

E-cigarette Nicotine Delivery: Data and Learnings from Pharmacokinetic Studies

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Objectives: E-cigarettes could potentially play a major role in tobacco harm reduction by delivering nicotine in a vapor containing significantly fewer toxicants than cigarette smoke and may aid smoking behavior changes such as reduction or cessation. **Methods:** We examined blood nicotine levels in smokers who were non-accustomed to e-cigarette use (Study 1) and accustomed e-cigarette users (Study 2). We compared nicotine levels when participants used a closed modular system e-cigarette to those when participants smoked a cigarette. **Results:** In Study 1, C_{\max} (geometric mean (CV)) during a 5-minute puffing period (10 puffs, 30 seconds apart) was 13.4 (51.4%) ng/ml for a regular cigarette. The e-cigarette C_{\max} was significantly lower ($p < .05$) at

2.5 (67.8%) ng/ml. In Study 2, during a 5-minute *ad libitum* puffing period, cigarette C_{\max} was 7.2 (130.8%) ng/mL, and it was 7.8 (108.2%) ng/mL for the e-cigarette. **Conclusions:** Our data demonstrate heterogeneity of nicotine deliveries both between products and also with the same products used by different cohorts, eg, accustomed users versus smokers. Such differences must be taken into account when determining the likely behavioral impact, on smoking reduction and cessation, of nicotine delivery data and when planning e-cigarette nicotine pharmacokinetic studies.

Key words: nicotine; e-cigarette; vaping; pharmacokinetics

Am J Health Behav. 2017;41(1):16-32

DOI: <https://doi.org/10.5993/AJHB.41.1.2>

Smoking is a leading cause of numerous human diseases including lung cancer, chronic obstructive pulmonary disease, and atherosclerotic cardiovascular disease. The addictive properties of cigarette smoking are primarily due to nicotine, a chemical found naturally in tobacco leaf that transfers into cigarette smoke. Nicotine is absorbed rapidly into the bloodstream during cigarette smoking,¹ from where it is distributed causing both systemic and central effects. In the central nervous system, nicotine acts at neuronal nicotinic receptors and this interaction may underpin its effects on mood and relaxation. The pharmacoki-

netic profile of nicotine during cigarette smoking is a rapid rise and fall in plasma nicotine concentrations. Correspondingly, the delivery of nicotine to the brain, and the consequent pleasurable effects experienced by the smoker, are also rapid.

Whereas nicotine dependence underpins the addiction to cigarette smoking, nicotine itself is not thought to convey a risk of smoking-related disease. It is now generally acknowledged that the harmful effects of smoking are caused by exposure to some of the 6500 or more identified chemical constituents² in the particulate and vapor phases of cigarette smoke. The health risks associated

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with cigarette smoking are correlated with duration of smoking and degree of daily cigarette consumption.^{3,4} Therefore, behavioral changes of either substantially reducing cigarette consumption or complete cessation are likely to have a positive impact on a smokers' health by reducing their relative risks of smoking-related diseases. This hypothesis is a key principle underpinning the concept of tobacco harm reduction;⁵ moreover, in a committee-led evaluation of the available science, the US Institute of Medicine reported: "...for many diseases, reducing tobacco smoke exposure can result in decreased disease incidence with complete abstinence providing the greatest benefit."⁶ The potential for such behavior change in reducing harm by reducing toxicant exposure depends however on the user's behavior, such as frequency and intensity of use and their co-use with other tobacco products.⁶

E-cigarettes are electronic devices that aerosolize a solution, either with or without nicotine, into an inhalable vapor. For nicotine-containing e-liquids, in the absence of both tobacco and combustion as a means of transferring nicotine into the inhaled matter, e-cigarettes deliver a vapor that is considered considerably less toxic than cigarette smoke,^{7,8} due to reductions in exposure to chemical toxicants.⁹⁻¹¹ Indeed, an independent scientific expert panel utilized a multi-criteria decision analysis approach, incorporating numerous aspects of harm to users, to demonstrate the potential reduction in harm of e-cigarettes compared to combustible cigarettes,¹² a conclusion recently endorsed by both Public Health England and the UK Royal College of Physicians (RCP).^{13,14} The use of e-cigarettes in helping smokers either reduce or quit smoking has been proposed as having the potential to play a major role in tobacco harm reduction,^{11,13,14} and this potential is further supported by data from large cross-sectional and longitudinal survey studies in the United Kingdom (UK).^{15,16} The cross-sectional data also suggest that e-cigarettes are a more effective aid to cessation than more traditional nicotine replacement therapy (NRT) products,¹⁵ though the reasons for this are not fully clear. However, in general, the delivery of nicotine from NRT products is relatively slow and the pharmacokinetic profile does not fully resemble that of cigarettes.^{17,18} The time to maximum blood nicotine concentration (T_{max}) tends to be longer and the maximum nicotine level (C_{max}) is not characterized by a sharp peak, but by a lower and flatter peak. The e-cigarette pharmacokinetic profile, however, is closer to that of a cigarette, in terms of both T_{max} and C_{max} ,¹⁹⁻²² and the development of new-generation devices has improved this even further.^{23,24} The potential role of such nicotine delivery in supporting cessation and preventing relapse was highlighted by the UK RCP as one of the features of e-cigarettes, along with replacing behavioral components of smoking, which has contributed to their popularity as a quitting aid and

their effectiveness relative to NRT.¹⁴ Furthermore, although data point to a lower efficacy of NRT as a cessation aid compared to e-cigarettes,¹⁵ NRT clearly does aid smoking reduction and cessation and a role for nicotine delivery in this support has also previously been suggested.^{25,26}

Historically, numerous studies have been performed examining blood nicotine levels in participants smoking a cigarette or using other tobacco products such as snus.¹⁸ More recently, similar studies also have been performed in users of e-cigarettes. Such studies are critical to determine the harm reduction potential of e-cigarettes because they allow the determination of nicotine delivery from e-cigarettes relative to combustible cigarettes, a key factor in supporting behavior change in smokers and facilitating smoking reduction or cessation. In this paper, we describe data from 2 nicotine pharmacokinetic studies in which we examined elevations in blood nicotine when participants used e-cigarettes under different puffing schedules and also in different cohorts of users (smokers who were familiar with but not current users of e-cigarettes, and accustomed e-cigarette users who were occasional cigarette smokers). We discuss the implications of these findings on our knowledge of the potential for e-cigarettes to contribute to tobacco harm reduction by facilitating smokers' behavior change.

METHODS

Participants

Study 1. This study was conducted at the clinical facilities of Celerion, Inc, Belfast, UK in accordance with the principles of Good Clinical Practice. Participants were familiar with the use of e-cigarettes but not currently dual-using cigarettes and e-cigarettes. Thirty-seven participants underwent screening procedures to ensure that they met the requirements for inclusion, within 30 days prior to participation in the study. We used the REC-approved informed consent form (ICF) to collect written informed consent from all participants prior to completion of the screening or other study procedures. Twenty-four participants (17 men and 7 women) were enrolled for participation in the study; 2 participants withdrew from the study and the remaining 22 participants properly completed the study and were included in the analyses. All participants were required to be between 21 and 55 years of age and had smoked 10 or more manufactured cigarettes per day for at least 12 months prior to the study start. Participants' current brand of cigarette was required to be of a machine-smoked International Organization for Standardization (ISO) tar yield of 8-10 mg. All participants tested positive for urine cotinine (≥ 200 ng/mL; One-Step) and had an exhaled breath carbon monoxide (eCO) measurement of ≥ 10 ppm (Smokerlyzer, Bedfont Scientific Ltd, Maidstone, UK) at screening. Participants were in good health as determined by medical history, vital signs, blood biochemistry,

Table 1
Study Products Used in Each Study

Study	Product number	Form	Product and manufacturer	Nicotine content of liquid solution	Nicotine yield	Other ingredients
1	1.1	Combustible tobacco cigarette	John Player Special Blue; Imperial Tobacco	NA	1.0 mg/cig ^a	NA
	1.2	Closed modular system e-cigarette	Vype vPro ePen; Nicov-entures Ltd ^b	1.86% w/w	0.6 mg/puff ^c	Glycerol, propylene glycol, water, 0.3 MEq organic acid, tobacco flavor
2	2.1	Combustible tobacco cigarette	Marlboro Ultralights; Philip Morris USA	NA	0.5 mg/cig ^a	NA
	2.2	Closed modular system e-cigarette	Vype vPro ePen; Nicoventures Ltd ^b	1.86% w/w	0.6 mg/puff ^c	Glycerol, propylene glycol, water, 0.3 MEq organic acid, tobacco flavor
	2.3	First-generation e-cigarette	Nicolites; Nicocigs Limited	1.33% w/w	ND	Glycerol, propylene glycol, water, flavorings

Note.

a=ISO nicotine yield; b=ePen power setting of 4.55W (voltage 3.6V, coil resistance 2.85ohms) in Study 1 and 5.6W in Study 2 (voltage 4.0V, coil resistance 2.85ohms); c=machine yield with 80/3/30 regimen with 1s pre-heat; NA=not applicable; ND=not determined

haematology, urinalysis and physical examination. Exclusion criteria included a history of clinically active significant mental or physical health condition, current pregnancy or breastfeeding, clinically significant blood pressure abnormalities, positive urinary drugs of abuse screen, use of tobacco or nicotine-containing products other than manufactured cigarettes and e-cigarettes, and use of any prescription smoking cessation treatments within 30 days prior to Day 1 product administration and throughout the study. We also excluded persons using medications known to interact with cytochrome P450 enzymes within 28 days prior to Day 1 product administration. Appendix 1 of the Supplementary Information shows the full inclusion and exclusion criteria.

