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The association of e-cigarette use with exposure to nickel and chromium: A preliminary study of non-invasive biomarkers

Angela Aherrera[a,*], Pablo Olmedo[a,b], Maria Grau-Perez[a,b], Stefan Tanda[c], Walter Goessler[c], Stephanie Jarmul[a], Rui Chen[a], Joanna E. Cohen[e,f], Ana M. Rule[a], Ana Navas-Acien[a,b,d]

[a] Johns Hopkins Bloomberg School of Public Health, Department of Environmental Health and Engineering, Johns Hopkins University, Baltimore, MD, USA
[b] Columbia University Mailman School of Public Health, Department of Environmental Health Sciences, Columbia University, New York, NY, USA
[c] Institute of Chemistry Analytical Chemistry, University of Graz, Austria
[d] Johns Hopkins Bloomberg School of Public Health, Department of Epidemiology, Johns Hopkins University, Baltimore, MD, USA
[e] Institute for Global Tobacco Control, Johns Hopkins University, Baltimore, MD, USA
[f] Johns Hopkins Bloomberg School of Public Health, Department of Health, Behavior, and Society, Johns Hopkins University, Baltimore, MD, USA

ABSTRACT

Background: Nickel (Ni) and chromium (Cr) are components of e-cigarette heating coils. Whether e-cigarettes increase metal internal dose, however, is unknown. We assessed the association of e-cigarette use patterns and of e-liquid and aerosol metal concentrations with Ni and Cr biomarker levels in e-cigarette users from Maryland.
Methods: We recruited 64 e-cigarette users from December 2015 to March 2016. We collected urine, saliva, and exhaled breath condensate (EBC), data on e-cigarette use, and samples from their e-cigarette device (dispenser e-liquid, aerosol, and tank e-liquid).
Results: Median Ni and Cr levels were 0.73 and 0.39 μg/g creatinine in urine, 2.25 and 1.53 μg/L in saliva, and 1.25 and 0.29 μg/L in EBC. In adjusted models, tertiles 2 and 3 of aerosol Ni concentrations were associated with 16% and 72% higher urine Ni and 202% and 321% higher saliva Ni compared to the lowest tertile. Tertile 3 of aerosol Cr levels were associated with 193% higher saliva Cr. An earlier time to first vape in the morning and more frequent coil change were associated with higher urine Ni. Tertile 2 of e-liquid consumption per week and voltage were associated with higher saliva Ni levels than tertile 1.
Conclusion: Positive associations of Ni and Cr aerosol concentrations with corresponding Ni and Cr biomarker levels indicate e-cigarette emissions increase metal internal dose. Increased e-cigarette use and consumption were also associated with higher Ni biomarker levels. Metal level standards are needed to prevent involuntary metal exposure among e-cigarette users.

1. Introduction

Electronic cigarette (e-cigarette) use is increasing worldwide (White et al., 2015), yet, relatively little is known about its long-term health effects (Zhu et al., 2014). Few studies have evaluated the e-cigarette device itself, and whether the e-liquid changes once it comes into contact with the device. The heating coils, for instance, are primarily made up of metals (Lippi et al., 2014; Grana et al., 2014; Oh and Kacker, 2014; Sussan et al., 2015). Once the coil is heated, the e-liquid is aerosolized and inhaled by the user. In a recent study, metal concentrations in the aerosol and e-liquid from the tank were markedly higher compared to e-liquid from the refilling dispenser, demonstrating metals were transferred from the coil to the liquid in the tank and the aerosol (Olmedo et al., submitted for publication). Nickel (Ni) and chromium (Cr) are major metal components of Nichrome and Kanthal (Williams et al., 2013, 2015; Farsalinos et al., 2015), commonly used e-cigarette coils, and both metals have been detected in e-cigarette cartomizers and aerosols (Olmedo et al., submitted for publication; Williams et al., 2013; Goniewicz et al., 2014; Saffari et al., 2014; Lerner et al., 2015; Palazzo et al., 2017). As there are no metal biomarker studies of e-cigarette use, this study was planned to establish the link between metals in e-cigarette aerosol and metal internal dose in e-cigarette users.

We aimed to assess whether e-cigarette use is associated with increased Ni and Cr exposure, determined by non-invasive biomarkers (urine, saliva, and exhaled breath condensate (EBC) (Table 1)) (Nordberg, 2007; Agaoglu et al., 2001; Burguera et al., 1998; Gil et al.,

* Correspondence to: Department of Environmental Health and Engineering, Johns Hopkins Bloomberg School of Public Health, 615N Wolfe St., Office W7017, Baltimore, MD 21205, USA.
E-mail address: aaherre2@jhu.edu (A. Aherrera).

