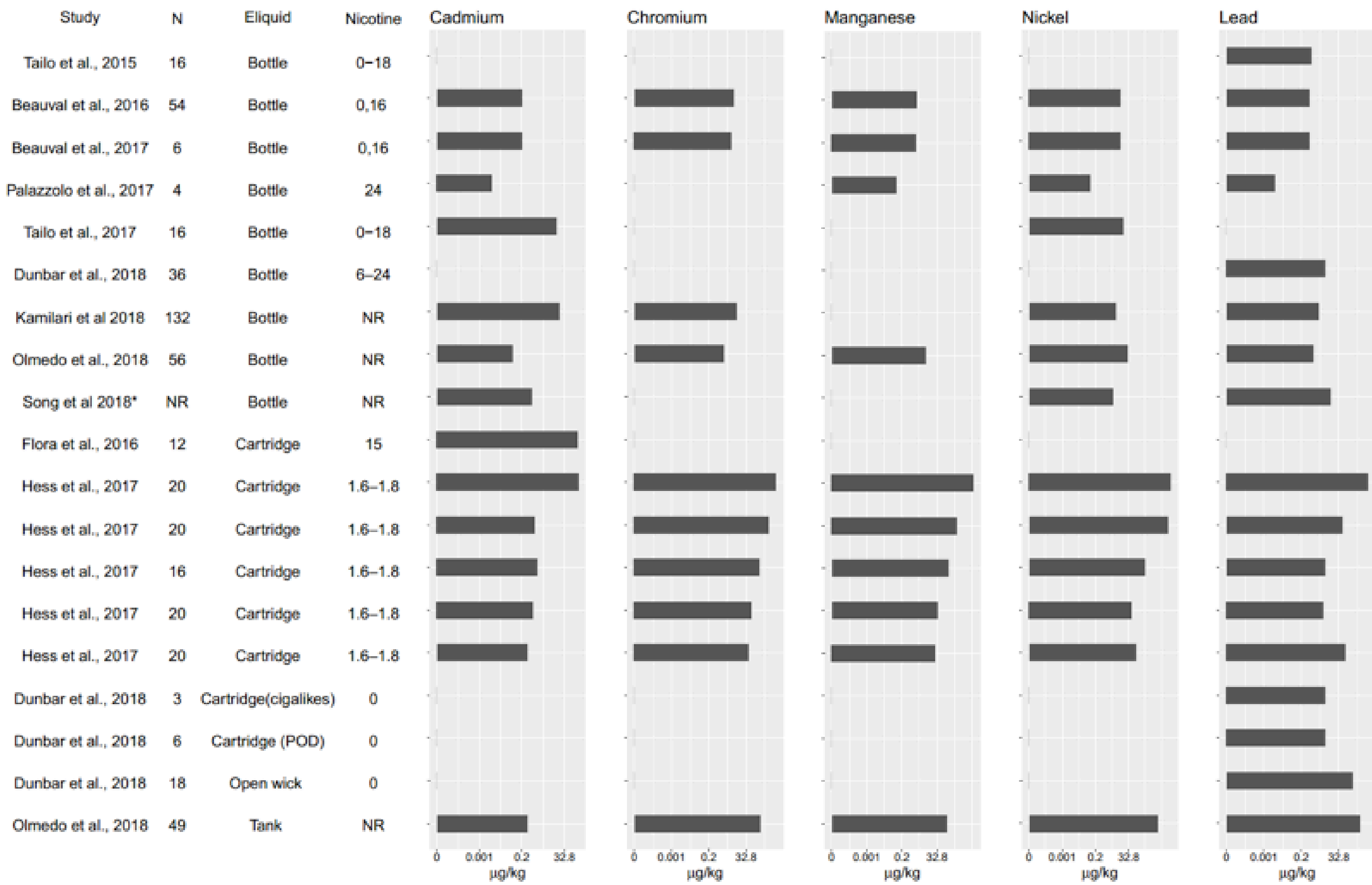
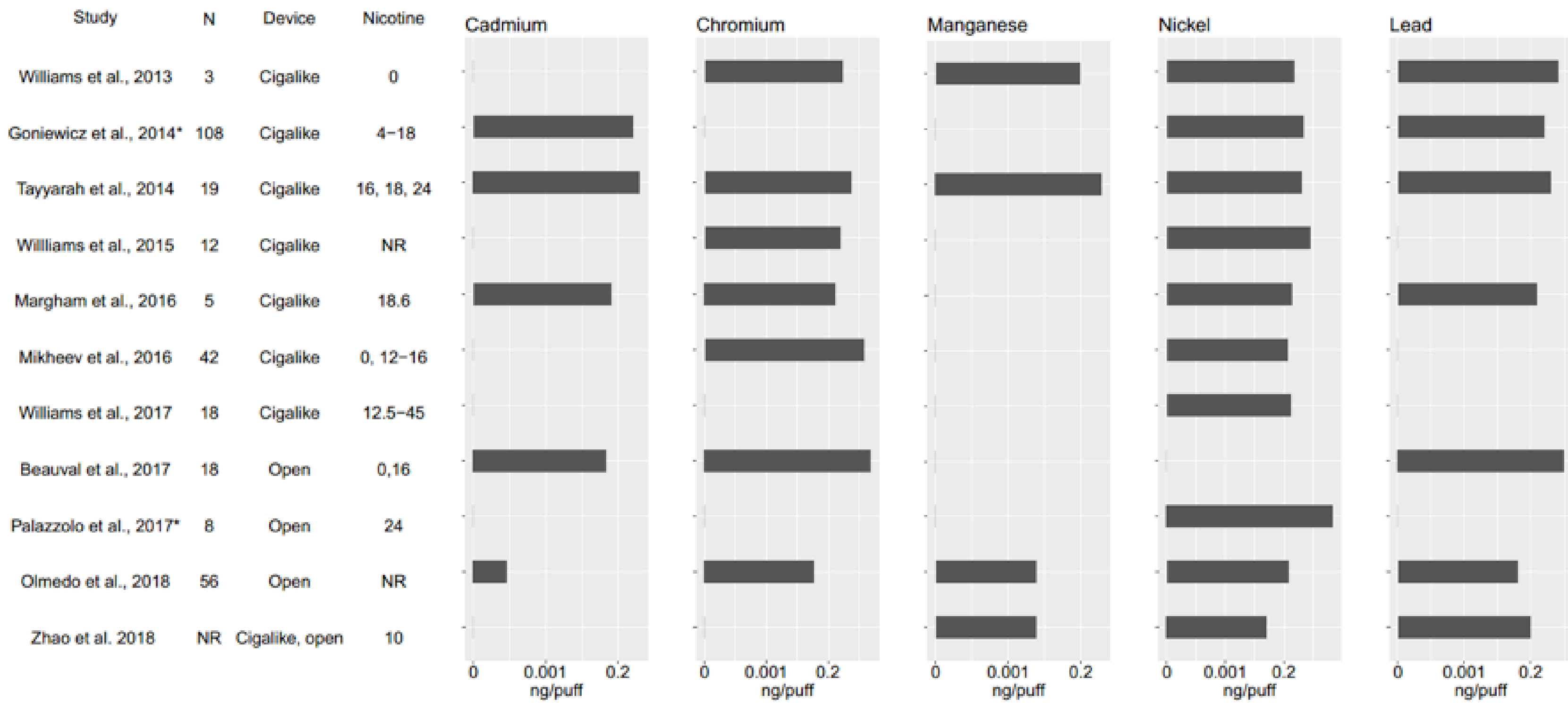


Figure 2. Mean metal concentrations in e-liquid samples (μg/kg) used in e-cigarette devices.



Footnote: The study by Song et al. (2018) measured chromium but did not report the concentrations. Other missing metals in other studies were not measured. The unit of nicotine was mg/ml.

Figure 3. Mean metal concentrations in aerosol samples (ng/puff) collected from e-cigarette devices.



Footnote: The study by Goniewicz et al. (2014) measured chromium and manganese concentrations and the study by Palazzolo et al. (2017) measured cadmium, manganese, and lead concentrations. Other missing metals in other studies were not measured. The unit of nicotine was mg/ml.

SUPPLEMENTAL MATERIAL

Supplemental File 1. Search Strategies

Search Strategy A (Developed by DZ, ANA, and MH)

PubMed (N=257):

(("electronic nicotine delivery systems"[MeSH Terms] OR ("electronic"[All Fields] AND "nicotine"[All Fields] AND "delivery"[All Fields] AND "systems"[All Fields]) OR "electronic nicotine delivery systems"[All Fields] OR "e cigarette"[All Fields]) OR ("electronic nicotine delivery systems"[MeSH Terms] OR ("electronic"[All Fields] AND "nicotine"[All Fields] AND "delivery"[All Fields] AND "systems"[All Fields]) OR "electronic nicotine delivery systems"[All Fields] OR "e cigarettes"[All Fields]) OR ("electronic nicotine delivery systems"[MeSH Terms] OR ("electronic"[All Fields] AND "nicotine"[All Fields] AND "delivery"[All Fields] AND "systems"[All Fields]) OR "electronic nicotine delivery systems"[All Fields] OR ("electronic"[All Fields] AND "cigarette"[All Fields]) OR "electronic cigarette"[All Fields]) OR ("electronic nicotine delivery systems"[MeSH Terms] OR ("electronic"[All Fields] AND "nicotine"[All Fields] AND "delivery"[All Fields] AND "systems"[All Fields]) OR "electronic nicotine delivery systems"[All Fields] OR ("electronic"[All Fields] AND "cigarettes"[All Fields]) OR "electronic cigarettes"[All Fields]) OR ("electronic nicotine delivery systems"[MeSH Terms] OR ("electronic"[All Fields] AND "nicotine"[All Fields] AND "delivery"[All Fields] AND "systems"[All Fields]) OR "electronic nicotine delivery systems"[All Fields] OR "e cig"[All Fields]) OR ecig[All Fields] OR ecigs[All Fields] OR (("nicotine"[MeSH Terms] OR "nicotine"[All Fields]) AND ("delivery, obstetric"[MeSH Terms] OR ("delivery"[All Fields] AND "obstetric"[All Fields]) OR "obstetric delivery"[All Fields] OR "delivery"[All Fields])) OR (("nicotine"[MeSH Terms] OR "nicotine"[All Fields]) AND ("equipment and supplies"[MeSH Terms] OR ("equipment"[All Fields] AND "supplies"[All Fields]) OR "equipment and supplies"[All Fields] OR "device"[All Fields])) OR (("electronics"[MeSH Terms] OR "electronics"[All Fields] OR "electronic"[All Fields]) AND ("nicotine"[MeSH Terms] OR "nicotine"[All Fields]) AND ("delivery, obstetric"[MeSH Terms] OR ("delivery"[All Fields] AND "obstetric"[All Fields]) OR "obstetric delivery"[All Fields] OR "delivery"[All Fields])) OR (("electronics"[MeSH Terms] OR "electronics"[All Fields] OR "electronic"[All Fields]) AND ("nicotine"[MeSH Terms] OR "nicotine"[All Fields]) AND ("equipment and supplies"[MeSH Terms] OR ("equipment"[All Fields] AND "supplies"[All Fields]) OR "equipment and supplies"[All Fields] OR "device"[All Fields])) OR ("vaping"[MeSH Terms] OR "vaping"[All Fields] OR "vape"[All Fields]) OR ("vaping"[MeSH Terms] OR "vaping"[All Fields]) OR e-liquid[All Fields]) AND (("metals"[MeSH Terms] OR "metals"[All Fields]) OR ("metals"[MeSH Terms] OR "metals"[All Fields] OR "metal"[All Fields]) OR metallic[All Fields] OR metalloid[All Fields] OR ("aluminium"[All Fields] OR "aluminum"[MeSH Terms] OR "aluminum"[All Fields]) OR ("arsenic"[MeSH Terms] OR "arsenic"[All Fields]) OR ("cadmium"[MeSH Terms] OR "cadmium"[All Fields]) OR ("chromium"[MeSH Terms] OR "chromium"[All Fields]) OR ("cobalt"[MeSH Terms] OR "cobalt"[All Fields]) OR ("copper"[MeSH Terms] OR "copper"[All Fields]) OR ("iron"[MeSH Terms] OR "iron"[All Fields]) OR ("manganese"[MeSH Terms] OR "manganese"[All Fields]) OR ("nickel"[MeSH Terms] OR "nickel"[All Fields]) OR ("Physician's Bull"[Journal] OR "pb"[All Fields]) OR ("tin"[MeSH Terms] OR "tin"[All Fields]) OR ("zinc"[MeSH Terms] OR "zinc"[All Fields]))

Web of Science (n=264):
TOPIC: (((((((((((((e-cigarette OR e-cigarettes) OR electronic cigarette) OR electronic cigarettes) OR e-cig) OR ecog) OR ecogs) OR nicotine delivery) OR nicotine device) OR electronic nicotine delivery) OR electronic nicotine device) OR vape) OR vaping) OR e-liquid) AND ((((((((((((((metals OR metal) OR metallic) OR metalloid) OR aluminum) OR arsenic) OR cadmium) OR chromium) OR cobalt) OR copper) OR iron) OR manganese) OR nickel) OR Pb) OR tin) OR zinc))

Embase (n=212):
((((((('e cigarette'/exp OR 'e cigarette' OR 'e cigarettes'/exp OR 'e cigarettes' OR electronic) AND ('cigarette'/exp OR cigarette) OR electronic) AND cigarettes OR 'e cig' OR ecig OR ecigs OR 'nicotine'/exp OR nicotine) AND ('delivery'/exp OR delivery) OR 'nicotine'/exp OR nicotine) AND ('device'/exp OR device) OR electronic) AND ('nicotine'/exp OR nicotine) AND ('delivery'/exp OR delivery) OR electronic) AND ('nicotine'/exp OR nicotine) AND ('device'/exp OR device) OR vape OR 'vaping'/exp OR vaping OR 'e liquid') AND ('metals'/exp OR metals OR 'metal'/exp OR metal OR metallic OR 'metalloid'/exp OR metalloid OR 'aluminum'/exp OR aluminum OR 'arsenic'/exp OR arsenic OR 'cadmium'/exp OR cadmium OR 'chromium'/exp OR chromium OR 'cobalt'/exp OR cobalt OR 'copper'/exp OR copper OR 'iron'/exp OR iron OR 'manganese'/exp OR manganese OR 'nickel'/exp OR nickel OR pb OR 'tin'/exp OR tin OR 'zinc'/exp OR zinc)

<u>Search Strategy B (developed by AA1, AA2, AR)</u>

Embase (n=171):
('electronic cigarettes':ti,ab,kw OR 'electronic cigarette':ti,ab,kw OR 'e cig':ti,ab,kw OR 'ecigs':ti,ab,kw OR 'vaping':ti,ab,kw OR 'e-cigarette':ti,ab,kw OR 'e-cigarettes':ti,ab,kw OR 'nicotine delivery system':ti,ab,kw OR 'nicotine delivery systems':ti,ab,kw OR 'nicotine inhaler':ti,ab,kw OR 'nicotine inhalers':ti,ab,kw OR 'nicotrol':ti,ab,kw OR 'smokeless cigarette':ti,ab,kw OR 'smokeless cigarettes':ti,ab,kw OR 'electronic nicotine':ti,ab,kw OR 'nicotine inhalator':ti,ab,kw OR 'vapor device':ti,ab,kw OR 'vapor devices':ti,ab,kw OR 'vapour device':ti,ab,kw OR 'vapour devices':ti,ab,kw OR 'alternative cigarette':ti,ab,kw OR 'alternative cigarettes':ti,ab,kw OR 'digital cigarette':ti,ab,kw OR 'digital cigarettes':ti,ab,kw OR 'vapor smoking':ti,ab,kw) AND ('metal':ti,ab,kw OR 'metals':ti,ab,kw OR 'nickel':ti,ab,kw OR 'raney alloy':ti,ab,kw OR 'np 2':ti,ab,kw OR 'nichel italian':ti,ab,kw OR 'ni 4303t':ti,ab,kw OR 'ni 270':ti,ab,kw OR 'ni 0901 s':ti,ab,kw OR '58ni':ti,ab,kw OR '7440-02-0':ti,ab,kw OR 'chromium':ti,ab,kw OR 'chrome':ti,ab,kw OR '7440-47-3':ti,ab,kw OR '52cr':ti,ab,kw OR '14092-98-

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Pubmed and TOXLINE (149):

