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Evaluation of the safety profile of an electronic vapour product used for two years by smokers in a real-life setting

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ABSTRACT

The safety profile of Puritane™, a closed system electronic vapour product (EVP), was evaluated when used by smokers of conventional cigarettes (CCs) for 24 months in a real-life setting. The study was a two-centre ambulatory clinical study with 209 healthy volunteers. Outcome measures included adverse events (AEs), vital signs, electrocardiogram, lung function tests, exposure to nicotine and selected smoke constituents, nicotine withdrawal effects and smoking desire. No serious AEs related to EVP use were observed. The most frequently reported AEs were headache, nasopharyngitis, sore throat and cough, reported by 28.7%, 28.7%, 19.6% and 16.7% of subjects, respectively, which dissipated over time. Small decreases in lung function were not considered clinically relevant. No clinically relevant findings were observed in the other safety parameters. From Month 2, nicotine withdrawal symptoms decreased. Smoking desire and CC consumption steadily decreased over time in all subjects. EVP use was associated with reduced exposure to cigarette smoke constituents, whereas urinary nicotine levels remained close to baseline. Body weight did not increase in CC subjects switching to the EVP. In conclusion, the aerosol of the EVP at study was well tolerated and not associated with any clinically relevant health concerns after usage for up to 24 months.

1. Introduction

Electronic vapour products (EVPs) are becoming an increasingly popular alternative to conventional tobacco cigarettes among smokers worldwide. EVPs are battery-powered devices that deliver vaporized nicotine, propylene glycol and/or glycerol and flavourings to users from an “e-liquid”. EVPs simulate the visual, sensory, and behavioural aspects of smoking, which conventional nicotine replacement therapy (NRT) products do not (Hajek et al., 2014; Nelson et al., 2015; Nides et al., 2014) and have also been found to deliver sufficient levels of nicotine to satisfy users (Goniewicz et al., 2017; McNeill et al., 2015; Polosa et al., 2014a; Vansickel and Eissenberg, 2013; Walele et al., 2016a, 2016b). There is also evidence that EVPs can encourage quitting or cigarette consumption reduction even among those smokers not intending to quit or rejecting other forms of cessation (Caponnetto et al., 2013; Hartmann-Boyce et al., 2016; McNeill et al., 2015; McRobbie et al., 2014).

Current data indicate that, for smokers, switching to EVPs triggers few, if any, adverse physiological changes (Callahan-Lyon, 2014; Farsalinos et al., 2014; Flouris et al., 2012, 2013; Vansickel et al., 2010; Vardavas et al., 2012) and no serious side effects have been reported with short- to medium-term EVP use (McNeill et al., 2015). Some changes in lung function parameters, blood pressure and heart rate, consistent with those observed after smoking cessation, have been observed in conventional cigarette (CC) smokers switching to an EVP for five days (D'Ruiz and O'Connell, 2016). Long-term safety data (from 6 to 24 months) are currently available from five studies (Bullen et al., 2013; Caponnetto et al., 2013; Farsalinos et al., 2016; Manzoli et al., 2016; Polosa et al., 2011, 2014b), which show that few persistent adverse events (AEs) occur in smokers of CCs who switch to using EVPs. Similar to NRT products, the reported transient AEs relate predominantly to mouth and throat irritation (Ferguson et al., 2011; Ossip

Abbreviations: AE, adverse event; BoBE, biomarker of biological effect; BoE, biomarker of exposure; CC, conventional cigarette; COHb, carboxyhaemoglobin; CPD, cigarettes per day; ECG, electrocardiogram; FAS, full analysis set; FEF_{25-75}, forced expiratory flow 25–75%; FEV_1, forced expiratory volume in one second; FVC, forced vital capacity; eCO, exhaled carbon monoxide; EoS, end of study; EVP, electronic vapour product; HPHC, harmful and potentially harmful constituent; MWS-R, Revised Minnesota Nicotine Withdrawal Scale; QSU-Brief, Brief Questionnaire of Smoking Urges; PEF, peak expiratory flow; SAF, safety analysis set; SD, standard deviation; SEM, standard error of the mean

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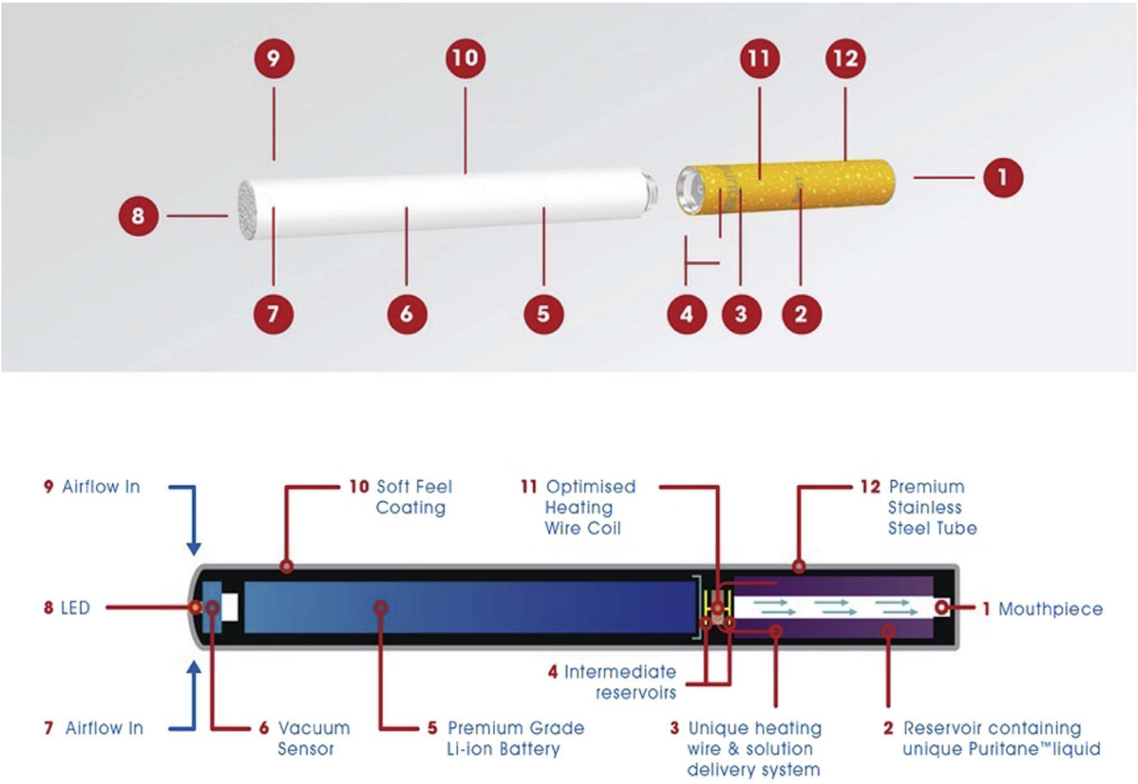

