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Assessment of the Nicotine Pharmacokinetics When Using Two Types of E-Cigarettes in Healthy Adults Who Smoke: Results From Two Randomized, Crossover Studies *

by

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SUMMARY

The nicotine pharmacokinetics (PK) of non-combustible tobacco and nicotine products, including e-cigarettes, have been extensively studied, with lower or similar nicotine exposure reported for most products compared with combustible cigarettes (CC). We conducted two clinical studies to evaluate nicotine PK and assess nicotine consumption when using two types of e-cigarettes with different flavor variants in U.S. healthy adults who smoke, under similar study protocols.
Study 1 was a randomized, 6-period crossover study conducted in healthy adults who smoke. The primary objective was to evaluate nicotine PK following use of a cig-a-like e-cigarette (eDNC1.0a) with three flavor variants, subjects' own brand of CC, a nicotine gum, and a reference e-cigarette. Study 2 was a randomized, 7-period crossover study conducted in healthy adults who smoke. The primary objective was to evaluate nicotine PK following use of a closed-tank e-cigarette (eDNC2.0a) with four flavor variants, subjects' own brand of CC, a nicotine inhaler, and a reference e-cigarette.
In summary, the results of the present studies indicate that nicotine exposure from eDNC1.0a with three flavor variants and eDNC2.0a with four flavor variants was less than that from subjects' own brand of CC, similar to or less than that from reference e-cigarettes, but similar to or greater than that from pharmaceutical nicotine replacement products. It was observed that the nicotine consumption, estimated based on e-liquid consumption, was generally directly proportional to the level of nicotine exposure as indicated by nicotine PK parameter measurements in each study. Furthermore, linear relationships were found between estimated nicotine consumption and plasma nicotine PK parameters following e-cigarette use. Our findings suggest that mixed effects modelling can be used as a non-invasive method to provide insights of nicotine PK parameters (AUC and C_{max}) from e-liquid nicotine consumption data. [Contrib. Tob. Nicotine Res. 33 (2024) 173–188]

KEYWORDS

Clinical study; e-cigarette; nicotine pharmacokinetics

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ZUSAMMENFASSUNG

Die Pharmakokinetik (PK) von nicht verbrennbaren Tabak- und Nikotinprodukten, einschließlich E-Zigaretten, wurde umfassend untersucht, wobei für die meisten Produkte eine geringere oder ähnliche Nikotinexposition im Vergleich zu verbrennbaren Zigaretten (CC) berichtet wurde. Wir haben zwei klinische Studien durchgeführt, um die Nikotin-PK zu bewerten und den Nikotinkonsum bei der Verwendung von zwei Arten von E-Zigaretten mit unterschiedlichen Geschmacksvarianten bei gesunden, rauchenden Erwachsenen in den USA unter ähnlichen Studienprotokollen zu beurteilen.

Bei Studie 1 handelte es sich um eine randomisierte, 6-periodige Crossover-Studie, die an gesunden, rauchenden Erwachsenen durchgeführt wurde. Das primäre Ziel war die Bewertung der Nikotin-PK nach dem Gebrauch einer Zigaretten-ähnlichen E-Zigarette (eDNC1.0a) mit drei Geschmacksvarianten, der eigenen CC-Marke der Probanden, eines Nikotinkaugummis und einer Referenz-E-Zigarette. Bei Studie 2 handelte es sich um eine randomisierte Crossover-Studie über 7 Perioden, die an gesunden, rauchenden Erwachsenen durchgeführt wurde. Das primäre Ziel war die Bewertung der Nikotin-PK nach dem Gebrauch einer E-Zigarette mit geschlossenem Tank (eDNC2.0a) mit vier Geschmacksvarianten, der eigenen CC-Marke, eines Nikotininhalators und einer Referenz-E-Zigarette.

Zusammenfassend deuten die Ergebnisse der vorliegenden Studien darauf hin, dass die Nikotinbelastung durch die eDNC1.0a mit drei Geschmacksvarianten und die eDNC2.0a mit vier Geschmacksvarianten geringer war als die durch die eigene CC-Marke der Testpersonen, ähnlich oder geringer als die durch die Referenz-E-Zigaretten, aber ähnlich oder höher als die durch pharmazeutische Nikotinersatzprodukte. Es wurde festgestellt, dass der Nikotinkonsum, der auf der Grundlage des E-liquid-Konsums geschätzt wurde, im Allgemeinen direkt proportional zur Höhe der Nikotinexposition war, wie aus den Messungen der Nikotin-PK-Parameter in beiden Studien hervorgeht. Außerdem wurden lineare Beziehungen zwischen dem geschätzten Nikotinkonsum und den Nikotin-PK-Parametern im Plasma nach dem Konsum von E-Zigaretten festgestellt. Unsere Ergebnisse deuten darauf hin, dass die Modellierung gemischter Effekte als nicht-invasive Methode verwendet werden kann, um Erkenntnisse über Nikotin-PK-Parameter (AUC und C_{max}) aus Daten zum Nikotinkonsum von E-liquids zu gewinnen. [Contrib. Tob. Nicotine Res. 33 (2024) 173–188]

RESUME

La pharmacocinétique des produits du tabac non combustibles et de la nicotine, y compris les e-cigarettes, a été largement étudiée, avec une exposition à la nicotine plus faible ou similaire pour la plupart des produits par rapport aux cigarettes combustibles (CC). Nous avons mené deux études cliniques pour évaluer la pharmacocinétique de la nicotine et la consommation de nicotine lors de l'utilisation de deux types d'e-cigarettes avec différentes variantes d'arômes chez des adultes américains en bonne santé qui fument, dans le cadre de protocoles d'étude similaires.

L'étude 1 était une étude croisée randomisée de 6 périodes menée chez des adultes fumeurs en bonne santé. L'objectif principal était d'évaluer la pharmacocinétique de la nicotine après l'utilisation d'une e-cigarette cig-a-like (eDNC1.0a) avec trois variantes d'arômes, la propre marque de CC des sujets, une gomme à la nicotine et une e-cigarette de référence. L'étude 2 était une étude croisée randomisée de 7 périodes menée auprès d'adultes fumeurs en bonne santé. L'objectif principal était d'évaluer la pharmacocinétique de la nicotine après l'utilisation d'une e-cigarette à réservoir fermé (eDNC2.0a) avec quatre variantes d'arômes, la propre marque de CC des sujets, un inhalateur de nicotine et une e-cigarette de référence.

En résumé, les résultats des présentes études indiquent que l'exposition à la nicotine de l'eDNC1.0a avec trois variantes d'arômes et de l'eDNC2.0a avec quatre variantes d'arômes est inférieure à celle de la propre marque de CC des sujets, similaire ou inférieure à celle des e-cigarettes de référence, mais similaire ou supérieure à celle des produits pharmaceutiques de remplacement de la nicotine. Il a été observé que la consommation de nicotine, estimée sur la base de la consommation d'e-liquide, était généralement directement proportionnelle au niveau d'exposition à la nicotine, comme l'indiquent les mesures des paramètres pharmacocinétiques de la nicotine dans chaque étude. En outre, des relations linéaires ont été constatées entre la consommation estimée de nicotine et les paramètres pharmacocinétiques de la nicotine dans le plasma après l'utilisation de l'e-cigarette. Nos résultats suggèrent que la modélisation des effets mixtes peut être utilisée comme une méthode non invasive pour donner un aperçu des paramètres pharmacocinétiques de la nicotine (AUC et C_{max}) à partir des données sur la consommation de nicotine dans les e-liquides. [Contrib. Tob. Nicotine Res. 33 (2024) 173–188]

ABBREVIATIONS

AE	Adverse event
AUC	Area under the concentration-time curve
CC	Combustible cigarettes
C_{max}	Maximum blood concentration
E_{max}	Maximum VAS score
LS	Least square
mPES	Modified product evaluation scale
PEAE	Product-emergent AE
PK	Pharmacokinetics
VAS	Visual analog scale

INTRODUCTION

Cigarette smoking is a cause of serious diseases including lung cancer, coronary heart disease, emphysema and chronic bronchitis (1, 2). Over the past decade, rapid advances in the availability of non-combustible tobacco and nicotine products, such as e-cigarettes, have accumulated scientific evidence suggesting that, using e-cigarettes have the potential to reduce the health risks associated with smoking (3–5). The e-cigarette aerosol that is inhaled is qualitatively and quantitatively different compared to cigarette smoke containing both fewer compounds and lower levels of toxicants compared to the aerosol generated from a conventional cigarette (4, 6, 7).