((((((("Electronic Cigarettes"[Mesh] OR "Vaping"[Mesh] OR electronic cigarette*[tw] OR e cig*[tw] OR ecig*[tw] OR vaping[tw] OR "nicotine delivery system"[tw] OR "nicotine delivery systems"[tw] OR "nicotine inhaler"[tw] OR "nicotine inhalers"[tw] OR nicotrol[tw] OR "smokeless cigarette"[tw] OR "smokeless cigarettes"[tw] OR "electronic nicotine"[tw] OR "nicotine inhalator"[tw] OR "vapor device"[tw] OR "vapor devices"[tw] OR "vapour device"[tw] OR "vapour devices"[tw] OR "alternative cigarettes"[tw] OR "digital cigarettes"[tw] OR "vapor smoking"[tw]))))) AND ((((("Metals"[Mesh] OR metal[tw] OR metals[tw])))) OR ((((((((((((tin[tw] OR Stannum[tw] OR stannium[tw] OR "14314-35-3"[tw] OR "14314-35-3"[rn] OR "7440-31-5"[tw] OR "7440-31-5"[rn]))) OR ((antimony[tw] OR stibium OR "antymon polish " "antimony black" OR “7440-36-0”[rn] OR antimonic OR antimonium OR “Sb 122” OR “14374-79-9”[rn]))) OR ((Copper [tw] OR cda 122 [tw] OR cda 110 [tw] OR cda 102 [tw] OR cda 101 [tw] OR Cu62 [tw] OR Cu 63 [tw] OR Cu 64 [tw] OR Cu 67 [tw] OR arwood copper [tw] OR anac 110 [tw] OR 1721 gold [tw] OR bronze powder [tw] OR ci 77400 [tw] OR raney copper [tw] OR ofhc cu [tw] OR kafar copper [tw] OR gold bronze [tw] OR 7440-50-8 [rn]))) OR ((iron[tw] OR 56Fe OR Fe OR ferro OR ferrum OR "iron polymaltose" OR "suy b 2" OR pzh2m OR loha OR "ferrovac e" OR "eo 5a" OR "armco iron" OR “53858-86-9”[rn] OR “7439-89-6”[rn] OR “14093-02-8”[rn]))) OR ((Manganese [tw] OR 19768-33-3 [tw] OR mangan polish [tw] OR colloidal manganese [tw] OR 7439-96-5 [tw] OR 7439-96-5 [rn] OR Mn 54 [tw] OR Mn 55 [tw]))) OR ((zinc[tw] OR "zinc dust" OR "zinc powder" OR merrillite OR "granular zinc" OR "blue powder" OR “7440-66-6”[rn] OR 64Zn OR zincum OR “Zn 64” OR “14378-32-6”[rn]))) OR ((Aluminum [tw] OR ci 77000" [tw] OR "pap 1" [tw] OR metana [tw] OR "jisc 3110" [tw] OR "jisc 3108" [tw] OR "ao a1" [tw] OR alumina fibre" [tw] OR alumina fibre [tw] OR alaun german [tw] OR ad1m [tw] OR ad 1 [tw] OR Al derivative [tw] OR ci 77000 [tw] OR pap 1 [tw] OR jisc 3110 [tw] OR jisc 3108 [tw] OR Al 26 [tw] OR Al 27 [tw] OR 7429-90-5 [RN] OR 7429-90-5 [tw]))) OR ((lead[tw] OR "olow polish " OR "lead s2" OR "lead flake" OR "ks 4" OR “7439-92-1”[rn] OR 208Pb OR plumbum OR “13966-28-4”[rn]))) OR ((cadmium[tw] OR cadmium OR Cd 109[tw] OR 7440-43-9[rn] OR 7440-43-9[tw]))) OR ((chromium[tw] OR chrome OR “7440-47-3”[rn] OR 52Cr OR “14092-98-9”[rn] OR “16065-83-1”[rn]))) OR ((Nickel[tw] OR "raney alloy" OR "np 2" OR "nichel Italian" OR "ni 270" OR "ni 0901 s" OR "58Ni" OR "7440-02-0"[tw] OR "7440-02-0"[rn]))))))

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CHAPTER 3

E-cigarette use behaviors and device characteristics of daily sole e-cigarette users in Maryland

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Target journal: Nicotine & Tobacco Research

ABSTRACT

Background: The use of electronic cigarettes (e-cigarettes) has steadily increased, yet few studies have characterized daily exclusive e-cigarette users, their device characteristics, and use behaviors. This study aims to describe daily e-cigarette user characteristics and compare their health status to non-users, as well as assess the association of use behaviors with e-cigarette user demographics.

Methods: From December 2015 to October 2017, 150 participants (100 daily sole e-cigarette users and 50 non-users) were recruited in Maryland, USA. Data on sociodemographic characteristics, overall health status, e-cigarette use behaviors and tobacco use history, device characteristics, and primary reasons for e-cigarette use was collected by interview.

Results: Majority of daily sole e-cigarette users were men, white, former smokers, used open system devices (MODs/tanks), and vaped an average of 365 puffs/day (SD: 720 puffs). Close to a third of users first vape within 5 minutes of waking in the morning, and more than half vape all throughout the day. The most commonly used heating coils were Kanthal or some combination with Kanthal (58%), stainless steel (18%), and Nichrome (16%), which were replaced an average of 3 times/month (SD: 2). The mean voltage used was 4.21 V (SD: 1.2) with men more likely to vape at a higher voltage than women. E-liquid consumption ranged from 5-240 ml/week (median: 32.5), with an average nicotine concentration of 5.3 mg/ml. Together with individuals of lower education, men also consumed more e-liquid/week. Older individuals used e-liquids with higher nicotine concentrations but vaped fewer puffs/day. Compared to non-users, e-cigarette users were more likely to report symptoms of wheezing and whistling in the chest as well as having

hypertension, although this was not statistically significant after adjustment. While e-cigarette use was reported as an aid to quit smoking and as a healthier alternative to cigarettes, less than half planned to quit vaping.

Conclusion: This research reports relevant information regarding use behaviors of daily exclusive e-cigarette users. With chronic use and no intention to quit vaping, these users may be at risk for increased toxic exposures. Further research is needed to characterize the long-term health effects of daily e-cigarette use.

INTRODUCTION

Electronic cigarettes (e-cigarette) have significantly increased in use, particularly among youth and young adults [1, 2]. The number of current e-cigarette users among middle and high school US student has increased from 2.1 million in 2017 to 3.6 million in 2018 [3]. E-cigarettes are comprised of a battery, a cartridge containing e-liquid, and an atomizer, which heats and aerosolizes the e-liquid. There are various types of e-cigarette devices and they can be classified into closed and open systems [4]. Closed system devices, which include first-generation *cig-a-likes* and the recent *PODs* (including Juul), consist of a disposable cartridge that contains the e-liquid and low-capacity re-chargeable batteries. PODs, in particular, are commonly used by new e-cigarette users and youth [5, 6]. Open system devices, which include e-pen models and tank-like systems, are common among former smokers [7]. These devices are typically larger in size with a more powerful battery and adjustable voltage/wattage delivery (modifiable e-cigarettes (MODs)), a re-fillable e-liquid reservoir, and replaceable heating coils which are typically made up of metal alloys; commonly used coils include Kanthal (chromium, aluminum, iron), Nichrome (nickel and chromium), and stainless steel (nickel, chromium, carbon) [8, 9].

Many studies have focused on the prevalence of e-cigarette use [2, 10] or on the characteristics of e-cigarette cartomizers [11-13]. Few studies, however, have characterized daily e-cigarette users and their perceptions of e-cigarette safety. Daily e-cigarette users represent a small subgroup (19%) of the e-cigarette population compared to intermittent (29%) and occasional (51%) e-cigarette users [14]. Moreover, while nationally representative studies such as the Population Assessment of Tobacco and

Health (PATH) study and the National Health Interview Survey (NHIS) have begun including questions pertaining to the prevalence of e-cigarette use, they are limited in asking questions pertaining to e-cigarette device characteristics (including voltage, power, and the type of heating coil used) and use behaviors (including amount of e-liquid consumed per week, the number of times the heating coil is replaced per month, puffs taken per day). Understanding daily use is critical given the concern that chronic exposure could potentially result in long-term health effects. The purpose of this study was to evaluate daily sole e-cigarette users' demographic characteristics, e-cigarette use behaviors and reasons for use, self-reported health status, and to compare with non-users (those who do not vape e-cigarettes and smoke combustible cigarettes). We describe e-cigarette device characteristics, vaping frequency, and e-liquid nicotine concentrations in association with user demographics among e-cigarette users in Maryland to better identify the types of users at risk and to understand the practices that may influence potential toxicity of e-cigarettes among daily users.

METHODS

Study Population and Recruitment

E-cigarette users were recruited through advertisements and flyers posted in universities, local newspapers (City Paper), social media platforms, e-cigarette (vape) shops and conventions between December 2015 and October 2017 in Maryland. Participants were residents of Maryland, at least 18 years old and non-pregnant at the time of recruitment. The goal was to recruit 50 daily exclusive e-cigarette users during the first wave of recruitment (December 2015 to March 2016), and 50 daily exclusive e-cigarette users and 50 non-users during the second wave (March 2017 to October 2017). Sole e-cigarette users were defined as non-tobacco cigarette smokers or former smokers who had quit at least 6 months prior to enrollment and vaped daily for at least 6 weeks. Users could either bring an open or closed system e-cigarette device to the study. It should be noted at the time of recruitment, none of the participants were POD users. Non-users were defined as non-tobacco cigarette smokers and non-e-cigarette users or former smokers who quit at least 6 months prior to enrollment. To aid in the comparability between the two groups, non-users were matched according to age (within 5 years), sex, and race of e-cigarette users. The study protocol was approved by the Institutional Review Board at Johns Hopkins University (Baltimore, Maryland). All participants provided written informed consent.

Data collection

After confirming eligibility, e-cigarette user participants were asked to carry out their normal vaping routine and bring their e-cigarette device to the study visit, which took place at Johns Hopkins Bloomberg School of Public Health in Baltimore, MD. At the time of their appointment, participants responded to an interviewer-based questionnaire addressing sociodemographic characteristics, previous tobacco use, current e-cigarette use (including e-liquid consumed/week, preferred voltage, e-liquid nicotine concentrations), overall health status, and beliefs/perceptions on e-cigarette safety. Additional questions on e-cigarette use (including number of puffs/day, average seconds/puff, days since last coil change) were added in the second year of recruitment. Intensity of nicotine addiction was assessed adapting the Fagerstorm Test for nicotine dependence [15], while sensory and respiratory symptoms were addressed using a questionnaire commonly used in studies regarding tobacco smoking and exposure to tobacco smoke [16].

Statistical Analysis

We compared e-cigarette users and non-users by demographic characteristics, rules about smoking and vaping indoors, and health characteristics using Chi-squared for categorical variables and Student t-test for continuous variables (Tables 1, 5, and 6). We also compared male and female e-cigarette users by primary reasons for vaping, their intention to reduce nicotine, and intention to quit vaping using Chi-squared (Table 4). Lastly, we conducted linear regression models to analyze the association of age, sex, education, race, and previous smoking status with preferred voltage, preferred nicotine concentration, e-liquid consumed/week, puff count/day, seconds/puff before and after adjusting for those same indicators (Table 3). Statistical analyses were conducted in Stata 14 (Stata Corp, College Station, TX). The level of statistical significance was set at alpha 0.05.

RESULTS

Participant characteristics

One hundred and fifty participants (100 e-cigarette users and 50 non-users) were recruited (Table 1). Their mean age was 30.1 years (SD: 9.6), 64% were men, and 82.7% were white. Compared to e-cigarette users, most non-users had a higher level of education (90%) and were never smokers (90%). Eighty nine percent of e-cigarette users were former smokers; they had an earlier age to first smoke cigarettes, and smoked more cigarettes per day before quitting (mean: 17 cigarettes/day; range: 1- 80 cigarettes/day) compared to non-users who were former smokers.

E-cigarette use patterns, device characteristics, and reasons for vaping Among e-cigarette users, the mean (SD) age at first vape was 28 (9) years (data not shown). By device type, only 2 participants used first-generation devices while 98 users used 2nd or 3rd generation devices. More than a third (41%) of users first vape within 15 minutes of waking in the morning, with 30% vaping within 5 minutes (Table 2). Most participants (54%) owned two or more devices, with about half of the users (56%) vaping continuously throughout the day. Most users (85%) were knowledgeable about their coil, with Kanthal or some combination with Kanthal (58%), stainless steel (18%), and Nichrome (16%) being the most commonly used coils. Users' coils were last changed at an average of 16 (SD 19) days prior to coming to the study session, and replaced at an average of 3 (SD 2) times per month. The reported mean voltage was 4.21 V (range: 2.12 – 12.50 V), and 85% reported periodically changing the voltage of the device. For other characteristics, men used a higher voltage than women, and former smokers used a lower voltage than never smokers (Table 3). According to e-liquid characteristics and use, 79% of the study population purchased their e-liquid from a vape shop, 14% online, and the remaining 8% from "other" sources, such as making it on their own or receiving it from a friend. E-liquid consumption varied greatly, ranging from 5 to 240 ml/week (median: 32.5 ml/week), with women and individuals with higher level of education consuming less per week than men and individuals with lower level of education, respectively. The average (SD) nicotine concentration of e-liquid was 5.3 (5.3) mg/ml. The median (IQR) number of puffs per day was 200 (90, 360) puffs, with each puff lasting an average of 4 (SD 2) seconds. Older aged participants preferred higher nicotine concentrations in e-liquid and fewer puffs/day. Seconds/puff was not associated with demographic characteristics.

The primary reasons for vaping were to quit smoking cigarettes (34%) and as a healthier alternative than cigarettes (32%) (Table 4). Women reported they were less likely intending to reduce nicotine levels than men. Overall, less than 50% of e-cigarette users reported the intention to quit vaping.

Self-reported health status and home rules with tobacco/e-cigarette use

Regarding general health characteristics, e-cigarette users were more likely to report symptoms of wheezing and whistling in the chest (15% vs. 2%, $p = 0.02$) as well as having hypertension (22% vs. 4%, $p = 0.007$) than non-users (Table 5). After running additional analyses, adjusting for age, sex, and previous smoking status, this was not statistically significant. Twenty-seven of the e-cigarette users reported sensory and respiratory symptoms (sore throat, runny nose, bringing up phlegm, and coughing) occurring with e-cigarette use. While there was no difference with banning cigarette smoking inside the home between users and non-users, most e-cigarette users (89%) had no rules on banning vaping indoors than non-users (Table 6).

DISCUSSION

In our study sample from Maryland between 2015 and 2017, the majority of the daily sole e-cigarette users were men, white, former smokers, and used open system devices (MODs/tanks). This is consistent with the nationally representative Population Assessment of Tobacco and Health (PATH) study (Waves 1 and 2), where exclusive use of e-cigarettes was more prevalent among non-Hispanic whites compared to non-Hispanic Black and Hispanics [17], and those who reported using open-system devices were more likely to report daily use as compared to those who did not use this type of device [18]. Prior studies have also found ever use of e-cigarettes to be higher among men than women [19, 20], although other studies have reported the opposite [21, 22]. This study differs from prior e-cigarette research as it focuses on daily exclusive e-cigarette users, the behaviors that may influence toxic exposures from daily e-cigarette use, and the differences in health characteristics and house rules of tobacco use between users and non-users.

According to e-cigarette behaviors, close to a third of our participants first vape within 5 minutes of waking in the morning and more than half vape throughout the day, indicating a high level of dependence of the product. Older aged individuals vaped e-liquids of higher nicotine concentration but at lower total puffs per day. These finding are consistent with a study of nicotine dependence and consumption among vapers mostly based in United Kingdom, Australia, Finland, Ireland and the United States, which found that older vapers employed a high nicotine-concentration and low power style of vaping [23]. With a higher level of nicotine, fewer puffs would be necessary for the nicotine delivery. Men were more likely to vape at a higher voltage and consume more e-liquid per week than women. This higher intensive use among men has also been reported in other studies [24, 25], and is concerning given that increasing the voltage, and subsequently increasing the power, shifts the particle mass distribution towards micron-sized particles and increases the respirable fraction of aerosol to enter ciliated airways [26]. Increasing power and closed-system device use has also been associated with higher metal release into the aerosol, which is a major health concern given the toxicity of metals[27]. Users in our study vaped at an average voltage of 4.21 V (median: 4.20 V), with men vaping at a higher voltage compared to women; users also vaped an average of 365 puffs/day (median: 200 puffs/day), with individuals of lower education levels (< HS) reporting higher number of puffs compared to individuals of higher education levels. This is concerning as users with a vaping regimen of 250 puffs/day with a tank device of voltages from 3.8 to 4.8 V were predicted to inhale formaldehyde (up to 49 mg/day), acrolein (up to 10 mg/day) and diacetyl (up to 0.5 mg/day), at levels that exceeded U.S. occupational limits [28].

E-cigarette users in our study changed their coils on average 3 times per month. No previous studies have reported on the frequency of coil change. This is an important behavior as several studies [1, 29, 30] have found elements from coil alloys such as nickel and chromium in the aerosol that is inhaled by the user, and an increased frequency of coil change has been associated with higher metal biomarker levels [29]. The most frequently reported coil types in this study (Kanthal, stainless steel, and Nichrome) contain chromium (Cr) and/or nickel (Ni). Our group has found that the levels of these two metals in the aerosol correlate with metal levels in urine or saliva from the same participants [29]. We also found that metal levels are, in general, higher in the aerosol than in the original liquid [8], supporting the finding that metal exposure from e-cigarette devices is likely derived, at least in part, from the heating coils. This is concerning as inhalation of nickel and chromium has been shown to cause airway irritation and obstruction, as well as lung, nasal, and sinus cancer[31].

Participants reported using e-cigarettes primarily as an aid to quit smoking (35%) and because it is healthier than cigarettes (32%). An online survey conducted from April and June 2014 among US adults similarly found cessation- and health-related factors as primary reasons for e-cigarette use [32], and adult current established e-cigarette users from Wave 1 of the PATH study (2013-2014) also reported using e-cigarettes as an alternative to cigarettes [33]. Interestingly, women in our study less likely intended to reduce their nicotine e-liquid concentrations compared to men. It could be that women have higher nicotine dependence and a lower likelihood of abstinence in tobacco dependence, which has been reported in several smoking cessation studies [34-38]. Alternatively, it could perhaps be due to a lower nicotine flux, which is the nicotine emitted per puff second (mg/s) that is not only determined by the e-liquid concentration used but also the device characteristics (i.e. voltage or power settings) and use puff topography (i.e. seconds/puff, puffs/day) [39]. While women in our study had the same preferred mean e-liquid nicotine concentration (5.3 mg/ml), they vaped their devices at a lower voltage (mean: 3.88 vs. 4.34 volts), longer seconds/puff (mean: 4.23 vs. 3.89 secs/puff), but at lower number of puffs/day (mean: 292 vs. 396 puffs/day), indicating that the amount of nicotine they receive is relatively lower than men and is enough to suppress nicotine withdrawal. Overall, 48.5% of our study population intended to quit vaping altogether, which is lower than the findings from the PATH study (Wave 3: 2015-2016) where nearly two-thirds of e-cigarette users (62.38%) planned to quit e-cigarettes [40]. While a sizable percentage of users report plans to quit, most of these users' timeframe for quitting is long-drawn-out (8% plan to quit within the next 7 days, 7.7% in the next month, 13% in the next 6 months, 33% in the next year, 38% longer than that). Moreover, more than 25% reported quit attempts to e-cigarettes in the past year signifying that quitting e-cigarette use may be a challenge, similar to quitting traditional cigarettes [40].

