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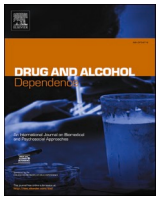

Sensory appeal and puffing intensity of e-cigarette use: Influence of nicotine salts versus free-base nicotine in e-liquids

Charlotte G.G.M. Pauwels [a], Wouter F. Visser [a], Jeroen L.A. Pennings [a], Ernesto P. Baloe [a], Arnout P.T. Hartendorp [a], Loes van Tiel [b], Mirjam van Mourik [b], Wim Vaessen [b], Sanne Boesveldt [c], Reinskje Talhout [a,*]

[a] National Institute for Public Health and the Environment (RIVM), Centre for Health Protection, Bilthoven, the Netherlands
[b] Essensor BV, Marijkeweg 22a, Wageningen 6709 PG, the Netherlands
[c] Division of Human Nutrition and Health, Wageningen University & Research, P.O. Box 17, Wageningen 6700 AA, the Netherlands

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ABSTRACT

Background: In the US, nicotine salts (with protonated nicotine instead of free-based nicotine) have been reported to lower the harshness and bitterness of e-cigarette aerosols, making it easier to inhale high levels of nicotine. This study aimed to determine whether nicotine salts also increase sensory appeal at lower concentrations (< 20 mg/mL). Moreover, and novel, inhalation intensity of both types of e-liquids was compared.

Methods: In a randomized, double-blinded, within-participants design, healthy adults who use e-cigarettes (n=68) vaped tobacco-flavored e-liquids containing 12 mg/mL of free-based nicotine or nicotine salt *ad libitum*, using their own device, during two online sessions (June-July 2021, Utrecht, The Netherlands). The sensory parameters perceived liking, nicotine intensity, harshness, and pleasantness were rated on a 100-unit visual analog scale. The intensity of use was determined by the recorded puff number, duration and interval.

Results: Test scores on appeal, harshness and puffing behavior parameters showed no significant differences between the nicotine salt and the free-base condition. The average inhalation time was 2.5 seconds. Additional analyses found no significant effect of liquid order, age, gender, smoking status, vaping frequency and familiarity with nicotine salts. Significant positive correlations were found between the sensory parameters except for harshness.

Conclusions: Contrary to a previous study that used higher nicotine concentrations and standardized puffing conditions in a laboratory setting, we did not observe the effects of nicotine salts on sensory appeal in our real-life study paradigm. Moreover, we did not see effects on study parameters related to puffing intensity.

1. Introduction

In the United States (US), marketing of JUUL and similar e-cigarettes led to a fast increase in e-cigarette use among young people who have never smoked; JUUL entered the US market in 2015, capturing more than half of the e-cigarette market in 2018 (Ramamurthi et al., 2018; Kavuluru et al., 2019). Product characteristics that may play a role in this rapid increase in use are JUUL's attractive and discrete device, many different flavors, high aerosol nicotine levels, and liquids containing nicotine salts (protonated nicotine) instead of free-base nicotine (Jackler and Ramamurthi, 2019).

In this study, carried out in the Netherlands, we investigated the effects of nicotine salts versus free-base nicotine on both sensory appeal and puffing intensity. Nicotine salts are less harsh and bitter to inhale than free-base nicotine, making inhalation of high amounts of nicotine more palatable (Duell et al., 2020). As such, the use of nicotine salts could ultimately lead to a highly addictive vaping product (Prochaska et al., 2021). Effects of protonated nicotine on nicotine blood delivery levels have already been studied by several groups (Prochaska et al., 2021; Hajek et al., 2020; O'Connell et al., 2019; Reilly et al., 2019). Some studies show that nicotine salts, unlike e-cigarettes filled with free-base nicotine liquid, result in nicotine blood profiles similar to those of tobacco cigarettes (Prochaska et al., 2021; Hajek et al., 2020; O'Connell et al., 2019; Reilly et al., 2019). These findings suggest that protonated nicotine leads to higher nicotine delivery via more intense puffing behavior, as this is the most likely explanation of higher blood

* Correspondence to: National Institute for Public Health and the Environment, Antonie van Leeuwenhoeklaan 9, Bilthoven 3721 MA, the Netherlands.
E-mail address: Reinskje.talhout@rivm.nl (R. Talhout).

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levels, given that the protonation state of the nicotine was the only product variable. However, blood nicotine levels are also dependent on the total nicotine levels in e-liquids. A study funded by JUULLABS showed that higher levels of protonated nicotine give rise to significantly higher plasma nicotine levels and relief from craving than lower levels of nicotine salts (Goldenson et al., 2021). In Europe, nicotine concentrations in liquids are generally lower than in the US, since in the European Union (EU) a nicotine maximum limit of 20 mg/mL is prescribed by the European Tobacco Products Directive (TPD) (European Union, 2014). An EU version of the JUUL, with nicotine levels of 18 mg/mL, showed lower nicotine delivery and a lower reduction of urge to smoke/vape upon usage in comparison to tobacco cigarettes (Mallock et al., 2021), as well as in comparison to the US JUUL with 59 mg/mL (Phillips-Waller et al., 2021). Thus, to address potential differences between the EU and the US due to legislation, we carried out the current study in the Netherlands, an EU country where the EU TPD has been fully implemented. We studied a nicotine concentration well below 20 mg/mL, which is the highest concentration allowed.