Study 2. This study was conducted at the clinical facilities of Los Angeles Clinical Trials (Burbank, CA, USA.) in accordance with the principles of Good Clinical Practice. Participants were required to be between 21 and 55 years of age and current users of second-generation (eg, cartomiser or tank-type) e-cigarettes, and had been using these devices for at least 3 months. Participants were required to be occasional smokers of combustible cigarettes (lower limit of 1 cigarette per month; upper limit of 5 cigarettes per week). Twenty-five participants underwent the screening procedures to ensure that they met the requirements for inclusion within 30 days prior to par-

ticipation in the study. The IRB-approved ICF was used to collect written informed consent from all participants prior to completion of the screening or other study procedures. Eighteen participants (12 men and 6 women) enrolled for participation in the study; no participants withdrew and data from all participants was included in the analyses. All participants tested positive for urine cotinine (≥ 200 ng/mL; NicAlert) and negative for urinary drugs of abuse at screening. Participants were in good health as determined by medical history, vital signs, blood biochemistry, haematology, urinalysis and physical examination. Exclusion criteria were as for Study 1. Appendix 1 of the Supplementary Information shows the full inclusion and exclusion criteria.

Study Products

During clinic visits, each participant received a test product (market-typical combustible cigarette or e-cigarette) to use according to the study randomisation schedule. Tables 1 shows details of each product, including their machine smoked nicotine yields.

Study Design and Procedures

Study 1. This was a part-randomized, part-blinded, crossover study conducted in 24 healthy male and female volunteer smokers. For each study visit, we asked participants to abstain from using

Table 2
Participant Demographics for Study 1

		Overall (N = 24)	Male (N = 17)	Female (N = 7)
Age (years)	Mean (SD)	34.0 (7.6)	33.2 (6.5)	36.0 (10.2)
	Min–Max	24.0–51.0	24.0–45.0	25.0–51.0
Height (cm)	Mean (SD)	174.6 (10.5)	179.9 (6.4)	161.6 (5.5)
	Min–Max	154.0–190.0	165.0–190.0	154.0–169.0
Weight (kg)	Mean (SD)	76.4 (16.9)	84.3 (12.4)	57.2 (8.7)
	Min–Max	46.2–107.5	58.4–107.5	46.2–72.2
BMI (kg/m ²)	Mean (SD)	24.7 (3.1)	25.9 (2.6)	21.8 (2.4)
	Min–Max	18.0–29.8	21.5–29.8	18.0–25.3
Race/Ethnicity, N (%)	White	24 (100)	17 (100)	7 (100)

Note.

BMI=body mass index; N=number of participants; SD=standard deviation

any tobacco or nicotine products and avoid specified foods and drinks for 12 hours before product administration until after the visit was complete. Compliance with tobacco product abstinence was confirmed by eCO measurement <15ppm.

On the morning of Visit 1, we asked participants to smoke a single cigarette by taking a single puff every 30 seconds (10 puffs in total) before extinguishing the cigarette. If a participant reached the end of their cigarette before 10 puffs had been taken, they were required to light another cigarette to take the remaining puffs. After a time of 15 minutes from the first cigarette puff, we allowed participants to smoke cigarettes *ad libitum* for a period of one hour. During both smoking periods, we drew blood samples at specific time points (see section on plasma nicotine measurement) for plasma nicotine analysis. Additionally, we administered a single-item questionnaire to examine the urge to smoke a cigarette by asking: “Right now, how much would you like a cigarette?” at specific time points (-2, 1.25, 3.25, 5.5, 7.5, 10.5, 14.5, 30, 45, 60, 75 and 90 minutes). Smoking urge was rated on a 7-point Likert scale, ranging from 1 (not at all) to 7 (a great deal).

At the end of Visit 1, we provided participants with an e-cigarette and cartridges and were asked to use the e-cigarette so that they became familiar with it before returning to the clinic for Visit 2. The time between Visits 1 and 2 was 2 days. During Visit 2, we followed the same procedures described for Visit 1, but nicotine pharmacokinetics were examined when participants used the e-cigarette instead of smoking a regular cigarette. The same puffing regimens (1 puff every 30 seconds for 10

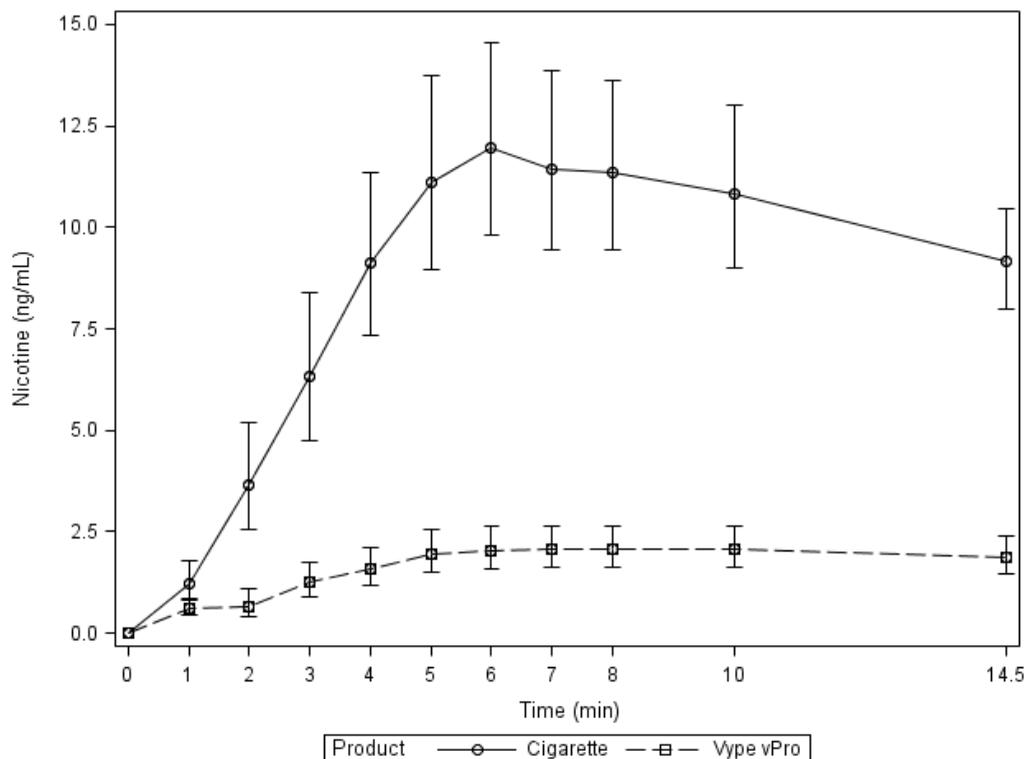
puffs then *ad libitum* use between 15 minutes and 1 hour) were used for both cigarettes and e-cigarettes.

Study 2. This was a part-randomized, open-label, crossover study conducted in 18 healthy male and female volunteer participants, who were accustomed and regular users of e-cigarettes and who occasionally smoked conventional cigarettes. For each study visit, we asked participants to abstain from using any tobacco or nicotine products and to avoid specified foods and drinks for 12 hours before product administration until after the visit was complete. Compliance with tobacco product abstinence was confirmed by eCO measurement <10ppm.

On the morning of Visit 1, we asked participants to smoke a single cigarette, taking *ad libitum* puffs for a period of 5 minutes. If the participant finished the cigarette before the end of the 5-minute period, no more cigarettes were allowed to be smoked. The smoking period was video-recorded to determine the number of puffs taken during the smoking period. Before, during, and after the 5-minute smoking period, we drew blood samples at specific time points (see section on nicotine measurement) for plasma nicotine analysis. At the end of Visit 1, participants were provided with an e-cigarette, selected from the 4 different e-cigarettes at random according to a computer-generated randomization schedule. We asked participants to use the e-cigarette so that they became familiar with that e-cigarette, before returning to the clinic for Visit 2. The time between Visits 1 and 2 was at least 2 days.