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Table 1
Nickel and chromium sources of exposure, metabolism, biomonitoring and potential health effects.[a]

Nickel	
Sources of exposure	Air pollution (combustion of fossil fuels), food (cacao, nuts), tobacco smoking and occupational settings
Absorption/Distribution	20% of nickel from inhaled sources is absorbed by respiratory tract, with approx. 30% inhaled nickel deposited in the lungs; kidney is the primary target for nickel retention followed by lung, brain, and pancreas. Chelation with edetate disodium markedly increases nickel excretion in the urine supporting that nickel is stored in the body
Excretion	Primarily excreted in urine, with salivary and sweat excretion being secondary
Biological Half-time	Ranges from 15 to 40 h in plasma and urine; lung clearance of particles such as nickel oxide can take several months.
Levels in human fluid/tissues	The reference values for nickel in healthy adults are 0.2 μg/L in serum and 1–3 μg/L in urine (Agency for Toxic Substances and Diseases Registry, 2005)
Biomarkers of exposure	Urinary and serum nickel measurements are considered useful biomarkers of environmental or occupational exposures. Little is known about nickel in saliva or exhaled breath condensate
Potential health effects	Acute effects include gastrointestinal distress, pulmonary fibrosis and edema; non-cancer chronic effects include asthma, decreased lung function and bronchitis (Environmental Protection Agency, 2016); lung and nasal sinus cancers (Agency for Toxic Substances and Diseases Registry, 2015)
Chromium	
Sources of exposure	Chromium (III) is the dominating species in the environment and may be an essential element by ingestion. Chromium (VI) is highly toxic and rapidly reduced to chromium (III) in the lung and intestinal tract. The main source of chromium (VI) is air pollution and water pollution from industrial sources, smoking and occupational settings.
Absorption/Distribution	Cr concentrations are generally highest in lung tissue, where the concentration tends to increase with age
Excretion	Chromium is excreted through the urine and feces (predominantly through the urine)
Biological Half-time	Ranges from 15 to 41 h in urine. The elimination curve as measured by whole-body counting, has an exponential form.
Levels in human fluid/tissues	In the general population, the mean levels of chromium in serum and urine are 0.10–0.16 and 0.22 μg/L, respectively (Agency for Toxic Substances and Diseases Registry, 2012)
Biomarkers of exposure	Total chromium generally reflects chromium (III).
Potential health effects	Acute effects include shortness of breath, coughing, wheezing, gastrointestinal effects (abdominal pain, vomiting, hemorrhage); chronic non-cancer effects include perforations and ulcerations of the nasal septum, bronchitis, decreased pulmonary function, pneumonia, asthma, nasal itching and soreness (Agency for Toxic Substances and Diseases Registry, 2005); Cancer effects include lung, nasal, and sinus cancer (Agency for Toxic Substances and Diseases Registry, 2008)

[a] Source from Nordberg (2007) unless otherwise indicated.

2011; Menegário et al., 2001; Wang et al., 2008). We compared Ni and Cr biomarker levels first by variables related to e-cigarette use (Jarmul et al., in preparation) and second to the corresponding metal levels in e-liquid samples collected from the participants' devices. We hypothesized that e-liquid that has been in contact with the heating coil is the main source of metal exposure. To test this hypothesis, we obtained samples from the e-cigarette refilling dispenser (e-liquid never in contact with the device), aerosol (inhaled by the user) (Olmedo et al., 2016), and e-liquid remaining in the tank after vaping (in contact with the heating coil). We hypothesized a positive association between Ni and Cr biomarkers with increased e-cigarette use and with increased Ni and Cr concentrations in the aerosol and tank samples.

2. Methods

2.1. Study population and recruitment

E-cigarette users were recruited through vaping conventions, flyers posted in universities, and e-cigarette shops between December 2015 and March 2016 in Baltimore, Maryland. To be eligible, participants had to be 18 years of age or older, non-pregnant (for women), daily e-cigarette users, e-cigarette users for at least 6 weeks, and residents of Maryland. Our goal was to recruit 50 sole e-cigarette users, but dual users (e-cigarette users and cigarette smokers) interested in participating were not excluded. This resulted in a total of 64 participants, 50 sole users (never smokers or had quit smoking at least 3 months prior) and 14 dual users (used combustible cigarettes at least weekly). According to device type, 5 participants used first-generation devices (cigalikes), while 59 used 2nd or 3rd generation devices that operate using a customizable tank-like system and/or mechanical mods (modified e-cigarettes). The study protocol was approved by the Institutional Review Board at Johns Hopkins University (Baltimore, Maryland). All participants provided informed consent.

2.2. 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 and lifestyle factors, including e-cigarette use patterns (e-liquid consumed per week, time to first vape from waking in the morning, preferred voltage, heating coil used (Kanthal/Nichrome/other), coil change per month, and nicotine concentrations in e-liquid).

Following the interview, each participant provided three biospecimens: 1) Urine, in collection cups washed with 10% (v/v) nitric acid (HNO_3) overnight and rinsed with deionized water; 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 min. The Rtube consists of a condensing tube made of polypropylene, a silicon 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. (2016) 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