Until now, only one study assessed the sensory effects of nicotine salts as compared to free-base nicotine (Leventhal et al., 2021), and no studies have been carried out on their effect on puffing intensity. In the US, a randomized clinical trial by Leventhal et al. studied ratings of several sensory attributes in a controlled setting (Leventhal et al., 2021). Salt versus free-base nicotine formulations of 24 mg/mL resulted in significantly higher ratings of appeal, sweetness, and smoothness, and lower ratings of bitterness and harshness. Nicotine salts were concluded to improve the sensory experience -and thereby product attractiveness- of vaping, particularly among people who have never smoked unaccustomed to inhaling free-base nicotine. They did not study the effects on puffing intensity but rather used a fixed puff protocol. It is however likely that people who use e-cigarettes in the 'real world' would change their puffing behavior to adapt to the new product and their preferences, as also suggested by the nicotine blood profile studies described above (Prochaska et al., 2021; Hajek et al., 2020; O'Connell et al., 2019; Reilly et al., 2019). Moreover, one might expect a negative correlation between sensory attributes such as harshness and puffing intensity. Thus, puffing topography is also a parameter of interest when studying the effects of nicotine salts on a person who uses e-cigarettes' ease of inhalation.

Based on the above considerations, we tested if vapor resulting from e-liquids with nicotine salts is more appealing, tastes milder and is easier to inhale than vaping from e-liquids with free-base nicotine. To resemble real-life conditions as closely as possible, we used an in-home paradigm, where our participants used their own refillable e-cigarette device, and vaped in a naturalistic manner (e.g., without a fixed puffing pattern and *ad libitum*). Since flavors other than tobacco will be banned in The Netherlands in the future, (Overheid.nl, 2021) tobacco aroma was selected as a test flavor for this study. Lastly, we prepared the study e-liquids with a nicotine concentration of 12 mg/mL, which is typical for the Dutch market. A previous study showed that of all e-liquids notified to the Dutch regulator, 13% contained nicotine salts or acid additives, with a median nicotine concentration of 12 mg/mL (Pennings et al., 2022).

2. Material and methods

2.1. Materials and equipment

We used commercially available products intended for do-it-yourself preparation of e-liquids by people who use e-cigarettes. Concentrated aroma ('FR tobacco', CirKus aromas), 20 mg/mL stock solutions of free-base and salt form nicotine ('Fusion 50PG/50VG Nicotine booster' and 'Fusion 50PG/50VG Nic Salt booster', both HALO brand), and 50:50 mixture of propylene glycol and glycerol ('pharma clear base', Savevape) were purchased from local web shops. We also bought empty e-liquid refill bottles with a child-proof cap and a nozzle tip ('Unicorn Mix bottle 30 mL', Chubby Gorilla). Nicotine analytical standard (Acros) was purchased from Thermo Scientific (Waltham MA, USA), and N-heptadecane (used as an internal standard), isopropanol and methanol from Merck KGaA (Darmstadt, Germany). Nicotine analyses were performed on a Shimadzu GC2010 instrument (Shimadzu, 's-Hertogenbosch, The Netherlands). Nicotine protonation analyses were performed using a Spinsolve 60 MHz NMR Spectrometer (Magritek GmbH, Aachen, Germany).

2.2. Selection of tobacco aroma, preparation and labeling of test liquids

2.2.1. Selection of the tobacco aroma

A sensory pilot experiment using twelve different tobacco aromas was conducted to select an aroma that resembles tobacco aroma and has a pleasant flavor. Each aroma solution was diluted with distilled water to a final concentration of 10 or 15 vol% (v/v) (according to the manufacturer's recommendation). One mL of the diluted aroma was placed into a capped glass vial. Six volunteers scored on a 100-unit visual analogue scale (VAS) the pleasantness and tobacco-like character by smelling the solutions. We found that aromas that most strongly resembled tobacco were not considered pleasant, and vice versa. We, therefore, selected an aroma that was average in both pleasantness (44 points) and tobacco-like character (23 points) according to the six volunteers.