Fig. 1. Schematic diagram of Puritane™ and its component parts.

et al., 2009). EVPs have been characterised by Public Health England as being around 95% less harmful than CCs (McNeill et al., 2015). In addition, in its 2016 report, The Royal College of Physicians stated that "*Although it is not possible to quantify the long-term health risks associated with e-cigarettes precisely, the available data suggests that they are unlikely to exceed 5% of those associated with smoked tobacco products, and may well be substantially lower than this figure*" (RCP, 2016).

Herein, we present the results of a 2-year clinical study aimed at evaluating the long-term effects of an EVP (ClinicalTrials.gov, #NCT02143310).

2. Material and methods

2.1. Study design

This study was designed as an open-label, ambulatory clinical trial conducted in two centres in the UK (Covance Clinical Research Unit Ltd, Leeds and Simbec Research Ltd, Merthyr Tydfil). The same subjects who participated in our previous clinical trial (ClinicalTrials.gov, #NCT02029196) conducted in the same centres, with another EVP (Cravo et al., 2016), were invited to participate in this study. All volunteering subjects were assigned to switch to using Puritane™, a closed system EVP, for two years, starting on the last day of the previous trial (End of Study [EoS] visit), which corresponded to the baseline visit of this study.

All relevant study documents were approved by the Wales Research Ethics Committee 2 on 11 April 2014. The study is registered with the US National Institutes of Health (ClinicalTrials.gov, #NCT02143310). All procedures were performed in accordance with the Declaration of Helsinki and with the International Conference on Harmonisation (ICH) Harmonised Tripartite Guideline for Good Clinical Practice (GCP). Written informed consent was obtained from each subject before any procedures or assessments commenced.

2.2. Study population

The study population consisted of subjects who had participated in a previous 12-week clinical trial with another EVP (Cravo et al., 2016). In that study, 419 subjects were randomised in a 3:1 ratio to use either an EVP prototype (EVP arm) or continue using their usual CC brand (CC arm). Detailed population characteristics and inclusion criteria to the previous study are outlined in Cravo et al. (2016). In brief, male or female subjects were aged between 21 and 65 years and had a body mass index in the range of 18–35 kg/m^2. Subjects had to be smokers of 5–30 cigarettes per day for at least one year (self-reported) and to be in good general health.

To participate in this study, subjects were assessed by the principal investigator as being compliant in the previous study (*e.g.* having attended out-patient visits and having been compliant with study procedures). Subjects had to be willing to use the study product as the only nicotine-containing product for the duration of the study, and, as deemed by the principal investigator, had to have no clinically significant abnormalities in 12-lead electrocardiogram, vital signs, spirometry and clinical laboratory assessments in the preceding study. In addition, subjects who were assigned to the CC arm in the previous study had to be established smokers of CCs, which was assessed by urinary cotinine levels (a score of 3 and above on a NicAlert™ test strip was considered positive), exhaled carbon monoxide (eCO) levels (a readout greater than 6 ppm was considered positive) and by review of a smoking history questionnaire. Subjects who had taken or received any form of NRT, snuff or chewing tobacco during the previous study or intended to use it during this study, as well as female subjects who were of childbearing potential and who were not intending to use an acceptable contraceptive method for the duration of this study, were excluded. Subjects with a history of any relevant or clinically significant disorder or illness, as judged by the principal investigator, were also excluded. Withdrawn subjects were not replaced.

2.3. Product used in this study

Commercially available Puritane™, representative of a typical closed system EVP, consists of a lithium-ion rechargeable battery and a replaceable cartomiser comprising of an e-liquid reservoir pre-filled by the manufacturer, a heating element and a mouthpiece (Fig. 1). The battery can be recharged at least 100 times, and one single cartomiser provides 300–350 puffs depending on the user's puffing behaviour. The cartomisers contain 1 mL of e-liquid, which is comprised of 67.5–69.0% (w/w) propylene glycol (PG), 30.0% (w/w) glycerol, 1.6% nicotine (16 mg/g) and 0.54–1.0% (w/w) flavouring. During the study, the e-liquid was available in two different flavours: tobacco or menthol.

2.4. Study schedule and procedures

Two weeks before baseline, subjects were invited to consent to the study and participated in a familiarisation session with Puritane™, where they could see and try the EVP. At baseline, subjects were trained on how to use Puritane™, and were given 24-h urine collection containers, a container to collect their used products and a diary to record product usage throughout the duration of the study. Once all baseline procedures were completed, subjects could start using Puritane™.