Compared to non-users, e-cigarette users were more likely to report symptoms of wheezing and whistling in the chest as well as having hypertension, although after further analysis adjusting for sex, age, and former smoking status, this was not statistically significant. An assessment of Wave 2 of the PATH study also found an increased risk of wheezing and related respiratory symptoms among current e-cigarette users compared to non-users, but a lower risk in wheezing and related respiratory symptoms than current smokers or dual users [41], which are groups we did not recruit in this study. Lastly, while there was no difference in house rules on banning smoking cigarettes indoors, e-cigarette users were less likely to have rules in place for vaping indoors as compared to non-users. This may pose a concern to both users and bystanders as e-cigarette aerosols consist of small particles (PM2.5 and ultrafine particles (UFP)) and a mixed composition of organic (formaldehyde and acrolein levels) [28] as well as inorganic (nickel and chromium) [8] compounds, which have been linked to an increased risk of respiratory and cardiac events [42-44]. Moreover, nicotine contained in the aerosol can also be deposited on various surfaces, and contribute to thirdhand exposure [45].

This study has several limitations. While both groups (e-cigarette users and non-users) were matched according to sex, age, and race, the majority of non-users (90%) had a higher level of education and were current students compared to e-cigarette users (59%). This study could be affected by selection bias, due to convenience sampling. Moreover, our report of e-cigarette use behaviors are based on self-report and it is possible that participants could display recall bias or social desirability bias. As this study only looked at participants aged 18 and older, and as use of PODs (Juul, Suorin, etc) rose in popularity towards the tail end of our recruitment in 2017 (especially among adolescents and youth), we are likely missing an important population of e-cigarette use, particularly among middle and high school-aged youth.

CONCLUSIONS

Despite these limitations, this study provides relevant information regarding use behaviors of daily sole e-cigarette users. Most daily e-cigarette users were male, white, former smokers, owned an average of 2 open-system devices and vaped an average of 365 puffs/day, all throughout the day. Men were more likely to vape at a higher voltage than women. Together with individuals of lower education, men consumed more e-liquid/week, suggesting a higher likelihood of intensive use. Older individuals used e-liquids with a higher nicotine concentration but vaped fewer puffs/day. Women expressed less desire to further lower nicotine levels in their e-liquid and to quit vaping altogether compared to men. Lastly, while e-cigarette use was reported as an aid to quit smoking and as a healthier alternative to cigarettes, e-cigarette users were more likely to report symptoms of wheezing and whistling in the chest as well as having hypertension compared to non-users. With chronic use and no intention to quit vaping, daily sole e-cigarette users may be at risk for long-term health effects from potential toxic exposures of e-cigarettes. Future research should document the practices of daily e-cigarette users, particularly related to the coil, voltage, and nicotine in e-liquid. Given the heterogeneity of e-cigarettes in the market and ability of users to modify these devices, research studies looking at health effects and e-cigarette constituents should include a comprehensive characterization of e-cigarette use patterns and device characteristics.

TABLES

Table 1. Participant characteristics by vaping category

General Characteristics	N	Total (n = 150)	E-cig users (n = 100)	Non-users (n = 50)	*p*-value[a]
Age, mean (SD)	150	30.1 (9.6)	30.3 (9.2)	29.7 (10.5)	0.7
Gender %					
Male	97	64	67	60	0.60
Female	59	36	33	40	
Education level %					
≤ High School	46	30.7	41	10	<0.001
> High School	104	69.3	59	90	
Race %					
White	124	82.7	87.0	74.0	0.05
Non-White	26	17.3	13.0	26.0	
Employed %					
Yes	99	66	75.0	48.0	0.001
No	51	34	25.0	52.0	
Current Student	29	19.3	9.00	40.0	
Tobacco Use					
Smoking status %					
Ever smoker	94	62.7	89.0	10.0	<0.001
Never smoker	56	37.3	11.0	90.0	
Ever smoker Age, mean (SD)	94	31.2 (9.4)	30.3 (9.2)	31.6 (12.0)	
Never smoker Age, mean (SD)	56	28.2 (9.6)	30.3 (8.7)	29.7 (10.6)	
Age first smoked (tobacco cigarettes), mean (SD)	94	15.4 (2.9)	15.1 (2.5)	19.8 (5.7)	<0.001
Time in months since quit cigarettes, mean (SD)	91	23.7 (18.2)	23.2 (18.1)	33.5 (19.8)	0.27
Cigarettes smoked daily before quitting, mean (SD)	92	16.3 (11.9)	16.8 (11.9)	4.5 (3.8)	0.04

[a]Comparing sole e-cig users vs. non-users

Table 2. E-cigarette Use Behaviors and Patterns

E-cigarette Use Behaviors	N	E-cig users (n = 100)
Time to first vape %		
Less than 5 min	27	27
6- 15 min	9	9
16 -30 min	29	29
31 – 60 min	24	24
More than 1 hour	11	11
# Different devices used %		
1	45	46.0
2	25	26.0
3	13	13.0
4	15	15.0
Number of puffs/day* Mean (SD)	50	365.1 (720)
Portion of the day to vape*		
Morning	4	8.0
Afternoon	6	12.0
Evening	12	24.0
Most of the day	28	56.0
Average seconds/puff (secs)*	50	4.0 (2.0)
E-liquid purchase location %		
Vape shop	77	79
Online	14	14
Other	6	6
Preferred nicotine concentration (mg/ml) Mean (SD)	98	5.3 (5.3)
E-liquid consumed per week (ml), mean (SD)	98	53.3 (48.4)
Power of Device (watts), Mean (SD)	96	56.3 (30.8)
Voltage of Device (Volts), Mean (SD)	92	4.21 (1.2)
Change Voltage %		
Yes	85	87
No	13	13
How often change coil/month, mean (SD)	96	2.5 (2.4)
Last time of coil change (days)*	50	15.9 (19.4)
Knowledge of coil composition %		
Yes	83	86
No	13	14
Type of coil used		
Kanthal	39	48.0
Nichrome	13	16.0
Pure nickel	2	4.0
Stainless steel	15	18.0
Titanium	4	5.0
Combination with Kanthal	8	10.0

* Year 2 data only

Table 3. Mean difference (95% CI) in e-cigarette use patterns by demographic characteristics analyzed using linear regression

		Voltage (volts)			Nicotine Use (mg/ml)			E-liquid/wk (ml)			Puff count/day*			Seconds/puff*	
	N	Crude (95% CI)	Adjusted† (95% CI)	N	Crude (95% CI)	Adjusted† (95% CI)	N	Crude (95% CI)	Adjusted† (95% CI)	N	Crude (95% CI)	Adjusted† (95% CI)	N	Crude (95% CI)	Adjusted† (95% CI)
Age (per year)	92	-0.01 (-0.04, 0.02)	-0.01 (-0.03, 0.02)	98	0.24 [0.13, 0.34)	0.24 (0.12,0.36)	98	-0.04 (-1.10, 1.02)	-0.46 (-1.55, 0.63)	50	-20.3 (-43.0, 2.39)	-25.1 (-49.9, -0.25)	50	0.01 (-0.05, 0.08)	0.02 (-0.06, 0.09)
Gender															
Male	65	0.00 (ref)	0.00 (ref)	66	0.00 (ref)	0.00 (ref)	67	0.00 (ref)	0.00 (ref)	35	0.00 (ref)	0.00 (ref)	35	0.00 (ref)	0.00 (ref)
Female	27	-0.46 (-1.00, 0.07)	-0.54 (-1.04, -0.03)	32	0.04 (-2.24, 2.33)	0.20 (-1.95, 2.34)	31	-23.5 (-43.9, -3.11)	-22.7 (-42.9, -2.46)	15	-103.4 (-553, 347)	-132.2 (-49.9, -0.25)	15	0.34 (-0.88, 1.57)	0.40 (-0.92, 1.73)
Education															
≤ HS	38	0.00 (ref)	0.00 (ref)	41	0.00 (ref)	0.00 (ref)	41	0.00 (ref)	0.00 (ref)	19	0.00 (ref)	0.00 (ref)	19	0.00 (ref)	0.00 (ref)
> HS	54	-0.13 (-0.63, 0.37)	-0.12 (-0.60, 0.36)	57	-0.90 (-3.07, 1.27)	-0.02 (-2.09, 2.04)	57	-20.3 (-39.6, -0.93)	-20.4 (-39.7, -1.09)	31	202.8 (-219, 625)	134.1 (-301, 570)	31	-0.23 (-1.38, 0.93)	-0.25 (-1.50, 1.01)
Race															
White	84	0.00 (ref)	0.00 (ref)	86	0.00 (ref)	0.00 (ref)	87	0.00 (ref)	0.00 (ref)	43	0.00 (ref)	0.00 (ref)	43	0.00 (ref)	0.00 (ref)
Non-white	8	-0.13 (-1.00, 0.75)	-0.19 (-1.03, 0.65)	12	-2.22 (-5.47, 1.01)	-1.12 (-4.22, 1.98)	11	-25.0 (-55.5, 5.48)	-25.5 (-55.8, 4.73)	7	-245 (-837, 346)	-342 (-968, 284)	7	0.09 (-1.53, 1.71)	0.07 (-1.74, 1.87)
Previous smoker															
No	10	0.00 (ref)	0.00 (ref)	11	0.00 (ref)	0.00 (ref)	11	0.00 (ref)	0.00 (ref)	6	0.00 (ref)	0.00 (ref)	6	0.00 (ref)	0.00 (ref)
Yes	82	-1.28 (-2.03, -0.54)	-1.31 (-2.10, -0.53)	87	0.75 (-2.64, 4.14)	-1.09 (-4.38, 2.19)	87	0.17 (-30.7, 31.1)	-3.10 (-33.9, 27.7)	44	39.5 (-597, 676)	247 (-423, 919)	44	-1.04 (-1.84, 1.63)	-0.14 (-2.08, 1.79)

† Adjusted for age, gender, education level, race, and previous smoking status
*Only year 2 data

Table 4. Primary reasons for vaping and intention to quit

Characteristic	**N**	**Total**	**Men**	**Women**	***p*-value**
Primary reasons for vaping, %	97				
Aid to quit smoking cigarettes	34	35	35.9	33.3	0.65
Healthier than cigarettes	32	33	34.4	30.3	
It is enjoyable	20	21	21.9	18.2	
Cheaper than cigarettes	5	5	3.10	9.10	
Other	6	6	4.70	9.10	
Intention to reduce nicotine %					
Yes	60	61	70.4	48.9	0.004
No	30	30	16.7	46.7	
Don't know	9	9	12.9	4.40	
Intention to quit vaping? %					
Yes	48	48.5	47.0	51.5	0.11
No	27	27.3	33.3	15.2	
Don't know	24	24.2	19.7	33.3	

Table 5. Health characteristics among study population

Health Characteristics %	N	Total	E-cig users	Non-users	*p*-value
Asthma					
Yes	22	14.7	14.0	16.0	0.74
No	128	85.3	86.0	84.0	
Respiratory Disease %					
Yes	10	6.70	8.00	4.00	0.36
No	140	93.3	92.0	96.0	
Allergies %					
Yes	40	26.7	27.0	26.0	0.90
No	110	73.3	73.0	74.0	
Irritated Eyes %					
Yes	32	21.3	22.0	20.0	0.78
No	118	78.7	78.0	80.0	
Runny nose %					
Yes	60	40.0	42.0	36.0	0.48
No	90	60.0	58.0	64.0	
Sore throat %					
Yes	32	21.3	20.0	24.0	0.57
No	118	78.7	80.0	76.0	
Wheezing/whistling in the chest %					
Yes	16	10.7	15.0	2.00	0.02
No	134	89.3	85.0	98.0	
Shortness of breath %					
Yes	27	18.0	21.0	12.0	0.18
No	123	82.0	79.0	88.0	
Coughing in the morning %					
Yes	21	16.0	16.0	10.0	0.32
No	129	84.0	84.0	90.0	
Coughing in the evening %					
Yes	29	19.3	18.0	22.0	0.56
No	121	80.7	82.0	78.0	
Bringing up phlegm %					
Yes	25	16.7	15.0	20.0	0.44
No	125	83.3	85.0	80.0	
Hypertension % *					
Yes	13	13	22.0	4.0	0.007
No	87	87	78.0	96.0	
Diabetes/cholesterol % *					
Yes	9	9	8.00	10.0	0.73
No	91	91	92.0	90.0	
Dental discoloration % *					
Yes	24	24	32.0	16.0	0.061
No	76	76	68.0	84.0	
Gingival inflammation % *					
Yes	19	19	24.0	14.0	0.20
No	81	81	76.0	86.0	
Symptoms with e-cig use %					
Yes	27	27.3	27.3	-	-
No	69	69.7	69.7	-	
Don’t know	3	3.00	3.00	-	

*Year 2 data only

Table 6. Home rules about smoking and vaping indoors

Characteristic	**N**	**Sole e-cig user**	**Non-user**	***p*-value**
Rule banning vaping indoors, %				
Yes	32	10.2	44.0	
No	113	89.0	50.0	<0.001
Don't know	3	0	6.0	
Rule banning smoking indoors, %				
Yes	94	63.6	62.0	0.37
No	54	36.4	36.0	
Don't know	1	0	2.0	

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TRANSITIONAL CHAPTER 4

Connecting daily e-cigarette user demographics and use behaviors with metal exposure

In chapter 3, we evaluated daily sole e-cigarette users' demographic characteristics, device characteristics, and their use behaviors. Daily e-cigarette users represented, at the time of this study (December 2015 to October 2017), a small subgroup (19%) of the e-cigarette user population (1) yet they may be at most risk for potential long-term health effects from chronic use. It is important to understand daily use behaviors as this may influence potential exposure to toxic chemicals. In chapter 5, we narrow our focus on exposure to metals – nickel, chromium, lead, manganese –and whether certain use behaviors and device settings are associated with increased levels. Studies have found sources of metal exposure may be derived, at least in part, from the e-liquid, the heating coil used to aerosolize the e-liquid (2, 3), and soldered joints of the device (4, 5). Metals and metalloids emitted in the e-cigarette aerosols may pose a concern as exposure to metals has been linked to different negative health effects such as lung cancer (6, 7) and cardiovascular disease (8-10). This next chapter determines whether daily sole e-cigarette users have increased metal exposure measured in biospecimen samples (urine, saliva, and exhaled breath) as compared to non-users, and assesses whether certain use behaviors previously described in chapter 3 may augment exposure.

Both chapters provide detailed salient information on e-cigarette use, which are currently not captured in nationally representative studies such as the Population Assessment of Tobacco and Health (PATH) study, the National Health Interview Survey (NHIS), or the National Health and Nutrition Examination Survey (NHANES). The PATH study, which is a national longitudinal study of tobacco use, was initiated in 2013 by the US Food and

Drug Administration (FDA) in collaboration with the National Institutes of Health (NIH) (11). Using a four-stage stratified area probability sample design, more than 49,000 participants enrolled in the study in 2013 (12). This is the first federal instrument to ask detailed information on e-cigarette use (i.e. use of a disposable or replaceable battery, disposable or refillable cartridge, and nicotine level)(11). NHIS, which is an annual, nationally representative in-person survey on the health of the civilian non-institutionalized population, was initiated in 1957 by the Centers for Disease Control and Prevention (CDC). Using an area probability design that permits a representative sampling of households, NHIS was administered to a sample of 33,028 adults aged ≥18 years in 2016 (13). And lastly, NHANES is a program of studies, which began in the early 1960s used to assess the health and nutritional status of both adults and children (14). Using a multistage probability sampling design, this survey examines a nationally representative sample of about 5,000 persons per year asking demographic, socioeconomic, dietary and health-related questions (14). In this dissertation, chapters 3 and 5 specifically provide information on e-cigarette device characteristics (including voltage, power, and the type of heating coil used), use behaviors (including amount of e-liquid consumed per week, the number of times the heating coil is replaced per month, puffs taken per day), and nickel and chromium urine levels, which are currently not measured in PATH, NHIS, nor NHANES.

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CHAPTER 5

Characterization of metal exposure from e-cigarette use: a study of non-invasive biomarkers

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ABSTRACT

Background: Metals have been detected in electronic cigarette (e-cigarette) aerosol that is inhaled by the user. Few studies have looked at metal biomarkers from e-cigarette use. We compared metal biomarker levels between e-cigarette users and non-users, and assessed the association of e-cigarette use characteristics as well as metal concentrations in e-liquid samples collected from the participants' devices with metal biomarker concentrations.

Methods: We recruited 148 participants, 98 e-cigarette users and 50 non-users from December 2015 to October 2017. We collected urine, saliva, and exhaled breath condensate (EBC), and, particularly for e-cigarette users, we also collected data on e-cigarette use, and samples from their e-cigarette device (dispenser e-liquid, condensed aerosol, and e-liquid in the tank). Cr, Ni, Pb, Mn concentrations were measured using ICP-MS.

Results: Median Cr, Ni, Pb, and Mn levels were 0.66, 0.71, 0.23, and 0.92 μg/g creatinine in urine, respectively; 1.03, 0.77, 0.66, 12.2 μg/l in saliva; 0.23, 0.13, 0.03, 0.09 μg/l in EBC. In fully adjusted models, e-cigarette users were associated with 212%, 222%, 129% higher Ni EBC, Pb saliva, Mn EBC levels, respectively. Users had 99% and 247% higher Cr and Mn saliva levels with more e-liquid consumed per week, 84% and 132% Cr and Ni saliva levels with a more frequent coil change, 67% higher Ni urine levels with a shorter time to first vape when waking in the morning, and 40-70% lower Cr, Ni, Mn saliva and EBC levels when using a coil (titanium, stainless steel, nichrome) other than Kanthal. Tertile 2 of Cr in aerosol samples and tank samples were associated with a 123% and 101% higher Cr urine levels, respectively. Ni in saliva was also positively associated with Ni concentrations in the aerosol (p-trend 0.001).