2.2.2. Preparation of the test liquids

We prepared a study e-liquid with free-base nicotine and a study e-liquid with nicotine salts. Both e-liquids contained equal concentrations of nicotine, aroma and solvents (propylene glycol and glycerol). First, a stock solution was made by pooling the aroma from different bottles. Second, a free-base nicotine stock solution and a nicotine salt stock solution were made. Third, the nicotine concentrations of the stock solutions ('boosters') were verified (23 mg/mL and 19 mg/mL respectively), using the method as described in TobLabNet SOP 11 (World Health Organization, 2021). Fourth, a single large batch of each e-liquid was prepared by dilution to 12 mg/mL nicotine, 10% v/v aroma, with the remainder being a 50:50 (v/v) mixture of propylene glycol and glycerol. The nicotine content of the final free-base and the nicotine salt e-liquids was found to be 11.9 and 12.2 mg/mL respectively. The protonation state of the nicotine in the free-base and nicotine salt e-liquids was 3% and 90%, respectively, as determined by NMR spectroscopy (Hartendorp et al., 2021), using a method derived from that used by Duell et al. (Duell et al., 2018). The e-liquids were transferred to refill vials and provided with an information leaflet, in accordance with the Dutch Tobacco Act (Dutch Tobacco Act).

2.3. Recruitment of participants

The recruitment was carried out by the research agency called Essensor (Wageningen, The Netherlands), which is experienced with participants conducting in-home tests. A sample size calculation using an effect size half that of the average effect size in Leventhal et al. (Leventhal et al., 2021), a standard deviation equal to that reported in Leventhal et al. (Leventhal et al., 2021), and a p-value of 0.05/9 (Bonferroni correction for the number of sensory parameters) indicated a sample size of 49 would be sufficient to have 90% statistical power. In accordance with Essensor guidelines and the NEN-ISO 11136:2014 guideline (International Organisation of Standardisation, 2014), the minimum required number of participants was 60. The latter sample size was used and to account for attrition, we aimed to recruit 84 healthy vapers from the Essensor database. To find participants representing the healthy Dutch vaping population, a screening questionnaire with questions about vaping status, nicotine concentration and (ever-) use of flavors was sent to all individuals in the Essensor database. The respondents who meet the inclusion criteria were invited to our in-home study. Selection criteria were adults (aged 18–70 years), regular

vapers (vaping at least more than once a week), used to vaping e-liquids with nicotine (>1 mg/mL nicotine concentration), generally healthy as self-reported, able to use refill e-liquids (owning and using a refillable e-cigarette), able to attend an online session at home with sound and webcam images (owning and familiar with using a computer with webcam and microphone). Exclusion criteria were being pregnant or lactating, experiencing negative health effects of vaping or smoking, and suffering from asthma or other lung disease.

2.4. Study design and procedure

This study had an experimental within-participants design and consisted of four consecutive test days (e.g., Monday/Day 1 receiving and testing e-liquid 1, Tuesday/Day 2 experiment e-liquid 1, Wednesday/Day 3 receiving and testing e-liquid 2, Thursday/Day 4 experiment e-liquid 2). Half of the group used the nicotine salts e-liquid during the first two days, followed by the free-base nicotine e-liquid on Day 3 and 4, and vice versa. The two e-liquids were presented blinded: products were labeled with a three-digit pseudo-random code, known by the investigators but not shared with the Essensor team that interacted with the participants. The Medical Ethical Review Committee Maastricht UMC+ evaluated the research protocol (NL77340.068.21/ METC 21–026) and concluded that the study was exempt from ethical approval.

Before the start of the tests, informed consent was obtained from each study participant. After inclusion, the pick-up or shipping of study materials (i.e., the first study e-liquid, written instructions) was scheduled one day before the in-home test. Both study samples were not provided at the same time to prevent them from being mixed up or tested both on Day 1 out of curiosity. Another reason is that the study e-liquids appeared to differ in color (transparent vs. yellowish), and we minimized the chance of the difference in color influencing the study results. The in-home test was hosted by Essensor via an online live connection (Zoom). Participants used their own refillable e-cigarettes for the in-home test with the study e-liquids. Both liquids were vaped two times: first as a try-out to become acquainted with the flavor (Day 1, Day 3) and second during the online session (Day 2, Day 4) (Fig. 1). The adjustable settings (if applicable) of the e-cigarette could be chosen by the participant before the first session and they were instructed to use the same settings for both online sessions. Participants were asked to refrain from vaping and consuming nicotine two hours prior to the online sessions to create similar circumstances between participants. To prevent the influence of the remaining taste of consumed products, participants were asked to refrain from using chewing gum, brushing their teeth and eating or drinking anything besides water for at least one hour prior to the online session.

At the scheduled time on Day 2 or 4, each participant participated in an individual online session with an Essensor researcher. After a short introduction during which the aim and procedure of the session were explained, the participant started vaping study e-liquid 1. While vaping, the participants watched a video of five minutes (see supplementary information). After five and ten minutes, liking, nicotine intensity (buzz), harshness of throat hit, pleasantness of throat hit and willingness to use again were assessed on a 100-unit VAS scale. The entire session was audiovisual recorded, to determine the puffing topography (i.e., puff number, puff duration, puff interval) afterwards by an Essensor researcher. Briefly, the recording was tagged with timestamps that were extracted for further analysis (see supplementary information for further details). At the end of the online session at Day 4, the participant filled in an additional questionnaire with questions about their vaping behavior. More details about the study design can be found in the supplementary information.