On Visit 2, we followed the same procedures de-

Figure 1
Plasma Nicotine Levels in Smokers Smoking a Cigarette or Using an E-cigarette in Study 1



Note.

Participants smoked a combustible cigarette or used the Vype vPro ePen e-cigarette under controlled conditions (10 puffs, each separated by 30 seconds). We drew blood samples at the indicated time points and analyzed plasma nicotine levels. Data are geometric means \pm 95% confidence intervals for between 23 and 24 participants in each case (Table 3).

scribed for Visit 1, but participants used their assigned e-cigarette, instead of a regular cigarette, for nicotine pharmacokinetic analysis. At the end of Visit 2, participants were given a different e-cigarette according to their randomization schedule to use before returning for nicotine pharmacokinetic analysis.

Plasma Nicotine Measurement

Study 1. We drew blood samples from a cannula placed in a forearm vein, at the following times from start of product use: -5, 1, 2, 3, 4, 5, 6, 7, 8, 10, 14.5, 30, 45, 60, 75 and 90 minutes. One 6 mL K₂EDTA Vacutainer® tube was collected at each time point for analysis of nicotine. We centrifuged the collection tubes at 2500 RPM for 15 minutes at 4°C within 60 minutes of collection. After centrifugation, the plasma was transferred to 2 methanol-prewashed polypropylene screw cap tubes and stored at 20°C (+/- 10°C) or below within 90

minutes from collection. We shipped the samples on dry ice for analysis to Celerion Bioanalytical Services (Lincoln, NE, USA.). An aliquot of plasma containing the analyte and internal standard was extracted using a solid phase extraction procedure. The extracted samples were analysed by an HPLC equipped with an AB SCIEX API 4000™ triple quadrupole mass spectrometer using an ESI source.

Study 2. We drew blood samples (3 mL) into 2 K₂EDTA Vacutainer® tubes from a cannula placed in a forearm vein at the following times from start of product use: -5, 1, 3, 5, 6, 7, 9, 15, 40 and 60 minutes. The number and timing of samples drawn was different than in Study 1 because we were able to take data and learnings from Study 1 and apply them to Study 2 to minimize blood drawing without affecting study outcomes. The first drawn sample at each time point (labeled A) was kept at room temperature and shipped to a central labora-

Table 3
Summary of the Pharmacokinetic Parameters for Nicotine, Study 1

Parameter	Statistic	Period ^a	Cigarette (N = 24)	Vype vPro ePen e-cigarette (N = 23)
C_{\max} (ng/ml)	LS Geometric mean	Controlled	13.0	2.5 ^b
	Geometric mean		13.4	2.5
	Geometric CV (%)		51.4	67.8
	Median		13.5	2.5
	Min		5.3	0.5
	Max		35.5	6.9
C_{\max} (ng/ml)	LS Geometric mean	<i>Ad libitum</i>	14.1	5.8 ^b
	Geometric mean		14.9	5.9
	Geometric CV (%)		45.7	61.1
	Median		14.7	6.5
	Min		6.9	1.6
	Max		40.6	12.5
t_{\max} (min)	Median	Controlled	7.0	6.0 ^c
	Median	<i>Ad libitum</i>	75	75
$AUC_{0-14.5}$ (ng.h/ml)	LS Geometric mean	Controlled	2.1	0.4 ^b
	Geometric mean		2.2	0.4
	Geometric CV (%)		45.5	60.5
	Median		2.2	0.4
	Min		1.0	0.1
	Max		5.0	1.2

Note.

a=Controlled 0–14.5 minutes; ad libitum 14.5–90 minutes; $AUC_{0-14.5}$, area under the plasma concentration-time curve from time zero to 14.5 minute sample; BLQ=below limit of quantification; C_{\max} , maximum observed plasma concentration; CV=coefficient of variation; LOQ= limit of quantification; t_{\max} , time to maximum observed plasma concentration; N=number of participants; b=p < .05 compared to cigarette; c=p > .05 compared to cigarette

tory (LabCorp; Durham, NC, USA) for analysis of nicotine by liquid chromatography/tandem mass spectrometry (LC/MS-MS).²⁷ The second drawn samples (labeled B) were centrifuged at 2500 RPM for 15 minutes at room temperature within 60 minutes of collection. After centrifugation, the plasma was transferred to a polypropylene screw cap tube and stored at 20°C (+/ 10°C) or below within 90 minutes from collection. These samples provided backup in case samples need to be retested.

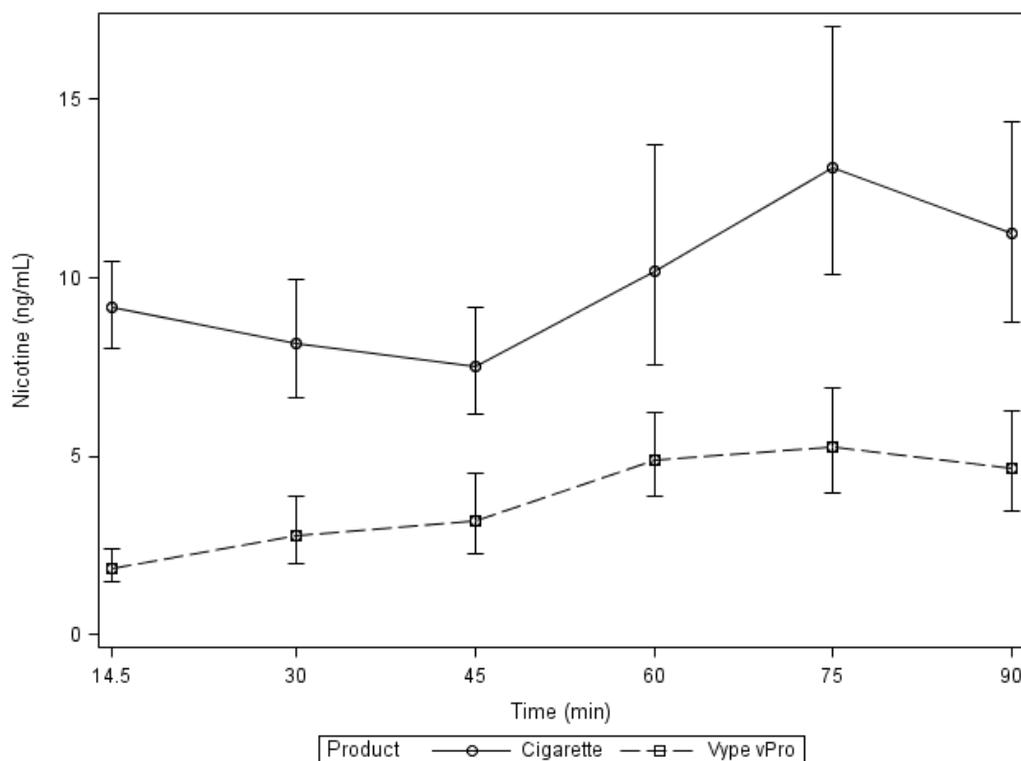
Data Analysis and Statistics

We carried out pharmacokinetic analysis was using SAS® Version 9.3 (SAS Institute Inc., Cary, NC, USA.). Pharmacokinetic parameters were determined from the plasma concentrations of nicotine using non-compartmental procedures. Baseline nicotine-level adjustment was used to account for residual plasma nicotine and levels below the

limit of quantification (BLQ) were imputed as one-half lower limit of quantification (LOQ; 0.5 ng/ml). We obtained C_{\max} and t_{\max} directly from the plasma concentration-time profiles. Area under the curve ($AUC_{0-14.5}$) was calculated using the linear trapezoidal method.

Study 1. To determine whether there were statistically significant differences between pharmacokinetic parameters, we determined the sample size for a paired t-test using Minitab® v17 (Minitab Inc., State College, PA, USA), with a power value of 0.8, alpha = .05, and standard deviations of 1 and 3.4 (obtained from published data when participants used e-cigarettes or smoked cigarettes).^{18,24,28-32} $AUC_{0-14.5}$ was defined as the area under the curve from t=0 sample collection time-point until the 14.5 minute sample collection. C_{\max} and t_{\max} were independently determined for the controlled puffing period (1 - 14.5 minutes) and the subsequent

Figure 2
Plasma Nicotine Levels in Smokers Smoking a Cigarette or Using an E-cigarette Ad Libitum in Study 1



Note.