E-cigarettes are battery-powered devices that heat a liquid (e-liquid), usually containing propylene glycol, glycerin, water, flavorings, and nicotine, to produce an inhalable aerosol. The nicotine pharmacokinetics (PK) of e-cigarettes have been widely assessed, particularly maximum blood concentration (C_{max}) and area under the concentration-time curve (AUC) as indicators of level of nicotine exposure, as well as time-to-maximum blood concentration (T_{max}). These PK studies of e-cigarettes have been reported to have lower or similar nicotine exposure (C_{max} and AUC) compared to combustible cigarettes (8–12). However, these studies cover only a subset of currently marketed products, and e-cigarettes are a relatively new product category that continues to evolve rapidly, both in terms of product design and performance. There is a wide variety of e-liquids, including various nicotine concentrations, in different forms (either salt or freebase forms of nicotine or a mixture of the two), coupled with a wide choice of flavors. As a result, the scientific understanding of this product category is still incomplete and continued research is needed on these currently marketed products. In addition to the variety of this product category, it has been also reported that some other factors such as user experience affect the nicotine uptake (13–15). While a limited number of studies suggested that e-liquid flavors affect nicotine exposure, the findings are inconsistent and inconclusive (12, 16–20). It is known that puffing topography has an effect on the delivery of aerosol, including nicotine (21–23), and it has also been suggested that nicotine consumption of e-cigarette users may vary as they change their use behavior (11, 24–26). Although it has been suggested that differences in nicotine consumption estimated based on e-liquid consumption may lead to differences in the level of nicotine exposure as indicated by nicotine PK parameters (C_{max} and AUC), much less is known about how the two are related when assessing nicotine PK.

In this article, we describe data from two nicotine PK studies in which we evaluated nicotine PK and estimated nicotine consumption when using two types of e-cigarettes with several different flavor variants in healthy adults who smoked under similar study protocols. The primary objective of Study 1 was to evaluate the nicotine PK after the use of a cig-a-like e-cigarette (eDNC1.0a = the electronic direct heating nicotine system platform 1 generation 0 version a) with three flavor variants compared to subjects' own brand of CC. The primary objective of Study 2 was to evaluate the nicotine PK after the use of a closed-tank e-cigarette (eDNC2.0a = the electronic direct heating nicotine system platform 2 generation 0 version a) with four flavor variants compared to subjects' own brand of CC.

MATERIALS AND METHODS

Study design

• *Study 1.* This was a randomized, open-label, six-period, six-sequence, crossover study conducted at a single clinic in Florida, USA, with 7 days of confinement. The study was approved by the Institutional Review Board (IRB) responsible for review and approval (Midlands IRB, Kansas, USA) and adhered to the ethical standards of the Declaration of Helsinki, applicable sections of the U.S. Code of Federal Regulations, and ICH E6 Good Clinical Practices. All participants gave written informed consent to participate in the study.

• *Study 2.* This was a randomized, open-label, seven-period, 14-sequence, crossover study conducted at a single clinic in Florida, USA, with 8 days of confinement. The study was approved by the IRB responsible for review and approval (Midlands IRB, Kansas, USA) and adhered to the ethical standards of the Declaration of Helsinki, applicable sections of the U.S. Code of Federal Regulations, and ICH E6 Good Clinical Practices. All participants gave written informed consent to participate in the study.

Study Products

The study products are listed in Table 1.

• *Study 1.* A commercially available cig-a-like type of e-cigarette device (eDNC1.0a, Logic Power manufactured by Logic Technology Development, LLC, Princeton, NJ, USA) with three flavor variants containing freebase nicotine was evaluated as the test product in this study. The eDNC1.0a consists of a replaceable cartomizer (cartridge) assembly containing e-liquid and an atomizer unit, and a rechargeable battery unit. Three cartridges with different flavors were tested; the cartridges were Product A: tobacco flavor, 2.4% nicotine (*w/w*); Product B: menthol flavor, 2.4% nicotine (*w/w*); and Product C: cherry flavor, 2.4% nicotine (*w/w*). The subjects' own brand of commercial filter cigarettes (Product D) was used as a comparator. The U.S. FDA-approved nicotine replacement products, Nicorette® Gum, White Ice Mint, 2 mg (GlaxoSmithKline Consumer Healthcare, L.P., Brentford, UK: Product E) and a reference e-cigarette similar in composition and freebase nicotine content to the eDNC1.0a (Blu PLUS+ Tanks, Classic Tobacco (2.4% nicotine (*w/w*)) manufactured by Fontem US, LLC, Charlotte, NC, USA: Product F) were also evaluated.

• *Study 2.* A commercially available closed-tank e-cigarette device (eDNC2.0a, Logic Pro manufactured by Logic Technology Development, LLC) with four flavor variants containing freebase nicotine was evaluated as the test product in this study. The eDNC2.0a is a three-piece product consisting of a battery unit, a replaceable e-liquid capsule, and a capsule case. Four different flavored capsules were tested; the capsules were Product A: tobacco flavored, 1.8% nicotine (*w/w*); Product B: menthol flavored, 1.8% nicotine (*w/w*); Product C: cherry flavored, 1.4% nicotine (*w/w*); and Product D: berry mint flavored, 1.49% nicotine (*w/w*). The subjects' own brand of commercially available filter cigarettes (Product E) was used as a comparator. The U.S. FDA-approved nicotine replacement products, Nicotrol® Inhaler, 10 mg per cartridge (Pfizer Inc., New York, NY, USA: Product F) and a reference e-cigarette similar in composition to the eDNC2.0a (Vuse Vise, Original (3.0% nicotine (freebase nicotine)(*w/w*)) manufactured by R.J. Reynolds Vapor Company, Winston-Salem, NC, USA: Product G) were also evaluated.

Table 1. Study product characteristics.

Study	Product	Short code	Article	Brand	Category	Device type	Flavor	Nicotine content of e-liquid (%)	Regimen
Study 1	A	eDNC1.0a	Test	Logic Power	E-cigarette	Cig-a-like	Tobacco	2.4% (*w/w*)	*ad libitum*, 5 min use
	B	eDNC1.0a	Test	Logic Power	E-cigarette	Cig-a-like	Cherry	2.4% (*w/w*)	*ad libitum*, 5 min use
	C	eDNC1.0a	Test	Logic Power	E-cigarette	Cig-a-like	Menthol	2.4% (*w/w*)	*ad libitum*, 5 min use
	D	CC	Comparator	N/A	Cigarette	N/A	N/A	N/A	*ad libitum*, one cigarette (up to 5 min)
	E	Nicotine gum	Benchmark	Nicorette® Gum, White Ice Mint, 2 mg	Nicotine gum	N/A	N/A	N/A	*ad libitum*, 30 min use
	F	Reference e-cigarette	Benchmark	blu PLUS+	E-cigarette	Cig-a-like	Tobacco	2.4% (*w/w*)	*ad libitum*, 5 min use
Study 2	A	eDNC2.0a	Test	Logic Pro	E-cigarette	Closed tank	Tobacco	1.80% (*w/w*)	*ad libitum*, 5 min use
	B	eDNC2.0a	Test	Logic Pro	E-cigarette	Closed tank	Menthol	1.80% (*w/w*)	*ad libitum*, 5 min use
	C	eDNC2.0a	Test	Logic Pro	E-cigarette	Closed tank	Cherry	1.49% (*w/w*)	*ad libitum*, 5 min use
	D	eDNC2.0a	Test	Logic Pro	E-cigarette	Closed tank	Berry Mint	1.49% (*w/w*)	*ad libitum*, 5 min use
	E	CC	Comparator	N/A	Cigarette	N/A	N/A	N/A	*ad libitum*, one cigarette (up to 5 min)
	F	Nicotine inhaler	Benchmark	Nicotrol® Inhaler, 10 mg per cartridge	Nicotine inhaler	N/A	N/A	N/A	*ad libitum*, 20 min use
	G	Reference e-cigarette	Benchmark	Vuse Vibe	E-cigarette	Closed tank	Tobacco	3.0% (*w/w*)	*ad libitum*, 5 min use

Abbreviations: CC = combustible cigarettes; eDNC1.0a = electronic direct nicotine heating system platform 1 generation 0 version a; eDNC2.0a = electronic direct nicotine heating system platform 2 generation 0 version a; N/A = not applicable.