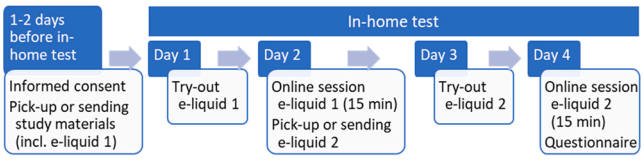

Fig. 1. Schematic overview of study design.

2.5. Statistical analysis

Analyses were carried out using the statistical software program R (version 4.0.2). The sensory study parameters analyzed were: perceived liking, nicotine intensity (buzz), harshness/throat hit, pleasantness of throat hit, and willingness to use again. These were measured on a 100-unit VAS scale at five and ten minutes, except for a willingness to use again, which was only measured at ten minutes. Puffing study parameters analyzed were: the number of puffs, average puff duration, total puff duration, and average puff interval.

Study parameters (nine sensory and four puffing) were compared between nicotine free-base and nicotine salts e-liquids by means of a paired t-test.

Additionally, we used a mixed model ANOVA on the study parameters to determine the potential (interaction) effects of other (co)variables. The model combined fixed/categorical variables (liquid type, liquid order, gender, smoking status, familiarity with nicotine) and random/continuous variables (age, four vaping frequency classes (1, > 15 x; 2, 11–15 x; 3, 5–10 x; 4, < 5 x) and an age-gender interaction term. The participant was not included as a variable here as we wanted to determine if the covariables by themselves could influence the results.

Changes over time (five vs ten minutes) for liking, nicotine intensity, harshness, and pleasantness were analyzed by a mixed model ANOVA on the ratings with participant, formulation, time as well as and formulation*time interaction as variables.

Correlations between study parameters were determined as Pearson correlation coefficients with the corresponding p-value. All p-values were adjusted for multiple testing using the Benjamini-Hochberg False Discovery Rate (FDR) (Benjamini and Hochberg, 1995). FDR values <5% were considered significant (see Tables S1 and S2 in the supplementary information).

3. Results

3.1. Participants and study

The study took place in June and July 2021, whereby several restrictions were in place regarding the COVID-19 pandemic. Due to recruitment challenges in finding suitable participants, in addition to participants from the Essensor database, participants were recruited via social media. Furthermore, we had a significant number of no-shows of participants that successfully completed the selection and were scheduled for online sessions. Nevertheless, 69 participants successfully finished the in-home test leading to 68 complete datasets (puffing parameter recording of one participant was unsuccessful). Our study included an approximately equal number of men and women aged 19–69 years old, all used to vape on a daily basis. Table 1 shows the participant characteristics including vaping status.

3.2. Sensory and puffing topography results

A comparison of sensory parameters between nicotine salts versus free-base e-liquids showed that these were all somewhat lower in nicotine salt e-liquids (Table 2). This difference ranged from −0.9 points (nicotine intensity at 5 minutes) to −6.8 points (pleasantness throat hit at 10 minutes). The total puff duration was somewhat higher in e-liquids with nicotine salts (43.7) than in those with free-base nicotine (41.6) (Table 2). However, none of the study parameters differed significantly between the two types of e-liquids at a 5% FDR.

Additional analyses found no significant effect of liquid order, age,

Table 1
Participant characteristics.

Item	Answers	Participants (n=69)	
		Absolute	Percentage
Gender	Male	36	52%
	Female	33	48%
Age (years)	Average	42	
	Range	19–69	
E-cigarette use per day	< 5 times	22	32%
	5–10 times	6	9%
	11–15 times	0	0%
	> 15 times	41	59%
Other product use	Cigarettes or RYO	28	41%
	None[1]	41	59%
Flavor use[2]:	Tobacco	69	100%
	Menthol	18	26%
	Sweet (e.g., fruit, vanilla, candy)	51	74%
	Not sweet (nutty, herbs, coffee)	0	0%
	No flavor	0	0%
Nicotine concentration used[2]	0 mg	4	6%
	1–4 mg	19	28%
	5–8 mg	24	35%
	9–12 mg	18	26%
	13–16 mg	8	12%
	17–20 mg	11	16%
Nicotine salts used before	Yes	8	12%
	No	24	35%
	Don't know	37	54%
Only for "Yes" on previous question: Why do you use nicotine salts?	It's more satisfying than an e-liquid without nicotine salts		5 out of 8
	It's more like the feeling of smoking a cigarette than an e-liquid without nicotine salts		3 out of 8
	It feels milder in my throat than an e-liquid without nicotine salts		3 out of 8
	I like the taste better than the taste of an e-liquid without nicotine salts		0 out of 8
	My friends also use e-liquids with nicotine salts		0 out of 8
	I don't know/no reason		0 out of 8

[1] no use of cigar, cigarillo, pipe, heated tobacco, waterpipe, snus, nicotine pouch.
[2] multiple answers could be given ("check all that apply").