Participants smoked a combustible cigarette or used the Vype vPro ePen e-cigarette by taking *ad libitum* puffs. We drew blood samples at the indicated time points and analyzed plasma nicotine levels. Data are geometric means \pm 95% confidence intervals for between 23 and 24 participants in each case (Table 3).

ad libitum period (30 - 90 minutes), whereas $AUC_{0-14.5}$ was calculated for the controlled period only. We compared differences between products using C_{max} and $AUC_{0-14.5}$. Changes in concentration were analysed using a mixed-model analysis, including product, sequence, and sex as fixed effects and participant (sequence) as a random effect. Statistical comparisons of log parameters were carried out between groups using ANOVA and *post hoc* t-test comparisons. Urge to smoke data analysis was also performed using ANOVA from the mixed procedure in SAS®.

Study 2. Sample size determination for a t-test with paired data was carried out with the power and sample size function in Minitab® v17 using a power value of 0.8, alpha = .05, and standard deviations from Study 1. Therefore, this sample size was powered to detect a minimum difference in C_{max} values of 3.6 ng/mL between a conventional combustible cigarette and e-cigarettes, and a difference of 1.1 ng/mL between e-cigarettes. AUC_{0-60}

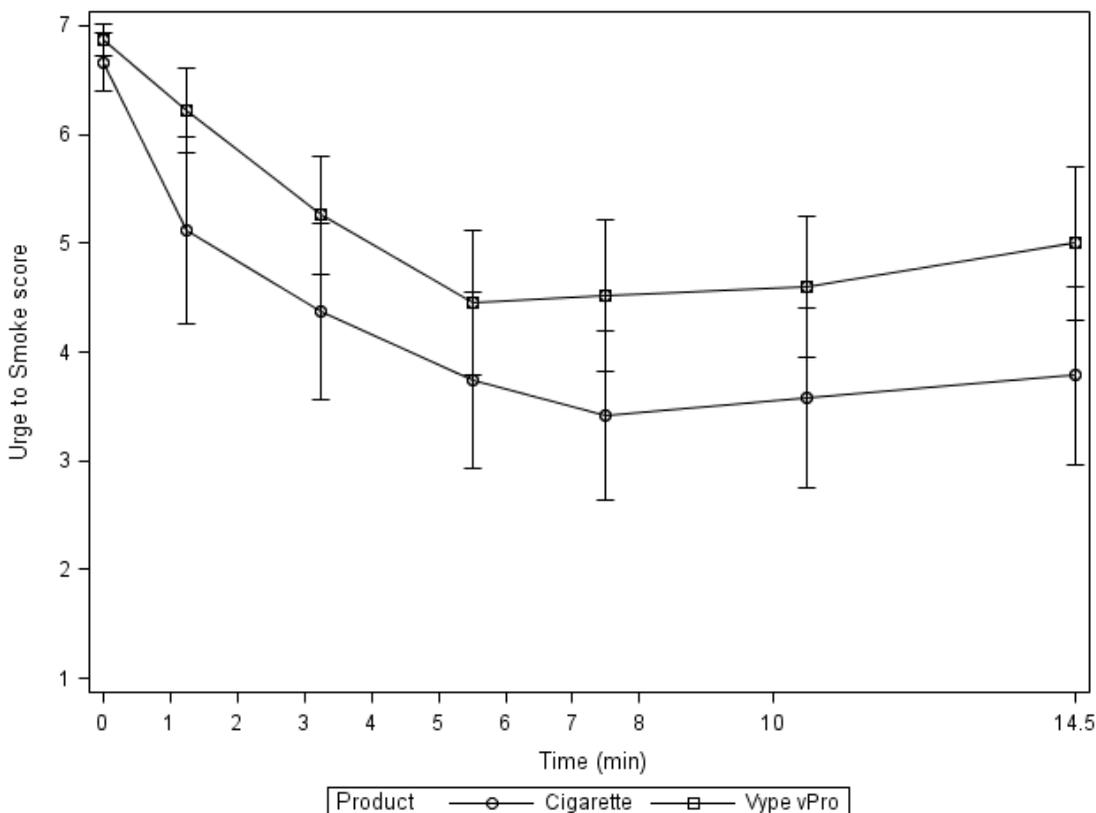
was defined as the area under the curve from t=0 sample collection time-point until the 60-minute sample collection. We compared differences between products using logarithmic transformed C_{max} and AUC_{0-60} . Changes in concentration were analysed using a mixed-model analysis, including product as fixed effect and participant as a random effect. *Post hoc* adjustments for multiple comparisons were made using Dunnett's test for comparisons versus the control cigarette.

RESULTS

Compliance

In both studies compliance was high. No participants in either study failed exhaled carbon monoxide (eCO) screening at each clinic visit. Furthermore, baseline plasma nicotine levels for each participant at all clinic visits were low, typically <2 ng/ml. This suggests that participants complied with the study protocol requirements for both cigarette and other nicotine product abstention.

Figure 3
Changes in Smoking Urges Over Time in Participants Smoking a Cigarette or Using a Vype vPro ePen E-cigarette in Study 1



Note.

We assessed urges by asking the question: ‘Right now, how much would you like a cigarette’ at the indicated time points. Smoking urges were rated on a 7-point Likert scale, ranging from 1 (not at all) to 7 (a great deal). Data are median values with 95% confidence intervals, with 23 or 24 participants in each case (Table 4).

Study 1

Study sample. Table 2 presents the demographic characteristics of the study sample. Of the 37 persons who underwent screening, 24 participated in the study. Of these, 22 participants completed all test product administration visits and the follow-up. Two participants discontinued the study; one participant was withdrawn from the study following a failed urinary drug screening test at Visit 2 and one participant withdrew from the study for personal reasons following Visit 3. Of the 24 participants who entered the study, 17 were male, 7 were female, and all participants were white. Participants were aged between 24 and 51 years inclusive and had a BMI between 18.0 and 29.8 kg/m² inclusive. At screening there were no findings of clinical concern in the medical history for any participant. In addition, there were no baseline signs or symptoms of clinical concern prior to test prod-

uct administration for any participants.

Nicotine pharmacokinetic parameters. During the initial period of controlled puffing while smoking a cigarette, as expected, plasma nicotine levels rose rapidly and reached a mean C_{max} value of 13.4 (CV 51.4%) ng/ml (Figure 1 and Table 3). Plasma nicotine levels then declined during the non-smoking phase but rose again in the *ad libitum* puffing period, reaching a level of 14.9 (45.7%) ng/ml (Figure 2 and Table 3). When participants used the closed modular Vype vPro ePen e-cigarette, plasma nicotine values during both the controlled and *ad libitum* puffing periods rose also, but to a lesser extent than that seen for the combustible cigarette. Mean C_{max} for the controlled puffing period was 2.5 (67.8%) ng/ml while in the *ad libitum* period this value was 5.9 (61.1%) ng/ml (Figures 1 and 2). For both the controlled and *ad libitum* periods, C_{max} values were significantly lower

Table 4
Minimum Urge to Smoke Scores in Study 1

	N	Mean (SD)	Score	
			95% CI	Min–Max
Controlled period				
Cigarette	24	3.0 (1.8)	2.2, 3.7	1–7
e-cigarette	23	4.2 (1.7)	3.4, 4.9	2–7
Controlled and ad libitum periods				
Cigarette	24	2.3 (1.5)	1.7, 3.0	1–6
e-cigarette	23	3.6 (1.8)	2.8, 4.4	1–7

Note.

A single-item questionnaire was administered to examine the urge to smoke a cigarette by asking ‘Right now, how much would you like a cigarette?’ at specific time points (-2, 1.25, 3.25, 5.5, 7.5, 10.5, 14.5, 30, 45, 60, 75 and 90 minutes). Smoking urge was rated on a 7-point Likert scale, ranging from 1 (not at all) to 7 (a great deal). Participants had abstained from any form of tobacco or nicotine product use for at least 12 hours prior to these measurements. We assessed urges to smoke for both a cigarette and the Vype vPro ePen e-cigarette. CI=confidence interval; SD=standard deviation; N=number of participants

than those seen for the combustible cigarette ($p < .001$ in each case). Mean area under the curve for the e-cigarette during the controlled puffing period ($AUC_{0-14.5}$) was 0.4 (60.5%) ng.h/ml, a value significantly lower than that measured for the cigarette (2.2 (45.5%) ng.h/ml; $p < .05$). Mean t_{max} values were not significantly different between the ciga-

rette and the e-cigarette.