Conclusion: We found higher metal biomarker levels in e-cigarette users compared to non-users, and positive associations of metal aerosol concentrations with corresponding metal biomarker levels, indicating e-cigarette emissions increase metal internal dose. Certain device characteristics and behaviors of increased use were also associated with higher metal biomarker levels. Metal level standards and best practice for device use are needed to prevent involuntary metal exposure among e-cigarette users.

INTRODUCTION

Electronic cigarette (e-cigarette) use has significantly increased over the years, particularly among youth and young adults [1, 2]. As of 2017, 2.1 million middle (3.3%) and high school students (11.7%)[3], and 6.9 million (2.8%) adults [4] currently use e-cigarettes. While the perception of safety and variety of appealing flavours contribute to its popularity [5-7], e-cigarettes are not toxic-free. Metals and metalloids in e-cigarette aerosol, in particular, pose as a major health concern given that exposure to metals has been linked to lung cancer [8, 9], cardiovascular and kidney disease [10-12], and neural toxicity [13]. Studies have shown sources of metal exposure may be from the heating coil used to aerosolize the e-liquid [5, 14] as well as soldered joints and other parts of the device [15, 16]. Heating coils, which are commonly made up of metal alloys, include Kanthal (chromium, aluminum, iron), Nichrome (nickel and chromium), and stainless steel (nickel, chromium, carbon) [5, 14, 17]. We previously showed that metal concentrations (including nickel, chromium, lead, and manganese) in the aerosol and e-liquid in the tank were markedly higher compared to the e-liquid from the refilling dispenser [14]; that power settings and device type may affect metal release [18]. Only a few studies have looked at the metal biomarkers of e-cigarette users, including two using national datasets (the Population Assessment of Tobacco and Health (PATH) study, the National Health and Nutrition Examination Survey (NHANES)) in the United States [19-21]. Neither PATH nor NHANES, however, measure nickel or chromium, nor ask detailed questions pertaining to e-cigarette device characteristics, including voltage, power, and type of coil used, as well as use behaviors, including how much e-liquid is consumed per week and how often the heating coil is changed.

In this study, we aimed to assess whether e-cigarette use is associated with increased exposure to Ni, Cr, Pb, Mn as determined by non-invasive biomarkers (urine, saliva, exhaled breath condensate (EBC). We first compared metal biomarker levels between e-cigarette users and non-users. We then assessed the association of e-cigarette use behaviours as well as metal concentrations in e-liquid samples collected from the participants' devices with metal biomarker concentrations. Previously, we conducted a preliminary analysis to evaluate Ni and Cr biomarkers from e-cigarette use but this lacked statistical power (small sample size) as well as a referent group of non-users/non-smokers. This current study aims to address these limitations with an increased sample size, a control group, additional e-cigarette use/device questions, and questions on other sources of metal exposure from work or recreational activity.

METHODS

Study Population and Recruitment

E-cigarette users were recruited through vaping conventions, flyers posted in universities and e-cigarette shops, ads on newspapers and social media between December 2015 and October 2017 in Maryland. To be eligible, participants had to be 18 years of age or older, non-pregnant, and residents of Maryland. The goal was to recruit 50 daily exclusive e-cigarette users during the first wave of recruitment (December 2015 to March 2016), and 50 daily exclusive e-cigarette users and 50 non-users during the second wave (March 2017 to October 2017). E-cigarette users were defined as non-tobacco cigarette smokers or former smokers who had quit for at least 6 months prior to enrollment and vaped daily using open-system devices for at least 6 weeks. Closed-system devices include e-pen models and tank-like systems, which allow modification of voltage/wattage/temperature (MODs) and are refillable. Closed-system devices include cig-a-likes and PODs, which are comprised of disposable cartridges and low-capacity re-chargeable batteries. From the 100 e-cigarette users recruited, 2 used closed-system devices and were excluded in the metal biomarker analysis. Non-users were defined as non-tobacco cigarette smokers and non-e-cigarette users or former smokers who quit at least 6 months prior to enrollment. To aid in the comparability, non-users were matched according to age (within 5 years), sex, and race of e-cigarette users. The study protocol was approved by the Institutional Review Board at Johns Hopkins University (Baltimore, Maryland). All participants provided written informed consent.

Data and Sample Collection

After confirming eligibility, participants were asked to carry out their normal vaping routine and bring their e-cigarette device to the study visit. The interviewer-based questionnaire collected data on sociodemographic factors, tobacco use history (if applicable), e-cigarette characteristics and use behaviors (e-liquid consumed per week, time to first vape from waking in the morning, preferred voltage, number of puffs/day, seconds/puff, heating coil used (Kanthal/Nichrome/other), coil change per month, and nicotine concentrations in e-liquid), and lifestyle factors (work and/or recreational activity) that may be potential sources of metal exposure.

Following the interview, each participant provided three biospecimen samples: 1) Urine, in collection cups; 2) Saliva, by chewing on a cotton swab (Salivette®, Sarstedt AG, Germany) until saturated; 3) Exhaled breath condensate (EBC), by exhaling through a chilled collection system (RTube™, Respiratory Research Inc, Austin TX) for 10 minutes. The Rtube consists of a condensing tube made of polypropylene, a silicone one-way valve, a t-connector with a closed bottom, which acts as a saliva trap, and an attached mouthpiece. An aluminum sleeve, which is kept in the freezer prior to use, cools the sample as it is being collected in the condensing tube. All samples were stored at -20°C until analysis.

For each participant, we collected three types of samples from their device and dispenser. First, we pipetted a minimum of 0.25 mL directly from the dispenser containing the refilling e-cigarette liquid (no contact with the coil) into a 1.5 mL centrifuge tube. Second, we collected 0.2-0.5 mL of the aerosol generated by the e-cigarette device using the methodology described in Olmedo et al (2018). Briefly, a peristaltic pump, placed inside a fume hood, puffs the e-cigarette and the generated aerosol is collected in a 1.5 mL centrifuge tube via deposition in a series of conical pipette tips and plastic tubing (1 L/min, 4 s per puff and 30 s inter-puff time). Approximately 20% of the generated aerosol remains in the tubing and around 10% is lost through the venting groove of the collection device. The collected aerosol sample is then ready for analysis. Third, a minimum of 0.25 mL of the e-liquid remaining in the mouthpiece tank after puffing the e-cigarette with the peristaltic pump was pipetted into a third centrifuge tube. The three sample types were analyzed using similar analytic methods, allowing a direct comparison between samples.

Metal Biomarker Analysis

Biospecimen samples were diluted into 2% HNO_3 and 0.5% HCl solution. Calibration curves were built using standard solution (Multi-element Aqueous CRM, QC Standard 21, VHG Labs, Manchester, NH, USA). Ten ppb (v/v) internal standard (CPI International, Santa Rosa, CA, USA) was added to samples and calibration curves to control potential drifts in the signal. Metal concentrations were measured using inductively coupled plasma mass spectrometry (ICP-MS, Agilent 7500ce Octopole ICP-MS, Agilent Technologies, Santa Clara, CA, USA). The limit of detection was 0.04 μg/L for both Ni and Cr and 0.01 μg/L for Pb in urine, saliva, and EBC (Supplementary Tables 1-3). The limit of detection for Mn was 0.03 μg/L in urine and 0.02 μg/L in saliva and EBC (Supplementary Table 4). The percentage of participants with metal concentrations below the limit of detection in urine, saliva and EBC was 1.4%, 0.7% and 0% for Cr, 0% 6.8%, and 1.4% for Cr, 0%, 0%, and 0% for Pb, 0%, 0%, and 0.7% for Mn (Supplementary Tables 1-4). Samples below the limit of detection were substituted by the limit of detection divided by the square root of 2. All urine cups were acid-washed with 10% nitric acid overnight and rinsed with deionized water before collecting samples in order to eliminate potential metal contamination. For urine cups and Rtubes, blank biomarker samples consisted of rinsing collection vessels with Milli-Q water and the rinsates were analyzed for metals (n=6). The concentrations of nickel, chromium, lead, and manganese in blank samples were non-detectable in urine cups and Rtubes. Blank saliva samples were collected by saturating the cotton swab and rinsing the vessels, followed by centrifuging to get the rinsate for analysis. We corrected our saliva results by subtracting the average blank concentrations Ni: 0.19 ug/L, Cr: 0.26 ug/L, Mn: 11.2 ug/L, Pb: 0.01 ug/L). For quality control, 10% duplicates and 10% blanks of each sample type were analyzed.

E-cigarette Sample Metal Analyses

E-liquid samples were sent to the Institute for Chemistry, University of Graz (Graz, Austria) for metal analysis. Methods for metal analysis in e-cigarette samples have been reported in detail [14]. In brief, multi-element analysis, including Ni, Cr, Pb, and Mn, in all samples and calibration standards were performed on an Agilent 8800 triple quadrupole inductively coupled plasma mass spectrometer (ICPQQQMS, Agilent Technologies, Santa Clara, USA). Concentrations were reported in a weight/weight basis (µg/kg) due to the difficulty to measure the volumes of thick and sticky e-liquid samples. A solution of propylene glycol (High purity grade, Amresco, Solon, OH, USA) and glycerol (Ultrapure, ICN Biochemicals, Aurora, OH, USA) (70 % propylene glycol, 30 % glycerol) was analyzed (n=5) as blank e-liquid to study possible matrix effects. Five blank e-liquid samples were also passed through the conical pipette tips and plastic tubing using the peristaltic pump in the lab to account for potential background air contamination as well as contamination within the sampling device. The median of the 5 aerosol blanks was used to correct aerosol samples while the median of the 5 e-liquid blanks was used to correct the dispenser and tank samples. More details on quality control are reported in Olmedo et al (2018).

Statistical Analysis

Urine, saliva, and EBC metal levels were right skewed and log-transformed to improve normality. Linear regression models on log-transformed metal biomarkers were used to compute geometric mean ratios (GMR) and the 95% confidence intervals (95% CI) by exponentiation of the beta coefficient. GMR and 95% CI were used to compare metal biomarkers of e-cigarette users versus non-users. Model 1 was adjusted for age, sex, race (white/non-white), and education (<HS, ≥ HS). Model 2 was further adjusted by previous smoking status and other sources of metal exposure. GMR and 95% CI of metal biomarkers were used to estimate their association with e-cigarette use behaviors, and metal concentrations in the dispenser, aerosol and tank samples in separate models. The main variables used as potential determinants of metal biomarker levels included the following data on e-cigarette use: e-liquid consumption per week (5-30 ml/35-240 ml), time to first vape from waking (within 15 / more than 15 minutes), preferred voltage for e-cigarette use (tertiles), coil change per month (1-2 / 3 times or more per month), coil composition (Kanthal, Nichrome, Kanthal + Nichrome), nicotine (0-3 mg/ml/ 6-24 mg/ml) as well as the corresponding metal levels in samples obtained from the dispenser, aerosol, and tank (tertiles). The analyses were restricted to users of tank-style/mods devices (n=98), as information on coil change and e-liquid consumed, and collection of e-liquid from the dispenser and/or tank did not apply to cig-a-like devices (n=2). These estimations were carried out to compare metal concentrations in the different categories of the explanatory variables; each tertile was compared to the bottom tertile explanatory variable or the highest level of a dichotomous variable was compared to the lowest one. Urine metal concentrations (µg/L) were divided by urine creatinine (g/L) and expressed in µg/g creatinine. For e-cigarette use or e-liquid metal levels categorized in tertiles, P-values for linear trend were obtained by including in the regression model a continuous variable with the medians of each tertile [22]. All analyses were performed using Stata

13.1 (StataCorp, College Station, TX). The level of statistical significance was 0.05 and all tests were 2-sided.

RESULTS

Participant characteristics

Participant characteristics have been reported in detail in another article [17]. In brief, the mean age (SD) was 30 (SD 9.6) years, 64% were men, and 82.7% were white.

Most e-cigarette users were former smokers (89%) and smoked a mean of 17 cigarettes/day (range: 1-80 cigarettes) prior to quitting. Compared to e-cigarette users, most non-users had a higher level of education (90% > HS) and were never smokers (90%). Most participants (65%) reported other sources of metal exposure and there was no significant difference between the two participant categories.

E-cigarette use behaviors and device characteristics

Among e-cigarette users, the mean (SD) age at first vape was 28 (9) years (data not shown). More than half (54%) owned two or more devices and vaped continuously throughout the day (56%). Users vaped an average of 365 (SD 720) puffs per day, with each puff lasting an average of 4 (SD 2) seconds (Table 1). More than a third (41%) of users first vape within 15 minutes of waking in the morning, with 30% vaping within 5 minutes. The reported mean voltage was 4.21 V (range: 2.12 – 12.50 V), and 85% reported periodically changing the voltage of the device. The most commonly used coils were Kanthal or some combination with Kanthal (58%), stainless steel (18%), and Nichrome (16%). Users' coils were last changed on average 16 (SD 19.4) days prior to coming to the study session, and replaced at an average of 3 (SD 2) times per month. E-liquid consumption varied greatly, ranging from 5 to 240 ml/week (median: 32.5 ml/week). The average nicotine concentration of e-liquid was 5.1 (SD 5.2) mg/ml.

Metal levels by participant category

Median (interquartile range) Cr, Ni, Pb, and Mn levels were 0.66 (0.35, 2.20), 0.71 (0.40, 1.54), 0.23 (0.10, 0.41), and 0.92 (0.06, 0.20) μg/g creatinine in urine, respectively (Supplementary Tables 1-4); 1.03 (0.29, 2.45), 0.77 (0.13, 2.37), 0.66 (0.17, 1.41), 12.2 (0.09, 47.1) μg/l in saliva; 0.23 (0.15, 0.47), 0.13 (0.13, 1.23), 0.03 (0.02, 0.32), 0.09 (0.09, 0.67) μg/l in EBC.

Compared to non-users, e-cigarette users had statistically significant higher urine (GMR 2.06, 95% CI 1.25, 3.41; p-trend: 0.005) and EBC (GMR 1.61, 95% CI 1.10, 2.36; p-trend: 0.02) Cr levels in model 1, although in fully adjusted models this was not statistically significant (Table 2). Ni saliva (GMR 2.60, 95% CI 1.33, 5.10) (Model 1) and EBC levels (GMR 3.12, 95% CI 1.53, 6.35; p-trend 0.002) in fully adjusted models as well as Pb saliva (GMR 3.22, 95% CI 1.69, 6.15) (Model 1) and urine levels (GMR 3.00, 95% CI 1.66, 5.41) in fully adjusted models were significantly higher among e-cigarette users than non-users. Lastly, users had significantly higher Mn EBC levels than non-users (GMR 2.29, 95% CI 1.24, 4.21; p-trend: 0.008) after further adjustment.

Metal levels by variables related to e-cigarette use characteristics

For Cr biomarkers, higher e-liquid consumption per week (35-240 ml) was associated with 99% higher Cr levels (p-trend 0.01) and a more frequent coil change per month (3 or more times/month) was associated with 84% higher Cr levels in saliva (p-trend 0.03) (Table 3). Although Cr levels in saliva and in EBC increased as voltage increased, Cr urine levels were 52% lower in the 2^{nd} tertile (3.87- 4.24 volts) compared to the lowest tertile. While Cr saliva levels were 60 and 40% lower when using nichrome and other coils (Titanium, stainless steel), compared to kanthal, respectively, Cr urine levels were 112 % higher when using a nichrome coil as compared to kanthal.

While the two highest to the lowest Cr levels measured in the e-liquid dispenser were associated with 48% and 53% lower Cr EBC levels (p-trend 0.01), the two highest to the lowest Cr levels were also associated with a 208% and 246% higher Cr urine levels (p-trend <0.001). Tertile 2 of Cr in aerosol samples and tank samples were associated with a 123% and 101% higher Cr urine levels, respectively. Tertile 3 of Cr in tank was also associated with 209% higher Cr saliva levels.

For Ni biomarkers, having an earlier time to first vape from waking in the morning (≤ 15 minutes) was associated with 67% higher urine Ni levels (p trend 0.02) and a more frequent coil change per month (3 or more times/month) was associated with 132% higher Ni levels in saliva (p-trend 0.04) (Table 4). Using other coils (Titanium or stainless steel) as compared to Kanthal was associated with 70% lower EBC Ni levels (p-trend 0.004) and using a higher nicotine concentration (6-24 mg/ml) was associated with 52% lower EBC Ni levels (p-trend 0.01). Increasing tertiles of Ni in aerosol tended to be associated with higher urinary, saliva, and EBC Ni levels, although this was only statistically significant for saliva Ni (p-trend 0.001). Higher Ni saliva levels were also associated with increasing tertiles of Ni in the dispenser (p-trend 0.023) and in the tank (p-trend 0.01).

For Pb biomarkers, although not statistically significant, increasing tertiles of Pb in the dispenser, aerosol, and tank tended to be associated with higher Pb levels in urine (Table

5). Increasing tertiles of Pb in the dispenser were associated with a decrease in Pb levels in EBC (p-trend 0.003).

For Mn biomarkers, increased e-liquid consumption/week was associated with 247% higher Mn saliva levels. While increasing voltage was associated with decreasing urinary Mn levels (p-trend 0.02), it was also associated with increasing EBC Mn levels (p-trend 0.04). Using nichrome and other coils (Titanium/Stainless steel) was associated with lower Mn saliva (p-trend 0.02) and EBC levels (p-trend 0.002) as compared to using Kanthal. While increasing Mn levels in dispenser samples were associated with lower Mn saliva (p-trend 0.03) and EBC levels (p-trend 0.01), increasing Mn levels in the tank tended to be associated with higher Mn saliva and EBC levels, although this was not statistically significant.