Table 2
Study parameter comparison.

Sensory parameters	Free-base	Nicotine salt	Difference
Like taste (5 min)	50.7 ± 2.9	48.5 ± 3.2	-2.2 ± 3.2
Like taste (10 min)	51.8 ± 3.2	47.8 ± 3.1	-4.1 ± 3
Nicotine intensity (5 min)	52.5 ± 2.7	51.6 ± 2.6	-0.9 ± 2.8
Nicotine intensity (10 min)	54.3 ± 2.9	49.6 ± 2.5	-4.7 ± 2.8
Harshness throat hit (5 min)	51.8 ± 3.1	48.2 ± 3.3	-3.6 ± 4.7
Harshness throat hit (10 min)	54.2 ± 3	50.4 ± 3	-3.8 ± 4.2
Pleasantness throat hit (5 min)	47.5 ± 2.9	43.2 ± 2.7	-4.3 ± 3.5
Pleasantness throat hit (10 min)	46.2 ± 3.3	39.4 ± 2.8	-6.8 ± 3.2
Willingness to use again	43.4 ± 3.6	40.6 ± 3.5	-2.8 ± 3.2
Puffing topography			
Number of puffs	19.2 ± 2	18.4 ± 1.4	-0.8 ± 1
Average puff duration (sec)	2.5 ± 0.1	2.5 ± 0.1	0.1 ± 0.1
Total puff duration (sec)	41.6 ± 3.4	43.7 ± 3.1	2.1 ± 2.3
Average puff interval (sec)	34.3 ± 2	34.2 ± 1.9	0.0 ± 1.5

Note: Values are given as average ± SEM.

gender, smoking status, vaping frequency and familiarity with nicotine salts on the study parameters. Similarly, perceived liking, nicotine intensity, harshness and pleasantness did not differ significantly between 5 and 10 minutes after the start of the session.

Finally, we assessed the internal consistency for parameters over time and between parameters. Sensory parameters showed good correlations between 5 and 10 minutes for perceived liking (R = 0.83), nicotine intensity (R = 0.77), harshness (R = 0.75) and pleasantness (R = 0.61). Generally, all sensory parameters showed significant positive correlations with each other, except for harshness, which was only significantly correlated to the nicotine intensity but not to other sensory parameters (Fig. 2). Also, among puffing parameters significant correlations were found, such as a positive correlation between the total puff duration and both the number of puffs and the average puff duration.

4. Discussion

Our study compared the effects of 12 mg/mL nicotine salts versus free-base nicotine in a tobacco-flavored e-liquid on sensory appeal and puffing behavior. The measured protonation states of the salt and the free-base nicotine were 90% and 3%, respectively, indicating that using a nicotine salt resulted in a close to 100% protonation grade, and the free-base liquid is almost completely unprotonated. In our study paradigm, test scores on appeal, harshness and puffing parameters did not show any significant differences between the nicotine salt and the free-base condition. Thus, we found no evidence that at concentrations of 12 mg/mL, nicotine salts are easier to inhale than free-base nicotine.

While JUUL is not on the Dutch market, many liquids with nicotine salts or acid additives have been notified to the Dutch regulator (Pennings et al., 2022). Overall, 13% of all liquids notified contained nicotine salts or acid additives, with a median nicotine concentration of 12 mg/mL. According to the screening questionnaire sent out before the study, this is also the average nicotine concentration used by vapers in the research agency's database. In our study, only 28% of the participants reported using higher concentrations. Other studies on Dutch adults who use e-cigarettes reported even lower average nicotine concentrations of 9.7 mg/mL (Gucht et al., 2017) and 8.9 mg/mL (Smets et al., 2019). The screening also showed that 54% of the vapers (ever) used a tobacco-flavored liquid, which supported our choice of a tobacco-flavored liquid. In our study, all participants reported ever-use of tobacco-flavored e-liquids (Table 1), even though this was not an inclusion criterion.

4.1. Strengths and limitations

Our study had several strengths. We used a nicotine concentration relevant to the European market to study the effects of nicotine salts versus free-base nicotine on sensory appeal and puffing intensity. Our within-participants design excluded potential confounding influences of participant characteristic factors such as device on the parameter effect size. Furthermore, the in-home paradigm enabled participants to vape in a naturalistic setting, using their own device, and vaping *ad libitum*, with no fixed puffing pattern. This experimental setting resembles real-life conditions as closely as possible and is therefore a strength. But at the same time, it could be a limitation, as vaping without a fixed puffing pattern introduces an additional variable, and thus may have introduced additional variation.