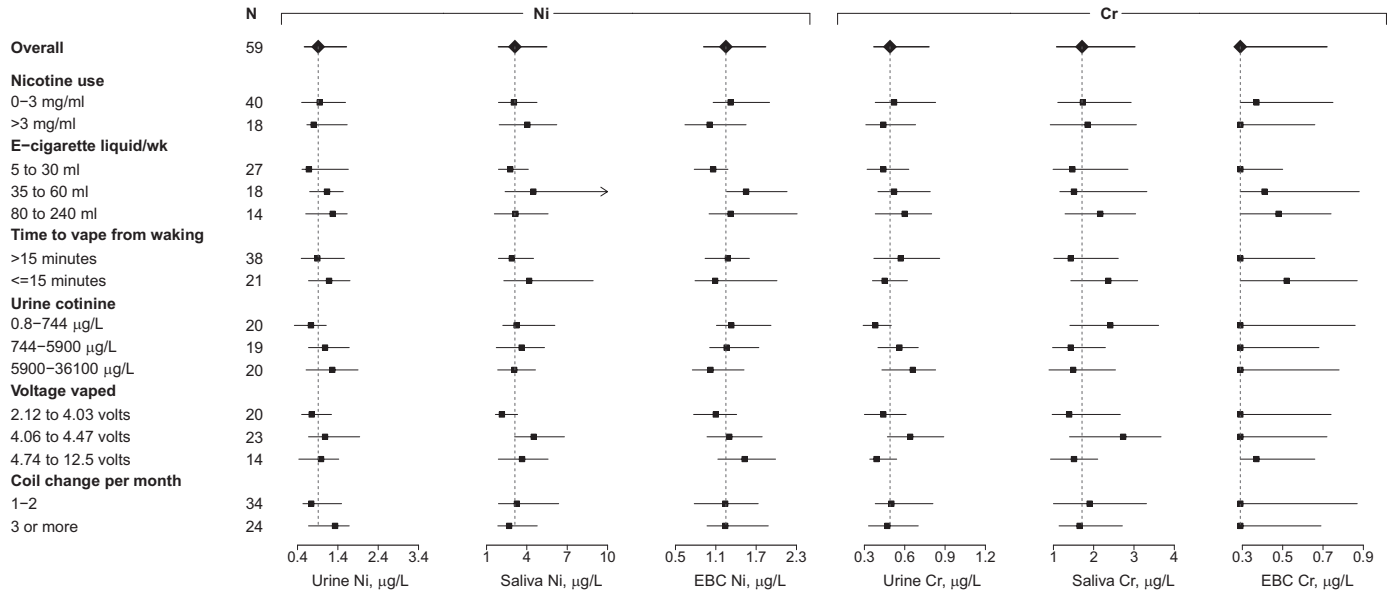

Fig. 1. Median (interquartile range) urine, saliva, and exhaled breath condensate (EBC) nickel and chromium levels by variables related to e-cigarette use patterns. Legends: Horizontal lines, interquartile ranges; squares and diamonds, medians; dotted vertical line, the geometric mean for the study sample.

peristaltic pump was pipetted into a third centrifuge tube. The 3 sample types are analyzed using similar analytic methods, allowing a direct comparison between samples.

2.3. Metal and cotinine 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 in urine, saliva, and EBC (Supplementary Table 1). The percentage of participants with metal concentrations below the limit of detection in urine, saliva and EBC was 4.7%, 3.2% and 3.1% for Ni, and 7.8%, 1.6% and 56.3% for Cr (Supplementary Table 1). Those samples were substituted by the limit of detection divided by the square root of 2. All urine cups were acid-washed with 10% nitric acid 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 and chromium 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, and centrifuging to get the rinsate for analysis. We corrected our saliva results by subtracting the average blank concentrations (Ni: 0.860 µg/L and Cr: 0.187 µg/L). For quality control, 10% duplicates and 10% blanks of each sample type were analyzed.

Urinary cotinine was analyzed using Cotinine ELISA Kits (CALBIOTECH, Spring Valley, CA). To account for urine dilution, creatinine was measured using Creatinine Colorimetric Assay Kits (Cayman Chemical Company, Ann Arbor, MI).

2.4. 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 (Olmedo et al., submitted for publication). In brief, multi-element analysis, including Ni and Cr, in all samples and calibration standards were performed on an Agilent 8800 triple quadrupole inductively coupled plasma mass spectrometer (Agilent 8800 Triple Quadrupole ICP-MS (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 = 6) as blank e-liquid to study possible matrix effects. Three 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 3 aerosol blanks was used to correct aerosol samples while the median of the 6 e-liquid blanks was used to correct the dispenser and tank samples. More details on quality control are reported in Olmedo et al. (submitted for publication).

2.5. Statistical analysis

Urine, saliva, and EBC metal levels were right skewed and log-transformed to improve normality. The main variables used as potential determinants of Ni and Cr biomarker levels included the following data on e-cigarette use: e-liquid consumption per week (tertiles), time to first vape from waking (within 15 / more than 15 min), preferred voltage for e-cigarette use (tertiles), coil change per month (1–2 / 3 times or more per month), and urinary cotinine (tertiles), as well as the corresponding metal levels in samples obtained from the dispenser, aerosol, and tank (tertiles). These analyses were restricted to users of tank-style/mods devices (n = 59), as information on coil change and e-liquid consumed, and collection of e-liquid from the dispenser and/or tank did not apply to cigalike devices.

Linear regression models on log-transformed Ni and Cr biomarkers were used to estimate their association with e-cigarette use patterns, urine cotinine, and Ni and Cr concentrations in dispenser, aerosol and tank samples in separate models. Using linear regression models on log-transformed metal levels, we computed geometric mean ratios (GMR) and the 95% confidence intervals (95% CI) of urine, saliva, and EBC Ni

