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Are Urine Propylene Glycol or Vegetable Glycerin Markers of E-cigarette Use or Abstinence?

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Abstract

Objectives: Urine propylene glycol (PG) and vegetable glycerin (VG) were evaluated as potential markers for discriminating ECIG users from non-users and verifying ECIG abstinence.

Methods: Urine samples from 51 ECIG users (collected pre/post 12-hours ECIG abstinence), and 50 controls (who do not use nicotine/tobacco) were analyzed for urine cotinine, PG, and VG concentration.

Results: Of 42 ECIG users with pre-abstinence urine cotinine indicating nicotine use, mean (SD) urine cotinine concentration was 1053.7 ng/ml (874.5) and for controls was 1.93 ng/ml (0.4); after abstinence, ECIG users' mean cotinine decreased to 615.4 ng/ml (753.0). For ECIG users, mean urine PG pre-abstinence was 25.6 mcg/ml (20.0) and was 9.8 mcg/ml (13.5) for controls; after abstinence, ECIG users' mean urine PG decreased to 9.7 mcg/ml (15.0; ps < .05). For ECIG users, mean urine VG pre-abstinence was 7.5 mcg/ml (7.1) and was 13.2 mcg/ml (25.0) for controls; after abstinence, ECIG users' mean VG decreased to 5.0 mcg/ml (4.4; ps < .05).

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Human Subjects Statement

This study was approved by Virginia Commonwealth University's Institutional Review Board.

Conflict of Interest Statement

Dr. Eissenberg is a paid consultant in litigation against the tobacco industry and electronic cigarette industry and is named on a patent application for a device that measures the puffing behavior of electronic cigarette users. All other authors have no disclosures.

Conclusions: ECIG users' mean urine PG was greater than controls and decreased after 12-hours ECIG abstinence suggesting urine PG may be useful for discriminating ECIG users from non-users and verifying short-term abstinence.

Keywords

electronic cigarette; e-cigarette; propylene glycol; vegetable glycerin; biomarker

Electronic cigarettes (ECIGs) heat a liquid solution to produce an aerosol for users to inhale for users to inhale. ECIGs share common features including a battery, heating element, and a liquid comprised of propylene glycol (PG) and vegetable glycerin (VG), flavorants, and nicotine. Biomarkers of ECIG exposure would have utility for verifying ECIG use status in clinical and research settings and to inform policymaking and regulation, though no such biomarkers have been identified[1,2].

Biomarkers are used in research and clinical settings to determine tobacco use status[3,4]. For example, biomarkers of cigarette smoking, such as cotinine, tobacco specific nitrosamines (TSNAs), and expired air carbon monoxide (CO) can distinguish smokers from non-smokers, verify self-reported tobacco use status, assess abstinence status[3–8], and facilitate smoking cessation[9]. However, these biomarkers are not found in exclusive ECIG users and biomarkers of exclusive ECIG use and abstinence have not been identified[10]. Nicotine exposure can be detected following ECIG use through plasma nicotine or saliva/urine cotinine; however, nicotine is not specific to ECIG use[3,5].

Two ECIG-liquid constituents, PG and VG, can comprise up to 95% of ECIG-liquid[11], and these solvents may be useful markers to discriminate ECIG users from non-users and to determine short-term ECIG abstinence. However, because PG and VG also are contained in many commercially available products (eg, foods, beverages, and personal care products), determining whether ECIG users have higher exposure to PG and VG relative to individuals who do not use ECIGs is important. The purpose of this study was to examine if urine PG and/or VG concentrations are greater in ECIG users relative to individuals who do not use ECIGs/tobacco and assess whether urine PG and/or VG can be used as a biomarker for recent (ie, past 12–24 hour) ECIG use or abstinence.

METHOD

Participants & Study Procedures

Data were collected as a part of a broader study assessing nicotine and toxicant exposure in ECIG users conducted from Fall of 2016- Spring 2019. Experienced ECIG users and individuals who reported no use of ECIGs/tobacco-containing products (controls) were eligible to participate if they reported being healthy and between 18–55 years of age.