Baseline procedures included:

- confirmation of eligibility criteria;
- eCO levels (Bedfont Micro + Smokerlyzer) and blood carboxyhaemoglobin (COHb) levels;
- return of 24-h urine sample (same sample as 24-h urine sample for EoS of the previous study);
- body weight;
- vital signs (sitting blood pressure and heart rate);
- AEs;
- lung function test (measured through spirometry);
- 12-lead electrocardiogram (ECG);
- blood and urine sampling for clinical haematology, chemistry and urinalysis parameters;
- pregnancy test for females of childbearing potential;
- administration of the revised Minnesota Nicotine Withdrawal Scale (MWS-R) questionnaire to document nicotine withdrawal symptoms (Hughes, 2007) and the Brief Questionnaire of Smoking Urges (QSU-Brief) (Cox et al., 2001) to document smoking desire.

Subjects attended the study centres for assessments at Months 1, 2, 3, 6, 9, 12, 15, 18, 21 and 24 (EoS). At each visit, eCO and blood COHb was analysed, AEs were recorded, and the subjects had to complete the MWS-R (to assess nicotine withdrawal) and QSU-Brief (to assess smoking desire) questionnaires. Subjects also returned their product use diary and their containers with the used products. A pregnancy test was performed on all female subjects of childbearing potential (females with a positive pregnancy test were withdrawn from the study).

Weight, vital signs, 12-lead ECG, lung function, clinical chemistry, haematology and urinalysis were checked at Months 1, 3, 6, 12, 18 and at EoS. At these visits, subjects also returned 24-h urine samples (acquired the day before the visit and kept in a refrigerator or in a cool bag), for the analysis of urinary biomarkers of exposure. At EoS, subjects were given verbal smoking cessation advice by the principal investigator or the study team.

For the first three months, all subjects used the tobacco-flavoured e-liquid, as this was the only flavour available. From Month 3, Puritane™ was also available in menthol flavour, and subjects could choose their preferred flavour from that visit onwards. Subjects were allowed to change flavour during the course of the study, and flavour changes were recorded. Use of CCs during the study would not lead to termination although subjects were reminded to use only Puritane™.

2.5. Study outcomes

The primary outcomes of this study were the frequency of AEs (AEs were coded using the Medical Dictionary for Regulatory Activities, version 16.1, 2013), 12-lead ECG parameters, vital signs, lung function tests and clinical laboratory parameters (clinical chemistry, haematology and urinalysis).

All AEs were recorded and assessed by the investigator for their seriousness and severity (mild, moderate, severe). The investigator also judged their relationship to study product as unrelated, unlikely related, possibly related, probably related, or definitely related by assessing: the existence of an alternative cause; temporal sequence from use of study product; known patterns of response to study product; subject's medical history or concomitant medication; clinical response on withdrawal from/re-exposure to study product (if available).

Secondary outcomes included the level of selected biomarker of exposure (BoE) in urine (to harmful and potentially harmful constituents [HPHCs] typically found in CC smoke, and for which a BoE in urine has been identified), the level of selected biomarkers of biological effect (BoBE) in blood, nicotine withdrawal symptoms and desire to smoke.

More details on each of these outcome measures and the bioanalytical methods used are given in Cravo et al. (2016).

2.6. Statistical methods

The duration of this study and the sample size were guided by the Council for International Organizations of Medical Sciences (CIOMS) guideline (Dollery and Bankowski, 1983), the ICH E1 guideline (ICH, 1994) and published studies on a similar product (Caponnetto et al., 2013; Polosa et al., 2014b). The ICH E1 guideline states that exposing 100 subjects for a minimum of one year is considered acceptable for detecting any adverse product reactions with a true incidence of 3% and over (ICH, 1994). Therefore, all 387 subjects who completed the previous study were invited to participate.

Where deemed appropriate, data were stratified by the study arm of the previous clinical trial. Subjects who had used the EVP in the previous study are referred to as “pre-EVP subjects”, and subjects who had used the CC are referred to as “pre-CC subjects”. Product compliance was used as an additional stratification factor where considered appropriate: “EVP-compliant subjects” were defined as subjects who were abstinent from CCs for at least 80% of the completed study days. On a particular study day, a subject was judged abstinent if the subject reported not having smoked any CC that day. On study visit days, a subject was considered abstinent if, in addition to not having smoked CCs, the eCO level was below or equal to 8 ppm. Finally, the data are also shown for the subset of subjects who completed the study, referred to as “completers”.

The data were summarised using descriptive statistics for vital signs, lung function parameters, ECG parameters, clinical laboratory parameters, levels of BoE and BoBE and questionnaire scores. AEs were presented using frequency tables. A repeated measures analysis of covariance (RMANCOVA) was used to assess if the changes from baseline in the lung function test parameters were different from zero. Statistical significance was set at p-value $< .05$. The analysis was conducted using SAS® version 9.3.