Subjects

In both Studies 1 and 2, healthy U.S. males and females who were daily smokers of commercially manufactured filter cigarettes were eligible for enrollment. Healthy participants aged 22 to 65 years with a body mass index (BMI) of 18.5 to 35.0 kg/m^2 were eligible if they had smoked at least 10 commercial filter cigarettes per day for at least one year prior to screening. Individuals were ineligible if they self-reported the use of any nicotine replacement therapy or tobacco products other than commercially manufactured filter cigarettes within 14 days of screening, had a urinary cotinine level (One-step Cotinine Test Device, Confirm Biosciences, San Diego, CA, USA) of less than 200 ng/mL at screening, or planned to quit smoking during the study period, as well as pregnant or lactating women. At screening and check-in, general health assessments comprised standard physical and medical examinations (including vital signs, electrocardiogram, and spirometry), medical history (including concomitant medications), and clinical laboratory tests (chemistry, hematology, urinalysis, virology, and drug and alcohol screening) to confirm health status. Although an attempt was made to balance the sexes, no specific minimum ratio was required in the study.

These were the first clinical studies involving eDNC1.0a and eNDC2.0a, and the hypothesized difference in PK parameters (C_{max} and AUC) between the test e-cigarettes and subjects' own brand of CC were unknown. Therefore, referring to bioequivalence criteria, the sample size was calculated to give greater than 90% probability that the estimated geometric mean ratio was within 0.15 of the upper and lower bounds of the 90% confidential interval (CI) in the log scale, assuming that the intra-subject coefficient of variation is 30% for C_{max} of plasma nicotine based on previous studies (27). A total of approximately 60 subjects in Study 1 and 56 subjects in Study 2 were selected as target enrollments.

Procedures

Eligible adult cigarette smokers checked into the clinic on Day −2 and underwent check-in assessments and confirmation of eligibility. On Day −1, subjects were required to abstain from all tobacco and nicotine products for one day prior to the initial first assessment day to reduce the effect of potential carryover of plasma nicotine on the PK results.

• *Study 1.* Subjects were randomly assigned to one of six product-use sequences based on a Williams design by using a computer-generated pseudorandom permutation procedure. On each assessment day (Days 1 to 6), subjects received a study product according to the randomized product-use sequence and used it once daily. On the morning of each day, subjects were asked to smoke a single cigarette, use either the test or reference e-cigarette *ad libitum* for 5 min, or use nicotine gum for 30 min according to package instructions. Follow-up was conducted in the week following discharge.

• *Study 2.* Subjects were randomly assigned to one of 14 product-use sequences based on a Williams design by using a computer-generated pseudorandom permutation procedure. On each assessment day (Days 1 to 7), subjects received a study product according to the randomized product-use sequence and used it once daily. In the morning of each day, subjects were asked to smoke a single cigarette, use either the test or reference e-cigarette *ad libitum* for 5 min, and use the nicotine inhaler for 20 min according to package instructions. Follow-up was conducted in the week following discharge.

Measures

• *Baseline characteristics.* Subjects' baseline characteristics, including sex, age, race, BMI, daily cigarette consumption, and smoking history, the Fagerström Test for Cigarette Dependence (28, 29), were recorded at screening.

• *Estimation of nicotine consumption.* Before and after use of each test and reference e-cigarette, the weight of the e-cigarette (device and cartridge or capsule *in situ*) was measured to determine mass loss as the amount of e-liquid consumed by each subject. The theoretical nicotine consumption was estimated based on the amount of e-liquid used and the nicotine content (*w/w*%) of the e-liquid.

• *Pharmacokinetics*. On each study day (Days 1 to 6 in Study 1, Days 1 to 7 in Study 2), before, during, and after product use, 14 blood samples were collected either by direct venipuncture or from a cannula placed in a forearm vein, for measurement of plasma nicotine concentrations, at the following times: 5 min before product use (at t = 0 min, time of start product use), and then at 1, 3, 5, 7, 10, 15, 30, 45, 60, 90, 120, 180, and 360 min. Blood samples were collected into K_2EDTA tubes, and the tubes were gently mixed by inverting 8 times. Within a maximum of 60 min from sample collection, tubes were centrifuged at approximately 1000–1300 relative centrifugal force at 5 °C for approximately 10 min. Plasma nicotine was analyzed by liquid chromatography-tandem mass spectrometry using validated analytical methods with appropriate quality controls at Celerion (Lincoln, NE, USA) (30). The lower limit of quantification (LLOQ) was determined to be 0.200 ng/mL. The following PK parameters were calculated from the individual plasma nicotine concentrations applying non-compartmental methods using Phoenix WinNonlin version 6.3 (Certara LP, Princeton, NJ, USA): the maximum observed plasma concentration (C_{max}), the area under the plasma concentration *versus* time curve from time zero to the last time point with a measurable concentration ($AUC_{0\text{-last}}$), and the time of maximum observed plasma concentration (T_{max}). When a baseline nicotine concentration above the LLOQ was observed, the PK parameters (C_{max} and $AUC_{0\text{-last}}$) were adjusted by subtracting the portion attributable to the baseline nicotine concentration by using the baseline concentration and primary terminal elimination rate constant obtained from each subject. Baseline-adjusted PK parameters were calculated according to the following equations:

$$C_{max} = C_{max,\ unadjusted} - C_0 \times e^{K_{el} \cdot T_{max}}); \text{ and}$$

$$AUC_{0\text{-last}} = AUC_{0\text{-last},\ unadjusted} - C_0/K_{el},$$

where C_0 is the concentration before product use and K_{el} is the terminal elimination rate constant.

Table 2. Demographic characteristics by study group, Study 1.

	AFBECD (N = 9)		BACFDE (N = 10)		CBDAEF (N = 9)		DCEBFA (N = 8)		EDFCAB (N = 9)		FEADBC (N = 7)		TOTAL (N = 52)	
Sex														
Male, *n* (%)	5	(55.6%)	5	(50.0%)	7	(77.8%)	4	(50.0%)	6	(66.7%)	4	(57.1%)	31	(59.6%)
Female, *n* (%)	4	(44.4%)	5	(50.0%)	2	(22.2%)	4	(50.0%)	3	(33.3%)	3	(42.9%)	21	(40.4%)
Age, years Mean (SD)	40.6	(9.17)	37.9	(10.19)	40.8	(7.90)	44.5	(8.73)	38.0	(11.91)	39.7	(9.23)	40.1	(9.43)
Race														
White, *n* (%)	9	(100.0%)	8	(80.0%)	9	(100.0%)	6	(75.0%)	7	(77.8%)	5	(71.4%)	44	(84.6%)
African American, *n* (%)	0	(0.0%)	2	(20.0%)	0	(0.0%)	2	(25.0%)	2	(22.2%)	2	(28.6%)	8	(15.4%)
Other, *n* (%)	0	(0.0%)	0	(0.0%)	0	(0.0%)	0	(0.0%)	0	(0.0%)	0	(0.0%)	0	(0.0%)
BMI, kg/m^2 Mean (SD)	27.0	(4.55)	24.9	(2.61)	27.3	(4.23)	24.9	(2.75)	24.8	(3.46)	25.3	(2.36)	25.7	(3.45)
CPD, Mean (SD)	15.9	(4.70)	18.8	(5.47)	18.6	(6.23)	17.6	(3.50)	18.8	(8.45)	15.3	(4.72)	17.6	(5.68)
Regular smoking flavor														
Non-menthol, *n* (%)	5	(55.6%)	6	(60.0%)	7	(77.8%)	5	(62.5%)	3	(33.3%)	3	(42.9%)	29	(55.8%)
Menthol, *n* (%)	4	(44.4%)	4	(40.0%)	2	(22.2%)	3	(37.5%)	6	(66.7%)	4	(57.1%)	23	(44.2%)
FTCD, Mean (SD)	3.9	(0.93)	5.3	(1.42)	5.2	(1.39)	5.0	(1.77)	4.8	(1.72)	5.0	(2.00)	4.9	(1.55)

Abbreviations: BMI = body mass index; CPD = cigarettes per day; FTCD = Fagerström Test for Cigarette Dependence; SD = standard deviation;

Products: Product A = eDNC1.0a, Tobacco flavor; Product B = eDNC1.0a, Cherry flavor; Product C = eDNC1.0a, Menthol flavor; Product D = Usual brand of combustible cigarettes; Product E = Nicotine gum; Product F = reference e-cigarette.