ECIG users.—ECIG users were eligible if they reported using: an ECIG daily for ≥1 month, ≥1 ml of ECIG-liquid/cartridge daily, and a liquid containing ≥3 mg/ml nicotine. Exclusion criteria included use of: other tobacco products >3 times weekly, NRT in the past 7 days, marijuana ≥10 days in the past 30 days, or illicit drugs in the past 30 days. Self-reported chronic disease was also exclusionary. Eligible ECIG users provided a urine

sample on 2 separate visits completed in a fixed order. Visit 1 was preceded by participants' use of their own ECIG/liquid (ie, normal use visit) and visit 2 was preceded by ≥12 hours abstinence from ECIG/tobacco use. Prior to Visit 1, ECIG users were instructed to use their ECIG/liquid as they normally do; however, the time course of use was not controlled. During each visit, abstinence from combustible tobacco was verified via participants' expired air CO (<10 ppm; BreathCO monitor; Vitalograph, Lenexa, KS) and during visit 2 a bogus pipeline saliva test was administered[12] to improve participant compliance with ECIG abstinence requirements.

Controls: Controls were eligible if they reported no tobacco use (ie, cigarettes, ECIGs, smokeless, little cigars) in the past year and reported lifetime use of ≤100 cigarettes and ≤100 ECIGs. Exclusion criteria related to marijuana, other illicit drugs, and chronic disease were the same as for ECIG users. Eligible controls completed one visit in which they provided a urine sample. A semi-quantitative test for urine cotinine (NicAlert; had to be <3; ie, 0–200 ng/ml) and an expired air CO sample (<3 ppm) were used to confirm non-ECIG/tobacco use status.

Outcome Measures and Sample Analysis

All urine samples were stored at −80° C until analysis. Urine concentrations of cotinine (to verify tobacco use status), PG, and VG were determined using liquid chromatography tandem mass spectrometry (LC-MS/MS); limit of quantitation for PG and VG was 1.0 mcg/ml. A method for extracting and measuring PG and VG in urine was developed and validated using a subset of participants from this study (without regard to normal ECIG use or abstinence status when reporting results) and is detailed elsewhere[13]. Urine PG and VG concentrations were adjusted for creatinine.

Statistical Analyses

Differences in urine cotinine, PG, and VG concentration between ECIG users and controls were assessed using independent samples *t*-tests. For ECIG users, within-participant differences in urine cotinine, PG, and VG concentrations pre- and post-12 hours ECIG abstinence were assessed using paired samples *t*-tests. For ECIG users, who were the only participants to provide data for an abstinence condition, difference scores were generated by subtracting post-abstinence from pre-abstinence concentrations for cotinine, PG and VG.

Prior to conducting analyses, urine cotinine concentration for 51 ECIG users was inspected to confirm ECIG use status using an inclusion criterion of urine cotinine ≥200 ng/ml (following normal ECIG use for visit 1); nine had urine cotinine <200 ng/ml) and their data were therefore excluded.

RESULTS

Participant Characteristics

ECIG users.—The 42 confirmed ECIG users' had a mean age of 27.8 (SD=7.9) and 24% were women; 73.8% self-identified as White/Caucasian, 10.5% as African-American/Black (AA), 10.5% as Asian, and the remaining 5.2% as more than one race or 'other.'

On average, they had been using their ECIG for 1.8 years (SD=1.2), used an ECIG of 58.4 watts (SD=44.9), and used 6.5 ml of ECIG-liquid daily (SD=5.3) with a mean nicotine concentration of 8.2 mg/ml (SD=7.7). Average expired air CO was 2.1 ppm (SD=1.6), consistent with no recent combustible tobacco use, although 4 ECIG users reported occasional smoking, averaging 0.2 cigarettes/week (SD=1.6).