3. Results

3.1. Subjects

Out of the 387 subjects who completed the previous clinical study, 209 were enrolled into this study (Safety Analysis Set or SAF), of which 147 had used the EVP in the previous study and 62 the CC. Two hundred and six subjects used the product at least once (Full Analysis Set or FAS). A total of 102 subjects (48.8%) went on to use Puritane™ for at

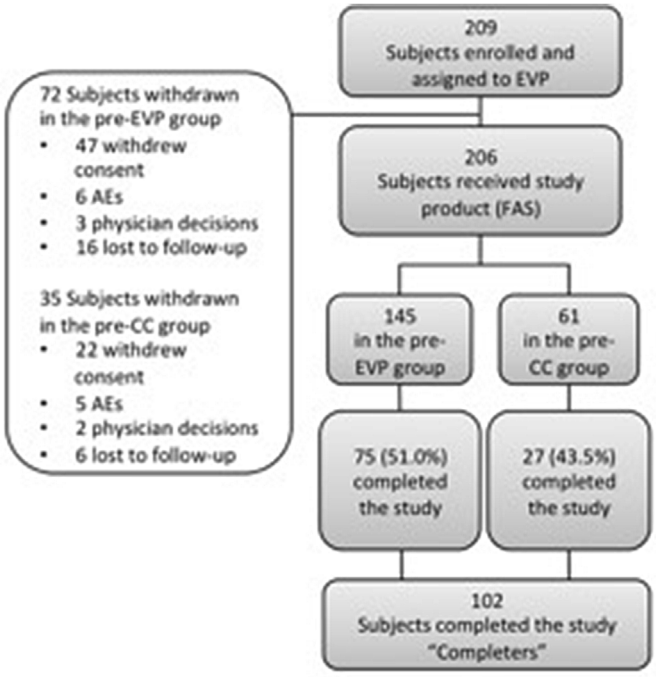

Fig. 2. Flow of subjects.

least 24 months ("completers": 51.0% of pre-EVP subjects and 43.5% of pre-CC subjects). Eleven subjects were withdrawn due to an AE (unrelated or unlikely to be related to the study product) and 96 were withdrawn for other reasons (withdrawal of consent, lost to follow-up or based on investigator's decision) (Fig. 2).

Table 1 shows the subject's baseline characteristics. Pre-EVP subjects and pre-CC subjects had similar baseline characteristics for age, BMI, weight and urine cotinine and nicotine levels, but differed in eCO and COHb levels. The baseline characteristics for EVP-compliant subjects and completers were similar to the overall study population.

3.2. Product usage and compliance

The mean number of cartomisers used per day, based on diary data, is reported in Fig. 3A. Subjects used a mean (± SD) of 0.85 (± 0.84) cartomisers per day during the first study month. The cartomiser usage steadily decreased to 0.57 (± 0.42) per day at Month 8. From Month 9 to Month 23, the usage remained stable at 0.54–0.60 cartomisers used per day. During the last study month, the usage slightly increased, to reach 0.68 (± 0.58) cartomisers per day at EoS. The usage pattern was similar for pre-EVP and pre-CC subjects, with an initial decrease and subsequent stable use. However, pre-CC subjects used a higher number of cartomisers per day from baseline to Month 20. From Month 1 to Month 7, completers used a higher mean number of cartomisers per day than the overall study population. From Month 8–24, the EVP consumption of completers was similar to the whole population.

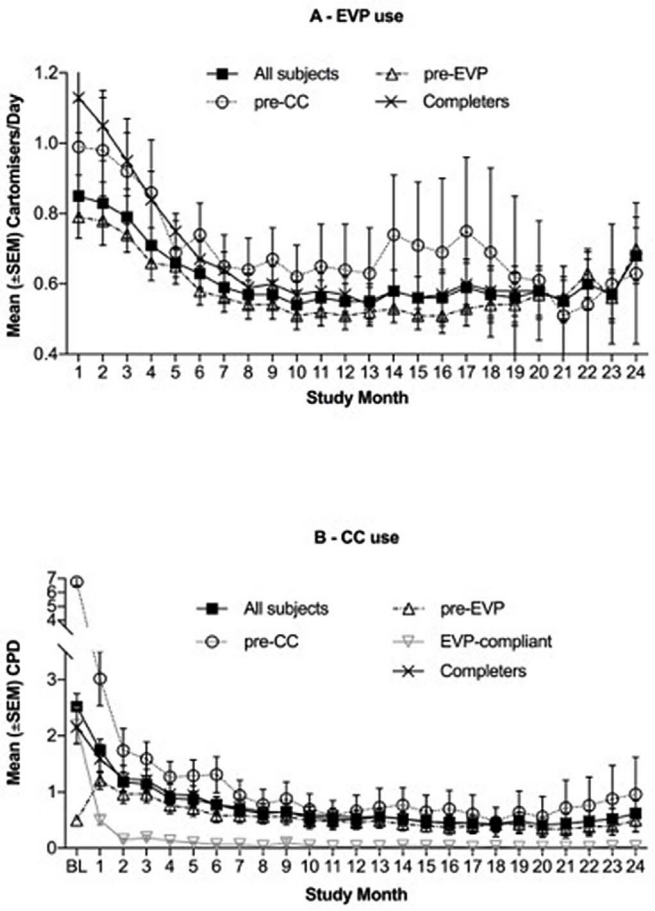

Fig. 3. Product consumption by study month, based on self-reported data on subjects' diary cards. (**A**) Mean (± SEM) cartomisers started per day from Month 1 to Month 24, (**B**) mean (± SEM) number of CCs smoked per day from baseline (BL) to Month 24. Data are shown for all subjects, for pre-EVP and pre-CC subjects, and for EVP-compliant subjects.; *Abbreviations*: CC: conventional cigarette; CPD: cigarettes per day; EVP: e-vapour product; SEM: standard error of the mean.