Table 2
Geometric Mean Ratios (95% CI) of nickel levels in urine, saliva, and exhaled breath condensate (EBC) of mod e-cigarette users by variables related to e-cigarette use patterns and by nickel 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	
		Model 1	Model 2	Model 1	Model 2	Model 1	Model 2
E-cigarette use pattern							
E-cig liquid/wk							
5–30 ml	27	1.00 (ref)	1.00 (ref)	1.00 (ref)	1.00 (ref)	1.00 (ref)	1.00 (ref)
35–60 ml	18	1.36 (0.80, 2.30)	1.29 (0.76, 2.19)	2.59 (0.99, 6.80)	2.88 (1.11, 7.51)	1.68 (1.02, 2.74)	1.66 (1.01, 2.72)
80–240 ml	14	1.53 (0.87, 2.71)	1.43 (0.81, 2.52)	0.91 (0.32, 2.59)	1.05 (0.37, 2.97)	1.56 (0.91, 2.65)	1.51 (0.88, 2.59)
p -trend		0.12	0.19	0.84	0.64	0.06	0.08
Wake vape time							
> 15 min	38	1.00 (ref)	1.00 (ref)	1.00 (ref)	1.00 (ref)	1.00 (ref)	1.00 (ref)
≤ 15 min	21	1.78 (1.12, 2.84)	1.79 (1.14, 2.82)	2.35 (0.97, 5.68)	2.33 (0.97, 5.59)	1.03 (0.65, 1.65)	1.03 (0.65, 1.64)
p -trend		0.02	0.01	0.06	0.06	0.90	0.90
Voltage vaped							
2.12–4.03 V	20	1.00 (ref)	1.00 (ref)	1.00 (ref)	1.00 (ref)	1.00 (ref)	1.00 (ref)
4.06–4.47 V	23	1.60 (0.94, 2.70)	1.52 (0.90, 2.57)	3.33 (1.32, 8.37)	3.65 (1.47, 9.07)	1.03 (0.62, 1.73)	0.98 (0.58, 1.65)
4.74–12.5 V	14	1.46 (0.79, 2.70)	1.54 (0.82, 2.88)	1.32 (0.45, 3.85)	1.21 (0.40, 3.62)	1.18 (0.65, 2.15)	1.16 (0.62, 2.18)
p -trend		0.17	0.14	0.44	0.48	0.60	0.67
Coil change/month							
≤ 2	34	1.00 (ref)	1.00 (ref)	1.00 (ref)	1.00 (ref)	1.00 (ref)	1.00 (ref)
3 or more	24	1.66 (1.06, 2.60)	1.91 (1.23, 2.98)	1.20 (0.50, 2.88)	1.05 (0.43, 2.60)	0.97 (0.62, 1.53)	1.06 (0.67, 1.70)
p -trend		0.03	0.01	0.68	0.91	0.90	0.79
Cotinine in urine							
0.34–1000 μg/L	20	1.00 (ref)	1.00 (ref)	1.00 (ref)	1.00 (ref)	1.00 (ref)	1.00 (ref)
1020–4500 μg/L	19	1.08 (0.62, 1.86)	1.38 (0.79, 2.42)	1.02 (0.37, 2.82)	0.70 (0.24, 2.03)	0.93 (0.55, 1.58)	1.08 (0.62, 1.90)
4780–31,500 μg/L	20	1.56 (0.88, 2.75)	1.80 (1.03, 3.14)	0.47 (0.16, 1.36)	0.38 (0.13, 1.09)	0.78 (0.45, 1.34)	0.84 (0.48, 1.47)
p -trend		0.12	0.04	0.16	0.07	0.35	0.51
Nickel levels in e-cigarette samples from[a]							
Dispenser							
0.7 μg/kg	25	1.00 (ref)	1.00 (ref)	1.00 (ref)	1.00 (ref)	1.00 (ref)	1.00 (ref)
1.06 – 27.5 μg/kg	16	0.92 (0.56, 1.52)	0.83 (0.51, 1.38)	1.28 (0.45, 3.61)	1.52 (0.53, 4.37)	1.18 (0.69, 2.02)	1.09 (0.62, 1.90)
40.2 – 152 μg/kg	16	1.07 (0.64, 1.78)	0.94 (0.56, 1.56)	0.79 (0.27, 2.29)	0.99 (0.33, 2.93)	1.32 (0.76, 2.29)	1.20 (0.68, 2.13)
p -trend		0.86	0.74	0.74	0.93	0.30	0.51
Aerosol							
0.7–12.4 μg/kg	19	1.00 (ref)	1.00 (ref)	1.00 (ref)	1.00 (ref)	1.00 (ref)	1.00 (ref)
12.7 – 189 μg/kg	19	1.21 (0.75, 1.95)	1.16 (0.72, 1.88)	2.62 (0.96, 7.11)	3.02 (1.16, 7.83)	1.30 (0.77, 2.17)	1.25 (0.74, 2.12)
219 – 6820 μg/kg	19	1.80 (1.11, 2.90)	1.72 (1.05, 2.80)	3.62 (1.33, 9.86)	4.21 (1.59, 11.2)	0.97 (0.57, 1.62)	0.92 (0.53, 1.58)
p -trend		0.02	0.03	0.01	0.01	0.89	0.74
Tank							
3.64 – 105 μg/kg	17	1.00 (ref)	1.00 (ref)	1.00 (ref)	1.00 (ref)	1.00 (ref)	1.00 (ref)
124 – 468 μg/kg	17	1.38 (0.79, 2.40)	1.13 (0.64, 1.99)	1.08 (0.35, 3.35)	1.66 (0.54, 5.11)	1.41 (0.79, 2.52)	1.24 (0.67, 2.30)
629–54600 μg/kg	16	1.20 (0.69, 2.10)	1.03 (0.58, 1.82)	2.41 (0.77, 7.57)	3.12 (1.01, 9.69)	0.70 (0.39, 1.26)	0.62 (0.33, 1.15)
p -trend		0.54	0.95	0.12	0.04	0.22	0.10