DISCUSSION

This study quantified biomarkers of metal exposure, as assessed in urine, saliva, and EBC in daily e-cigarette users and non-users from Maryland. Cr, Ni, Mn, and Pb, which have been measured in e-liquid and e-cigarette aerosol [14-16, 23-28], are metals that have been linked to lung, nasal, sinus cancer, cardiovascular and kidney disease, and neurotoxicity [29-33]. Compared to non-users, we found that e-cigarette users had higher Cr and Pb levels in urine, higher Cr, Ni, Mn levels in EBC, and higher Ni and Pb levels in saliva. Among e-cigarette users, we found higher Cr and Mn saliva levels with higher e-liquid consumed per week, higher Cr and Ni saliva levels with a more frequent coil change, higher Ni urine levels when having a shorter time to first vape from waking in the morning, and higher Mn and Cr EBC but lower Mn and Cr urine with increasing voltage. Compared to the use of Kanthal coil, lower Cr, Ni, Mn saliva and EBC levels were found when using Titanium, stainless steel, and nichrome coils. Lastly, we found increasing Cr urine, Ni urine and saliva, Pb urine with increasing corresponding metal concentrations in the aerosol. These findings support that e-cigarette use contributes to increased metal exposure as shown in comparison to non-users and that certain use/device characteristics further increase this exposure.

This is the first study to measure and compare metal biomarkers among e-cigarette users and non-users. There are only a few metal biomarker studies on e-cigarette use [19-21, 34]. Two of these studies, which are based on US nationally representative datasets, drew comparisons between e-cigarette users and cigarette smokers [20, 21] and found no statistically significant difference in urinary Ba, Be, Co, Mo, Mn, Sb, Sn, Tl levels between the two groups, except for urinary Sr levels, which were higher among e-cigarette users compared to smokers [21], and urinary Cd levels, which were lower in e-cigarette users [20]. One Romanian-based study found e-cigarette users' serum Ag, Se, and V levels were higher compared to cigarette smokers [34]. The National Health and Nutrition Examination Survey (NHANES) and Population Assessment of Tobacco and Health (PATH) study, which provide biomonitoring data for metals and tobacco use, have available data on Mn and Pb biomarkers on non-tobacco product users and among e-cigarette users, respectively [20, 35]. While both geometric means of urine Pb concentrations of non-users and e-cigarettes users were lower compared to national levels, both geometric means of urine Mn concentrations of non-users and e-cigarette users were higher in our study sample (Supplementary Table 5). Currently, urine Ni and Cr biomarkers are not available in both NHANES and PATH. The Agency for Toxic Substances and Disease Registry (ATSDR), however, has provided toxicological reference guides (ToxGuides^{TM}) containing the arithmetic mean of urine Cr [36] and Ni levels [37] of healthy adults. While our study group samples had comparable levels of urine Ni, e-cigarette users in our study had a higher mean urine Cr level.

Compared to our preliminary study [19], our findings of positive associations between Ni levels in the aerosol and the Cr levels in the tank with urine Ni and saliva Cr levels, respectively, remain even after increasing the sample size. Increasing Cr levels in the aerosol was also positively associated with increased Cr urine levels, further providing direct support that metals in the aerosol are absorbed by the e-cigarette user.

E-cigarette use behaviors may also influence metal exposure as it has been reported that being a "daily" e-cigarette user versus a "some day" user had significantly higher urinary Pb and Sr levels [20]. In our study, consuming more e-liquid per week and more frequently changing the coil were associated with increased metal levels in the saliva. Larger volumes of e-liquid introduced into the tank can facilitate the entry of e-liquid to the coil chamber [38], and numerous studies have shown that e-liquids in contact with heating coils facilitate leaching metals into the liquid [14-16, 26-28]. Indeed, some e-cigarette users have reported a metallic taste when vaping [39], supporting metal transfer from the device to the user. The type of coil used has never before been analyzed and we found that compared to using a kanthal coil, the use of nichrome, Titanium, or stainless steel was associated with lower Cr and Mn salivary levels as well as lower Mn and Ni EBC levels. Other device components, such as brass clamps and copper wires with silver coatings, may also transfer metals into the e-liquid as the presence of these components have been associated with increased Zn, Cu, Ag, and Al in the aerosol. Furthermore, the quality of manufacturing techniques may contribute to the potential impurities as the presence of substandard or frayed solder joints were associated with higher Sn levels in the aerosol [15, 16, 27]. Lastly, using e-cigarettes at a higher voltage could also influence metal transfer as aerosol generation and thermal degradation byproducts have previously been found to increase linearly with increasing voltage [38]. In our study, while increasing voltage was associated with higher Mn and Cr EBC levels, it was also associated with lower Mn and Cr urine levels. While we have found an inverse relationship (Supplementary Figure 1), other studies that have looked at Cr and Mn levels in chrome-plating workers [40, 41] and in welders [42] have found either a weak or lack of correlation between these two matrices. One possible explanation for this is that urinary Cr levels reflect all three routes of exposure (inhalation, dermal, and ingestion), while EBC mainly reflects inhalation exposure, with some contribution from what is present in the mouth. A positive correlation between saliva and EBC further demonstrates this (Supplementary Table 6).

Our findings of higher EBC Mn levels among e-cigarette users compared to non-users provide support to the growing literature [40-45] of using EBC as a biomarker for toxic metals and transition elements. Because systemic homeostasis of Mn, which is an essential element, is tightly maintained under normal dietary consumption through its intestinal absorption and removal by the liver, the use of blood or urine as biomarkers may be unreliable [46-50]. The collection of exhaled breath may be a more reliable biomarker to link exposure via inhalation and the burden of Mn on the lungs. Elevated urinary Ni and Cr levels, on the other hand, are useful biomarkers of exposure as these metals are absorbed and their main excretory pathway is via urine [37, 51, 52]. Pb is also excreted from the body mainly in the urine and is an indicator of recent Pb intake [51]. In our study, daily e-cigarette users had higher Pb urine levels compared to non-users. Similarly, using PATH data, Goniewicz et al (2018) found higher urine Pb levels among "daily" e-cigarette users compared to "some day" users. Although urinary Pb levels have been used to assess Pb exposure [51], these measurements are limited to long-term occupational monitoring programs or monitoring patients during chelation therapy [53], and are not as reliable as whole blood, which has been the primary biological fluid to assess Pb exposure throughout the last five decades [46, 53, 54].

Participants in our study reported using their devices daily, all throughout the day, at a few hundreds of puffs per day [17]. Long-term use of these devices poses as a concern as these metals, which are rapidly absorbed through the respiratory tract [55, 56], have been associated with serious adverse health effects. For instance, Ni and Cr (VI) are established inhalation carcinogens [8, 9] and are associated with decreased lung function, bronchitis, increased risk of asthma [56], and cardiovascular disease [57]. While we report total Cr in this study, there is still concern for Cr (III)'s carcinogenic potential due to the possible oxidation of Cr (III) to Cr (VI) within the lungs, which is an oxygen-rich environment [58]. Pb only requires low levels of exposure to result in health effects [11] and is associated with increased risk for cardiovascular and kidney disease; it is also a major neurotoxicant especially among children and the aging population [10, 12]. Lastly, if inhaled, Mn is associated with manganism, which is an irreversible Parkinson-like disease [13].

This study has several limitations. First, we only obtained single measurements of metal biomarkers. Second, we did not collect blood samples, which serves as the most reliable biomarker for Pb, and may provide complimentary toxicokinetic information to urine Ni and Cr biomarkers. Third, given the limited amount of sample available, we did not conduct elemental speciation. While it is likely that the Cr in the aerosol is composed of both states as valence can change given the oxidation and reduction reactions in the airways, speciation would be needed to determine if it is mainly composed of non-soluble and non-reactive Cr (III) or highly soluble, corrosive, and highly toxic Cr (VI). Cr speciation is also possible in the blood as Cr (VI) is known to enter red blood cells (RBCs) but Cr (III) does not and should be carried out. Fourth, our findings on e-cigarette users behaviors and device characteristics were based on self-report and it is possible that participants could display recall or social desirability bias. Fifth, as majority of our non-users (90%) had a higher level of education and were current students compared to e-cigarette users, this study could be affected by selection bias due to convenience sampling. It is possible that while we asked an exhaustive list of other sources of metal exposure, we may have missed other sources, including food, nutrient and herbal intake, medication and history of metal allergy, orthodontic appliances, and place of residence (i.e. urban, rural) [59-62]. Lastly, as the use of PODs (i.e. Juul, Suorin) rose in popularity towards the tail end of our recruitment, our characterization of metal exposure from e-cigarette use may not be as extensive without these new and emerging products.

Notwithstanding these limitations, this study has several strengths. It has measured non-invasive biomarker levels and e-liquid concentrations of select metals, such as Ni and Cr, pertinent to coil composition, which is not measured in NHANES and the PATH study. Moreover, it details e-cigarette use behaviors (puffs/day, how soon you first vape from waking) and device characteristics (voltage, e-liquid consumed/week, coil change/month, type of coil used), which are also not detailed in these national datasets. The collection of e-cigarette samples from each participant's device is a major strength as this assigns each participant with his/her own source of exposure and also reflects levels of exposure from the most commonly used devices at the time study was conducted. Our study not only provides a direct comparison between metal levels in the aerosol and biomarkers of internal dose, but it also draws comparisons between e-cigarette users and non-users and factors in other sources of metal exposure, which were limitations we aimed to address in our preliminary study [19]. Other strengths include utilizing a standardized study protocol and rigorous laboratory procedures to measure both biospecimen and e-cigarette samples.

CONCLUSIONS

This study demonstrates that daily e-cigarette use represents a relevant contribution to metal exposure as users had statistically significant higher metal levels in urine, saliva, and EBC compared to non-users in Maryland. From direct comparisons between source and metal biomarkers from e-cigarette use, Cr, Ni, and Pb in urine and Ni in saliva were positively associated with concentrations of corresponding metals in aerosol samples collected from personal devices of e-cigarette users, demonstrating that metals present in the aerosol are inhaled by the user. Furthermore, e-cigarette use behaviors and device characteristics may augment exposure as having a shorter time to first vape from waking, a more frequent coil change, more e-liquid consumed per week, a certain type of coil used, and vaping at a higher voltage, were associated with higher Ni, Cr, and Mn biomarker levels. Research, including those conducted at the national level, should consider including a more detailed set of e-cigarette use questions as well as a more comprehensive metal analysis to not only confirm these findings but also understand the health effects from metal exposure in the long term. This work may inform the FDA for product review and regulation, specifically implementing metal standards in e-cigarette emissions, adequate labeling of device components such as coils, and best practice for use, so as to inform users and prevent unwanted metal exposure.

TABLES

Table 1. Descriptive summary of e-cigarette use characteristics

E-cigarette characteristic	N	Mean (SD)	Range
E-liquid consumed/week (ml)	98	53.3 (48.4)	5 – 240
Preferred nicotine concentration (mg/ml)	96	5.10 (5.17)	0-24
Preferred voltage (volts)	92	4.21 (1.18)	2.12 – 12.5
Coil change/month	96	2.45 (2.37)	0-15
Coil number*	49	1.61 (0.67)	1-3
Last coil change (days)*	50	15.9 (19.4)	0-75
Puffs/day*	50	365 (720)	15-5000
Seconds/puff (secs)*	50	4.00 (1.96)	1-10
E-cigarette characteristic	**N**	**%**	
Time to first vape			
Less than 5 min	26	26.5	
6-15 min	9	9.20	
16-30 min	29	29.6	
31-60 min	23	23.5	
More than 1 hr	11	11.2	
Coil category			
Kanthal	40	48.8	
Nichrome/Nickel	16	19.5	
Both kanthal/nichrome	7	8.54	
Other (Ti or Stainless steel)	19	23.2	

*Only Year 2 data (n =50)

Table 2. Geometric mean ratios (95% CI) of chromium, nickel, lead, manganese in urine, saliva, and exhaled breath (EBC) of participants by vaping category (non-user to e-cigarette users)

	N	Crude GM	GMR (95%CI) Urine Model 1	GMR (95%CI) Urine Model 2	N	Crude GM	GMR (95%CI) Saliva Model 1	GMR (95%CI) Saliva Model 2	N	Crude GM	GMR (95%CI) EBC Model 1	GMR (95%CI) EBC Model 2
CHROMIUM												
Non-user	50	0.57	1.00 (ref)	1.00 (ref)	50	0.76	1.00 (ref)	1.00 (ref)	48	0.21	1.00 (ref)	1.00 (ref)
E-cigarette user	98	0.98	2.06 (1.25, 3.41)	1.96 (0.92, 4.17)	98	0.98	1.23 (0.75, 2.03)	1.17 (0.55, 2.49)	98	0.38	1.61 (1.10, 2.36)	1.06 (0.61, 1.86)
p –trend			0.005	0.08			0.403	0.673			0.015	0.831
NICKEL												
Non-user	50	0.65	1.00 (ref)	1.00 (ref)	50	0.39	1.00 (ref)	1.00 (ref)	48	0.17	1.00 (ref)	1.00 (ref)
E-cigarette user	98	0.83	1.23 (0.84, 1.78)	1.30 (0.74, 2.29)	98	1.00	2.60 (1.33, 5.10)	2.40 (0.86, 6.64)	98	0.56	3.13 (1.94, 5.06)	3.12 (1.53, 6.35)
p –trend			0.282	0.362			0.006	0.092			<0.001	0.002
LEAD												
Non-user	50	0.08	1.00 (ref)	1.00 (ref)	50	0.19	1.00 (ref)	1.00 (ref)	48	0.07	1.00 (ref)	1.00 (ref)
E-cigarette user	98	0.28	3.33 (2.24, 4.96)	3.00 (1.66, 5.41)	98	0.57	3.22 (1.69, 6.15)	1.62 (0.62, 4.24)	98	0.08	1.07 (0.56, 2.07)	0.75 (0.28, 1.97)
p –trend			<0.001	<0.001			<0.001	0.322			0.827	0.554
MANGANESE												
Non-user	50	0.11	1.00 (ref)	1.00 (ref)	50	2.24	1.00 (ref)	1.00 (ref)	48	0.10	1.00 (ref)	1.00 (ref)
E-cigarette user	98	0.11	1.07 (0.74, 1.53)	1.19 (0.69, 2.06)	98	4.53	1.94 (0.63, 6.01)	1.85 (0.34, 10.3)	98	0.28	2.83 (1.88, 4.27)	2.29 (1.24, 4.21)
p –trend			0.723	0.533			0.246	0.477			<0.001	0.008

Model 1: Adjusted for age, sex, race, education

Model 2: Further adjusted by previous smoking status and other sources of metal exposure†

† Other sources of metal exposure include: welding, spray painting, screen printing, metal work, machining metals, leather tanning, jewelry making or repair, painting with oil, construction of models, gardening, stained glass making, soldering/welding, pottery and ceramics, making/cutting/setting tile, recycling or fixing batteries, motor vehicle repair, hunting/firearm practice/casting bullets, making fishing weights, woodworking/furniture refinishing

Table 3. Geometric mean ratios (95% CI) of **chromium** in urine, saliva, and exhaled breath (EBC) of e-cigarette users by variables related to e-cigarette use patterns and by corresponding metal levels in samples from their personal e-cigarette devices

	N	GMR (95%CI) Urine Cr	GMR (95%CI) Saliva Cr	GMR (95%CI) EBC Cr
E-cig liquid/wk				
5 to 30 ml	49	1.00 (ref)	1.00 (ref)	1.00 (ref)
35 to 240 ml	49	0.98 (0.59, 1.64)	1.99 (1.18, 3.37)	1.29 (0.82, 2.04)
p –trend		0.93	0.01	0.27
Wake vape time				
> 15 minutes	63	1.00 (ref)	1.00 (ref)	1.00 (ref)
≤ 15 minutes	35	1.14 (0.67, 1.94)	1.03 (0.59, 1.81)	1.08 (0.67, 1.73)
p –trend		0.62	0.91	0.75
Voltage vaped				
2.12 to 3.80 volts	29	1.00 (ref)	1.00 (ref)	1.00 (ref)
3.87 to 4.24 volts	32	0.48 (0.26, 0.88)	1.64 (0.83, 3.23)	1.33 (0.75, 2.36)
4.33 to 12.5 volts	31	0.55 (0.30, 1.02)	1.23 (0.61, 2.46)	1.45 (0.81, 2.60)
p-trend		0.06	0.55	0.20
Coil change/month				
≤ 2	63	1.00 (ref)	1.00 (ref)	1.00 (ref)
3 or more	33	1.05 (0.62, 1.79)	1.84 (1.06, 3.19)	0.82 (0.51, 1.32)
p –trend		0.86	0.03	0.41
Coil comp				
Kanthal	40	1.00 (ref)	1.00 (ref)	1.00 (ref)
Nichrome	16	2.12 (1.10, 4.10)	0.40 (0.19, 0.88)	0.65 (0.33, 1.31)
Kanthal + Nichrome	7	0.65 (0.27, 1.55)	0.70 (0.25, 1.96)	2.11 (0.84, 5.31)
Other (Ti, Stainless)	19	1.68 (0.93, 3.04)	0.60 (0.30, 1.20)	0.65 (0.35, 1.20)
p –trend		0.22	0.16	0.41
Nicotine				
0 to 3 mg/ml	63	1.00 (ref)	1.00 (ref)	1.00 (ref)
6 to 24 mg/ml	33	0.86 (0.49, 1.48)	0.6 (0.34, 1.06)	0.72 (0.45, 1.15)
p-trend		0.57	0.08	0.17
Cr in dispenser				
0.40 to 1.02 μg/kg	32	1.00 (ref)	1.00 (ref)	1.00 (ref)
1.04 to 9.6 μg/kg	37	3.08 (1.79, 5.29)	0.66 (0.35, 1.27)	0.52 (0.31, 0.87)
9.7 to 41 μg/kg	27	3.46 (1.92, 6.22)	0.57 (0.28, 1.16)	0.47 (0.27, 0.83)
p-trend		<0.001	0.11	0.01
Cr in aerosol				
0.4 to 8.8 μg/kg	32	1.00 (ref)	1.00 (ref)	1.00 (ref)
9 to 29.4 μg/kg	32	2.23 (1.19, 4.19)	0.73 (0.37, 1.47)	0.71 (0.40, 1.28)
33 to 1901 μg/kg	31	1.24 (0.64, 2.40)	1.35 (0.65, 2.79)	1.06 (0.58, 1.96)
p-trend		0.53	0.41	0.84
Cr in tank				
1.5 to 25.2 μg/kg	29	1.00 (ref)	1.00 (ref)	1.00 (ref)
25.8 to 129 μg/kg	28	2.01 (1.05, 3.88)	0.97 (0.47, 1.99)	0.82 (0.43, 1.56)
132 to 2808 μg/kg	28	0.68 (0.35, 1.34)	3.09 (1.47, 6.50)	1.14 (0.59, 2.22)
p-trend		0.26	0.004	0.67