• *Subjective effect measures.* Three subjective effects questionnaires were administered on each study day (Days 1 to 6 in Study 1, Days 1 to 7 in Study 2). The product liking question ("Do you like the product effect?") was administered as a 100-mm visual analog scale (VAS) ranging from "not at all (0 mm)" to "extremely (100 mm)" at 5, 15, 30, 45, 60, and 120 min after product use, and the percentage of subjects likely to prefer the product (VAS score > 50 to 100 mm) was evaluated from the observed maximum VAS score (E_{max}). The intent to use again question ("If given the opportunity, would you use this product again?") was administered as a 100-mm line VAS rating scale with "Definitely would not (0 mm)," "Don't care (50 mm)," to "Definitely would (100 mm)" at 60 min post product use, and the percentage of subjects likely to use the product again (VAS score > 50 to 100 mm) was evaluated. The modified Product Evaluation Scale (mPES) questionnaire, consisting of 12 questions, was administered at the end of product use with scoring on a 7-point scale (ranging from 1 (not at all) to 7 (extremely)) at completion of product use, and the following subscales scores were evaluated: Satisfaction (average score of 4 questions); Psychological reward (average score of 5 questions); Aversion (average score of 2 questions); and Relief (score of 1 question). The mPES questionnaire was adapted from the modified Cigarette Evaluation Scale (31) to evaluate all study products by replacing the words "smoking" and "cigarette" with "it".

• *Safety.* Safety outcomes, including an assessment of adverse events (AEs), were recorded. These included any abnormal clinical findings from vital signs, clinical laboratory tests, and physical examinations throughout the study period after randomization. A product-emergent AE (PEAE) was also identified as an AE that occurred after the first product use or that was present before the first product use and became more severe after the first product use.

Data analyses

Data were analyzed for all randomized subjects who did not have major protocol deviations that could invalidate or bias the PK results and who completed all product-use sequences. Statistical analyses were performed using SAS® version 9.4 (SAS Institute Inc., Cary, NC, USA).

The primary objective was assessed by comparing the PK parameters (C_{max} and $AUC_{0\text{-}last}$) for the test products (Study 1: Products A, B, and C / Study 2: Products A, B, C, and D) with the comparator (Study 1: Product D / Study 2: Product E). After log-transformation, C_{max} and $AUC_{0\text{-}last}$ were evaluated separately for each study in a linear mixed-effects analysis of variance (ANOVA) model with fixed effects for period, sequence, product, and a random effect for subject nested within sequence.

Descriptive statistics are presented for the estimated nicotine consumption of each test and reference e-cigarette and the VAS questionnaire E_{max} and mPES subscale scores observed during the use of each product.

RESULTS

Study 1

• *Study population.* Fifty-eight subjects were screened and randomized to one of six product-use sequences, with 52 subjects completing the study in accordance with the protocol (Table 2). Of the six subjects who withdrew from the study, two were physician-decided withdrawals and four withdrew from the study themselves. The physician-withdrawn subjects were withdrawn because of poor venous access or because of disruptive and argumentative behavior.

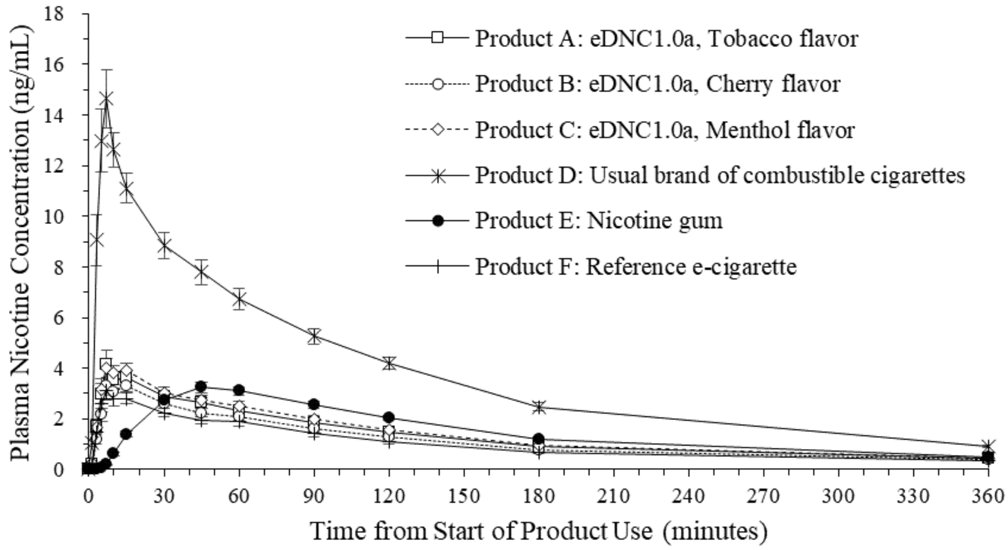

Figure 1. Mean plasma nicotine concentration profiles in Study 1. Error bars correspond to standard error.

• *Nicotine pharmacokinetics.* The mean plasma nicotine - concentration-time curve following single use of the study products is shown in Figure 1. The profile of the plasma nicotine concentration-time curves obtained after use of eDNC1.0a with three flavor variants (Products A, B, and C) was similar. Following the use of eDNC1.0a with three flavor variants (products A, B, and C, respectively), analysis of the geometric LS means showed that C_{max} values of 3.71, 3.44, and 4.18 ng/mL, were reached after 15.0 min of product use, and similar $AUC_{0\text{-last}}$ values of 6.24, 5.62, and 7.04 hr × ng/mL were observed (Table 3). In comparison, the profile of the nicotine concentration-time curves obtained after the use of the usual brand of CC (Product D) showed higher concentrations throughout the 360-minute sampling period, with a higher C_{max} value (15.32 ng/mL) that was reached earlier (7 min), and a higher $AUC_{0\text{-last}}$ value (21.09 hr × ng/mL) observed. The nicotine gum (Product E) differed from that of the test e-cigarettes in that the C_{max} value of 3.26 ng/mL was reached later (45 min), and a slightly higher $AUC_{0\text{-last}}$ value (8.21 hr × ng/mL) was observed. The reference e-cigarette (Product F) was found to be similar to the test e-cigarettes in terms of the profile of the nicotine concentration-time curve and PK parameters. Nicotine consumption from a

Table 2. Demographic characteristics by study group, Study 1.

	AFBECD	BACFDE	CBDAEF	DCEBFA	EDFCAB	FEADBC	TOTAL
	(*N* = 9)	(*N* = 10)	(*N* = 9)	(*N* = 8)	(*N* = 9)	(*N* = 7)	(*N* = 52)
Sex							
Male, *n* (%)	5 (55.6%)	5 (50.0%)	7 (77.8%)	4 (50.0%)	6 (66.7%)	4 (57.1%)	31 (59.6%)
Female, *n* (%)	4 (44.4%)	5 (50.0%)	2 (22.2%)	4 (50.0%)	3 (33.3%)	3 (42.9%)	21 (40.4%)
Age, years Mean (SD)	40.6 (9.17)	37.9 (10.19)	40.8 (7.90)	44.5 (8.73)	38.0 (11.91)	39.7 (9.23)	40.1 (9.43)
Race							
White, *n* (%)	9 (100.0%)	8 (80.0%)	9 (100.0%)	6 (75.0%)	7 (77.8%)	5 (71.4%)	44 (84.6%)
African American, *n* (%)	0 (0.0%)	2 (20.0%)	0 (0.0%)	2 (25.0%)	2 (22.2%)	2 (28.6%)	8 (15.4%)
Other, *n* (%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
BMI, kg/m^2 Mean (SD)	27.0 (4.55)	24.9 (2.61)	27.3 (4.23)	24.9 (2.75)	24.8 (3.46)	25.3 (2.36)	25.7 (3.45)
CPD, Mean (SD)	15.9 (4.70)	18.8 (5.47)	18.6 (6.23)	17.6 (3.50)	18.8 (8.45)	15.3 (4.72)	17.6 (5.68)
Regular smoking flavor							
Non-menthol, *n* (%)	5 (55.6%)	6 (60.0%)	7 (77.8%)	5 (62.5%)	3 (33.3%)	3 (42.9%)	29 (55.8%)
Menthol, *n* (%)	4 (44.4%)	4 (40.0%)	2 (22.2%)	3 (37.5%)	6 (66.7%)	4 (57.1%)	23 (44.2%)
FTCD, Mean (SD)	3.9 (0.93)	5.3 (1.42)	5.2 (1.39)	5.0 (1.77)	4.8 (1.72)	5.0 (2.00)	4.9 (1.55)

Abbreviations: BMI = body mass index; CPD = cigarettes per day; FTCD = Fagerström Test for Cigarette Dependence; SD = standard deviation;

Products: Product A = eDNC1.0a, Tobacco flavor; Product B = eDNC1.0a, Cherry flavor; Product C = eDNC1.0a, Menthol flavor; Product D = Usual brand of combustible cigarettes; Product E = Nicotine gum; Product F = reference e-cigarette.

Table 3. Summary of the pharmacokinetic parameters for nicotine, Study 1.