Controls.—The 50 controls had a mean age of 26.0 (SD=9.9) and 78% were women; 58% self-identified as White/Caucasian, 18% as Asian, 12% as AA/Black, 10% as Hispanic, and 2% as 'other.' Average expired air CO was 1.5 ppm (SD=0.5).

Urine Cotinine

Mean urine cotinine concentration for ECIG users', following regular ECIG use, was 1053.7 ng/ml (SD=874.5), consistent with recent nicotine/tobacco use. Relative to ECIG users, controls had significantly lower mean urine cotinine concentration of 1.93 ng/ml (SD=0.4; $p < .05$), consistent with no nicotine/tobacco use. Following 12 hours of self-reported abstinence, ECIG users' mean cotinine concentration decreased significantly to 615.4 ng/ml (SD=753.0; $p < .05$). ECIG users' mean decrease in urine cotinine pre-to-post abstinence was 467.7 ng/ml (SD=459.6).

Urine Propylene Glycol (PG)

Results for urine PG are presented in Table 1. For ECIG users', mean urine PG concentration, following normal ECIG use, was 25.6 mcg/ml (SD=20.0) and was significantly lower for controls whose mean urine PG was 9.8 mcg/ml (SD=13.5; $p < .05$; see Figure 1). Following 12 hours self-reported abstinence, ECIG users' mean urine PG concentration decreased significantly to 9.7 mcg/ml (SD=15.0; $p < .05$) and no longer differed significantly from controls. ECIG users' mean decrease in urine PG pre-to-post abstinence was 16.7 mcg/ml (SD=20.8).

Urine Vegetable Glycerin (VG)

ECIG users' mean urine VG concentration, following normal ECIG use, was 7.5 mcg/ml (SD=7.1). Controls' mean urine VG concentration was significantly higher: 13.2 mcg/ml (SD=25.0; $p<0.05$, see Figure 1). Following 12 hours of self-reported abstinence, ECIG users' mean urine VG concentration decreased significantly to 5.0 mcg/ml (SD=4.4; $p< 0.05$). ECIG user's mean decrease in urine VG pre-to-post abstinence was 2.9 mcg/ml (SD=6.5).

There were 2 outliers among controls with urine VG concentrations 5–6 times greater than the mean, possibly due to greater than average exposures to VG-containing food and/or products. Upon removal of these 2 outliers, mean urine VG concentration was 8.1 mcg/ml (SD=10.9) for controls (N = 48) and was no longer significantly different compared to non-abstinent ECIG users.

CONCLUSIONS

This is the first observational study to characterize urinary PG and VG concentration in daily ECIG users compared to non-ECIG/tobacco using controls. Daily ECIG users, following normal use of their own ECIG and liquid, had higher concentrations of urinary PG relative to controls. Median urine PG concentration was almost 7 times greater in ECIG users (median=20.9 mcg/ml) relative to controls (median=3.2 mcg/ml), suggesting that urine PG may be useful for discriminating ECIG users from non-users. Following ≥12 hours of self-reported ECIG abstinence, ECIG users' mean urine PG concentration decreased such that it did not differ from controls, highlighting the possible utility of urine PG as a marker for verifying short-term ECIG abstinence. In contrast, compared with ECIG users, urinary VG concentration was higher in controls, suggesting that urine VG concentration may be ineffective at distinguishing ECIG users from non-users. These results confirm and expand upon those found in a controlled inpatient industry-sponsored study in which ECIG users used an ECIG that contained liquid spiked with a stable-isotope-labeled tracker[14]. Results showed that increased concentrations of urinary and plasma PG were associated with ECIG use while VG concentrations were not[14].