The mean consumption of CCs is reported in Fig. 3B. The

Table 1
Subjects' baseline characteristics.

		pre-EVP (N = 147)	pre-CC (N = 62)	EVP-compliant (N = 110)	Completers (N = 102)	Overall (N = 209)
Age (years)	Mean (SD)	36.8 (10.5)	36.1 (9.5)	35.6 (10.0)	38.7 (10.2)	36.6 (10.2)
Sex						
Males	n (%)	84 (57.1%)	31 (50.0%)	64 (58.2%)	57 (55.9%)	115 (55.0%)
Females	n (%)	63 (42.9%)	31 (50.0%)	46 (41.8%)	45 (44.1%)	94 (45.0%)
BMI (kg/m^2)	Mean (SD)	26.01 (3.68)	25.47 (3.69)	25.79 (3.73)	26.18 (4.03)	25.85 (3.68)
Body weight (kg)	Mean (SD)	76.00 (13.68)	73.81 (13.72)	75.38 (13.39)	75.83 (14.49)	75.35 (13.69)
eCO (ppm)	Mean (SD)	8.7 (6.5)	25.2 (11.1)	12.4 (9.7)	11.6 (9.1)	13.6 (11.1)
COHb (%)	Mean (SD)	4.25 (1.40)	7.09 (1.93)	4.77 (1.82)	4.79 (1.84)	5.09 (2.04)
NicAlert™ score	Mean (SD)	5.8 (0.7)	5.9 (0.3)	5.8 (0.7)	5.8 (0.7)	5.8 (0.6)
Urine cotinine (NicAlert™)						
Positive	n (%)	136 (92.5%)	62 (100%)	106 (96.4%)	99 (97.1%)	198 (94.7%)
Negative	n (%)	11 (7.5%)	0 (0.0%)	4 (3.6%)	3 (2.9%)	11 (5.3%)

Abbreviations: BMI: body mass index; COHb: carboxyhaemoglobin; eCO: exhaled carbon monoxide; EVP: e-vapour product; N: number of subjects; ppm: parts per million; pre-CC: subjects who used the conventional cigarette in the previous clinical study; pre-EVP: subjects who used the electronic vapour product in the previous clinical study; SD: standard deviation.

consumption of CCs decreased in all subjects from baseline to Month 8. From Month 8 to EoS, the CC consumption in all subjects remained low, ranging from 0.41 to 0.65 cigarettes per day (CPDs), and was similar for pre-CC and pre-EVP subjects. There was a small tendency to increase CC consumption at EoS in all subjects except in EVP-compliant subjects. During the first study months, the CC consumption was higher in pre-CC subjects compared with pre-EVP subjects. In pre-CC subjects, the use of CCs decreased from a mean (± SD) of 6.76 (± 2.21) CPDs at baseline to 3.02 (± 3.77) at Month 1, and continued to decrease steadily to reach 0.78 (± 1.55) at Month 8. Pre-EVP subjects reported using a mean (± SD) of 0.50 (± 0.64) CPDs at baseline, 1.21 (± 1.87) CPDs at Month 1 and 0.56 (± 1.23) CPDs at Month 8. The CC consumption of completers was similar to that of the whole population.

Regarding compliance, subjects abstained from smoking CCs for a mean (± SD) of 68.9% (± 33.3) of the total study days. A total of 110 subjects (53.4%) were compliant for over 80% of the completed study days. The proportion of compliant subjects was greater among pre-EVP users (56.6%) than among pre-CC users (45.9%). The mean number of cartomisers used per day by compliant subjects was similar to that of the whole population (data not shown). The proportion of compliant subjects among completers reached 69.6%.

At the Month 3 visit, when Puritane™ became available with menthol flavour, one subject (0.6% of all subjects) chose this flavour. At Month 4, 13.5% of subjects preferred menthol. From Month 5 to Month 20, the percentage of subjects who reported choosing the menthol flavour remained stable, between 21.2% and 27.2%. During the last four study months, fewer subjects chose the menthol flavour, with percentages ranging from 16.7% to 18.4%.

At Month 4, subjects who chose menthol reported using a mean (± SD) of 0.88 (± 0.49) cartomisers per day and subjects who chose the tobacco flavour used 0.69 (± 0.72) cartomisers per day. From Month 5–24, subjects who chose menthol used 0.54 (± 0.51) to 0.76 (± 0.55) cartomisers per day, and subjects who chose tobacco flavour used 0.52 (± 0.41) to 0.71 (± 0.59) cartomisers per day.

3.3. Safety outcomes

3.3.1. Adverse events

Throughout the study, 159 (76.1%) subjects reported a total of 971 AEs. Within EVP-compliant subjects, 90 (81.8%) subjects reported a total of 575 AEs, and within completers, 94 (92.2%) subjects reported a total of 640 AEs. Table 2 shows the proportion of mild, moderate or severe AEs, as well as the proportion of AEs by relationship to the study product, in each group. All AEs (regardless of relationship to study product) reported by 3% or more of subjects in either group are shown in Table 3.

Table 2
Number of AEs (%) by severity and by relationship to the study product.

	All Subjects (N = 209)	EVP-compliant subjects (N = 110)	Completers (N = 102)
Total	971 (100%)	575 (100%)	640 (100%)
SAEs	7 (0.7%)	3 (0.5%)	1 (0.2%)
AEs leading to study withdrawal	11 (1.1%)	6 (1.0%)	0
AEs by severity (% of AEs)			
Mild	323 (33.3%)	222 (38.6%)	236 (36.9%)
Moderate	503 (51.8%)	292 (50.8%)	318 (49.7%)
Severe	145 (14.9%)	61 (10.6%)	86 (13.4%)
AEs by relationship to study product (% of AEs)			
Almost definitely related	11 (1.1%)	7 (1.2%)	3 (0.5%)
Probably related	32 (3.3%)	27 (4.7%)	17 (2.7%)
Possibly related	401 (41.3%)	192 (33.4%)	259 (40.5%)
Unlikely related	207 (21.3%)	114 (19.8%)	122 (19.1%)
Unrelated	320 (33.0%)	235 (40.9%)	239 (37.3%)

Abbreviations: AE: adverse event; N: number of subjects; SAE: serious adverse event.