	Product A	Product B	Product C	Product D	Product E	Product F
	eDNC1.0a Tobacco flavor	eDNC1.0a Cherry flavor	eDNC1.0a Menthol flavor	Usual brand of combustible cigarettes	Nicotine gum	Reference e-cigarette
Parameter	(n = 52)	(n = 52)	(n = 52)	(n = 52)	(n = 52)	(n = 52)
C_{max} (ng/mL)						
Geometric LS mean (95% CI) [a]	3.71 (3.15, 4.37) [b]	3.44 (2.92, 4.05) [b]	4.18 (3.55, 4.92) [b]	15.32 (13.00, 18.04)	3.26 (2.77, 3.85)	2.90 (2.46, 3.42)
Min, Max	0.72, 17.20	0.65, 11.30	1.41, 13.50	5.52, 42.80	0.87, 10.10	0.66, 11.40
$AUC_{0\text{-}last}$ (ng × hr/mL)						
Geometric LS mean (95% CI) [a]	6.24 (5.37, 7.25) [b]	5.62 (4.84, 6.54) [b]	7.04 (6.06, 8.18) [b]	21.09 (18.15, 24.52)	8.21 (7.06, 9.54)	4.64 (3.99, 5.39)
Min, Max	0.92, 20.67	0.99, 14.53	2.22, 21.50	5.25, 45.33	1.98, 15.68	0.80, 12.77
T_{max} (min)						
Median	15.0	15.0	15.0	7.1	45.2	15.0
Min, Max	5.0, 60.0	7.0, 45.0	5.0, 60.0	3.0, 45.0	30.0, 90.0	5.0, 60.0
Amount of e-liquid used (mg)						
Mean (SD)	31.5 (16.31)	29.6 (13.25)	35.0 (19.44)	N/A	N/A	28.2 (16.77)
Min, Max	8.0, 73.7	7.1, 64.4	10.2, 104.2			5.9, 73.9
Estimated nicotine consumption (mg)						
Mean (SD)	0.75 (0.391)	0.71 (0.318)	0.84 (0.467)	N/A	N/A	0.68 (0.402)
Min, Max	0.19, 1.77	0.17, 1.55	0.24, 2.50			0.14, 1.77

[a] Geometric LS means were evaluated separately in a linear mixed-effects analysis of variance (ANOVA) model with fixed effects for period, sequence, product, and a random effect for subject nested within sequence.
[b] Significantly different from usual brand of combustible cigarettes (Product D); $p < 0.05$.

Abbreviations: SD = standard deviation; CI = confidential interval; N/A = not applicable; LS = least square.

single use was estimated to be 0.68–0.84 mg by measuring the weight of e-liquid used, with no marked differences between the test and reference e-cigarettes (Products A, B, C, and F, shown in Table 3).

• *Subjective effect measures.* Overall product liking, intent to use the product again, and subscales of mPES were markedly lower when using eDNC1.0a with three flavor variants (Products A, B, and C) compared to the usual brand of CC (Product D, shown in Table 4). These subjective measures were generally similar between the test e-cigarettes, nicotine gum (Product E), and reference e-cigarette (Product F).

• *Safety.* Thirty AEs were reported by 22 of the 58 subjects. All AEs were mild or moderate in severity; 14 PEAEs were considered to be possibly related or related to study product use. Nervous system disorders (e.g., headache) were the most common PEAEs reported during this study, with 14 events reported by 11 subjects, and there were no trends in PEAEs related to product use. All AEs were resolved without treatment, and there were no serious AEs or severe PEAEs reported during the study.

Study 2

• *Study population.* Fifty-five subjects were screened and randomized to one of 14 product-use sequences, with 51 subjects completing the study without any major protocol deviations (Table 5). Two subjects withdrew from the study themselves and two were excluded from the analysis because of multiple major protocol deviations due to poor venous access.

• *Nicotine pharmacokinetics.* The mean plasma nicotine concentration-time curves following single use of the study products are shown in Figure 2. The profile of the plasma nicotine concentration-time curves obtained after use of eDNC2.0a with four flavor variants (Products A, B, C, and D) was similar. Following the use of eDNC2.0a with four flavor variants (products A, B, C, and D, respectively), analysis of the geometric LS means showed that C_{max} values of 4.22, 4.11, 3.52, and 3.99 ng/mL, were reached after 10.0 or 15.0 min of product use, and similar $AUC_{0\text{-}last}$ values of 6.71, 6.62, 5.28, and 6.17 hr × ng/ mL were observed (Table 6). In comparison, the profile of the nicotine concentration-time curves obtained after the use of the usual brand of CC (Product E) showed higher concentrations throughout the 360-minute sampling period, with a higher C_{max} value (12.30 ng/mL) that was reached earlier (7 min), and a higher $AUC_{0\text{-}last}$ value (19.02 hr × ng/mL) observed. The nicotine inhaler (Product F) differed from the test e-cigarettes in that the C_{max} value of 1.95 ng/mL was reached later (40 min), and a slightly lower $AUC_{0\text{-}last}$ value (3.93 hr × ng/mL) was observed. The reference e-cigarette (Product G) showed higher nicotine concentrations throughout the 360-minute sampling period, with a higher C_{max} value (5.74 ng/mL) that was reached at a similar time (7 min) and a slightly higher $AUC_{0\text{-}last}$ value (7.69 hr × ng/mL) compared to the test e-cigarettes. Nicotine consumption from a single use of the test e-ciga-

Table 4. Summary of the subjective effects measures, Study 1.

Parameter	Product A	Product B	Product C	Product D	Product E	Product F
	eDNC1.0a Tobacco flavor	eDNC1.0a Cherry flavor	eDNC1.0a Menthol flavor	Usual brand of combustible cigarettes	Nicotine gum	Reference e-cigarette
	(n = 52)	(n = 52)	(n = 52)	(n = 52)	(n = 52)	(n = 52)
Product liking VAS [a]						
E_{max} – Mean (SD)	54.5 (34.44)	53.4 (30.32)	56.7 (33.03)	83.1 (22.80)	52.0 (31.60)	57.8 (31.58)
> 50 to 100 mm – n (%)	32 (61.5%)	32 (61.5%)	33 (63.5%)	47 (90.4%)	30 (57.7%)	36 (69.2%)
Intent to use the product again VAS [b]						
E_{max} – Mean (SD)	55.1 (33.70)	49.3 (31.22)	52.7 (36.15)	90.4 (17.37)	46.2 (36.19)	54.2 (33.20)
> 50 to 100 mm – n (%)	36 (69.2%)	31 (59.6%)	32 (61.5%)	51 (98.1%)	29 (55.8%)	31 (59.6%)
mPES, subscales [c]						
Satisfaction – Mean (SD)	3.4 (1.74)	3.4 (1.55)	3.7 (1.79)	5.3 (1.56)	3.5 (1.76)	3.6 (1.58)
Psychological reward – Mean (SD)	2.8 (1.49)	2.8 (1.37)	2.8 (1.35)	3.9 (1.67)	2.7 (1.67)	2.7 (1.42)
Aversion – Mean (SD)	1.4 (0.59)	1.3 (0.41)	1.4 (0.74)	2.1 (1.18)	1.4 (0.77)	1.2 (0.47)
Relief – Mean (SD)	3.7 (2.15)	4.0 (2.00)	3.9 (1.86)	5.5 (2.02)	3.0 (1.91)	4.0 (1.75)

[a] Product liking VAS questionnaire ("Do you like the product effect?") rating scales with "Not at all (0 mm)" to "Extremely (100 mm)".
[b] Intent to use product again VAS questionnaire ("If given the opportunity, would you use this product again?") rating scales with "Definitely would not (0 mm)", "Don't care (50 mm)", to "Definitely would (100 mm)".
[c] The subscales of modified Product Evaluation Scale (mPES):

- Satisfaction = average score from 4 questions ("Was it satisfying?", "Did it taste good?", "Did you enjoy the sensations in your mouth?" and "Did you enjoy it?");
- Psychological reward = average scores from 5 questions ("Did it calm you down?", "Did it make you feel more awake?", "Did it make you feel less irritable?", "Did it help you concentrate?", and "Did it reduce your hunger for food?");
- Aversion = average score from 2 questions ("Did it make you dizzy?" and "Did it make you nauseous?");
- Relief = score from a question ("Did it immediately relieve your craving for a cigarette?"). Each item was rated on a 7-point Likert scale ranging from 1 ("Not at all") to 7 ("Extremely").

Abbreviations: SD = standard deviation; mPES = modified Product Evaluation Scale; VAS = visual analog scale.