Model: Adjusted for age, sex, race, education, previous smoking status and other sources of metal exposure

Table 4. Geometric mean ratios (95% CI) of **nickel** in urine, saliva, and exhaled breath (EBC) of e-cigarette users by variables related to e-cigarette use patterns and by corresponding metal levels in samples from their personal e-cigarette devices

	N	GMR (95%CI) Urine Ni	GMR (95%CI) Saliva Ni	GMR (95%CI) EBC Ni
E-cig liquid/wk				
5 to 30 ml	49	1.00 (ref)	1.00 (ref)	1.00 (ref)
35 to 240 ml	49	0.76 (0.49, 1.17)	1.79 (0.8, 3.99)	1.4 (0.81, 2.41)
p –trend		0.21	0.16	0.22
Wake vape time				
> 15 minutes	63	1.00 (ref)	1.00 (ref)	1.00 (ref)
≤ 15 minutes	35	1.67 (1.08, 2.59)	2.07 (0.91, 4.74)	1.05 (0.60, 1.85)
p –trend		0.02	0.08	0.87
Voltage vaped				
2.12 to 3.80 volts	29	1.00 (ref)	1.00 (ref)	1.00 (ref)
3.87 to 4.24 volts	32	1.29 (0.76, 2.18)	1.60 (0.59, 4.36)	1.72 (0.89, 3.34)
4.33 to 12.5 volts	31	1.02 (0.59, 1.74)	1.81 (0.65, 5.02)	1.73 (0.88, 3.40)
p-trend		0.94	0.25	0.11
Coil change/month				
≤ 2	63	1.00 (ref)	1.00 (ref)	1.00 (ref)
3 or more	33	1.3 (0.83, 2.03)	2.32 (1.06, 5.11)	1.01 (0.58, 1.79)
p –trend		0.25	0.04	0.96
Coil comp				
Kanthal	40	1.00 (ref)	1.00 (ref)	1.00 (ref)
Nichrome	16	1.11 (0.58, 2.11)	0.53 (0.17, 1.66)	0.53 (0.26, 1.05)
Kanthal + Nichrome	7	0.57 (0.24, 1.34)	0.63 (0.14, 2.84)	1.75 (0.70, 4.40)
Other (Ti, Stainless)	19	1.23 (0.69, 2.18)	0.84 (0.3, 2.31)	0.30 (0.16, 0.57)
p –trend		0.75	0.67	0.004
Nicotine				
0 to 3 mg/ml	63	1.00 (ref)	1.00 (ref)	1.00 (ref)
6 to 24 mg/ml	33	0.84 (0.53, 1.33)	0.60 (0.25, 1.42)	0.48 (0.28, 0.84)
p-trend		0.45	0.24	0.01
Ni in dispenser				
0.01 to 1 μg/kg	35	1.00 (ref)	1.00 (ref)	1.00 (ref)
1.06 to 9.38 μg/kg	29	1.06 (0.63, 1.79)	1.06 (0.40, 2.84)	0.48 (0.25, 0.92)
14 to 370 μg/kg	32	0.98 (0.60, 1.63)	3.06 (1.19, 7.88)	1.37 (0.73, 2.56)
p-trend		0.96	0.023	0.38
Ni in aerosol				
0.7 to 23 μg/kg	32	1.00 (ref)	1.00 (ref)	1.00 (ref)
25 to 203 μg/kg	32	1.31 (0.79, 2.17)	1.78 (0.69, 4.63)	1.04 (0.54, 2.03)
219 to 15015 μg/kg	31	1.62 (0.98, 2.68)	4.73 (1.83, 12.2)	1.15 (0.59, 2.23)
p-trend		0.06	0.001	0.67
Ni in tank				
3.64 to 196 μg/kg	29	1.00 (ref)	1.00 (ref)	1.00 (ref)
203 to 940 μg/kg	28	1.18 (0.70, 2.01)	1.63 (0.58, 4.59)	0.73 (0.35, 1.54)
952 to 54608 μg/kg	28	1.00 (0.58, 1.72)	4.28 (1.47, 12.5)	0.70 (0.33, 1.51)
p-trend		0.96	0.01	0.37

Model: Adjusted for age, sex, race, education, previous smoking status and other sources of metal exposure

Table 5. Geometric mean ratios (95% CI) of **lead** in urine, saliva, and exhaled breath (EBC) of e-cigarette users by variables related to e-cigarette use patterns and by corresponding metal levels in samples from their personal e-cigarette devices

	N	GMR (95%CI) Urine Pb	GMR (95%CI) Saliva Pb	GMR (95%CI) EBC Pb
E-cig liquid/wk				
5 to 30 ml	49	1.00 (ref)	1.00 (ref)	1.00 (ref)
35 to 240 ml	49	0.84 (0.56, 1.26)	1.79 (0.99, 3.25)	0.76 (0.39, 1.45)
p –trend		0.38	0.06	0.40
Wake vape time				
> 15 minutes	63	1.00 (ref)	1.00 (ref)	1.00 (ref)
≤ 15 minutes	35	1.36 (0.90, 2.07)	1.40 (0.75, 2.61)	0.81 (0.41, 1.59)
p –trend		0.14	0.29	0.53
Voltage vaped				
2.12 to 3.80 volts	29	1.00 (ref)	1.00 (ref)	1.00 (ref)
3.87 to 4.24 volts	32	1.15 (0.70, 1.89)	1.24 (0.57, 2.66)	0.79 (0.35, 1.78)
4.33 to 12.5 volts	31	0.91 (0.55, 1.52)	1.04 (0.47, 2.28)	1.04 (0.45, 2.38)
p-trend		0.73	0.92	0.94
Coil change/month				
≤ 2	63	1.00 (ref)	1.00 (ref)	1.00 (ref)
3 or more	33	1.25 (0.82, 1.91)	1.15 (0.61, 2.16)	1.22 (0.62, 2.41)
p –trend		0.30	0.66	0.56
Coil comp				
Kanthal	40	1.00 (ref)	1.00 (ref)	1.00 (ref)
Nichrome	16	0.91 (0.49, 1.69)	0.53 (0.20, 1.40)	0.62 (0.23, 1.68)
Kanthal + Nichrome	7	0.94 (0.41, 2.13)	0.69 (0.19, 2.46)	1.63 (0.44, 6.12)
Other (Ti, Stainless)	19	0.70 (0.40, 1.22)	0.68 (0.29, 1.61)	0.65 (0.27, 1.59)
p –trend		0.22	0.36	0.51
Nicotine				
0 to 3 mg/ml	63	1.00 (ref)	1.00 (ref)	1.00 (ref)
6 to 24 mg/ml	33	0.78 (0.51, 1.2)	0.70 (0.38, 1.31)	0.96 (0.48, 1.91)
p-trend		0.26	0.26	0.91
Pb in dispenser				
0.002 to 0.482 µg/kg	30	1.00 (ref)	1.00 (ref)	1.00 (ref)
0.5 to 1.23 µg/kg	33	1.01 (0.63, 1.62)	0.88 (0.43, 1.79)	0.43 (0.20, 0.92)
1.3 to 109 µg/kg	33	1.14 (0.70, 1.85)	1.97 (0.95, 4.11)	0.30 (0.14, 0.64)
p-trend		0.60	0.07	0.003
Pb in aerosol				
0.1 to 3.6 µg/kg	33	1.00 (ref)	1.00 (ref)	1.00 (ref)
3.7 to 26.3 µg/kg	31	1.05 (0.66, 1.68)	1.23 (0.61, 2.51)	1.55 (0.71, 3.38)
28.2 to 4788 µg/kg	31	1.51 (0.94, 2.41)	0.82 (0.4, 1.66)	1.31 (0.60, 2.86)
p-trend		0.09	0.59	0.48
Pb in tank				
1.6 to 17.8 µg/kg	29	1.00 (ref)	1.00 (ref)	1.00 (ref)
18.04 to 110 µg/kg	28	0.95 (0.62, 1.45)	0.83 (0.36, 1.90)	1.80 (0.76, 4.25)
116 to 7317µg/kg	28	1.52 (0.97, 2.37)	1.02 (0.43, 2.42)	1.33 (0.54, 3.28)
p-trend		0.08	0.99	0.48

Model: Adjusted for age, sex, race, education, previous smoking status and other sources of metal exposure

Table 6. Geometric mean ratios (95% CI) of **manganese** in urine, saliva, and exhaled breath (EBC) of e-cigarette users by variables related to e-cigarette use patterns and by corresponding metal levels in samples from their personal e-cigarette devices

	N	GMR (95%CI) Urine Mn	GMR (95%CI) Saliva Mn	GMR (95%CI) EBC Mn
E-cig liquid/wk				
5 to 30 ml	49	1.00 (ref)	1.00 (ref)	1.00 (ref)
35 to 240 ml	49	0.82 (0.53, 1.29)	3.47 (1.04, 11.6)	1.17 (0.71, 1.91)
p –trend		0.39	0.04	0.54
Wake vape time				
> 15 minutes	63	1.00 (ref)	1.00 (ref)	1.00 (ref)
≤ 15 minutes	35	1.24 (0.79, 1.97)	1.32 (0.37, 4.71)	0.90 (0.54, 1.49)
p –trend		0.35	0.66	0.67
Voltage vaped				
2.12 to 3.80 volts	29	1.00 (ref)	1.00 (ref)	1.00 (ref)
3.87 to 4.24 volts	32	0.61 (0.36, 1.03)	2.34 (0.51, 10.8)	1.56 (0.85, 2.86)
4.33 to 12.5 volts	31	0.52 (0.31, 0.89)	1.24 (0.26, 5.88)	1.90 (1.02, 3.52)
p-trend		0.02	0.77	0.04
Coil change/month				
≤ 2	63	1.00 (ref)	1.00 (ref)	1.00 (ref)
3 or more	33	1.42 (0.89, 2.24)	2.62 (0.74, 9.26)	1.16 (0.70, 1.94)
p –trend		0.14	0.13	0.56
Coil comp				
Kanthal	40	1.00 (ref)	1.00 (ref)	1.00 (ref)
Nichrome	16	1.38 (0.71, 2.72)	0.06 (0.01, 0.35)	0.40 (0.21, 0.76)
Kanthal + Nichrome	7	1.02 (0.42, 2.49)	0.80 (0.08, 7.67)	1.92 (0.83, 4.43)
Other (Ti, Stainless)	19	1.38 (0.71, 2.72)	0.12 (0.03, 0.57)	0.28 (0.16, 0.50)
p –trend		0.39	0.02	0.002
Nicotine				
0 to 3 mg/ml	63	1.00 (ref)	1.00 (ref)	1.00 (ref)
6 to 24 mg/ml	33	0.90 (0.58, 1.4)	0.22 (0.06, 0.78)	0.66 (0.39, 1.1)
p-trend		0.64	0.02	0.11
Mn in dispenser				
0.003 to 0.71 μg/kg	30	1.00 (ref)	1.00 (ref)	1.00 (ref)
0.8 to 2.43 μg/kg	33	1.10 (0.64, 1.90)	0.07 (0.02, 0.30)	0.30 (0.17, 0.53)
2.46 to 113 μg/kg	33	0.72 (0.41, 1.24)	0.17 (0.04, 0.72)	0.45 (0.25, 0.78)
p-trend		0.20	0.03	0.01
Mn in aerosol				
0.003 to 1.5 μg/kg	32	1.00 (ref)	1.00 (ref)	1.00 (ref)
1.55 to 4.8 μg/kg	32	1.02 (0.59, 1.75)	0.34 (0.08, 1.52)	0.62 (0.34, 1.13)
4.9 to 109 μg/kg	31	1.40 (0.80, 2.46)	0.58 (0.12, 2.72)	1.08 (0.58, 2.00)
p-trend		0.23	0.48	0.82
Mn in tank				
0.7 to 17.5 μg/kg	29	1.00 (ref)	1.00 (ref)	1.00 (ref)
18.1 to 62.7 μg/kg	28	0.88 (0.49, 1.59)	1.73 (0.34, 8.86)	1.20 (0.62, 2.31)
64.7 to 1542 μg/kg	28	0.52 (0.28, 0.98)	4.49 (0.79, 25.6)	1.67 (0.83, 3.38)
p-trend		0.05	0.09	0.15

Model: Adjusted for age, sex, race, education, previous smoking status and other sources of metal exposure

Supplementary Table 1. Summary of chromium levels in urine, saliva, and exhaled breath condensate (EBC) of study participants (n= 148)

	Chromium												
	Total	Total	User	Non-user	*p*-value	Total	User	Non-User	*p*-value	Total	User	Non-user	*p*-value
	Urine (μg/l)	Urine (μg/g of creatinine)	Urine (μg/g of creatinine)	Urine (μg/g of creatinine)		Saliva (μg/l)	Saliva (μg/l)	Saliva (μg/l)		EBC (μg/l)	EBC (μg/l)	EBC (μg/l)	
Sample size	148	148	98	50		148	98	50		148	98	48	
Arithmetic mean	1.60	2.16	2.36	1.76		2.16	2.31	1.88		0.73	0.93	0.33	
Geometric mean	0.87	0.82	0.98	0.57	0.03	0.90	0.98	0.76	0.27	0.31	0.38	0.21	<0.001
Percentile													
10^{th}	0.15	0.14	0.30	0.06		0.15	0.15	0.15		0.15	0.15	0.15	
25th	0.41	0.35	0.38	0.11		0.29	0.39	0.15		0.15	0.15	0.15	
50th	1.14	0.66	0.66	0.60		1.03	1.20	0.62		0.23	0.29	0.15	
75^{th}	1.75	2.20	2.06	2.50		2.45	2.28	2.74		0.47	0.73	0.23	
90th	3.19	6.11	6.57	3.98		4.76	4.02	6.14		1.52	1.95	0.50	
Maximum	13.6	29.4	29.4	17.7		33.5	33.5	8.66		19.4	19.4	3.59	
LOD	0.04					0.04	0.04	0.04		0.04	0.04	0.04	
Percent < LOD	1.35					0.70	1.02	0		0	0	0	

Dilution factor for urine: 5, Dilution factor for saliva and EBC: 10

p-values obtained from t-test

Supplementary Table 2. Summary of nickel levels in urine, saliva, and exhaled breath condensate (EBC) of study participants (n= 148)

	Nickel												
	Total	Total	User	Non-user	*p*-value	Total	User	Non-User	*p*-value	Total	User	Non-user	*p*-value
	Urine (µg/l)	Urine (µg/g of creatinine)	Urine (µg/g of creatinine)	Urine (µg/g of creatinine)		Saliva (µg/l)	Saliva (µg/l)	Saliva (µg/l)		EBC (µg/l)	EBC (µg/l)	EBC (µg/l)	
Sample size	148	148	98	50		148	98	50		146	98	48	
Arithmetic mean	1.58	1.33	2.09	1.88		4.07	5.40	1.47		1.01	1.29	0.44	
Geometric mean	0.81	0.76	0.83	0.65	0.17	0.73	1.00	0.39	0.003	0.38	0.56	0.17	<0.001
Percentile													
10th	0.13	0.22	0.22	0.18		0.13	0.13	0.08		0.13	0.13	0.10	
25th	0.34	0.40	0.43	0.37		0.13	0.27	0.13		0.13	0.13	0.13	
50th	0.92	0.71	0.81	0.65		0.77	1.16	0.27		0.13	0.78	0.13	
75th	1.74	1.54	1.74	1.03		2.37	3.33	1.51		1.23	1.42	0.13	
90th	3.25	2.45	2.90	2.33		9.57	13.8	2.50		1.93	2.31	1.06	
Maximum	19.2	17.0	17.0	12.9		176	176	12.8		17.9	17.9	6.47	
LOD	0.04					0.04	0.04	0.04		0.04	0.04	0.04	
Percent < LOD	0					6.76	6.12	8.00		1.35	0	4.17	

Dilution factor for urine: 5, Dilution factor for saliva and EBC: 10
p-values obtained from t-test

Supplementary Table 3. Summary of lead levels in urine, saliva, and exhaled breath condensate (EBC) of study participants (n= 148)