Urge to smoke. Following their overnight abstinence of at least 12 hours, we administered a single-item questionnaire at various time points following the initiation of smoking a cigarette/using the e-cigarette. Figure 3 shows changes in the urge to smoke scores over time during the controlled

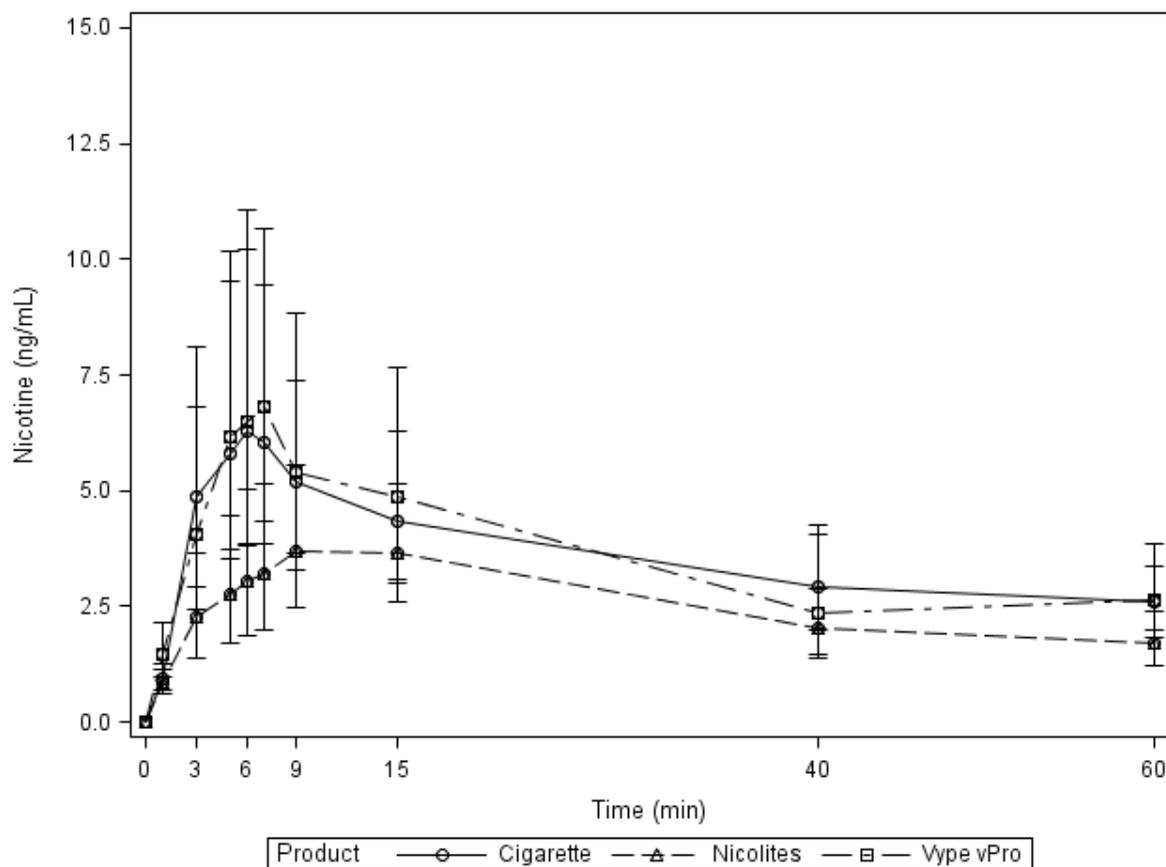
Table 5
Participant Demographics for Study 2

		Overall (N = 18)	Male (N = 12)	Female (N = 6)
Age (years)	Mean (SD)	33.5 (8.6)	29.5 (5.3)	41.5 (8.7)
	Min–Max	24 - 54	24 - 38	32 - 54
Height (cm)	Mean (SD)	172.1 (9.1)	175.8 (7.0)	164.7 (8.8)
	Min–Max	153.0-186.5	165.0-186.5	153.0-178.0
Weight (kg)	Mean (SD)	76.3 (14.0)	81.9 (10.3)	65.2 (14.3)
	Min–Max	49.0-95.5	65.0-95.5	49.0-84.0
BMI (kg/m²)	Mean (SD)	25.9 (3.2)	26.8 (2.3)	23.9 (4.2)
	Min–Max	19.1-29.9	23.0-29.9	19.1-29.1
Race / ethnicity, N (%)	White - Non-Hispanic	13 (72)	9 (50)	4 (22)
	White - Hispanic	3 (17)	1 (6)	2 (11)
	Black - Non Hispanic	1 (6)	1 (6)	0 (0)
	Asian - Non Hispanic	1 (6)	1 (6)	0 (0)

Note.

BMI=body mass index; N=number of participants; SD=standard deviation

Figure 4
Plasma Nicotine Levels in E-Cigarette Users Smoking a Cigarette or Using One of Two Different E-cigarettes in Study 2



Note.

Participants smoked a combustible cigarette or used their assigned e-cigarette (Vype vPro ePen or Nicolites) by taking ad libitum puffs over a period of 5 minutes. We drew blood sample at the indicated time points and analyzed plasma nicotine levels. Data are geometric means \pm 95% confidence intervals for 18 participants in each case.

puffing period. Smoking the cigarette or using the e-cigarette reduced participants' urges to smoke. Mean minimum urge to smoke score during the controlled use period (Table 4) was not significantly different between the combustible cigarette and the e-cigarette ($p = .23$).

Study 2

Study sample. Table 5 presents the demographic characteristics of the study sample. Of the 25 potential participants who underwent screening, 18 entered the study and all participants completed all test product administration visits and the follow-up. Of the participants who entered the study, 12 were male and 6 were female. Participants were aged between 24 and 54 years inclusive, had a BMI between 19.1 and 29.9 kg/m² inclusive, and were

of mixed race (Table 5). At screening there were no findings of clinical concern in the medical history for any participant. In addition, there were no baseline signs or symptoms of clinical concern prior to test product administration for any participants.

Nicotine pharmacokinetic parameters. As seen in Study 1, in this study plasma nicotine levels rose during a 5-minute *ad libitum* puffing period on a cigarette, reaching a mean C_{max} value (CV%) of 7.2 (130.8%) ng/ml (Figure 4 and Table 6). During this period, participants took an average of 14 \pm 4 puffs on the cigarette. For the closed modular (Vype vPro ePen) and first-generation rechargeable (Nicolites) e-cigarettes, mean C_{max} values were 7.8 (108.2%) ng/ml and 4.7 (93.6%)^{max} ng/ml respectively (Figure 4 and Table 6). These maximum plasma

Table 6
Summary of the Pharmacokinetic Parameters for Nicotine, Study 2

Parameter	Statistic	Cigarette (N = 18)	Vype vPro ePen e-cigarette (N = 18)	Nicolites e-cigarette (N = 18)
C_{max} (ng/mL)	LS Geometric mean	7.2	8.8 ^a	4.7 ^b
	Geometric mean	7.2	7.8	4.7
	Geometric CV (%)	130.8	108.2	93.6
	Median	6.2	9.2	5.1
	Min	0.7	0.0	1.2
	Max	37.6	40.2	18.2
t_{max} (min)	Median	6.0	6.0 ^a	9.0 ^a
AUC (ng.h/mL)	LS Geometric mean	3.4	2.9 ^a	2.2 ^b
	Geometric mean	3.4	2.9	2.2
	Geometric CV (%)	c	c	c
	Median	4.0	4.6	2.9
	Min	0.2	0.0	0.0
	Max	11.5	15.6	6.3

Note.

a=p > .05 compared to the cigarette; b=p > .05 compared to either the cigarette or the Vype vPro e-cigarette; c=unable to determine.

levels were attained from an average of 20 ± 6 puffs for the closed modular e-cigarette and 21 ± 7 puffs for the first generation e-cigarette, and were not significantly different either to the combustible cigarette or to each other ($p > .05$ in each case). Inter-participant variability was high for C_{max} , with values ranging, for example, from $<LOQ$ to $>40ng/ml$ for the same product (Vype vPro ePen) in 2 different participants. This gave rise to relatively large coefficients of variation and confidence intervals in the dataset (Table 6 and Figure 4). Area under the curve (AUC_{0-60}) was not significantly different between any of the 3 products examined (Table 6). Furthermore, although the t_{max} for the Nicolites e-cigarette was higher than that for the cigarette or the Vype vPro ePen e-cigarette, again there were no statistically significant differences for this parameter between any of the 3 products examined (Table 6).