Model 1: Adjusted for age sex race.
Model 2: Further adjusted by e-cigarette use category (dual vs. sole e-cigarette user), and previous smoking status (applies to sole e-cigarette users only).
[a] Samples obtained from bottle were pre-contact with coil; from aerosol condensate were post-contact with the coil; from tank were in contact with coil.

and Cr concentrations by exponentiation of the beta coefficient. Indeed, the mean difference in log-units corresponds to a geometric mean ratio after exponentiation to the original scale, what makes the results easily interpretable. 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. Model 1 was adjusted for sex, age, and race (white/non-white). Model 2 was further adjusted for smoking and e-cigarette use characteristics, including user category (sole/dual use), and previous smoking status (never/former, applies to sole e-cigarette users only). Urine Ni and Cr, and urine cotinine 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 (Agresti, 2002). All analyses were performed using Stata 13.1 (StataCorp, College Station, TX) and R software (R Project for Statistical Computing, Vienna, Austria). The level of statistical significance was 0.05 and all tests were 2-sided.

3. Results

Median (interquartile range) Ni and Cr levels were 0.73 (0.41, 1.41) and 0.39 (0.30, 0.54) μg/g creatinine in urine, 2.19 (1.02, 4.91) and 1.42 (0.81, 2.90) μg/L in saliva, and 1.25 (0.91, 1.88) and 0.29 (0.29, 0.75) μg/L in EBC (Supplementary Table 1). Compared to dual users, sole e-cigarette users had higher levels of Ni and Cr in urine (Supplementary Figure 1).

Increasing tertiles of e-liquid consumption per week tended to be associated with higher urinary, saliva and EBC Ni levels (Fig. 1), although in fully adjusted models the association was only statistically significant for the second tertile of e-liquid per week and saliva Ni (GMR 2.88, 95%CI 1.11, 7.51) (Table 2, model 2). Participants who vaped within 15 min from waking had 79% (95%CI 1.14, 2.82) higher urine Ni levels compared to those taking longer to vape. By self-reported voltage, there was a non-statistically significant trend with higher urine Ni levels (p for trend 0.14); with saliva, the association was only observed for the second tertile (4.06–4.47 V) (GMR 3.65, 95%CI 1.47, 9.07). Changing coils ≥3 times per month was associated with 91% (95%CI 1.23, 2.98) higher urinary Ni levels. Tertiles 2 and 3 of

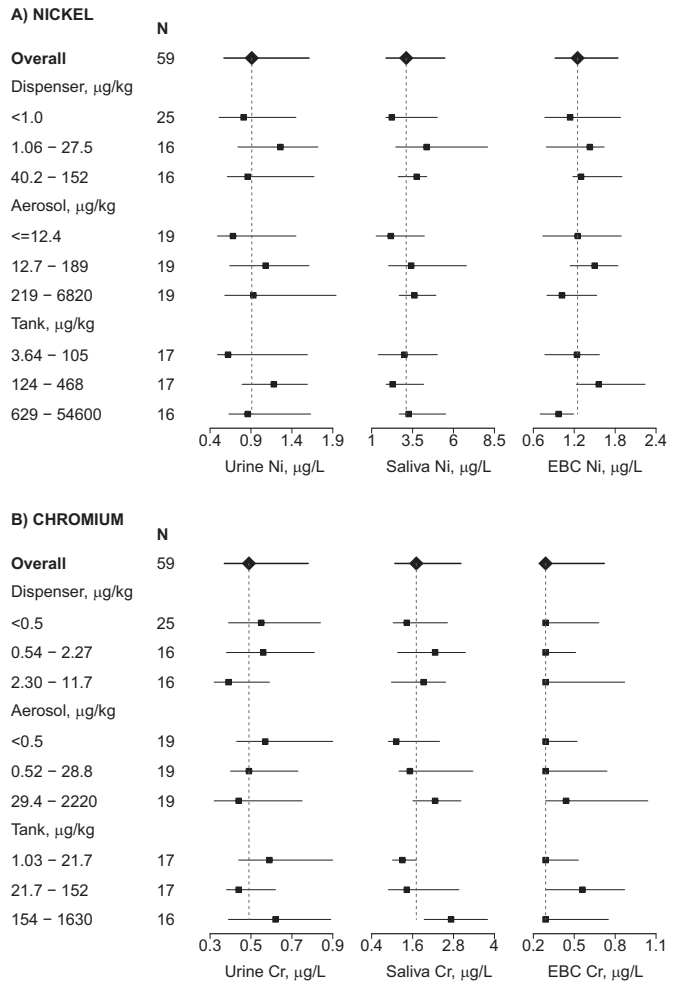

Fig. 2. Median (interquartile range) urine, saliva and exhaled breath condensate (EBC) nickel and chromium levels by the corresponding nickel and chromium levels in samples collected from the participants' personal e-cigarette devices. Legends: Horizontal lines, interquartile ranges; squares and diamonds, medians; dotted vertical line, the geometric mean for the sample.

urinary cotinine were associated with 38% and 80% higher urinary Ni levels (p-trend 0.04), respectively.