	Lead												
	Total	Total	User	Non-user	*p*-value	Total	User	Non-User	*p*-value	Total	User	Non-user	*p*-value
	Urine (µg/l)	Urine (µg/g of creatinine)	Urine (µg/g of creatinine)	Urine (µg/g of creatinine)		Saliva (µg/l)	Saliva (µg/l)	Saliva (µg/l)		EBC (µg/l)	EBC (µg/l)	EBC (µg/l)	
Sample size	148	148	98	50		148	98	50		146	98	48	
Arithmetic mean	0.41	0.36	0.45	0.16		1.30	1.52	0.86		0.33	0.25	0.48	
Geometric mean	0.20	0.18	0.28	0.08	<0.001	0.39	0.57	0.19	<0.001	0.07	0.08	0.07	0.63
Percentile													
10^{th}	0.02	0.03	0.09	0.01		0.02	0.05	0.02		0.02	0.02	0.02	
25th	0.09	0.10	0.14	0.03		0.17	0.27	0.02		0.02	0.02	0.02	
50th	0.31	0.23	0.30	0.10		0.66	0.68	0.27		0.03	0.06	0.02	
75^{th}	0.56	0.41	0.48	0.24		1.41	1.28	1.47		0.32	0.30	0.77	
90th	0.98	0.65	0.98	0.51		2.84	2.94	2.53		1.19	0.83	1.51	
Maximum	3.05	4.30	4.30	0.79		23.6	23.6	4.36		3.77	2.20	3.77	
LOD	0.01					0.01	0.01	0.01		0.01	0.01	0.01	
Percent < LOD	0					0	0	0		0	0	0	

Dilution factor for urine: 5, Dilution factor for saliva and EBC: 10
p-values obtained from t-test

Supplementary Table 4. Summary of manganese levels in urine, saliva, and exhaled breath condensate (EBC) of study participants (n= 148)

	Manganese												
	Total	Total	User	Non-user	*p*-value	Total	User	Non-User	*p*-value	Total	User	Non-user	*p*-value
	Urine (µg/l)	Urine (µg/g of creatinine)	Urine (µg/g of creatinine)	Urine (µg/g of creatinine)		Saliva (µg/l)	Saliva (µg/l)	Saliva (µg/l)		EBC (µg/l)	EBC (µg/l)	EBC (µg/l)	
Sample size	148	148	98	50		148	98	50		146	98	48	
Arithmetic mean	0.20	0.22	0.25	0.18		46.6	43.8	52.0		0.41	0.51	0.21	
Geometric mean	0.12	0.11	0.11	0.11	0.72	3.57	4.53	2.24	0.19	0.20	0.28	0.10	<0.001
Percentile													
10th	0.09	0.03	0.03	0.04		0.09	0.09	0.09		0.09	0.09	0.09	
25th	0.09	0.06	0.06	0.06		0.09	0.09	0.09		0.09	0.09	0.09	
50th	0.09	0.92	0.10	0.08		12.2	13.4	0.31		0.09	0.38	0.09	
75th	0.13	0.20	0.20	0.20		47.1	37.0	66.3		0.67	0.77	0.09	
90th	0.20	0.53	0.56	0.32		162	112	185		1.16	1.34	0.13	
Maximum	4.25	4.38	4.38	1.74		538	534	538		4.51	2.42	4.51	
LOD	0.03					0.02	0.02	0.02		0.02	0.02	0.02	
Percent < LOD	0					0	0	0		0.70	0	2.08	

Dilution factor for urine: 5, Dilution factor for saliva and EBC: 10

p-values obtained from t-test

Supplementary Table 5. Comparison of metal urinary concentrations in the study with data from US national surveys

	Study sample			US National reports and surveys	
Unit	µg/l	µg/l	µg/g creatinine	µg/l	µg/g creatinine
Summary Statistic	Arithmetic Mean (95% CI)	GM (95% CI)	GM (95% CI)	Mean	GM (95% CI)
CHROMIUM					
Non-users	1.26 (0.76, 1.74)	0.55 (0.37, 0.82)	0.57 (0.35, 0.93)	0.22[36]	
E-cigarette users	1.77 (1.34, 2.21)	1.10 (0.91, 1.33)	0.98 (0.77, 1.25)		
NICKEL					
Non-users	1.07 (0.72, 1.43)	0.62 (0.46, 0.85)	0.65 (0.49, 0.85)	1-3[37]	
E-cigarette users	1.84 (1.27, 2.42)	0.93 (0.73, 1.18)	0.83 (0.67, 1.02)		
LEAD					
Non-users	0.23 (0.12, 0.33)	0.08 (0.05, 0.12)	0.08 (0.06, 0.12)		0.32* [35] (0.29-0.36)
E-cigarette users	0.50 (0.40, 0.59)	0.31 (0.25, 0.39)	0.28 (0.23, 0.34)		0.43[20] (0.38, 0.49)
MANGANESE					
Non-users	0.11 (0.09, 1.22)	0.10 (0.09, 0.11)	0.11 (0.08, 0.14)		0.12* [35] (0.12-.14)
E-cigarette users	0.24 (0.12, 0.36)	0.13 (0.11, 0.15)	0.11 (0.09, 0.14)		0.14 [20] (0.12, 0.16)

GM: Geometric mean, CI: Confidence interval

*Non-smokers in the US Adult population; Cigarette nonsmokers who used other tobacco products were excluded

Supplementary Table 6. Correlation Matrix of metal biomarkers among e-cigarette users (n=98)

	UrineCr	UrineNi	UrinePb	UrineMn	Sal_Cr	Sal_Ni	Sal_Pb	Sal_Mn	EBC_Cr	EBC_Ni	EBC_Pb	EBC_Mn
Urine_Cr	1.0000											
Urine_Ni	0.1985	1.0000										
Urine_Pb	0.1525	0.4786*	1.0000									
Urine_Mn	0.5023*	0.3449*	0.3137*	1.0000								
Sal_Cr	-0.2731*	-0.0309	-0.0779	-0.1287	1.0000							
Sal_Ni	-0.3261*	0.1296	0.1306	-0.0620	0.3785*	1.0000						
Sal_Pb	-0.1218	-0.0494	-0.0855	-0.2396*	0.5731*	0.1576	1.0000					
Sal_Mn	-0.4486*	-0.0286	0.0283	-0.1004	0.6784*	0.3671*	0.5035*	1.0000				
EBC_Cr	-0.2675*	-0.0204	0.0525	-0.0361	0.1958	0.1611	-0.0033	0.2140*	1.0000			
EBC_Ni	-0.4080*	0.0627	0.1201	-0.1592	0.1727	0.1052	0.0733	0.3811*	0.4200*	1.0000		
EBC_Pb	-0.2728*	-0.0026	0.0469	-0.1077	0.0882	0.0111	0.0150	0.2015*	0.3997*	0.5720*	1.0000	
EBC_Mn	-0.5300*	0.0194	0.1267	-0.1665	0.2898*	0.2545*	0.1010	0.4757*	0.5343*	0.8066*	0.6137*	1.0000

Supplementary Figure 1. Scatter plots of urine metal concentrations against saliva and EBC metal concentrations of e-cigarette users (n = 98)

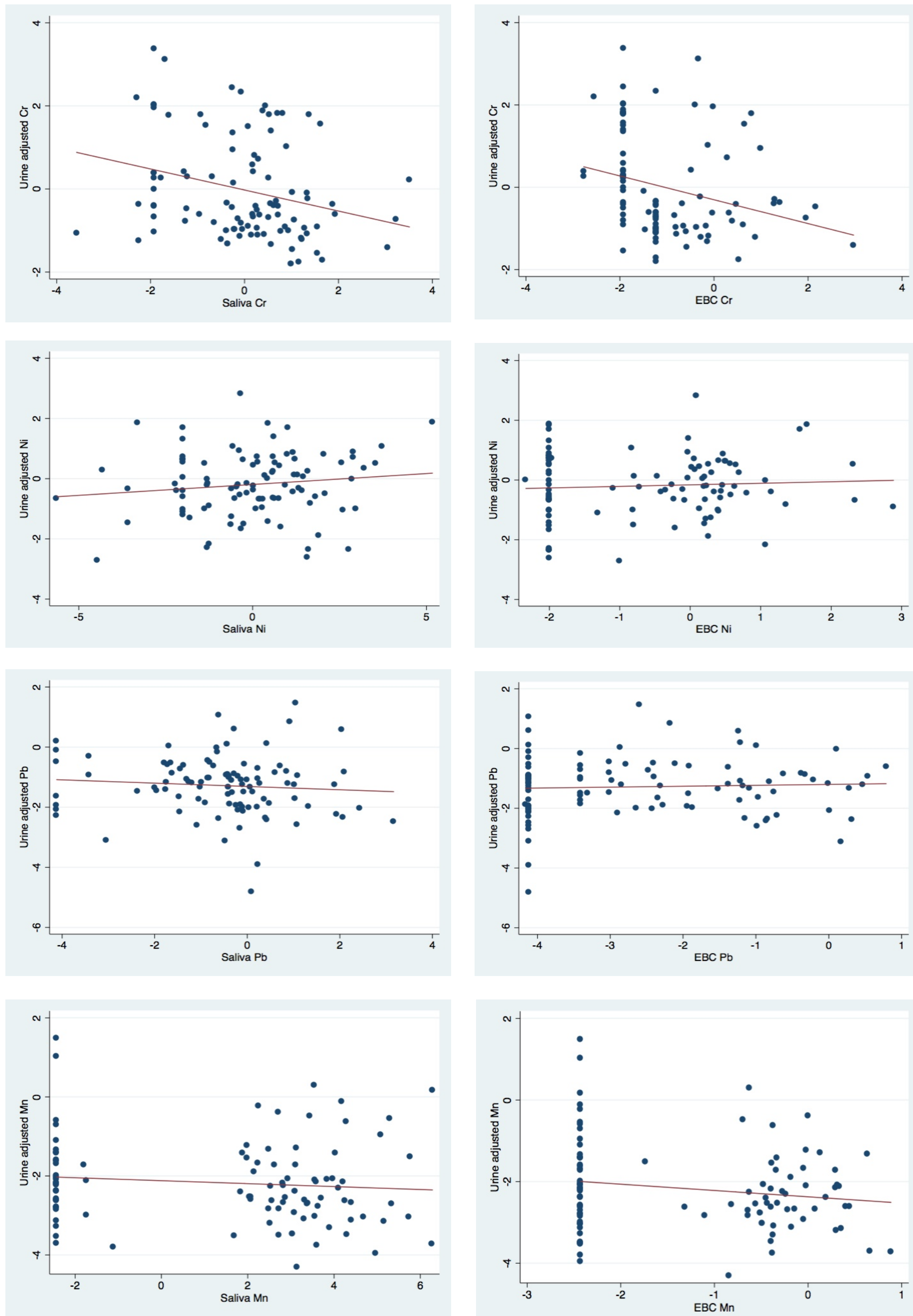
SALIVA EBC

Cr URINE

Ni URINE

Pb URINE

Mn URINE



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CHAPTER 6: DISCUSSION

Summary Findings

The main objectives of this dissertation were to (1) determine the range of metal concentrations in e-liquids (bottles, cartridges), e-cigarette aerosols, and biomarkers of e-cigarette users; (2) evaluate the demographic characteristics and self-reported health status among e-cigarette users in Maryland; and (3) compare metal biomarker concentrations between users and non-users and investigate the contribution of e-cigarette user patterns that are associated with increased metal exposure. For objectives (2) and (3), a total of 150 participants (100 e-cigarette users and 50 non-users) were recruited and information on demographic characteristics, tobacco history, and other potential sources of metal exposure as well as 3 biospecimen samples (urine, saliva, and EBC) were collected. E-cigarette users in the study were additionally asked questions about their e-cigarette use behaviors and device characteristics and samples of their e-liquid and condensed aerosol were collected.

From the systematic review (Chapter 2), I identified a total of 24 studies – 12 reported on metals in e-liquid, 12 on metals in e-cigarette aerosol, and 4 on metals in biomarkers of e-cigarette users [1-24]. These studies report several metals present in e-liquid and aerosol samples, (Al, Sb, As, Cd, Co, Cr, Cu, Fe, Pb, Mn, Ni, Se, Sn, and Zn) at widely varied concentrations. There are, however, some notable findings and differences according to type of sample (e-liquid vs. aerosol), source of sample (dispenser bottle, cartridge, open wick or tank), and device type (open/closed system device). In e-liquids, Al, Fe, Ni, and Zn were consistently found, and metal levels in e-liquid samples not in contact with the heating coil (dispenser bottle) were generally lower than most e-liquid samples that have been in contact with the coil (cartridges or open wicks/tanks). In aerosols, Al, Cr, Cu, Fe, Ni, Pb, and Zn were consistently found, and metal levels in samples from open system devices were more elevated as compared to those from closed-system devices (*cig-a-likes*). This difference may perhaps be influenced by the ability to modify the device's coil type and number and as well as power, voltage, and temperature settings, which is not possible in closed devices. Studies that have looked at metals in e-liquid dispenser samples and corresponding aerosols are key to this review as they reveal metal levels to be 6 to 25 times higher in the aerosol, indicating metal transfer from the device to the liquid, which is transferred to the aerosol that is subsequently inhaled by the user [14, 15, 23]. Of note, 10 studies reveal Cd levels to be low or <LOD in both e-liquid and aerosol samples, indicating e-cigarettes may be a lower source of Cd exposure than conventional cigarettes. However, exposure to other metals may still be a concern as levels of some metals in e-cigarette users were similar or even higher than conventional cigarette users (urinary Sr levels, serum Ag, Se, and V levels [2, 10]) or even higher than cigar users (blood Mn [10]). One study found that Ni and Cr levels in the aerosol levels were positively associated with corresponding urinary and salivary metal levels [1], providing direct support that metals in the aerosol are absorbed by the e-cigarette user.

After summarizing the state of the knowledge on metal levels present in e-cigarettes, I sought to describe behaviors and device characteristics of daily exclusive e-cigarette users as their chronic use may place them at higher risk of metal exposure (Chapter 3). As of October of 2017, the majority of the daily exclusive e-cigarette users were men (67%), white (87%), former smokers (89%) and used open system devices (MODs/tanks) (98%), which is consistent with the nationally representative Population Assessment of

Tobacco and Health (PATH) study (Waves 1 (2013- 2014) and 2 (2014-2015)) [25, 26]. Most e-cigarette users in our sample owned two or more devices (54%), vape continuously throughout the day (56%), and more than a third (41%) of the users first vape within 15 minutes of waking in the morning, indicating a high level of dependence of use to nicotine. These users also vaped a median (range) of 200 (15, 5000) puffs/day at an average voltage of 4.21 V (SD: 1.2), consumed between 5-240 ml of e-liquid/week (Median: 32.5 ml/week) at an average nicotine concentration of 5.3 (SD: 5.3) mg/ml), and used either a Kanthal (58%), stainless steel (18%) or nichrome (16%) heating coil. More intense and frequent use was found among men compared to women as men preferred to vape at a higher voltage and consume more e-liquid/week; individuals with a lower level of education were also found to consume more e-liquid per week compared to individuals with a higher level of education. This is a concern as the vaping regimen of daily exclusive users exposes them to levels of formaldehyde, acrolein, diacetyl [27], and metals [14] that exceed US occupational health limits, and with more intense users (men, individuals of higher education) preferring to vape at a higher voltage, this increases the respirable fraction allowing more particles to readily enter the ciliated airways [28]. While e-cigarette use was primarily reported as an aid to quit smoking (34%) and as a healthier alternative to cigarettes (32%), less than half planned to quit vaping.

After describing daily e-cigarette users, I determined whether their use behaviors and device characteristics as well as metal concentrations in e-liquid samples are associated with increased exposure to metals, specifically Ni, Cr, Pb, and Mn, as determined by non-invasive biomarkers (urine, saliva, and exhaled breath condensate (EBC)) (Chapter 5). I also compared metal biomarker levels between e-cigarette users and non-users, and found that users had statistically significantly higher Ni and Mn EBC levels as well as Pb urine levels as compared to non-users. According to use behaviors and device characteristics, there were several significant associations with biomarkers for each metal, with some findings in one biomarker opposing the findings of another biomarker of the same metal (for example, voltage is negatively associated with Mn in urine but positively associated with Mn in EBC). Given this, the findings with the most appropriate biomarker for each metal are prioritized and reported accordingly. In the case of Ni and Cr, these metals in urine are useful biomarkers of environmental or occupational exposure and are primarily excreted in this route while saliva is a secondary biomarker [29-31]. I found that users who had a shorter time to first vape from waking in the morning had higher Ni urine levels. Moreover, I found that Cr and Ni concentrations measured in the aerosol were positively associated with Cr and Ni urine levels, respectively, which provides direct support that metals in the aerosol are absorbed by the user. Secondary findings include higher Cr saliva levels with more e-liquid consumed/week (>30 ml/week), and higher Cr and Ni saliva levels with a more frequent coil change/month (>2/month). This may be because commonly used coils, such as Kanthal and Nichrome, release Cr and Ni, respectively, at higher levels after the 1st burn and then progressively lower as the number of burns increases [32]. In the case of Pb, this metal is mainly excreted in urine which is an indicator of recent Pb exposure [30]. However, whole blood is the primary biomarker to assess Pb exposure [33-35], and this may explain why no significant associations were found with use behaviors or device characteristics. Conversely, because systemic homeostasis of Mn is tightly maintained under normal dietary consumption, the use of urine or blood may not be reliable sources [33, 36-39]. There is literature support for using EBC to quantify Mn inhalation exposure [40-45] and in this study, I found lower Mn EBC levels when using a Nichrome, titanium, or stainless steel coil as opposed to using a Kanthal coil. I also found higher Mn EBC levels but lower urine levels as voltage increased, which may reflect different routes of exposure (i.e. dermal and ingestion for urine, and inhalation for EBC). Nevertheless, the e-cigarette user's preferred voltage must further be explored, and perhaps in interaction with other factors of the device, such as puffing topography, e-liquid and coil composition, as they do not work in isolation from one another.