DISCUSSION

Electronic cigarettes are gaining popularity as a replacement for smoking combustible tobacco cigarettes and, in the UK, their uptake and use during quit attempts has grown markedly since 2012.³³ Data such as these highlight not only why e-cigarettes are gaining support as a potentially much less harmful alternative to cigarette smoking due to their significantly lower toxicant yields,⁹⁻¹¹ but also why they are increasingly considered as an effective facilitator of behavioral change by aid-

ing smoking reduction and cessation.^{15-17,34} Understanding how e-cigarettes are supporting behavior change is critical to the development of effective products to help smokers quit, thus capitalizing on the harm reduction potential of e-cigarettes.³⁵

The delivery of nicotine, the major addictive element of cigarette smoking, from e-cigarettes can perhaps be considered as a key determinant of the efficacy of e-cigarettes in replacing combustible cigarettes in the hands of a quitting smoker. Effective nicotine delivery has long been considered to be important for the smoking reduction or cessation efficacy of NRT products,²⁵ and some data support this.²⁶ Regarding e-cigarettes as a facilitator of smokers' behavior change, some data also support the idea that e-cigarette nicotine delivery is equally important for their efficacy.³⁶ This notion is gaining support from healthcare agencies, such as the UK's Royal College of Physicians, that concluded in its 2016 report that "regulation of e-cigarettes and other similar products should....ensure that those that deliver nicotine do so in doses that smokers find satisfying, and encourage substitution for smoked tobacco."¹⁴

If effective nicotine delivery is central to the efficacy of e-cigarettes as a harm reduction tool, studies measuring this nicotine delivery, such as those we present here, are crucial to being able to understand this role and to examine the potential for any given product in supporting behavior change. Whereas laboratory machine puffing or mathemat-

ical modeling studies may give an indication of the potential nicotine delivery from an e-cigarette under human use conditions,^{37,38} nicotine delivery can only be accurately determined by blood sampling in clinical studies on volunteer participants. Our data show that e-cigarettes are effective at delivering nicotine during brief use periods. However, the predominant finding of our 2 studies was that the magnitude of this delivery, compared both to one another, and also to a market-typical combustible cigarette, was far greater in accustomed users during *ad libitum* puffing (Study 2) than in smokers who were not current users of e-cigarettes during a defined puffing period (Study 1).

This finding has important implications; foremost, it highlights the need for strong consideration of participant cohorts when designing a nicotine pharmacokinetic study. If nicotine delivery is considered a marker of potential efficacy, our data show that a study in smokers who are not accustomed to e-cigarette use may not support this potential (akin to a type II error) whereas a study in accustomed vapers may show nicotine delivery, in terms of C_{max} , to be equivalent to a cigarette. In support of considering such a study design, some literature contains a number of studies, each using different protocols and, in many cases, reporting different outcomes from studies using apparently similar e-cigarettes.^{23,39,40} Furthermore, although a potential limitation of our work reported here is the small number of studies on which our recommendation of cohort selection is based, such a proposal is supported by other studies which have highlighted the apparent behavioral adaptation when transitioning from smoking to vaping.^{22,41} Those studies reiterate the need for careful cohort selection when using nicotine pharmacokinetics to assess potential efficacy.

If design factors, such as study cohorts (in our case, current smokers who don't use e-cigarettes versus current vapers who only smoke occasionally) and instructions for product use (defined versus *ad libitum* puffing), can affect the experimental outcome of a nicotine pharmacokinetic study significantly, is there a potential need for standardization of study protocols between different research groups? Without such a standardized approach, nicotine delivery data from different studies may not be comparable. However, standardizing how pharmacokinetic studies are performed would have to take into account the cohort being recruited (smokers vs current e-cigarette users), the market in the location where the study will be performed (eg, whether low- or high-tar cigarettes are more commonly smoked), the desirability of a controlled 'dose' of nicotine by fixing the number of puffs taken during a study period as in Study 1, or whether a more real-world view of nicotine delivery is preferable by allowing participants to use e-cigarettes *ad libitum*, yet still in a clinical confinement setting, as in Study 2.

Our finding in Study 2 that nicotine delivery

from a newer-generation e-cigarette matched that of a market-typical cigarette, in terms of C_{max} and AUC, are in general agreement with those of Ramoa et al,⁴² who also observed relatively high nicotine deliveries in participants who were long-term (>1 year) users of e-cigarettes. However whereas that paper concluded that e-cigarettes appear capable of exceeding the nicotine delivery profile of a combustible tobacco cigarette, the study did not make a direct comparison to a regular cigarette.⁴² To do so would have been both ethically questionable because it would have involved requiring 13 of the non-smoker participants to smoke a cigarette, as well as being scientifically unsound, because vapers who are not current smokers may find cigarette smoking aversive, leading to low nicotine deliveries from a cigarette. In being able to compare a cigarette and an e-cigarette directly, our findings of comparable deliveries between these 2 types of products may help to alleviate concerns about dependency and cessation difficulty of those using e-cigarettes.⁴²

One further aspect for consideration when planning nicotine pharmacokinetic studies is the sample size. In the 2 studies reported here, variability of data obtained was greater in Study 2 than in Study 1. The reasons for the larger inter-individual differences in blood nicotine levels in the e-cigarette users in Study 2 cannot be ascertained. However, a similar finding has been reported previously, albeit in e-cigarette naïve smokers; the finding was potentially due to a large degree of variability in puffing topography parameters observed between participants.⁴³ Whatever the source of the variability, those existing data and our findings in experienced vapers suggest that the planning of nicotine pharmacokinetic studies on e-cigarette users should take into account this potential for variability when performing power calculations.

In summary, data from 2 pharmacokinetic studies showed that elevation of blood nicotine levels during e-cigarette use was greater in experienced users when compared to e-cigarette naïve smokers and under more real-world conditions with unconstrained, *ad libitum* use. Careful selection of cohorts as well as standardization of study protocols may be required for data from different studies to be comparable. Furthermore, this would facilitate an assessment of the potential efficacy of an e-cigarette in supporting behavioral change during smoking reduction or cessation and allow comparisons with nicotine delivery from combustible cigarettes to be made.

Human Subjects Statement

All participants provided written informed consent before undergoing any screening or study procedures. Study 1 was approved by the Health and Social Care Research Ethics Committee B (HSC REC B) of the Office for Research Ethics Committees Northern Ireland (Lisburn, U.K.; approval reference number 14/NI/1118) and regis-

tered on the International Standard Randomised Controlled Trial Number (ISRCTN) registry (ISRCTN74070762). Study 2 was approved by the Aspire Institutional Review Board (IRB; San-tee, CA, U.S.A.) with approval reference number BAT4215006 and registered on the ClinicalTrials.gov registry (NCT02474849).

Conflict of Interest Statement

IMF, AE, NG, CJS, MM, OMC, EM, KMCA and CP are or were paid employees of British American Tobacco (Investments) Ltd. MN is President of Los Angeles Clinical Trials, a clinical trials unit contracted to perform Study 2. The 2 studies described in this paper were funded by British American Tobacco (Investments) Ltd.

Acknowledgments

We gratefully acknowledge the assistance in designing, planning and conducting Study 1 by Dr Donald Graff and Mrs Kirsten Gill (Celerion, Inc). We further gratefully acknowledge the support given to the design, planning and conduct of both studies by Dr Krishna Prasad, Mr. Graham Errington, Miss Eleni Mavropoulou and Mrs Madeline Ashley (British American Tobacco (Investments) Limited).