A previous study by Leventhal et al. found that salt versus free-base nicotine formulations with an average nicotine concentration of 24 mg/mL appeared to increase product appeal and improve the sensory experience of vaping (Leventhal et al., 2021). The average value of the appeal score (100-unit VAS scale) over all flavors in the Leventhal study (mean [SE]: 55.6 [1.7] for nicotine salt vs 43.6 [1.7] for free-base) was similar to our scores for tobacco flavor (mean [SE] 48.5 [3.2] vs 50.7 [2.9]). However, the differences between the salt and the free-base condition are much higher and more significant in their study (12 points vs −2.2 points). This similar average value was also observed for the harshness attribute (Leventhal et al. found 36.0 [1.6] for salt vs 56.9 [1.6] for free-base, and we found 48.2 [3.3] vs 51.8 [3.1]).

Apart from the two times higher nicotine concentration, another important difference is that Leventhal et al. used standardized puff settings with four seconds of inhalation, while in our study participants

Correlation coefficients	Like taste (5 min)	Like taste (10 min)	Intensity (5 min)	Intensity (10 min)	Harshness (5 min)	Harshness (10 min)	Pleasantness (5 min)	Pleasantness (10 min)	Willingness to use again	Number of puffs	Average puff duration (sec)	Total puff duration (sec)	Average puff interval (sec)
Like taste (5 min)	1.00	0.83	0.44	0.38	-0.13	-0.09	0.47	0.62	0.76	-0.06	0.05	0.04	0.09
Like taste (10 min)	0.83	1.00	0.35	0.42	-0.04	-0.11	0.45	0.64	0.82	-0.06	0.07	0.07	0.11
Nicotine intensity (5 min)	0.44	0.35	1.00	0.77	0.35	0.40	0.22	0.41	0.25	0.00	0.04	0.08	0.00
Nicotine intensity (10 min)	0.38	0.42	0.77	1.00	0.31	0.36	0.34	0.42	0.32	0.05	0.01	0.08	-0.03
Harshness throat hit (5 min)	-0.13	-0.04	0.35	0.31	1.00	0.75	0.03	0.13	-0.10	-0.02	-0.10	-0.11	0.07
Harshness throat hit (10 min)	-0.09	-0.11	0.40	0.36	0.75	1.00	-0.02	0.12	-0.18	-0.02	-0.16	-0.15	0.05
Pleasantness throat hit (5 min)	0.47	0.45	0.22	0.34	0.03	-0.02	1.00	0.61	0.47	-0.01	-0.02	-0.07	0.11
Pleasantness throat hit (10 min)	0.62	0.64	0.41	0.42	0.13	0.12	0.61	1.00	0.54	-0.08	-0.04	-0.06	0.16
Willingness to use again	0.76	0.82	0.25	0.32	-0.10	-0.18	0.47	0.54	1.00	-0.01	-0.08	-0.03	0.01
Number of puffs	-0.06	-0.06	0.00	0.05	-0.02	-0.02	-0.01	-0.08	-0.01	1.00	-0.06	0.40	-0.63
Average puff duration (sec)	0.05	0.07	0.04	0.01	-0.10	-0.16	-0.02	-0.04	-0.08	-0.06	1.00	0.73	-0.04
Total puff duration (sec)	0.04	0.07	0.08	0.08	-0.11	-0.15	-0.07	-0.06	-0.03	0.40	0.73	1.00	-0.56
Average puff interval (sec)	0.09	0.11	0.00	-0.03	0.07	0.05	0.11	0.16	0.01	-0.63	-0.04	-0.56	1.00

Fig. 2. Correlations between study parameters. Red and blue cells indicate correlation coefficients that are significantly positive and negative, respectively (FDR 5%). Comparisons of self-versus self are shown as gray cells.

vaped in a naturalistic manner (i.e., without a fixed puffing pattern and *ad libitum)*. Moreover, in the Leventhal study, one single type of pod-style device was used, with no adjustable settings, whereas in our study participants used their own refillable e-cigarette and were instructed to use the same settings for both online sessions if their e-cigarette had adjustable settings. While this choice reduced experimental variation, it might also have diminished the real-life value of our study. For a future study, it would be recommended to record the adjustable settings of the refillable e-cigarette to study how these impact results. In addition, it is recommended to use a study design with e-cigarette settings as an additional study parameter, as this more closely reflects real-life conditions. On the one hand, controlled lab experiments reduce variation, but their external validity is necessarily limited and further away from reality. Field experiments such as ours have a higher external validity, but on the other hand also have more confounders and are therefore more limited in demonstrating the effect of a specific variable. Thus, it can be argued that both studies have their specific strengths and limitations. Leventhal also studied flavors other than tobacco but did not find significant interactions between nicotine formulation and flavor (except for chocolate on harshness). This suggests that the type of flavor, tobacco or other, does not determine effect size. In both studies, appeal scores are relatively low, in the neutral range of around 50 points. Apparently, all types of e-liquid flavors used in both studies are not liked very well. Similar neutral appeal scores were found previously (Krusemann et al., 2020).