The frequency of AEs (% of subjects reporting AEs) steadily decreased throughout the study, both for all AEs and AEs related to the study product (possibly related, probably related and almost definitely related) (Fig. 4). During the first 8–9 months on study, the frequency of AEs was higher in pre-CC subjects than in pre-EVP subjects. The frequency was subsequently similar in the two groups. Headache was the most common AE within the first months of EVP use, for all subjects as well as within each group. The frequency of AEs (both overall and related to the product) was similar in EVP-compliant subjects and in completers, and was lower than for all subjects, during the first 11–12 months of the study. From Month 12, the AE frequency within EVP-compliant subjects and completers was similar to the overall study population.

Eleven subjects were withdrawn from the study due to AEs, which were judged by the principal investigator as being non-related, or unlikely to be related to the product. These were abdominal pain, periodontal disease, depression, bipolar disorder, six product exposures during pregnancy and a serious adverse event (SAE) of right occipital stroke. Seven subjects experienced a total of seven SAEs (acute pancreatitis, concussion, community acquired pneumonia, right knee injury, right occipital stroke, and two abortions), which were judged by the investigator as not being related to the product, or unlikely to be related to the product. No deaths or life-threatening AEs occurred during the study.

3.3.2. Vital signs, ECG, clinical laboratory parameters and body weight

Mean vital signs and ECG parameter values are reported in Table 4. The mean systolic blood pressure, diastolic blood pressure and pulse rate remained stable throughout the study. Some instances of increased systolic and diastolic blood pressure (shifts from normal pressure at baseline to high pressure at one or more study visits) were observed in 16 (7.8%), and 11 (5.3%) subjects, respectively. However, none of these changes were reported by the investigator as an AE, or led to withdrawal of subjects from the study. ECG parameters also stayed stable for the 24 months of EVP use. There were no increases in QTcB from baseline of 60 ms or greater, and none of the subjects had any instance of a QTcB interval value greater than 480 ms. Some instances of abnormal ECG results were found, however none of them were clinically significant, as judged by the investigator. No differences were observed between EVP-compliant subjects, or completers, and the overall study population for vital signs and ECG parameters.

Regarding clinical laboratory parameters, several instances of out of range clinical chemistry or haematology values were observed, however, the vast majority were not clinically significant. Two subjects (1.0%) experienced both an AE of increased blood cholesterol and of increased low density lipoprotein (LDL) cholesterol. One subject (0.5%) experienced an AE of increased hepatic enzyme. None of these AEs were serious or led to the withdrawal of the subject from the study.

The mean body weight stayed stable throughout the study for all subjects. For males, the mean body weight ranged from 82.3 to 85.0 kg. For females, the mean body weight ranged from 68.2 to 69.2 kg. The mean body weight of EVP-compliant subjects and of completers was similar to that of the overall study population.

3.3.3. Lung function tests

Mean lung function test parameters are reported in Table 4. A small decrease from baseline in each mean lung function test parameter was observed from Month 1 to Month 24, namely of 4.0%, 5.4%, 8.3% and 2.5% for FVC, FEV1, FEF25-75 and PEF, respectively. These changes were statistically significant (p-value $< .05$) for all four parameters for almost all timepoints as from Month 3, however, they were not considered to be clinically significant. The lung function test parameter values for EVP-compliant subjects and for completers were similar to the overall study population.

Table 3
Adverse events reported by ≥ 3% of subjects in either group, by system organ class, regardless of relationship to study product.

	All Subjects (N = 209)			EVP-compliant subjects (N = 110)			Completers (N = 102)		
	Number of subjects	% of subjects	Number of AEs	Number of subjects	% of subjects	Number of AEs	Number of subjects	% of subjects	Number of AEs
Nervous system disorders									
Headache	60	28.7	211	34	30.9	104	44	43.1	150
Migraine	6	2.9	7	4	3.6	5	5	4.9	6
Infection and infestation									
Nasopharyngitis	60	28.7	95	37	33.6	61	44	43.1	75
Influenza	18	8.6	23	9	8.2	13	10	9.8	14
Urinary tract infection	12	5.7	13	5	4.5	5	8	7.8	9
Lower respiratory tract infection	10	4.8	13	6	5.5	7	8	7.8	11
Upper respiratory tract infection	8	3.8	8	5	4.5	5	5	4.9	5
Ear infection	7	3.3	7	5	4.5	5	6	5.9	6
Gastroenteritis	6	2.9	6	4	3.6	4	2	2.0	2
Tooth abscess	5	2.4	6	3	2.7	3	5	4.9	6
Sinusitis	5	2.4	8	3	2.7	3	4	3.9	4
Respiratory, thoracic and mediastinal disorders									
Sore throat	41	19.6	57	25	22.7	38	27	26.5	42
Cough	35	16.7	47	21	19.1	31	18	17.6	26
Nasal congestion	4	1.9	7	3	2.7	6	4	3.9	7
Psychiatric disorders									
Nicotine dependence*	25	12.0	29	16	14.5	20	8	7.8	11
Insomnia	8	3.8	8	7	6.4	7	7	6.9	7
Anxiety	3	1.4	3	3	2.7	3	2	2.0	2
Restlessness	4	1.9	4	4	3.6	4	3	2.9	3
Gastrointestinal disorders									
Toothache	17	8.1	25	7	6.4	12	13	12.7	19
Nausea	11	5.3	16	8	7.3	11	9	8.8	12
Vomiting	10	4.8	13	8	7.3	10	7	6.9	9
Dyspepsia	7	3.3	13	6	5.5	10	7	6.9	13
Abdominal pain	6	2.9	7	4	3.6	5	2	2.0	3
Diarrhoea	6	2.9	6	3	2.7	3	4	3.9	4
Musculoskeletal and connective tissue disorders									
Back pain	15	7.2	17	10	9.1	12	12	11.8	14
Musculoskeletal pain	7	3.3	7	5	4.5	5	5	4.9	5
Pain in extremity	5	2.4	5	4	3.6	4	4	3.9	4
Neck pain	6	2.9	6	3	2.7	3	5	4.9	5
General disorders and administration site conditions									
Fatigue	3	1.4	4	3	2.7	4	3	2.9	4
Injury, poisoning and procedural complications									
Exposure during pregnancy	7	3.3	7	5	4.5	5	1	1.0	1
Surgical and medical procedures									
Tooth extraction	6	2.9	6	5	4.5	5	6	5.9	6
Immune system disorders									
Seasonal allergy	14	6.7	15	5	4.5	6	7	6.9	8
Metabolism and nutrition disorders									
Increased appetite	9	4.3	11	6	5.5	7	3	2.9	3
Reproductive system and breast disorders									
Dysmenorrhoea	7	3.3	9	3	2.7	3	4	3.9	4
Investigations									
Weight increased	7	3.3	8	5	4.5	5	3	2.9	3