References

- Lunell E, Molander L, Ekberg K, et al. Site of nicotine absorption from a vapour inhaler – comparison with cigarette smoking. *Eur J Clin Pharm*. 2000;55(10):737-741.
- Rodgman A, Perfetti TA. *The Chemical Components of Tobacco and Tobacco Smoke*. 2nd ed. Boca Raton, FL: CRC Press; 2013.
- Doll R, Peto R, Wheatley K, et al. Mortality in relation to smoking: 40 years' observations on male British doctors. *BMJ*. 1994;309(6959):901-911.
- International Agency for Research on Cancer. *IARC Handbook of Cancer Prevention, Volume 11, Reversal of Risk after Quitting Smoking*. Geneva, Switzerland: WHO Press; 2007.
- McNeill A, Munafò MR. Reducing harm from tobacco use. *J Psychopharmacol*. 2013;27(1):13-18.
- Stratton K, Shetty P, Wallace R, Bondurant S. Clearing the smoke: the science base for tobacco harm reduction – executive summary. *Tob Control*. 2001;10(2):189-195.
- Azzopardi D, Patel K, Jaunky T, et al. Electronic cigarette aerosol induces significantly less cytotoxicity than tobacco smoke. *Toxicol Mech Meth*. 2016;26(6):477-491.
- Taylor M, Carr T, Oke O, et al. E-cigarette aerosols induce lower oxidative stress *in vitro* when compared to tobacco smoke. *Toxicol Mech Meth*. 2016;26(6):465-476.
- Margham J, McAdam K, Forster M, et al. Chemical composition of an e-cigarette aerosol: a quantitative comparison with cigarette smoke. *Chem Res Toxicol*. 2016. doi: 10.1021/acs.chemrestox.6b00188.
- Goniewicz ML, Knysak J, Gawron M, et al. Levels of selected carcinogens and toxicants in vapour from electronic cigarettes. *Tob Control*. 2014;23(2):133-139.
- McRobbie H, Phillips A, Goniewicz ML, et al. Effects of switching to electronic cigarettes with and without concurrent smoking on exposure to nicotine, carbon monoxide, and acrolein. *Cancer Prev Res*. 2015;8(9):873-878.
- Nutt DJ, Phillips LD, Balfour D, et al. Estimating the harms of nicotine-containing products using the MCDA approach. *European J Addict Res*. 2014;20(5):218-225.
- McNeil A, Brose LS, Calder R, et al. *E-cigarettes: An Evidence Update. A Report Commissioned by Public Health England*. Available at: https://www.gov.uk/government/uploads/system/uploads/attachment_data/file/457102/E-cigarettes_an_evidence_update_a_report_commissioned_by_Public_Health_England_FINAL.pdf. Accessed April 25, 2016.
- Royal College of Physicians (RCP). *Nicotine without Smoke: Tobacco Harm Reduction*. London, UK: RCP; 2016. Available at: <https://www.rcplondon.ac.uk/projects/outputs/nicotine-without-smoke-tobacco-harm-reduction-0>. Accessed October 25, 2016.
- Brown J, Beard E, Kotz D, et al. Real-world effectiveness of e-cigarettes when used to aid smoking cessation: a cross-sectional population study. *Addiction*. 2014;109(9):1531-1540.
- Brose LS, Hitchman SC, Brown J, et al. Is the use of electronic cigarettes while smoking associated with smoking cessation attempts, cessation and reduced cigarette consumption? A survey with a 1-year follow-up. *Addiction*. 2015;110(7):1160-1168.
- Bullen C, McRobbie H, Thornley S, et al. Effect of an electronic nicotine delivery device (e cigarette) on desire to smoke and withdrawal, user preferences and nicotine delivery: randomised cross-over trial. *Tob Control*. 2010;19(2):98-103.
- Digard H, Proctor C, Kulasekaran A, et al. Determination of nicotine absorption from multiple tobacco products and nicotine gum. *Nicotine Tob Res*. 2013;15(1):255-261.
- Schroeder MJ, Hoffman AC. Electronic cigarettes and nicotine clinical pharmacology. *Tob Control*. 2014;23(Suppl 2):ii30-ii35.
- Caldwell B, Sumner W, Crane J. A systematic review of nicotine by inhalation: is there a role for the inhaled route? *Nicotine Tob Res*. 2012;14(10):1127-1139.
- St Helen G, Havel C, Dempsey DA, et al. Nicotine delivery, retention and pharmacokinetics from various electronic cigarettes. *Addiction*. 2016;111(3):535-544.
- Hajek P, Goniewicz ML, Phillips A, et al. Nicotine intake from electronic cigarettes on initial use and after 4 weeks of regular use. *Nicotine Tob Res*. 2015;17(2):175-179.
- Farsalinos KE, Spyrou A, Tsimopoulou K, et al. Nicotine absorption from electronic cigarette use: comparison between first and new-generation devices. *Sci Rep*. 2014;4:4133.
- Eissenberg T. Electronic nicotine delivery devices: ineffective nicotine delivery and craving suppression after acute administration. *Tob Control*. 2010;19(1):87-88.
- Rennard SI, Glover ED, Leischow S, et al. Efficacy of the nicotine inhaler in smoking reduction: a double-blind, randomized trial. *Nicotine Tob Res*. 2006;8(4):555-564.
- Caldwell BO, Crane J. Combination nicotine metered dose inhaler and nicotine patch for smoking cessation: a randomized controlled trial. *Nicotine Tob Res*. 2016;18(10):1944-1951.
- Moyer TP, Charlson JR, Enger RJ, et al. Simultaneous analysis of nicotine, nicotine metabolites, and tobacco alkaloids in serum or urine by tandem mass spectrometry, with clinically relevant metabolic profiles. *Clin Chem*. 2002;48(9):1460-1471.
- Vansickel AR, Cobb CO, Weaver MF, et al. A clinical laboratory model for evaluating the acute effects of electronic "cigarettes": nicotine delivery profile and cardiovascular and participative effects. *Cancer Epidemiol Biomarkers Prev*. 2010;19(8):1945-1953.
- Rose JE, Turner JE, Murugesan T, et al. Pulmonary delivery of nicotine pyruvate: sensory and pharmacokinetic characteristics. *Exp Clin Psychopharmacol*. 2010;18(5):385-394.
- Dawkins L, Corcoran O. Acute electronic cigarette use: nicotine delivery and participative effects in regular users. *Psychopharmacology (Berl)*. 2014;231(2):401-407.

31. Nides MA, Leischow SJ, Bhattar M, et al. Nicotine blood levels and short-term smoking reduction with an electronic nicotine delivery system. *Am J Health Behav.* 2014;38(2):265-274.
32. Vansickel AR, Eissenberg T. Electronic cigarettes: effective nicotine delivery after acute administration. *Nicotine Tob Res.* 2013;15(1):267-270.
33. Beard E, West R, Michie S, Brown J. Association between electronic cigarette use and changes in quit attempts, success of quit attempts, use of smoking cessation pharmacotherapy, and use of stop smoking services in England: time series analysis of population trends. *BMJ.* 2016;354:i4645.
34. Camenga DR, Kong G, Cavallo DA, Krishnan-Sarin S. Current and former smokers' use of electronic cigarettes for quitting smoking: an exploratory study of adolescents and young adults. *Nicotine Tob Res.* 2016 Sep 23. pii: ntw248. [Epub ahead of print]
35. Bauld L. Electronic cigarettes and smoking cessation. *Nicotine Tob Res.* 2016;18(10):1925.
36. Tseng TY, Ostroff JS, Campo A, et al. A randomized trial comparing the effect of nicotine versus placebo electronic cigarettes on smoking reduction among young adult smokers. *Nicotine Tob Res.* 2016;18(10):1937-1943.
37. Shihadeh A, Eissenberg T. Electronic cigarette effectiveness and abuse liability: predicting and regulating nicotine flux. *Nicotine Tob Res.* 2015;17(2):158-162.
38. Farsalinos KE, Yannovits N, Sarri T, et al. Protocol proposal for, and evaluation of, consistency in nicotine delivery from the liquid to the aerosol of electronic cigarettes atomizers: regulatory implications. *Addiction.* 2016;111(6):1069-1076.
39. Yan XS, D'Ruiz C. Effects of using electronic cigarettes on nicotine delivery and cardiovascular function in comparison with regular cigarettes. *Regul Toxicol Pharmacol.* 2015;71(1):24-34.
40. Walele T, Sharma G, Savioz R, et al. A randomised, crossover study on an electronic vapour product, a nicotine inhalator and a conventional cigarette. Part A: pharmacokinetics. *Regul Toxicol Pharmacol.* 2016;74:187-192.
41. Farsalinos KE, Spyrou A, Stefopoulos C, et al. Nicotine absorption from electronic cigarette use: comparison between experienced consumers (vapers) and naïve users (smokers). *Sci Rep.* 2015;5:11269.
42. Ramôa CP, Hiler MM, Spindle TR, et al. Electronic cigarette nicotine delivery can exceed that of combustible cigarettes: a preliminary report. *Tob Control.* 2016;25(e1):e6-9.
43. Lopez AA, Hiler MM, Soule EK, et al. Effects of electronic cigarette liquid nicotine concentration on plasma nicotine and puff topography in tobacco cigarette smokers: a preliminary report. *Nicotine Tob Res.* 2016;18(5):720-723.

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Appendix 1

Supplementary Information

Inclusion and Exclusion Criteria

Study 1.

Inclusion Criteria.

To be confirmed at Screening Visits:

Participants will be males or non-pregnant, non-lactating females between 21 and 55 years of age inclusive.

- Women of child-bearing potential should be using one of the following acceptable methods of contraception, as defined in the MHRA document 'Recommendations related to contraception and pregnancy testing in clinical trials (http://www.mhra.gov.uk/idcm2/idcplg?IdcService=GET_FILE&dDocName=CON2033037&RevisionSelectionMethod=Latest): combined (oestrogen and progestogen containing) oral, intravaginal or transdermal hormonal contraception associated with inhibition of ovulation; progestogen-only hormonal contraception, either oral, injected or implanted, associated with inhibition of ovulation; progestogen-only oral hormonal contraception where inhibition of ovulation is not the primary mode of action; intrauterine device (IUD); intrauterine hormone-releasing system (IUS); bilateral tubal occlusion; male or female condom with or without spermicide; cap, diaphragm or sponge with spermicide.
- Women of non-childbearing potential may be included if they are either surgically sterile (hysterectomy and/or oophorectomy) or postmenopausal for more than 1 year (with follicle-stimulating hormone [FSH] level greater than or equal to 8 mIU/mL) and must have a negative serum pregnancy test result during screening. Women who are surgically sterile must provide documentation of the procedure by an operative report.
- Male participants must use an approved method of birth control during the entire study. These participants must not donate sperm during this time.
- Participants must be in good health as determined by medical history, vital signs, blood biochemistry, hematology, urinalysis and physical examination.
- Participants with negative HIV and Hepatitis B and C results.
- Participants must have a body mass index (BMI) between 18 and 30 kg/m² inclusive. Male participants must have a weight between 50 and 110kg and female participants between 40 and 90kg.
- No clinically significant abnormalities in blood pressure values (the differences between supine and standing BP are less than 20 mmHg) with no symptomatic evidence of postural hypotension. Exceptions can be made at the discretion of the Principal Investigator and the reason documented in the CRF.
- Participants will have negative results for the urinary drug of abuse screening and ethanol test.
- Participants will have given their written informed consent to participate in the study and to abide by the study restrictions.
- Prior to study start, participants must be daily smokers of at least 10 factory-produced cigarettes and have smoked continuously for a minimum of 1 year. Participants current brand of cigarette will have a machine-smoked ISO tar yield of 8-10 mg. Smoking status will be confirmed with a Smokerlyzer breath CO \geq 10ppm and a urinary cotinine level of \geq 200ng/ml (determined using One Step cotinine test kit) at screening. Participants should be familiar with the use of ecigarettes but not currently dual-using cigarettes and e-cigarettes.