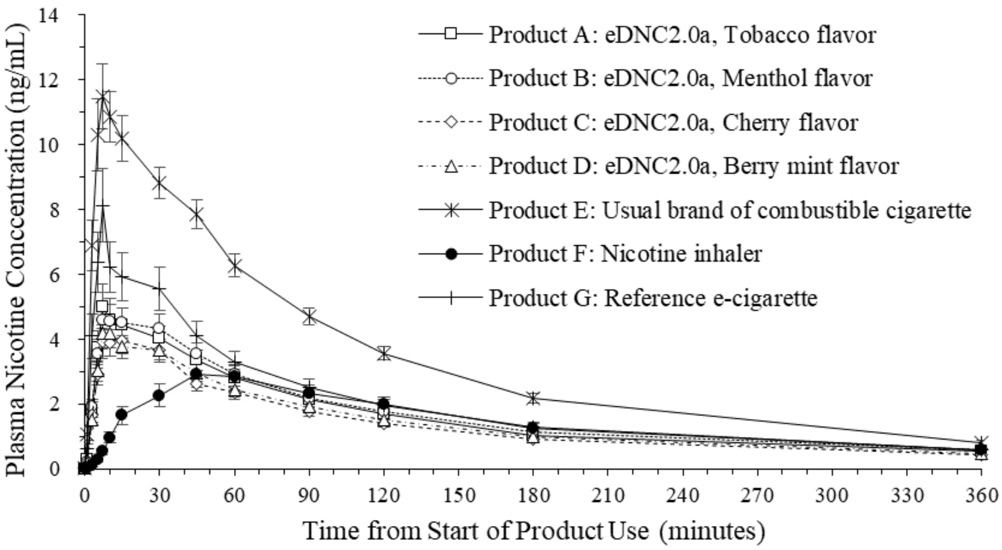

Figure 2. Mean plasma nicotine concentration profiles in Study 2. Errors bars correspond to standard error.

rettes (Products A, B, C, and D) was estimated to be 0.93 to 1.09 mg by measuring the weight of e-liquid used, which was less than that observed for the reference e-cigarette (Product G, 1.45 mg, shown in Table 6).

• *Subjective effect measures.* Overall product liking, intent to use the product again, and subscales of mPES were markedly lower with the use of eDNC2.0a with four flavor variants (Products A, B, C, and D) compared to the usual brand of CC (Product E, shown in Table 7). These subjective measures of the test e-cigarettes were generally

Table 5. Demographic characteristics by study group, Study 2.

	ABGCFDE	AGBFCED	BACGDFE	BCADGEF	CBDAEGF	CDBEAFG	DCEBFAG	DECFBGA	EDFCGBA	EFDGCAB	FEGDACB	FGEADBC	GAFBECD	GFAEBDC	TOTAL
	(*N* = 3)	(*N* = 2)	(*N* = 4)	(*N* = 4)	(*N* = 3)	(*N* = 4)	(*N* = 3)	(*N* = 4)	(*N* = 4)	(*N* = 4)	(*N* = 4)	(*N* = 4)	(*N* = 4)	(*N* = 4)	(*N* = 51)
Sex															
Male, *n* (%)	3 (100.0%)	2 (100.0%)	3 (75.0%)	2 (50.0%)	2 (66.7%)	4 (100.0%)	2 (66.7%)	2 (50.0%)	1 (25.0%)	3 (75.0%)	1 (25.0%)	3 (75.0%)	3 (75.0%)	2 (50.0%)	33 (64.7%)
Female, *n* (%)	0 (0.0%)	0 (0.0%)	1 (25.0%)	2 (50.0%)	1 (33.3%)	0 (0.0%)	1 (33.3%)	2 (50.0%)	3 (75.0%)	1 (25.0%)	3 (75.0%)	1 (25.0%)	1 (25.0%)	2 (50.0%)	18 (35.3%)
Age, years, Mean (SD)	27.3 (5.13)	26.5 (0.71)	41.0 (13.44)	28.0 (2.16)	39.3 (10.60)	39.0 (11.60)	29.7 (8.62)	43.3 (9.43)	31.8 (10.05)	39.0 (4.97)	41.8 (14.59)	45.8 (15.95)	46.8 (11.21)	42.3 (10.69)	38.0 (11.27)
Race															
White, *n* (%)	2 (66.7%)	2 (100.0%)	2 (50.0%)	4 (100.0%)	3 (100.0%)	4 (100.0%)	1 (33.3%)	4 (100.0%)	3 (75%)	4 (100.0%)	3 (75.0%)	4 (100.0%)	4 (100.0%)	4 (100.0%)	44 (86.3%)
African American, *n* (%)	0 (0.0%)	0 (0.0%)	2 (50.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	2 (66.7%)	0 (0.0%)	1 (25%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	5 (9.8%)
Other, *n* (%)	1 (33.3%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (25.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	2 (3.9%)
BMI, kg/m^2 Mean (SD)	23.3 (3.59)	31.6 (3.01)	28.6 (4.52)	24.8 (2.27)	28.4 (2.13)	29.9 (5.75)	25.8 (6.08)	27.8 (3.45)	25.7 (5.51)	28.5 (5.06)	23.7 (3.52)	27.3 (3.10)	27.8 (3.76)	26.1 (6.05)	27.0 (4.33)
CPD, Mean (SD)	17.3 (4.62)	20.0 (0.00)	15.5 (5.26)	13.8 (4.79)	14.0 (5.29)	17.5 (5.00)	12.0 (2.65)	18.8 (8.54)	11.5 (2.38)	17.5 (2.89)	20.0 (0.00)	13.5 (4.43)	17.5 (2.89)	19.5 (1.00)	16.3 (4.64)
Regular smoking flavor															
Non-menthol, *n* (%)	3 (100.0%)	0 (0.0%)	0 (0.0%)	2 (50.0%)	1 (33.3%)	2 (50.0%)	0 (0.0%)	2 (50.0%)	3 (75.0%)	4 (100.0%)	3 (75.0%)	1 (25.0%)	2 (50.0%)	4 (100.0%)	27 (52.9%)
Menthol, *n* (%)	0 (0.0%)	2 (100.0%)	4 (100.0%)	2 (50.0%)	2 (66.7%)	2 (50.0%)	3 (100.0%)	2 (50.0%)	1 (25.0%)	0 (0.0%)	1 (25.0%)	3 (75.0%)	2 (50.0%)	0 (0.0%)	24 (47.1%)
FTCD, Mean (SD)	3.3 (2.31)	5.5 (2.12)	4.8 (1.26)	3.0 (2.71)	5.0 (3.61)	5.0 (1.63)	5.3 (0.58)	5.3 (1.50)	5.0 (2.16)	5.0 (1.42)	5.0 (1.42)	3.8 (1.72)	5.5 (2.38)	5.5 (1.29)	4.8 (1.85)

Abbreviations: BMI = body mass index; CPD = cigarettes per day; FTCD = Fagerström Test for Cigarette Dependence; SD = standard deviation;
Products: Product A = eDNC2.0a, Tobacco flavor; Product B = eDNC2.0a, Menthol flavor; Product C = eDNC2.0a, Cherry flavor; Product D = eDNC2.0a, Berry mint flavor; Product E = Usual brand of combustible cigarettes; Product F = Nicotine inhaler; Product G = Reference e-cigarette.

Table 6. Summary of the pharmacokinetic parameters for nicotine, Study 2.

	Product A	Product B	Product C	Product D	Product E	Product F	Product G
	eDNC2.0a Tobacco flavor	eDNC2.0a Menthol flavor	eDNC2.0a Cherry flavor	eDNC2.0a Berry mint flavor	Usual brand of combustible cigarettes	Nicotine inhaler	Reference e-cigarette
Parameter	(n = 51)	(n = 51)	(n = 51)	(n = 50) [c]	(n = 51)	(n = 50) [c]	(n = 51)
C_{max} (ng/mL)							
Geometric LS mean (95% CI) [a]	4.22 (3.35, 5.31) [b]	4.11 (3.27, 5.18) [b]	3.52 (2.8, 4.44) [b]	3.99 (3.16, 5.03) [b]	12.30 (9.76, 15.49)	1.95 (1.55, 2.46)	5.74 (4.55, 7.22)
Min, Max	0.31, 24.10	0.36, 17.40	0.30, 21.60	0.36, 16.60	4.38, 34.00	0.23, 13.00	0.43, 36.4.0
$AUC_{0\text{-}last}$ (ng×hr/mL)							
Geometric LS mean (95% CI) [a]	6.71 (5.14, 8.78) [b]	6.62 (5.06, 8.66) [b]	5.28 (4.04, 6.9) [b]	6.17 (4.71, 8.08) [b]	19.02 (14.55, 24.87)	3.93 (3, 5.15)	7.69 (5.88, 10.05)
Min, Max	0.35, 23.17	0.53, 23.67	0.15, 17.33	0.58, 23.33	3.52, 50.33	0.02, 31.33	0.31, 33.33
T_{max} (min)							
Median	10.0	10.0	15.0	10.0	7.3	40.0	7.0
Min, Max	5.0, 41.0	3.0, 61.0	5.0, 60.0	5.0, 40.0	3.0, 40.0	7.0, 90.0	3.0, 40.0
Amount of e-liquid used (mg)							
Mean (SD)	58.2 (33.36)	60.7 (34.54)	62.4 (35.49)	64.8 (39.62)	N/A	N/A	48.2 (29.88)
Min, Max	2.0, 171.0	3.7, 126.9	2.0, 160.2	2.2, 203.2			1.7, 116.8
Estimated nicotine consumption (mg)							
Mean (SD)	1.05 (0.601)	1.09 (0.621)	0.93 (0.529)	0.96 (0.591)	N/A	N/A	1.45 (0.896)
Min, Max	0.04, 3.08	0.07, 2.28	0.03, 2.39	0.03, 3.03			0.05, 3.50