These findings may have implications for research and clinical settings. For example, urine PG could potentially be used as an ECIG biomarker to improve experimental rigor. Because researchers are currently unable to verify short-term abstinence from ECIGs, some ECIG users may not comply with protocol-mandated overnight ECIG abstinence[15,16,17]. Further, PG may be used to confirm current ECIG-use status, particularly in instances where individuals may be motivated to conceal their ECIG/tobacco use. Moreover, because most current ECIG users aged 18–34 want to quit[18] and thousands aged 13–24 have enrolled in ECIG-specific cessation programs[19], a biomarker, such as urine PG that can verify ECIG use and/or ECIG abstinence may be useful in facilitating ECIG cessation. If a more rapid urine PG test than the one used here were to be developed and validated (eg, a urine test strip comparable to that used to assess cotinine), it likely would have application in various research and clinical settings; though, given PG's short half-life (approximately 4 hours[20]), it likely would be used as a short-term marker in conjunction with other biomarkers, such as urine cotinine.

Findings from this study should be considered in the context of several limitations. These results were obtained from a small, predominantly Caucasian sample, thereby limiting generalizability. The uneven distribution of men and women across the ECIG and control groups raises concerns regarding possible sex differences in metabolism rates for PG and VG; future studies should include age- and sex-matched comparison groups[21]. Furthermore, the study did not include a comparison group of cigarette smokers and/or dual users (ie, cigarettes and ECIGs). Although previous studies have not detected increases in PG and VG in the urine and blood plasma of cigarette smokers[14], the majority of adult ECIG users are dual users and the results herein should be replicated in this population. Because these data were collected as a part of a broader study, analysis of PG and VG was limited to urine, 2 measurement timepoints (for ECIG users), and no control for non-ECIG sources of exposure to PG and VG (eg, foods, beverages, and personal care products). Future studies should consider use of inpatient facilities to control dietary sources of PG and VG and

employ controlled time-course of ECIG use to discern the level of exposure to PG and VG from ECIGs relative to other sources. Despite these limitations, the results from this study show that, under naturalistic use conditions, ECIG users' mean urine PG concentration was significantly higher relative to non-ECIG/tobacco using controls and ECIG user's urinary PG decreased after 12-hours ECIG abstinence.

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IMPLICATIONS FOR TOBACCO REGULATION

The scarcity of ECIG-specific biomarkers challenges assessment of ECIG use status. These results show that daily ECIG users, following normal use of their own ECIG/liquid, have higher concentrations of urine PG relative to non-ECIG-using controls. Following 12-hours ECIG abstinence, ECIG users' mean urinary PG concentration decreased and was lower than controls, suggesting PG's utility for verifying short-term ECIG abstinence. Discriminating ECIG users from non-users is important in a variety of research settings and can inform regulation of these products.