Ni levels in e-cigarette dispenser samples were not associated with any of the Ni biomarkers (Fig. 2a, Table 2). The Spearman correlation coefficients (p-value) for aerosol Ni levels with urine, saliva and EBC Ni levels were 0.23 (0.09), 0.24 (0.07) and −0.13 (0.34), respectively. The corresponding coefficients for tank Ni levels were 0.15 (0.30), 0.19 (0.18) and −0.20 (0.17). In adjusted models, the two highest compared to the lowest tertile of aerosol Ni were associated with 16% and 72% higher urine Ni (p-trend 0.03) and with 202% and 321% higher saliva Ni (p-trend 0.01). The highest tertile of tank Ni levels was associated with 212% higher saliva Ni (p-trend 0.04).

For Cr biomarkers, although the adjusted associations were not statistically significant, the two highest compared to the lowest tertile of liquid consumption per week were associated with 28% and 71% higher levels in EBC (p-trend 0.08), 21% and 56% higher levels in saliva (p-trend 0.26), and 14% and 30% higher levels in urine (p-trend 0.29) (Table 3). Cr levels in dispenser samples were not associated with Cr biomarkers (Fig. 2b, Table 3). The Spearman correlation coefficients (p-value) for aerosol Cr levels with urine, saliva and EBC Cr levels were −0.16 (0.24), 0.23 (0.09) and 0.11 (0.43), respectively. The corresponding coefficients for tank Cr levels were −0.04 (0.77), 0.32 (0.02) and 0.16 (0.28). In adjusted models, comparing the two highest to the lowest aerosol Cr levels were associated with 98% and 193% higher saliva Cr (p-trend 0.02). Tertile 3 of Cr in tank samples was associated with 178% (95%CI 1.19, 6.49) higher Cr levels in saliva.

4. Discussion

This study quantified biomarkers of Ni and Cr exposure, as assessed in urine, saliva, and EBC, in daily e-cigarette users from Maryland. Ni and Cr, both typical components of the heating coil, are highly toxic metals and established causes of lung, nasal, and sinus cancer (Table 1). We found higher urine Ni levels in participants who reported a shorter time to first vape from waking, more frequent change of coils, higher urinary cotinine, and in those with higher Ni levels in aerosol samples collected from their personal devices. Saliva Ni levels were also higher in participants in the intermediate category for the reported voltage and amount of e-cigarette liquid consumed per week, and in those with higher Ni levels in aerosol and tank samples. For Cr, we found higher EBC levels with higher e-cigarette liquid consumption per week and saliva Cr levels increased markedly with Cr concentrations in aerosol and tank samples from their personal devices. These findings support that e-cigarettes contribute to Ni and Cr exposure and that metal concentrations in the aerosol, likely coming from the devices themselves, are the most likely sources.

Some e-cigarette users have reported a metallic taste when vaping (Health and Safety Forum, 2016), supporting there is a transfer of metals from the device into the aerosol ultimately reaching the user. The lack of association with metal levels in the refilling dispenser confirms our initial hypothesis and supports that the main source of Ni and Cr exposure is not from the e-liquid but rather from the heating coil. Previous studies, mostly based on cigalikes, found Ni (Williams et al., 2013; Goniewicz et al., 2014; Saffari et al., 2014) and Cr (Williams et al., 2013) in aerosol generated by e-cigarettes and in e-liquid samples from the devices (Olmedo et al., submitted for publication; Olmedo et al., 2016; Hess et al., 2016). Heating coils are made of complex alloys and multiple metals have been found in aerosol and e-liquid samples beyond Ni and Cr, including lead, zinc, manganese, arsenic, copper, and tin (Olmedo et al., submitted for publication; Williams et al., 2013; Goniewicz et al., 2014; Saffari et al., 2014; Lerner et al., 2015; Palazzo et al., 2017; Olmedo et al., 2016; Hess et al., 2016). Additional studies are needed to assess metal exposure and internal dose among e-cigarette users, including the collection of blood samples to measure metals such as lead, manganese and zinc.

It is currently unknown how metals from the coil leach into the e-liquid in the tank and the generated aerosol although poor manufacturing techniques have been described (Lowenstein and Middlekauff, 2016). Larger volumes of e-liquid introduced into the tank could facilitate the entry of e-liquid to the coil chamber (Havel et al., 2016) and the transfer of metals onto e-liquid. In our study, participants who used more e-liquid solution per week tended to have higher Ni levels in all biomarkers and higher Cr levels in EBC, supporting larger volumes may facilitate metal transfer, although we had no data on tank size or e-liquid volume. Frequency of coil replacement could also influence metal transfer, with higher Ni levels among participants who changed the coils of their device more frequently. Lastly, using e-cigarettes at a higher voltage could also influence metal transfer as previous studies found that aerosol generation and thermal degradation byproducts increase linearly with increasing voltage (Havel et al., 2016). In our study, however, we found higher Ni levels in the intermediate voltage category. This finding needs to be confirmed in studies not relying solely on self-reported voltage data.

This is the first study measuring metal biomarkers and e-cigarette use. Ni and Cr biomarkers are not available in the current National Health and Nutrition Examination Survey of the United States, which provides biomonitoring data for 28 other metals and metalloids (Centers for Disease Control and Prevention, 2016). These two metals however, have been included in national survey data from other countries (Hoet et al., 2013; Fréry et al., 2011, 2016; Seifert et al.,

Table 3
Geometric Mean Ratios (95% CI) of chromium levels in urine, saliva, and exhaled breath condensate (EBC) of mod e-cigarette users by variables related to e-cigarette use patterns and by chromium levels in samples from their personal devices.