Abbreviations: AE: adverse event; N: number of subjects. * Desire to smoke.

3.4. Biomarkers of exposure

Fig. 5 shows the mean levels of nicotine equivalents (NEQ), 3-hydroxypropyl mercapturic acid (3-HPMA; BoE to acrolein), S-phenylmercapturic acid (S-PMA; BoE to benzene), total 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL; BoE to 4-[methylnitrosamino]-1-[3-pyridyl]-1-butanone [NNK]) and PG excreted in urine in 24 h (Ae_{24h}), during the course of the study. The mean (± SEM) Ae_{24h} values for each biomarker at each study visit, as well as changes from baseline, are presented in Table S1.

In all subjects, NEQ first decreased by 12.4% from baseline to Month 1, and subsequently showed a tendency to increase until the end of the study. At EoS, the mean NEQ level for all subjects was 10.1% higher than at baseline (however, the 95% CI for the mean change from baseline included 0.0). Pre-CC subjects had a higher baseline NEQ level by 3.3 mg, and showed a more pronounced decrease from baseline to Month 1, than pre-EVP subjects. The level of NEQ stayed higher in pre-CC subjects than in pre-EVP subjects at all study timepoints except at Month 12.

As expected, pre-CC subjects also had higher baseline urine levels of 3-HPMA, S-PMA and NNAL than pre-EVP subjects. The level of each of these three biomarkers rapidly decreased in pre-CC subjects, to reach levels similar to those in pre-EVP subjects at Month 1. In all subjects, the levels subsequently stayed stable from Month 1 to EoS, with a small tendency to increase from Month 18 to Month 24. Regarding PG, the baseline Ae_{24h} was lower in pre-CC subjects compared with pre-EVP subjects, and the level in pre-CC subjects rapidly increased by 166.7% from baseline to Month 1, to reach a similar level to that in pre-EVP subjects. From Month 1 to Month 12, the PG level was similar in both groups, and increased between Month 6 and Month 12. From Month 18

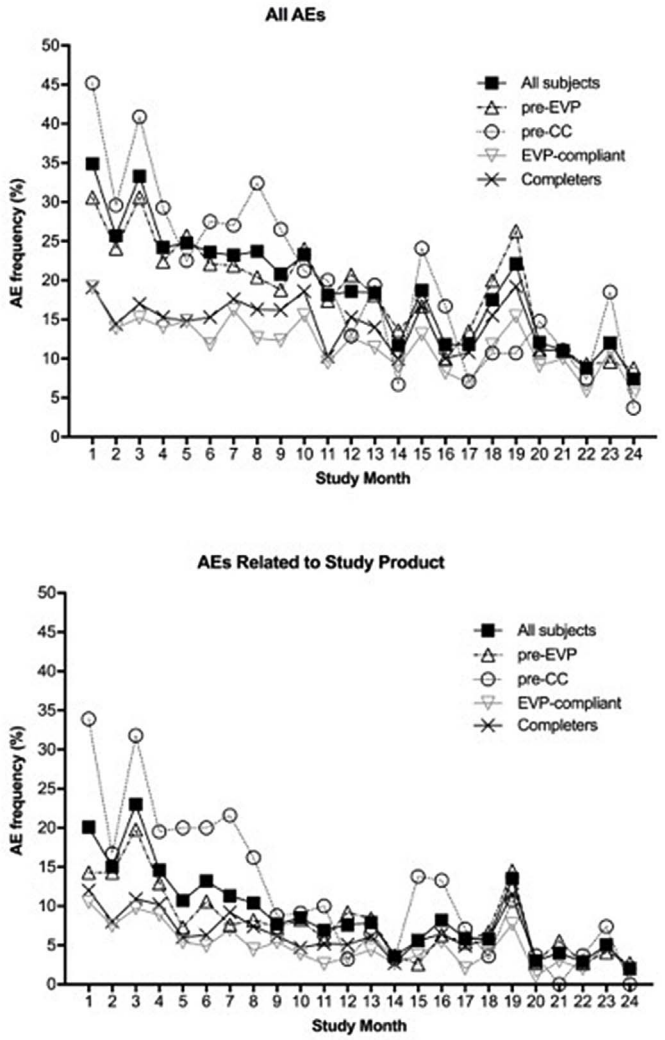

Fig. 4. Frequency of adverse events (AEs; % of subjects reporting AEs) by study month, for all AEs (upper panel), as well as AEs judged by the investigator as being related to the study product (possibly related, probably related and almost definitely related).

to Month 24, the PG levels further increased in all subjects, and pre-CC subjects showed higher levels of PG than pre-EVP subjects. The SEM was however large at Month 18 and Month 24 in pre-CC subjects.