4.2. Regulatory implications

Regarding regulatory implications, evidence that nicotine salt formulations enhance the appeal, sensory qualities and ease of inhalation of e-cigarette vape is important for public health researchers and policy makers. Product attractiveness (or appeal), and addictive potential (or dependence liability) lead to an increased risk of initiation and continuation of product use (WHO Framework Convention on Tobacco Control (WHO FCTC)). Specifically, our findings will inform EU regulators whether the use of nicotine salts in e-liquids should be prohibited. According to TPD Art. 20.3 (c) and 7.6 (d), additives that facilitate the inhalation of nicotine are prohibited (European Union, 2014). However, methods for assessing inhalation facilitation are not described in the TPD. We, therefore, advise that the TPD defines the concept of inhalation facilitation more concretely and in detail for research and regulatory purposes. Based on our methodology that combines sensory attributes scoring combined with measuring puffing intensity, we did not find evidence for inhalation facilitation by nicotine salts at concentrations of 12 mg/mL. Future research should investigate other nicotine concentrations and study designs varying in choices regarding laboratory setting versus in-home testing (online or with researchers) or another more naturalistic setting such as an apartment (Pauwels et al., 2020; Pauwels et al., 2020).

5. Conclusions

In our in-home study, we compared the effects of nicotine salts versus free-base nicotine in a tobacco-flavored e-liquid on sensory appeal and puffing intensity. Participants vaped an e-liquid containing nicotine concentrations of 12 mg/mL using their own e-cigarette device with an average inhalation time of 2.5 seconds. Using these e-liquids and this study paradigm, we did not observe that nicotine salts are easier to inhale than free-base nicotine. To gather more evidence on inhalation facilitation by nicotine salts, future research should address e-liquids with other nicotine concentrations and other study designs.

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CRediT authorship contribution statement

CP was the project coordinator, drafted the method, wrote and edited the manuscript. WouterV, JP and RT assisted with the conceptualization, interpretation of results and writing. AH provided expert consultation and coordinated laboratory results. EB executed laboratory analysis. JP was also responsible for sample size calculation and statistical analysis. MM was the study coordinator and provided expert consultation. LT, WimV and SB provided expert consultation and reviewed the article.

All authors approve the final article and its submission to Drug and Alcohol Dependence.

Declaration of Competing Interest

Authors have no conflict of interest.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.drugalcdep.2023.109914.

References

Benjamini, Y., Hochberg, Y., 1995. Controlling the false discovery rate: a practical and powerful approach to multiple testing. J. R. Stat. Soc. Ser. B (Methodol.) 57 (1), 289–300.

Duell, A.K., Pankow, J.F., Peyton, D.H., 2018. Free-base nicotine determination in electronic cigarette liquids by (1)H NMR spectroscopy. Chem. Res. Toxicol. 31 (6), 431–434.

Duell, A.K., Pankow, J.F., Peyton, D.H., 2020. Nicotine in tobacco product aerosols: 'It's deja vu all over again'. Tob. Control 29 (6), 656–662.

Dutch Tobacco Act, 1990.

European Union, 2014. Tobacco Products Directive 2014/40/EU of the European Parliament and of the Council. ⟨https://ec.europa.eu/health/sites/health/files/tobacco/docs/dir_201440_en.pdf⟩.

Goldenson, N.I., Fearon, I.M., Buchhalter, A.R., Henningfield, J.E., 2021. An open-label, randomized, controlled, crossover study to assess nicotine pharmacokinetics and subjective effects of the JUUL system with three nicotine concentrations relative to combustible cigarettes in adult smokers. Nicotine Tob. Res. 23 (6), 947–955.

Gucht, D.V., Adriaens, K., Baeyens, F., 2017. Online vape shop customers who use e-cigarettes report abstinence from smoking and improved quality of life, but a substantial minority still have vaping-related health concerns. Int. J. Environ. Res. Public Health 14, 7.

Hajek, P., Pittaccio, K., Pesola, F., Myers Smith, K., Phillips-Waller, A., Przulj, D., 2020. Nicotine delivery and users' reactions to Juul compared with cigarettes and other e-cigarette products. Addiction 115 (6), 1141–1148.

Hartendorp, A.P.T., Ahlal, I., Visser, W.F., Baloe, E.P., Nagtegaal-de Ruiter, H., Klerx, W. N., et al, 2021. Benchtop NMR Spectroscopy as E-liquid Analysis tool. (Manuscript in preparation).