	N	GMR (95%CI) Urine Cr		GMR (95%CI) Saliva Cr		GMR (95%CI) EBC	
		Model 1	Model 2	Model 1	Model 2	Model 1	Model 2
E-cigarette use characteristics							
E-cig liquid/wk							
5–30 ml	27	1.00 (ref)	1.00 (ref)	1.00 (ref)	1.00 (ref)	1.00 (ref)	1.00 (ref)
35–60 ml	18	1.19 (0.75, 1.90)	1.14 (0.71, 1.83)	1.17 (0.57, 2.37)	1.21 (0.58, 2.50)	1.37 (0.78, 2.39)	1.28 (0.73, 2.24)
80–240 ml	14	1.19 (0.75, 1.90)	1.30 (0.78, 2.17)	1.52 (0.70, 3.27)	1.56 (0.71, 3.42)	1.82 (0.99, 3.32)	1.71 (0.93, 3.13)
p -trend		0.19	0.29	0.28	0.26	0.05	0.08
Wake vape time[a]							
> 15 min	38	1.00 (ref)	1.00 (ref)	1.00 (ref)	1.00 (ref)	1.00 (ref)	1.00 (ref)
≤ 15 min	21	1.14 (0.74, 1.75)	1.15 (0.75, 1.76)	1.09 (0.57, 2.09)	1.08 (0.56, 2.09)	1.17 (0.70, 1.98)	1.19 (0.71, 2.00)
p -trend		0.54	0.51	0.80	0.82	0.54	0.49
Voltage vaped							
2.12–4.03 V	20	1.00 (ref)	1.00 (ref)	1.00 (ref)	1.00 (ref)	1.00 (ref)	1.00 (ref)
4.06–4.47 V	23	1.15 (0.72, 1.84)	1.12 (0.70, 1.81)	1.92 (0.96, 3.83)	1.84 (0.91, 3.71)	1.04 (0.59, 1.84)	1.06 (0.60, 1.88)
4.74–12.5 V	14	1.08 (0.62, 1.86)	1.15 (0.65, 2.03)	1.03 (0.46, 2.29)	0.89 (0.38, 2.07)	0.84 (0.43, 1.63)	0.97 (0.49, 1.93)
p -trend		0.74	0.61	0.76	0.99	0.64	0.96
Coil change/month							
≤ 2	34	1.00 (ref)	1.00 (ref)	1.00 (ref)	1.00 (ref)	1.00 (ref)	1.00 (ref)
3 or more	24	1.10 (0.73, 1.66)	1.16 (0.76, 1.78)	1.04 (0.56, 1.95)	1.14 (0.59, 2.21)	0.78 (0.48, 1.29)	0.71 (0.43, 1.19)
p -trend		0.64	0.48	0.90	0.70	0.33	0.19
Cotinine in urine							
0.34–1000 μg/L	20	1.00 (ref)	1.00 (ref)	1.00 (ref)	1.00 (ref)	1.00 (ref)	1.00 (ref)
1020–4500 μg/L	19	0.81 (0.50, 1.32)	0.93 (0.56, 1.55)	0.61 (0.30, 1.24)	0.59 (0.27, 1.27)	1.21 (0.67, 2.19)	1.36 (0.73, 2.54)
4780–31500 μg/L	20	1.14 (0.69, 1.88)	1.23 (0.74, 2.05)	0.44 (0.21, 0.93)	0.43 (0.20, 0.92)	1.10 (0.59, 2.04)	1.20 (0.65, 2.24)
p -trend		0.62	0.38	0.03	0.03	0.76	0.58
Chromium concentrations in E-cigarette sample obtained from[a]							
Dispenser							
< 0.5 μg/kg	31	1.00 (ref)	1.00 (ref)	1.00 (ref)	1.00 (ref)	1.00 (ref)	1.00 (ref)
0.54 – 2.27 μg/kg	13	0.91 (0.56, 1.48)	0.95 (0.57, 1.56)	1.22 (0.56, 2.67)	1.13 (0.50, 2.57)	0.75 (0.40, 1.40)	0.85 (0.44, 1.62)
2.30 – 11.7 μg/kg	13	1.22 (0.75, 1.99)	1.31 (0.80, 2.13)	1.06 (0.48, 2.33)	1.12 (0.50, 2.51)	1.04 (0.55, 1.95)	1.03 (0.55, 1.96)
p -trend		0.51	0.35	0.80	0.75	0.92	0.98
Aerosol							
0.4 μg/L	20	1.00 (ref)	1.00 (ref)	1.00 (ref)	1.00 (ref)	1.00 (ref)	1.00 (ref)
0.52 – 28.8 μg/kg	18	0.84 (0.52, 1.37)	0.93 (0.56, 1.55)	1.65 (0.77, 3.53)		0.95 (0.51, 1.75)	0.95 (0.50, 1.8)
29.4–2220 μg/kg	19	0.88 (0.51, 1.50)	0.89 (0.50, 1.57)	2.19 (0.94, 5.08)	2.93 (1.21, 7.06)	1.70 (0.86, 3.34)	1.51 (0.73, 3.10)
p -trend		0.60	0.67	0.06	0.02	0.14	0.28
Tank							
1.03 – 21.7 μg/kg	16	1.00 (ref)	1.00 (ref)	1.00 (ref)	1.00 (ref)	1.00 (ref)	1.00 (ref)
21.7 – 152 μg/kg	17	0.97 (0.56, 1.68)	1.00 (0.58, 1.71)	1.00 (0.46, 2.20)	0.93 (0.42, 2.07)	1.05 (0.51, 2.16)	1.18 (0.58, 2.38)
154 – 1630 μg/kg	17	0.76 (0.43, 1.37)	0.75 (0.42, 1.33)	2.98 (1.29, 6.88)	2.78 (1.19, 6.49)	1.11 (0.52, 2.39)	1.20 (0.57, 2.53)
p -trend		0.36	0.31	0.01	0.02	0.77	0.62