Strengths and Limitations

This dissertation has several strengths. The systematic review (Chapter 2) is the first to analyze metal levels in e-liquids, cartomizers and tanks, aerosols and biomarkers in such detail across several studies. I strove to include all information by standardizing units as much as possible, identify which sources of e-liquids and types of devices give off relatively higher metal levels, and compare levels to conventional cigarettes. The descriptive analysis (Chapter 3) provides relevant information on daily e-cigarette users, who represent a small subgroup of the e-cigarette population and who may be at higher risk for potential toxicity from chronic use. Compared to nationally representative datasets such as NHANES and PATH, this study provides more detailed information pertaining to e-cigarette device characteristics (including voltage, power, and the type of heating coil used) and use behaviors (including amount of e-liquid consumed per week, the number of times the heating coil is replaced per month, puffs taken per day). The biomarker analysis (Chapter 5) is the first study to compare metal biomarkers among e-cigarette users and non-users. There are only a few metal biomarker studies on e-cigarette use [1, 2, 8, 10]. This study has measured non-invasive biomarker levels and provided direct comparisons to the aerosol concentrations of select metals pertinent to coil composition, such as Ni and Cr, which are not measured in NHANES or the PATH study. The collection of e-cigarette samples from each participant's device is another major strength as this reflects the levels of exposure of the most commonly used devices at the time the study was conducted, and it assigns participants with their own source of exposure. Utilizing a novel aerosol-condensing device developed in one of our preliminary studies [46] also served as an easy-to-use aerosol collection system that directly collects the e-cigarette aerosol as it would be inhaled and re-aggregates into liquid form without having to dilute or extract from a collecting matrix. Lastly, collecting EBC serves as a promising tool as a biomarker for metals and transition metals, such as Mn, which may not be as reliable in other biomarker matrices [33, 36-39]. Other strengths include utilizing a standardized study protocol and rigorous laboratory procedures to measure both biospecimen and e-cigarette samples.

This dissertation is not without limitations. A major issue encountered while conducting the systematic review (Chapter 2) was the differing puffing protocols across all the different studies. Each study had different puff counts, seconds/puff, and puff volumes, and some studies left important aspects of their protocols unexplained, presented their findings using graphics as opposed to exact values, or did not specify if background correction after measuring blanks was conducted. In the descriptive analysis (Chapter 3), while e-cigarette users and non-users were matched according to age, sex, and race, majority of the non-users had a higher level of education and were current students compared to e-cigarette users, signifying the study could be affected by selection bias due to convenience sampling. For both Chapters 3 and 5, e-cigarette use behaviors were based on self-report and it is possible that participants could display recall bias or social desirability bias. In Chapter 5, the study only obtained single measurements of metal biomarkers and did not collect blood samples, which is the most reliable biomarker for Pb. This study also did not conduct elemental speciation for Cr (III vs. VI) as there was limited amount of sample available. While participants were asked an exhaustive list of questions to determine sources of metal exposure (hobbies, tattoos, occupational), it is possible I may have missed other potential sources such as food, medications, orthodontic appliances, and place of residence. It is also possible that this exhaustive list may have been too broad to account for work/lifestyle activities that specifically expose users to Ni, Cr, Mn, Pb in chapter 5. Lastly, at the tail end of completing this dissertation (October, 2017) newer e-cigarette devices were being introduced to the market that did not fit the definition of an open- nor closed-system device (i.e. newer POD systems which were initially defined as closed systems, now allow pod cartridges to be refilled with e-liquid). Thus, the nomenclature used in this dissertation may not apply to all devices and must be updated to account for the rapidly changing devices in the market.

Future research

One of the strengths of this dissertation was providing information from real world e-cigarette users, what they are presently using, and what they are being exposed to. At the time of recruitment of Maryland e-cigarette users, which was from December 2015 to October 2017, most were using open-system e-cigarette devices (MODs/Tanks). Towards the tail end of our recruitment, the use of pod mods (PODs) grew in popularity, particularly among college/university students [47, 48]. PODs are much smaller in size, similarly shaped to a USB drive, but have the same mechanics as cig-a-likes – the bottom part of the device includes a battery and temperature regulation system while the top part holds a pre-filled e-liquid cartridge that is disposable [49]. Compared to MODs/tank systems, which require the user to manually change heating coils and refill the tank with e-liquid, PODs simply require the replacement of a pod, which already has the pre-filled e-liquid and coil in it[50]. While earlier generations of e-cigarettes use a free-base type of nicotine, which passes quickly into the bloodstream when inhaled, nicotine salts are used in pod mods like Juul (JUULsalts™), which is a concentrated juice cocktail of salts and organic acids from tobacco leaves. Each pod contains 0.7 ml (or 59 mg/ml) of nicotine, which is equivalent to one pack of cigarettes, or 200 puffs. Moving forward, my next step is to recruit POD users to characterize their use behaviors and analyze for metal biomarkers. Major tobacco surveys such as the National Health Interview Survey (NHIS) and PATH are currently lacking specific questions about new and emerging products, including PODs [51] and many POD users who are mostly youth and young adults do not identify as e-cigarette users [52, 53]. This highlights the need to tailor recruitment and also add questions in interview-based studies appropriate to this type of e-cigarette users in future studies. Our research group intends to assess the metal concentrations from e-liquid and condensed aerosol samples of different POD brands I intend to explore the interaction of metals with other metals as well as the interaction of different device characteristics (nicotine concentration, voltage, puffing topography) in association to metal biomarker levels. In addition to urine, saliva, and EBC, blood will also be collected to not only measure metal concentrations but also conduct elemental speciation. Cr (VI) is known to enter red blood cells (RBC), while Cr (III) cannot [53], and thus measuring

Cr in RBC and serum can indicate whether e-cigarette users are exposed to Cr (VI). I will also factor the time since last vaped before coming to the study session in the biomarker analysis since metals have half-lives spanning few or several hours, and recent (or lapsed) e-cigarette use may affect metal exposure within the body.

Implications for policy and public health

Through the completion of this dissertation, I have found that there is considerable variability in the metal concentration from different e-cigarette device types, e-liquid formulations and aerosols. I have also found that exclusive e-cigarette users had higher metal biomarker levels compared to non-users, and that metal exposure is affected by user behaviors. As of June 2019, the FDA deadlines to meet certain requirements as a manufacturer and retailer of e-cigarettes have passed [54]. These requirements include (1) registering an establishment and submitting lists of products, including labeling and advertisements, (2) submitting tobacco health documents, (3) submitting ingredient listings, and (4) including a required warning statement on packages and advertisements for e-cigarettes stating "WARNING: This product contains nicotine. Nicotine is an addictive chemical." By May 12, 2020, manufacturers must file for premarket tobacco applications (PMTAs) with the FDA who will assess whether the product is appropriate for the protection of public health [55].

The FDA and Center for Tobacco Products (CTP) have called for research on e-cigarette toxicity and the findings from this dissertation add to the evidence base. The need for product review comes in a timely manner given the recent outbreak of lung disease and deaths related to using vaping products. As of October 8, 2019, 1299 lung injury cases in association with e-cigarette use have been reported to the CDC from 49 states, the

District of Columbia, and 1 U.S. territory, and 26 deaths have been confirmed in 21 states [56]. These illnesses, which include symptoms such as difficulty breathing, shortness of breath, and chest pain before hospitalization, have been found in patients that use e-cigarettes, most of which contain tetrahydrocannabinol (THC) [55]. Although the current investigation has not identified a specific product or substance, these illnesses appear to be associated with chemical exposure from e-cigarettes, emphasizing the need to scientifically evaluate the risks and benefits of the overall product.

Based on the dissertation findings, I have a few policy recommendations. First, I recommend that the FDA establish product standards for e-cigarette devices and e-liquids. From the systematic review, I identified that metals were present in the e-liquid even before use, especially in pre-filled cartridges where e-liquids are in contact with the coil. I also found that those same metals were elevated in the aerosol, which also included metals that were not listed as the makeup of the heating coil, suggesting they could have been from other parts of the device (i.e. solder joints, other wires). The FDA should consider finding and eliminating the sources of impurities in e-liquids as well as the specific materials in the device that have the potential for toxic metal generation to ultimately develop product specifications. They should also consider requiring manufacturers to abide by a quality control agreement from production in the manufacturing facility to transportation to the storefront to maintain the integrity of the product and supply chain. Second, based on several of our findings of (1) higher Cr and Ni in urine associated with higher Cr and Ni in the aerosol, (2) higher Cr urine associated with using nichrome as compared with a kanthal coil, and (3) lower levels of Cr, Ni, Mn in saliva and EBC with using titanium or stainless steel coils, I recommend the use of stainless steel or titanium coils as opposed to kanthal or nichrome in order to limit metal exposure. Third, I recommend that the FDA require packaging of e-cigarette devices and device paraphernalia (including heating coils and e-liquids) to include an ingredient listing in order to inform users of the chemical composition and whether some users might want to consider other alternatives in the case of a metal allergy (i.e. Ni). More importantly, I recommend that the packaging come with instructions for safe use. In the case of heating coils, I have found that more frequent coil replacement (>2 times/month) was associated with increased salivary metal biomarkers. One suggested practice for use would be to recommend maximum of 2 coil changes/month (i.e. replace heating coil every 2 weeks). I have also found that metal levels in the e-liquid remaining in the tank after vaping are the highest relative to the levels in the aerosol and in the dispenser before use. Another suggestion would be to frequently clean the tank so as to remove the residue that has accumulated from use. Lastly, because there are many different e-cigarette devices, components, use behaviors and preferences with vaping, I recommend that national studies or programs such as PATH and NHANES consider adding more questions on user behaviors (i.e. amount of e-liquid consumed/week, coil change/month, puffs/day) and device characteristics (preferred voltage, type of coil used), and also measure metals biomarkers, such as Ni and Cr, which are pertinent to coil composition and are currently only reported in PATH. The collection of such data would better characterize e-cigarette use behaviors and potential harms.

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CURRICULUM VITAE

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PROFILE

Under the guidance of Dr. Ana Rule, I am studying the fields of exposure science and environmental epidemiology through a state and federally funded study on exposure to toxic metals from electronic cigarette (e-cigarette) use.

EDUCATION

October 2019 — **Doctor of Public Health (DrPH)**
Department of Environmental Health and Engineering
Johns Hopkins Bloomberg School of Public Health, Baltimore, MD
Dissertation: Characterization of toxic metal exposure from electronic cigarette use (Dr. Ana Rule)

May 2015 — **Master of Public Health (MPH)**
Johns Hopkins Bloomberg School of Public Health, Baltimore, MD
Capstone project: Factors that Influence attitude and Enforcement of the Smoke-Free Law in Turkey: A Survey of Hospitality Venue Owners and Employees (Dr. Ana Navas-Acien)

May 2011 — **Bachelor of Arts in Behavioral Biology (BA)**
Johns Hopkins University, Baltimore, MD

RESEARCH INTERESTS

Exposure assessment, environmental epidemiology, biomarkers of exposure, tobacco control and regulatory science, secondhand smoke, new and emerging tobacco products

GRANTS

09/2018 – — NIEHS R01ES030025-01 “The Exposure to Metals from E-cigarettes (EMIT) Study (PI: Ana Rule)

07/2016 – 06/2019 — Maryland Cigarette Restitution Fund (CRF) “Electronic Cigarettes as a Pathway to toxic and carcinogenic metals” (PI: Ana Rule)

07/2016 – 06/2017 — NIOSH T42O008428 Johns Hopkins Occupational Safety and Health Education and Research Center Training grant (PI: Jacqueline Agnew)

09/2015 – 06/2017	1 P50 HL120163-01 American Heart Association Tobacco Regulation and Addiction Center (A-TRAC) research fellowship (PI: Aruni Bhatnagar, Michael Blaha)

PROFESSIONAL EXPERIENCE

08/2016 – 10/2019	Research program coordinator Department of Environmental Health and Engineering Johns Hopkins Bloomberg School of Public Health, Baltimore, MD
08/2015 – 06/2017	Research fellow Tobacco Regulation and Addiction Center, American Heart Association
08/2015 – 08/2016	Research assistant Department of Environmental Health and Engineering Johns Hopkins Bloomberg School of Public Health, Baltimore, MD
02/2012 – 06/2014	Research program coordinator Department of Pediatric Pulmonary Medicine Johns Hopkins School of Medicine, Baltimore, MD

HONORS

2017-2018	Centennial Scholarship Award
2011	University Honors, Johns Hopkins University

PUBLICATIONS (Peer Reviewed)

1. Zhao D, Aravindakshan A, Hilpert M, Olmedo P, Rule AM, Navas-Acien A, **Aherrera A**. Metals in electronic cigarettes: a systematic review. “Submitted for publication.”

2. Zhao D, Navas-Acien A, Ilievski V, Slavkovich V, Olmedo P, Adria-Mora B, Domingo-Relloso A, **Aherrera A**, Kleiman NJ, Rule AM, Hilpert M. Metal concentrations in electronic cigarette aerosol: Effect of open-system and closed-system devices and power settings. Environ Res. 2019 Jul;174:125-134. doi: 10.1016/j.envres.2019.04.003. Epub 2019 Apr 22.

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nickel and chromium: a preliminary study of non-invasive biomarkers. Environ Res. 2017 Aug 21;159:313-320. doi: 10.1016/j.envres.2017.08.014

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6. **Aherrera AD**, Susan J, Çarkoğlu A, Ergör G, Hayran M, Egrüder T, Kaplan B, Cohen J, Navas-Acien A. Factors that Influence attitude and Enforcement of the Smoke-Free Law in Turkey: A Survey of Hospitality Venue Owners and Employees. Tobacco Control. 2016 Sep 19. pii: tobaccocontrol-2016-053088. doi: 10.1136/tobaccocontrol-2016-053088.

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11. Collaco JM, **Aherrera AD**, Breysse PN, Winickoff JP, Klein JD, McGrath-Morrow SA. Hair nicotine levels in children with bronchopulmonary dysplasia. Pediatrics. 2015 Feb 2. pii: peds.2014-2501.

12. Collaco JM, **Aherrera AD**, Au Yeung KJ, Lefton-Greif MA, Hoch J, Skinner ML. Interdisciplinary pediatric aerodigestive care and reduction in health care costs and burden. JAMA Otolaryngol Head Neck Surg. 2015 Feb 1;141(2):101-5. doi: 10.1001/jamaoto.2014.3057.

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14. McGrath-Morrow SA, Hayashi M, **Aherrera AD**, Collaco JM. Respiratory outcomes of infants with bronchopulmonary dysplasia and gastric tube placement during the first two years of life. Pedatric Pulmonol. 2013 August 23. doi: 10.1002/ppul.22870

15. Collaco JM, **Aherrera AD**, Ryan T, McGrath-Morrow SA. Secondhand smoke exposure in preterm infants with Bronchopulmonary dysplasia. Pediatr Pulmonol. 2013 Jun 27. doi: 10.1002/ppul.22819

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ORAL AND POSTER PRESENTATIONS

1. **Aherrera AD**, Aravindakshan A, Chen R, Shao Y, Jarmul S, Olmedo P, Goessler W, Tanda S, Cohen J, Navas-Acien A, Rule AM. Characterization of Metal Exposure from E-cigarette use in Maryland: A study of non-invasive biomarkers. Poster presented at the 25th Annual meeting for the Society for Research on Nicotine and Tobacco; 20-23 February 2019; San Francisco, CA, USA.

2. **Aherrera AD**, Chen R, Aravindakshan A, Goessler W, Tanda S, Navas-Acien A, Rule AM. Metal exposure from e-cigarette users in Maryland. Oral presentation at The International Society for Exposure Science (ISES) and International Society for Environmental Epidemiology (ISEE) 2018 Joint meeting "Addressing Complex Local and Global Issues in Environmental Exposure and Health." 26-30 August 2018; Ottawa, Canada.

3. **Aherrera AD**, Rule A. Metal exposure from e-cigarette use: a systematic review. Poster presented at the 29th Conference of the International Society for Environmental Epidemiology "Healthy places, healthy people – where are the connections?"24-28 September 2017; Sydney, Australia.

4. **Aherrera AD**, Olmedo-Palma P, Tanda, S, Goessler W, Grau-Perez M, Jarmul S, Chen R, Cohen J, Rule A, Navas-Acien A. The Association of E-cigarette use with cotinine and exposure to metals: a study of non-invasive biomarkers. Poster presented at the 23rd Annual Meeting for the Society for Research on Nicotine and Tobacco; 11 March 2017. Florence, Italy.

5. **Aherrera AD**, Olmedo-Palma P, Tanda, S, Goessler W, Grau-Perez M, Jarmul S, Chen R, Cohen J, Rule A, Navas-Acien A. Biomarkers to assess exposure to nickel and chromium from e-cigarette use. Poster presented at the 2016 Annual meeting for the International Society for Exposure Science; 11 October 2016; Utrecht, Netherlands.

6. **Aherrera AD**, Susan J, Çarkoğlu A, Ergör G, Hayran M, Egrüder T, Kaplan B, Cohen J, Navas-Acien A. Factors that Influence attitude and Enforcement of the Smoke-Free Law in Turkey: A Survey of Hospitality Venue Owners and Employees. Poster presented

at: 28th Conference of the International Society for Environmental Epidemiology; 2016 Sep 3; Rome, Italy.

7. **Aherrera A**, Susan J, Çarkoğlu A, Ergör G, Hayran M, Egrüder T, Kaplan B, Cohen J, Navas-Acien A. Factors that influence support towards secondhand tobacco smoke legislation in Turkey. Poster presented at: 27th Conference of the International Society for Environmental Epidemiology; 2015 Sep 1; São Paulo, Brazil.

8. Smith D, **Aherrera A**, Lopez A, Collaco JM, Neptune E, Lazarus P, Chen G, McGrath-Morrow SA. Effects of prenatal and early postnatal e-cigarette exposure on adult behavior in male mice. Poster presented at: JHSPH Delta Alpha Omega Poster Competition; 2015 Feb 3; Baltimore, MD.