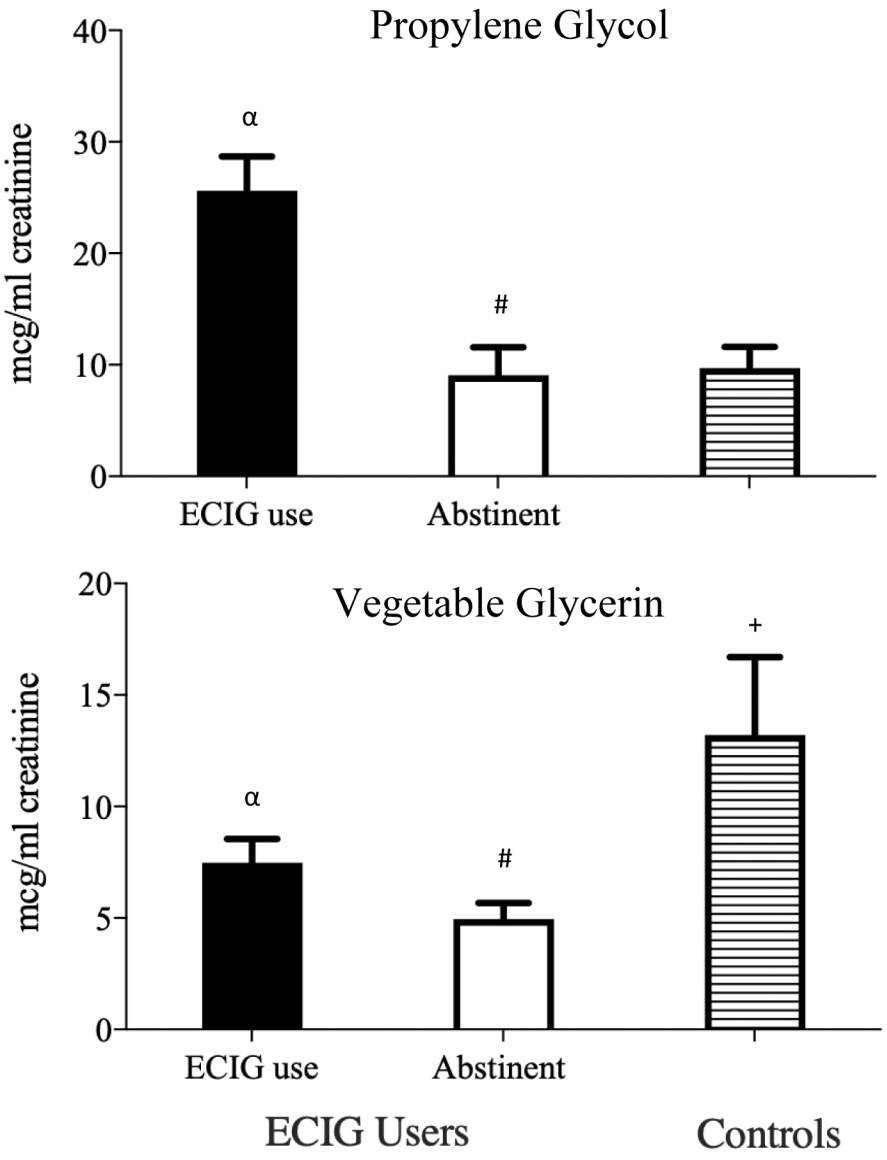

Figure 1. Mean (+SEM) Urine PG and VG Concentrations for Daily ECIG Users and Controls

Mean (+SEM) values for urine propylene glycol (PG) and vegetable glycerin concentration for experienced ECIG users pre- and post- 12-hours ECIG/nicotine abstinence and for controls. Among ECIG users, pound symbols (#) indicate significant differences pre- and post-ECIG/nicotine abstinence. Alpha symbols (α) indicate significant differences between non-abstinent ECIG users and controls; crosses (+) indicate significant differences between 12-hour abstinent ECIG users and controls. Note that, for controls, data for urine VG are presented with two outliers included whose urine VG concentrations were 5–6 times greater than the mean, possibly due to greater than average exposures to VG-containing food and/or products.

Table 1.

Mean (SD) and Median PG and VG Concentrations for Daily ECIG Users and Controls

	ECIG users (N = 42)	Controls (N = 50)
PG concentration (mcg/ml)		
Mean (SD)	25.6 (20.0)	9.8 (13.5)
Median	20.9	3.2
PG concentration abstinent (mcg/ml)		
Mean (SD)	9.7 (15.0)	n.a
Median	4.9	n.a.
VG concentration (mcg/ml)		
Mean (SD)	7.5 (7.1)	13.2 (25.0)
Median	5.1	4.4
VG concentration abstinent (mcg/ml)		
Mean (SD)	5.0 (4.4)	n.a.
Median	4.1	n.a.

Mean (SD) and median values for urine propylene glycol (PG) and vegetable glycerin (VG) concentration for daily ECIG users pre and post 12-hour ECIG/nicotine abstinence and for controls. Urine PG and VG normalized for creatinine. n.a. = not applicable/not measured; control group did not undergo an abstinence period as they were not users of ECIGs or nicotine/tobacco products. For controls, urine VG concentrations are presented with two outliers included whose urine VG concentrations were 5–6 times greater than the mean, possibly due to greater than average exposures to VG-containing food and/or products. Upon removal of these two outliers, mean urine VG concentration was 8.1 mcg/ml (SD=10.9) for controls (N=48).