Model 1: Adjusted for age sex race.
Model 2: Further adjusted by vape category (dual vs. sole e-cigarette user), and previous smoking status (applies to sole e-cigarette users only).
[a] Samples obtained from bottle were pre-contact with coil; from aerosol condensate were post-contact with the coil; from tank were in contact with coil.

2000; Health Canada, 2016). Geometric means of urine Ni concentrations from Belgium, France, Germany, and Canada were higher than that of our study sample (Supplementary Table 2). For Cr, however, the geometric mean was markedly higher in our study compared to geometric means of these countries (0.51 μg/L vs. 0.10–0.19 μg/L). Caution should be taken when interpreting these data, given that the differences in environment and diet may limit a robust comparison of metal exposure levels.

Participants in our study reported using their devices daily and all throughout the day (Jarmul et al., in preparation). Long-term inhalation of metals can result in serious adverse health effects, as metals are rapidly absorbed through the respiratory tract (Nordberg, 2007; Mansour, 2014). In mice, long-term inhalation of nickel hydroxide nanoparticles induced oxidative stress and inflammation in lung and cardiac tissues (Kang et al., 2011). In humans, in addition to cancer, chronic Ni and Cr exposure has been associated with decreased lung function and increased risk of asthma, bronchitis (Nordberg, 2007), and cardiovascular disease (Nigra et al., 2016; Zhihong et al., 2009). Inhaled Ni exposure can also induce rhinitis and sinusitis while exposure to Cr (VI) can induce mutations, chromosomal aberrations and DNA damage (Nordberg, 2007). Moving forward, it is pertinent that future studies investigate the long-term health effects of metal exposure from using e-cigarettes.

The study has several limitations. First, the small sample size, related to available funding, limited study power for some comparisons. Second, we did not collect blood samples and only obtained a single measurement of metal biomarkers. Third, as in most studies of urinary metals, we used urine creatinine to correct for urine dilution. Fourth, we did not conduct elemental speciation. Aerosol Cr speciation would be needed to determine if it is Cr (III), which is non soluble and non-reactive, or Cr (VI), which is highly soluble, corrosive and the main toxic form of Cr. Aerosol Cr, however, is likely composed of both states as the valence can change during oxidation and reduction reactions in the airways. Fourth, our findings of e-cigarette use were based on self-report and it is possible that participants could display recall bias or social desirability bias. Additional research is needed to estimate the accuracy and reliability of questionnaires used in e-cigarette research. Lastly, in this study we did not have a comparison group of none-cigarette users. Given limited resources for this research study and our concern for lack of comparability between e-cigarette users and non-users in other lifestyle characteristics, we prioritized making direct internal comparisons within e-cigarette users rather than comparing to

non e-cigarette users. Internal comparisons is a valid approach commonly used, for instance, in occupational research, where there is also often concern on the lack of comparability between workers and non-workers. Our study, moreover, provides a direct comparison between the metal level in the aerosol and the biomarker of internal dose, this is a powerful comparison that cannot be done in non-e-cigarette users. While residual confounding by socioeconomic status is still possible, it is likely minimized by internal comparisons. A major strength of our study is the collection of e-cigarette samples from each participant's own devices, which allowed us to make direct comparisons between metal levels in those samples and biomarker levels, as well as the comparison by e-cigarette use patterns. We recognize that e-cigarette devices vary by design, with some requiring longer contact of e-liquid with the coil to generate the same volume of aerosol. This is inherent to the characteristics of each device and thus also important in terms of human exposure. We believe it is one of the strengths of our approach to assign each participant to his/her own individual source of exposure. Those comparisons are important and cannot be done in a comparison group of non e-cigarette users. Additional strengths include the use of a standardized study protocol and rigorous laboratory procedures to measure metals in biospecimens and e-cigarette samples.

5. Conclusion

This study of daily e-cigarette users from Maryland indicates that metals in e-cigarette aerosol are inhaled and absorbed into the body of e-cigarette users, representing a relevant contributor to metal internal dose. As the first study to make direct comparisons between source and metal biomarkers from e-cigarette use, we found Ni in urine and saliva and Cr in saliva were positively associated with concentrations of the corresponding metals in aerosol samples collected from their personal devices, providing strong evidence that metals present in the aerosol are inhaled by the user. E-cigarette use patterns such as more e-liquid consumed per week, a shorter time to first vape from waking, and a higher voltage used were also associated with higher Ni biomarker levels. Research on metals in e-cigarettes is essential to understand their health effects and to inform the FDA for product review and regulation. From a policy perspective, metal standards in e-cigarette emissions and adequate labeling of coils are urgently needed to inform users and prevent unwanted metal exposure from e-cigarette use.

Conflicts of interest

The authors declare no potential conflicts of interest.

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Appendix A. Supplementary material

Supplementary material associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.envres.2017.08.014.

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