[a] Geometric LS means were evaluated separately in a linear mixed-effects analysis of variance (ANOVA) model with fixed effects for period, sequence, product, and a random effect for subject nested within sequence.
[b] Significantly different from usual brand cigarette; $p < 0.05$.
[c] No baseline-adjusted nicotine PK parameters were calculated for a subject because the terminal elimination phase was not well defined when using Product E and F.

Abbreviations: CI = confidential interval; N/A = not applicable; SD = standard deviation; LS = least square.

higher than the nicotine inhaler (Product F) and slightly lower or comparable to the reference e-cigarette (Product G), except for the mPES subscale "Aversion".

• *Safety*. Nineteen AEs were reported by 12 of the 55 subjects. All AEs were mild or moderate in severity; 11 PEAEs were considered to be possibly related or related to study product use. Nervous system disorders (e.g., headache) was the most common PEAEs reported during this study, with 12 events reported by 10 subjects, and there were no trends in PEAEs related to product use. All AEs were resolved without treatment, and there were no serious AEs or severe PEAEs reported during the study.

DISCUSSION

The e-cigarette product category is complex and diverse, with multiple factors including an individual product experience and behavior, contributing to user's nicotine exposure. It has been observed that several factors play a role in determining nicotine exposure, such as e-cigarette device characteristics, e-liquid composition, and user behavior, which remains complex and uniquely individual (including nicotine titration by users) (8–12, 24, 32). We conducted two clinical studies to evaluate the nicotine PK when using two types of e-cigarettes with several different flavor variants, a cig-a-like e-cigarette (eDNC1.0a) with three flavor variants and a closed tank e-cigarette (eDNC2.0a) with four flavor variants, in healthy adults who smoke under similar study protocols. These studies were not designed to assess the products based on a consistent level of nicotine consumption, but rather to estimate nicotine consumption under *ad libitum* use conditions.

In terms of the nicotine exposure during a single *ad libitum* use, C_{max} and $AUC_{0\text{-}last}$ results for a single use of the usual brand of CC were slightly higher in Study 1 than in Study 2, and data from both studies were within the range of the literature, with C_{max} values widely reported as 10–21 ng/mL (11, 33). The observed difference in nicotine exposure between Study 1 and Study 2 is within the range of variability observed in studies conducted with different subjects. This is consistent with the notion that nicotine intake from CC is influenced by individual smoking behavior differences, including demographics, smoking history, and various other factors (34). In Study 1 and 2, the C_{max} and $AUC_{0\text{-}last}$ were approximately 3-fold lower for any flavor variant of eDNC1.0a (Products A, B, and C in Study 1) and eDNC2.0a (Products A, B, C, and D in

Table 7. Summary of the subjective effects measures, Study 2.

Parameter	Product A	Product B	Product C	Product D	Product E	Product F	Product G
	eDNC2.0a Tobacco flavor	eDNC2.0a Menthol flavor	eDNC2.0a Cherry flavor	eDNC2.0a Berry mint flavor	Usual brand of combustible cigarettes	Nicotine inhaler	Reference e-cigarette
	(n = 50)	(n = 51)	(n = 51)	(n = 51)	(n = 51)	(n = 51)	(n = 51)
Product liking VAS [a]							
E_{max} – Mean (SD)	41.5 (27.55)	51.6 (28.00)	51.1 (27.00)	52.7 (28.73)	77.7 (26.12)	29.7 (28.56)	58.2 (26.00)
> 50 to 100 mm – n (%)	16 (32.0%)	27 (52.9%)	26 (51.0%)	30 (58.8%)	45 (88.2%)	16 (31.4%)	32 (62.7%)
Intent to use the product again VAS [b]							
E_{max} – Mean (SD)	44.8 (28.86)	53.1 (31.46)	54.2 (25.60)	55.3 (27.89)	83.9 (20.31)	24.3 (26.48)	59.6 (25.11)
> 50 to 100 mm – n (%)	31 (62.0%)	34 (66.7%)	39 (76.5%)	32 (62.7%)	48 (94.1%)	16 (31.4%)	43 (84.3%)
mPES, subscales [c]							
Satisfaction – Mean (SD)	2.9 (1.42)	3.6 (1.71)	3.4 (1.36)	3.9 (1.66)	5.2 (1.66)	1.9 (1.04)	3.8 (1.45)
Psychological Reward – Mean (SD)	2.7 (1.49)	2.7 (1.63)	2.7 (1.63)	2.9 (1.69)	3.6 (1.84)	2.1 (1.24)	2.9 (1.54)
Aversion – Mean (SD)	1.5 (0.89)	1.5 (0.64)	1.3 (0.65)	1.3 (0.54)	1.8 (0.94)	1.3 (0.66)	1.5 (0.72)
Relief – Mean (SD)	3.1 (1.75)	3.4 (1.84)	3.4 (1.71)	3.5 (1.91)	4.4 (1.96)	2.1 (1.45)	3.5 (1.70)

[a] Product liking VAS questionnaire ("Do you like the product effect?") rating scales with "Not at all (0 mm)" to "Extremely (100 mm)".
[b] Intent to Use Product Again VAS questionnaire ("If given the opportunity, would you use this product again?") rating scales with "Definitely would Not (0 mm)", "Don't care (50 mm)", to "Definitely would (100 mm)".
[c] The subscales of modified Product Evaluation Scale (mPES):

- Satisfaction = average score from 4 questions ("Was it satisfying?", "Did it taste good?", "Did you enjoy the sensations in your mouth?" and "Did you enjoy it?");
- Psychological reward = average scores from 5 questions ("Did it calm you down?", "Did it make you feel more awake?", "Did it make you feel less irritable?", "Did it help you concentrate?", and "Did it reduce your hunger for food?");
- Aversion = average score from 2 questions ("Did it make you dizzy?" and "Did it make you nauseous?");
- Relief = score from a question ("Did it immediately relieve your craving for a cigarette?"). Each item was rated on a 7-point Likert scale ranging from 1 ("Not at all") to 7 ("Extremely").

Abbreviations: SD = standard deviation; mPES = modified Product Evaluation Scale; VAS = visual analog square.

Study 2) compared to the usual brand of CC (Product D in Study 1, Product E in Study 2), and both reference e-cigarettes (Product F in Study 1, Product G in Study 2) were similarly lower compared to the usual brand of CC. These results are consistent with similar clinical studies of many types of e-cigarettes, which have shown that nicotine exposure following a single use of an e-cigarette is no higher than that of CC (35–39).