International Organisation of Standardisation, 2014. ISO 11136:2014 Sensory analysis — Methodology — General Guidance for Conducting Hedonic Tests with Consumers in A Controlled Area. Available from: ⟨https://www.iso.org/standard/50125.html⟩.

Jackler, R.K., Ramamurthi, D., 2019. Nicotine arms race: JUUL and the high-nicotine product market. Tob. Control 28 (6), 623–628.

Kavuluru, R., Han, S., Hahn, E.J., 2019. On the popularity of the USB flash drive-shaped electronic cigarette Juul. Tob. Control 28 (1), 110–112.

Krusemann, E.J.Z., Wenng, F.M., Pennings, J.L.A., de Graaf, K., Talhout, R., Boesveldt, S., 2020. Sensory evaluation of e-liquid flavors by smelling and vaping yields similar results. Nicotine Tob. Res. 22 (5), 798–805.

Leventhal, A.M., Madden, D.R., Peraza, N., Schiff, S.J., Lebovitz, L., Whitted, L., et al., 2021. Effect of exposure to e-cigarettes with salt vs free-base nicotine on the appeal and sensory experience of vaping: a randomized clinical trial. JAMA Netw. Open 4 (1), e2032757.

Mallock, N., Rabenstein, A., Gernun, S., Laux, P., Hutzler, C., Karch, S., et al., 2021. Nicotine delivery and relief of craving after consumption of European JUUL e-cigarettes prior and after pod modification. Sci. Rep. 11 (1), 12078.

O'Connell, G., Pritchard, J.D., Prue, C., Thompson, J., Verron, T., Graff, D., et al., 2019. A randomised, open-label, cross-over clinical study to evaluate the pharmacokinetic profiles of cigarettes and e-cigarettes with nicotine salt formulations in US adult smokers. Intern. Emerg. Med. 14 (6), 853–861.

Overheid.nl, 2021. Online Consultation Regulation E-cigarette Flavors / Online Consultatie Regulering Smaakjes E-sigaret (in Dutch). Available from: ⟨https://www.internetconsultatie.nl/smaakjes⟩.

Pauwels, C.G.G.M., Boots, A.W., Visser, W.F., Pennings, J.L.A., Talhout, R., Schooten, F. V., et al., 2020. Characteristic human individual puffing profiles can generate more TNCO than ISO and Health Canada regimes on smoking machine when the same brand is smoked. Int. J. Environ. Res Public Health 17, 9.

Pauwels, C.G.G.M., Hintzen, K.F.H., Talhout, R., Cremers, H., Pennings, J.L.A., Smolinska, A., et al., 2020. Smoking regular and low-nicotine cigarettes results in comparable levels of volatile organic compounds in blood and exhaled breath. J. Breath Res. 15 (1), 016010.

Pennings, J.L.A., Havermans, A., Pauwels, C., Krusemann, E.J.Z., Visser, W.F., Talhout, R., 2022. Comprehensive Dutch market data analysis shows that e-liquids with nicotine salts have both higher nicotine and flavour concentrations than those with free-base nicotine. Tob. Control.

Phillips-Waller, A., Przulj, D., Smith, K.M., Pesola, F., Hajek, P., 2021. Nicotine delivery and user reactions to Juul EU (20 mg/mL) compared with Juul US (59 mg/mL), cigarettes and other e-cigarette products. Psychopharmacology 238 (3), 825–831.

Prochaska, J.J., Vogel, E.A., Benowitz, N., 2021. Nicotine delivery and cigarette equivalents from vaping a JUULpod. Tob. Control.

Ramamurthi, D., Chau, C., Jackler, R.K., 2018. JUUL and other stealth vaporisers: hiding the habit from parents and teachers. Tob. Control.

Reilly, S.M., Bitzer, Z.T., Goel, R., Trushin, N., Richie, J.P., 2019. Free radical, carbonyl, and nicotine levels produced by juul electronic cigarettes. Nicotine Tob. Res. 21 (9), 1274–1278.

Smets, J., Baeyens, F., Chaumont, M., Adriaens, K., Van, Gucht, D., 2019. When less is more: vaping low-nicotine vs. high-nicotine e-liquid is compensated by increased wattage and higher liquid consumption. Int. J. Environ. Res Public Health 16, 5.

WHO Framework Convention on Tobacco Control (WHO FCTC), 2017. Partial Guidelines for Implementation of Articles 9 and 10 of the WHO Framework. Convention on Tobacco Control (Regulation of the Contents of Tobacco Products and Regulation of Tobacco Product Disclosures). Available from: http://wwwwho.int/fctc/guidelines/Decisions9and10.pdf.

World Health Organization, 2021. Standard Operating Procedure for Determination of Nicotine, Glycerol and Propylene Glycol in E-liquids. WHO TobLabNet Official Method SOP11. Geneva, Switzerland.