To be confirmed during each clinic visit:

- Abstention will be confirmed by an exhaled breath CO reading <15ppm.
- Participants will have negative results for the urinary drug of abuse and alcohol screen. Continued inclusion in the study will be at the discretion of the Principal Investigator.

Exclusion Criteria.

To be confirmed at Screening Visit:

- Participants who have a history of, or clinically active significant, neurological, gastrointestinal, renal, hepatic, cardiovascular, psychiatric, respiratory, metabolic, endocrine, haematological disease or other major disorders.
- Participants with significant allergies who in the opinion of the Principal Investigator should not be included.

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- Participants who have been diagnosed with urticaria or asthma.
- Participants with a recent history of or current drug or alcohol abuse who in the opinion of the Investigator should not be included. Excessive intake of alcohol within the last 6 months, defined as a regular maximum weekly intake of greater than 7 drinks for women or 14 drinks for men. One drink is defined as 1 pint of regular beer (5% alcohol), 200 ml of wine (12% alcohol), or 25 ml of distilled spirits (40% alcohol).
- Participants with an inability to communicate well with the Investigator/study staff (ie, language problem, poor mental development or impaired cerebral function).
- Participants who are participating in another clinical research study or who have participated in a clinical research study in the last 3 months.
- Participants who have had treatment with prescription medications within 21 days or over-the-counter medication within 72 hours of the planned first product use occasion. For female participants, oral contraceptives, hormonal contraceptive devices and replacement hormonal therapies are not included in the list of drugs leading to exclusion.
- Participants who have used any drugs or substances (except tobacco) known to be strong inducers or inhibitors of any CYP enzymes (formerly known as cytochrome P450 enzymes) within a 28-day period prior to first product administration. For a list of such drugs and substances, please refer to <http://medicine.iupui.edu/clinpharm/ddis/main-table/>.
- Participants who have had any treatment with smoking cessation medications (eg, Bupropion, Champix, any NRT or ENDS) within 30 days of the planned first product use occasion.
- Participants with any other clinically significant medical history, in the Investigator's opinion, including conditions which might affect drug absorption, metabolism or excretion.
- Female participants, who are pregnant or become pregnant during the course of the study.
- Participants who have lost or donated more than 450ml blood, plasma or platelets within the 3 months preceding the first product administration.
- Participants who are currently trying to stop smoking or considering stopping in the next 2 months.
- Participants who are unwilling or unable to comply with the study requirements.
- Participants who in the opinion of the Investigator should not participate in the study for any other reason.

To be re-confirmed during each clinic visit:

- Participant continues to meet all screening exclusion criteria.
- Receipt of any medication since screening visit that may have an impact on the safety and objectives of the study (at the Principal Investigator's discretion).

Study 2.

Inclusion Criteria.

To be confirmed at Screening Visits:

- Prior to study start, participants must be daily users of newer generation e-cigarette devices which they have used regularly for a minimum of 3 months. The solutions used in their e-cigarettes must contain nicotine. Participants must also be occasional smokers of combustible cigarettes (lower limit of 1 cigarette per month; upper limit of 5 cigarettes per week). Product use status will be confirmed with a urinary cotinine level of $\geq 200\text{ng/ml}$ (determined using NicAlert cotinine test kit) at screening.
- Participants will be males or non-pregnant, non-lactating females, and between 21 and 55 years of age inclusive. Age verification will be performed by checking of Federal or state-issued ID (eg, passport or driving licence) during screening.
- Women of child-bearing potential should be using one of the following acceptable methods of contraception, as defined in the MHRA document 'Recommendations related to contraception and pregnancy testing in clinical trials' (http://www.mhra.gov.uk/idcm2/idcplg?IdcService=GET_FILE&dDocName=CON2033037&RevisionSelectionMethod=Latest): combined (oestrogen and progestogen containing) oral, intravaginal or transdermal hormonal contraception associated with inhibition of ovulation; progestogen-only hormonal contraception, either oral, injected or implanted, associated with inhibition of ovulation; progestogen-only oral hormonal contraception where inhibition of ovulation is not the primary mode of action; intrauterine device (IUD); intrauterine hormone-releasing system (IUS); bilateral tubal occlusion; male or female condom with or without spermicide; cap, diaphragm or sponge with spermicide.
- Women of non-childbearing potential may be included if they are either surgically sterile (hysterectomy and/or oophorectomy) or postmenopausal for more than 1 year and must have a negative urine pregnancy test result during screening. Women who are surgically sterile must provide documenta-

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tion of the procedure by an operative report.

- Male participants must use an approved method of birth control during the entire study. These participants must not donate sperm during this time.
- Participants must be in good health as determined by medical history, vital signs, blood biochemistry, hematology, urinalysis and physical examination.
- Participants must have a body mass index (BMI) between 18 and 30 kg/m² inclusive. Male participants must have a weight between 60 and 120kg and female participants between 50 and 100kg.
- No clinically significant abnormalities in blood pressure values.
- Participants will have negative results for the urinary drug of abuse screening and ethanol test.
- Participants will have given their written informed consent to participate in the study and to abide by the study restrictions.

To be confirmed during each clinic visit:

- Abstinence from tobacco and nicotine products will be confirmed by an exhaled breath CO reading <10ppm.

Exclusion Criteria.

To be confirmed at Screening Visit:

- Participants who have a history of, or clinically active significant, neurological, gastrointestinal, renal, hepatic, cardiovascular, psychiatric, respiratory, metabolic, endocrine, haematological disease or other major disorders.
- Participants with significant allergies who in the opinion of the Principal Investigator should not be included.
- Participants who have been diagnosed with urticaria (hives) or asthma.
- Persons who have a history of urticaria (hives) in response to any product.
- Participants with a recent history of or current drug or alcohol abuse who in the opinion of the Investigator should not be included. Excessive intake of alcohol within the last 6 months, defined as a regular maximum weekly intake of greater than 7 drinks for women or 14 drinks for men. One drink is defined as 1 pint of regular beer (5% alcohol), 200 ml of wine (12% alcohol), or 25 ml of distilled spirits (40% alcohol).
- Participants with an inability to communicate well with the Investigator/study staff (ie, language problem, poor mental development or impaired cerebral function).
- Participants who are participating in another clinical research study or who have participated in a clinical research study in the last 3 months.
- Participants who have had treatment with prescription medications within 21 days or over-the-counter medication within 24 hours of the planned first product use occasion, except for those stated in the Inclusion Criteria. For all participants, prescribed use of blood pressure (beta blockers) and lipid lowering (statin) medications are permitted.
- Participants who have used any drugs or substances (except tobacco) known to be strong inducers or inhibitors of any CYP enzymes (formerly known as cytochrome P450 enzymes) within a 28-day period prior to first product administration. For a list of such drugs and substances, please refer to <http://medicine.iupui.edu/clinpharm/ddis/main-table/>.
- Participants who have had any treatment with smoking cessation medications (eg, Bupropion, Chantix or any NRT) within 30 days of the planned first product use occasion.
- Participants with any other clinically significant medical history, in the Investigator's opinion, including conditions which might affect drug absorption, metabolism or excretion.
- Female participants, who are pregnant or become pregnant during the course of the study.
- Participants who have lost or donated more than 450ml of blood within the 3 months preceding the first product administration.
- Participants who are currently trying to stop smoking or to stop using e-cigarettes, or considering stopping in the next 2 months.
- Participants who are unwilling or unable to comply with the study requirements.
- Participants who in the opinion of the Investigator should not participate in the study for any other reason.

To be re-confirmed during each clinic visit:

- Participant continues to meet all screening exclusion criteria.
- Receipt of any medication since screening visit that may have an impact on the safety and objectives of the study (at the Principal Investigator's discretion).