In both studies, the ranges of minimum to maximum estimated nicotine consumption from the same e-cigarette varied by nearly 10-fold or more (Table 3 and Table 6), and several clinical studies that investigated nicotine consumption in a similar method also found large inter-individual variation (32, 40–42). While no major differences in the mean estimated nicotine consumption values were observed in Study 1, a ranking attempt indicates the following order: Product F (reference e-cigarette), Product B (eDNC1.0a, Cherry flavor), Product A (eDNC1.0a, Tobacco flavor) and Product C (eDNC1.0a, Menthol flavor). Also in Study 2, a ranking of nicotine consumption values attempt indicates the following order: Product C (eDNC2.0a, Cherry flavor), Product D (eDNC2.0a, Berry mint flavor), Product A (eDNC2.0a, Tobacco flavor), Product B (eDNC2.0a, Menthol flavor) and Product F (reference e-cigarette) in the same order as the nicotine content of the e-liquid (Table 1). Although the mean values of estimated nicotine consumption differed very little between e-cigarettes, the ranking of estimated nicotine consumption generally reflected the ranking of the e-cigarettes with respect to $AUC_{0\text{-last}}$ and C_{max} values in each study. As an exploratory investigation, a linear mixed-effects ANOVA was performed on the integrated data from the two studies to assess the effects of nicotine consumption on $AUC_{0\text{-last}}$ and C_{max} values following e-cigarette use, with fixed effects for nicotine consumption, product, and the interaction between nicotine consumption and product, and random effects for subjects. The results showed that nicotine consumption [$F (1, 343) = 685.02$, $p < .001$ and $F (1, 343) = 172.31$, $p < .001$, respectively] significantly affected $AUC_{0\text{-last}}$ and C_{max} values, while product [$F (8, 343) = 1.15$, $p = 0.329$ and $F (8, 343) = 1.71$, $p = 0.095$, respectively] and the interaction between nicotine consumption and product [$F (8, 343) = 0.98$, $p = 0.454$ and $F (8, 343) = 2.62$, $p = 0.009$, respectively] had nonsignificant or small effects. It was concluded that the dose-response was generally consistent across product, device type and flavor. Using mixed effects models with a random slope and intercept adjusted by study, we characterized a good linear relationship between nicotine consumption and $AUC_{0\text{-last}}$ and C_{max} values, as shown in Figure 3. A similar high linearity between nicotine consumption and AUC values was recently reported by GAO *et al*. (42). Furthermore, it was confirmed that the estimated nicotine consumptions (0.25–1.63 mg) and C_{max} values (2.4–10.6 ng/mL) reported in

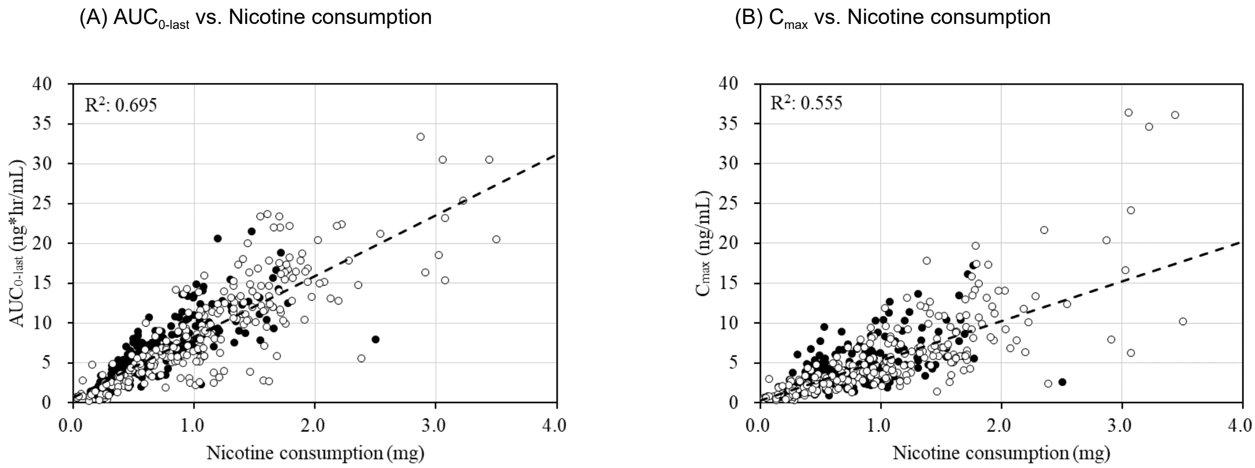

Figure 3. Linear regression of (A) $AUC_{0\text{-}last}$ *vs.* nicotine consumption and (B) C_{max} *vs.* nicotine consumption. Dot lines show the represented fixed effects regression between $AUC_{0\text{-}last}$ or C_{max} and estimated nicotine consumption derived a linear mixed-model that adjusted the intercepts and slopes by study. R^2 indicates the coefficient of determination derived from a simple linear regression (crude regression) taking all data of two studies.

several studies on other e-cigarettes generally overlap with the regression curve observed in this report (43–46). The results suggest that estimated nicotine consumption reflects nicotine exposure and that nicotine PK following e-cigarette use can be estimated non-invasively by examining nicotine consumption through human use.

Subjective effects of novel tobacco and/or nicotine product use are thought to be one of the informative elements for assessments of abuse liability assessment, which is of increasing interest to regulatory authorities (47–50). In these studies, subjective effects measures were assessed by using the product liking VAS, intent to use the product again VAS, and mPES questionnaires. As the subjects enrolled in the studies were long-term or established cigarette smokers, a general preference for the usual brand of CC was expected and was indeed observed in both studies as the higher values for product liking, intent to use the product again, and mPES subscale scores of positive reinforcing effects ("Satisfaction", "Psychological Reward", and "Relief"). In Study 1, the subjective effects of eDNC1.0a with three flavor variants (Products A, B, and C) as described by product ratings, were less than those of the usual brand of CC (Product D) and similar to those of nicotine gum (Product E) and reference e-cigarette (Product F). In Study 2, the subjective effects of eDNC2.0a with four flavor variants (Products A, B, C, and D) as described by product ratings, were less than those of the usual brand of CC (Product E), greater than those of the nicotine inhaler (Product F), and somewhat less than those of the reference e-cigarette (Product G). In addition, the difference in subjective effects scores among the e-cigarettes did not reflect the ranking of nicotine consumption, $AUC_{0\text{-}last}$, and C_{max} values. The fact that half of the subjects enrolled in the study were non-menthol cigarette smokers and half were menthol cigarette smokers (Table 2 and Table 5), suggests that general flavor preferences for the usual brand of CC may have influenced the results. In general, the results are consistent with the conclusions of others that the abuse liability of e-cigarettes as a category is less than that of CC but similar or greater than that of nicotine gum and inhaler (36, 37, 46).

The main limitation was that the enrolled subjects were cigarette smokers, as the intention was to compare the PK parameters of these e-cigarettes and CC. Several studies have reported the effect of product experience on nicotine exposure and subjective measures of effect from e-cigarette use (5, 8, 13, 26). However, it is also important to evaluate PK and subjective effects measures in experienced e-cigarette users. In addition, theoretical nicotine consumption was estimated based on the amount of e-liquid used and the nicotine content (*w/w*%) of the e-liquid, and the dose-response relationship was investigated in this report. The amount of nicotine absorption can also be calculated using AUC and the population mean clearance of nicotine, 1200 mL/min (51, 52). The mean amount of nicotine absorbed from the test e-cigarettes was estimated based on $AUC_{0\text{-}last}$ and was calculated as 0.40–0.58 mg in Study 1 and 0.52–0.80 mg in Study 2. This leads to an estimated nicotine absorption based on $AUC_{0\text{-}last}$ ranged from 54.0% to 73.5% in the test e-cigarettes, compared to the theoretical nicotine delivery estimated based on e-liquid consumption. Theoretical nicotine delivery may not match actual nicotine consumption due to a variety of factors, including the effects of device and component differences (53, 54), and it may be possible to improve the accuracy of predictions by measuring actual nicotine intake, accounting for product differences.

In summary, the results of the present studies indicate that nicotine exposure from eDNC1.0a with three flavor variants and eDNC2.0a with four flavor variants was less than that from CC, similar to or less than that from reference e-cigarettes, but similar to or greater than that from pharmaceutical nicotine replacement products. Overall, the use eDNC1.0a with three flavor variants and eDNC2.0a with four flavor variants by cigarette smokers resulted in subjective effects that were lower than reported after the use of the usual brand of CC, similar to or less than reference e-cigarettes, but similar or greater than pharmaceutical nicotine replacement products. Furthermore, linear relationships were found between nicotine consumption and plasma nicotine PK parameters following e-cigarette use. Our findings suggest that mixed effects modelling can be used as a non-invasive method to provide insights of nicotine PK parameters (AUC and C_{max}) from e-liquid nicotine consumption data.

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Dai Yuki: Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Visualization, Writing - original draft, Writing - review & editing.
Lesley Giles: Conceptualization, Investigation, Methodology, Project administration, Writing - review & editing.
Sylvain Larroque: Conceptualization, Data curation, Formal Analysis, Writing - review & editing.
Sam Harbo: Conceptualization, Funding acquisition, Project administration, Supervision, Writing - review & editing.
Anthony Hemsley: Conceptualization, Funding acquisition, Project administration, Writing - review & editing.
Javier Martinez: Conceptualization, Supervision, Writing - review & editing.

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CONFLICTS OF INTEREST

DY was an employee of JT International S.A. at the time the studies were designed and conducted. LG, SL, SH, AH, and JM are current employees of JT International SA. The views and conclusions contained herein are solely those of the authors and do not necessarily represent the views and conclusions of Japan Tobacco International.

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