In general, EVP compliant subjects had lower levels of NEQ, 3-HPMA, S-PMA, and total NNAL, and higher levels of PG, than the overall study population. Completers had similar levels of NEQ and PG to the whole study population throughout the study. The levels of 3-HPMA, S-PMA and total NNAL in urine of completers was similar to EVP compliant subjects from BL to Month 3, and similar to the whole population from Month 6 to EoS.

The levels of the biomarkers of exposure to carbon monoxide, eCO and COHb, are shown in Fig. 6. In pre-CC subjects, both eCO and COHb levels rapidly decreased from baseline to Month 1, where levels were similar to those in pre-EVP subjects. The mean (± SD) eCO level in all subjects at Month 1 was 8.7 ppm (± 6.5), and steadily decreased to reach 4.1 ppm (± 3.1) at Month 24. The mean (± SD) COHb level in all subjects at Month 1 was 4.33% (± 1.37), and stayed stable during the study; at Month 24, the COHb level in all subjects was 4.27% (± 0.87). Both eCO and COHb levels in EVP-compliant subjects and in completers was similar to the levels in the whole study population.

3.5. Biomarkers of biological effect

No clear, clinically significant tendencies in the changes from baseline were observed during the study for the investigated BoBE (Table 5). WBCs showed a small tendency to decrease, and were 4.2% lower at Month 24 than at baseline. In EVP-compliant subjects, the mean WBCs value was 6.2% lower at Month 24 compared to baseline. The 95% CI for the mean change did not cross 0.00 at Month 24 for the change in WBCs, both for EVP-compliant subjects and all subjects. The changes observed in completers were similar to those in all subjects, for all four BoBE.

3.6. Subjective effects

The overall mean (± SD) MWS-R extended total score was 4.6 (± 6.2) at baseline, and slightly raised to 5.4 (± 5.5) at Month 1. Scores subsequently showed a tendency to decrease, with a mean of 5.3 (± 6.2) at Month 3 and 3.3 (± 4.2) at Month 24 (Fig. 7A). At baseline, pre-EVP subjects reported higher MWS-R scores (5.6 ± 6.7) than pre-CC subjects (2.4 ± 3.7). The scores of the pre-CC subjects peaked to a mean (± SD) of 7.2 (± 6.8) at Month 1, and were then similar to pre-EVP subjects' scores as from Month 2, except for Months 12 and 15, when pre-CC subjects had lower scores than pre-EVP subjects.

Regarding smoking desire, the mean (± SD) overall QSU-Brief total score was 19.2 (± 11.5) at baseline, and decreased until Month 12, when it reached 13.3 (± 5.2). Scores then remained stable, and were at 12.4 (± 5.5) at Month 24 (Fig. 7B). At baseline, pre-EVP subjects scored lower than pre-CC subjects on the QSU-Brief questionnaire, with a difference of 3.6 points between the two groups. The difference between the groups steadily subsided as from Month 2, and by Month 12, both groups reported similar scores.

EVP-compliant subjects reported similar scores to the whole study population, both on the MWS-R and QSU-Brief questionnaires. Completers scored similar to the whole population on the MWS-R questionnaire, throughout the study. Regarding the QSU-Brief questionnaire, completers had lower scores than the whole population and than EVP compliant subjects from BL to Month 3, and similar scores to the whole population for the rest of the study.

4. Discussion

This paper presents data from a clinical study designed to evaluate the safety profile of a typical closed system EVP when used for 24 months in a real-life setting. The study population consisted of subjects who participated in and completed a previous clinical trial, in which they either used a similar EVP, or continued smoking CCs for a period of 12 weeks (Cravo et al., 2016). All subjects enrolled in the current study switched to use commercially available Puritane™, from the last day of the previous trial.

Overall, study participation and compliance to Puritane™ was satisfactory. More than half of all subjects were on study at the Month 12 visit, and 48.8% of subjects completed the 2-year study (102 subjects; "completers"). This is lower than the 57.5% rate of subjects completing the 24-month, real-life study by Polosa and colleagues (Polosa et al., 2014b), however, the subjects in our study had already completed a 3-month clinical trial before this one. Moreover, more than half of the subjects in our study (53.4%) were judged to be compliant (abstinent from CC use for over 80% of all study days).

Few SAEs or withdrawals due to AEs occurred during the 24 months of Puritane™ use, none of which were related to use of the EVP. Headache, nasopharyngitis, cough, sore throat and nicotine dependence (desire to smoke) were the most common AEs, and were more frequently reported early after product switch. Such AEs are commonly experienced in CC smokers switching to use EVPs (Chen, 2013; D'Ruiz et al., 2015; Polosa et al., 2014a; Polosa et al., 2011; Polosa et al., 2014b), or using NRT products (Ferguson et al., 2011; Hjalmarson et al., 1997; Murray et al., 1996; Ossip et al., 2009). The frequency of AEs steadily declined with time, which confirms earlier findings (Caponnetto et al., 2013; Cravo et al., 2016; Polosa et al., 2011). After

Table 4
Mean (SD) vital signs, electrocardiogram and lung function test parameters at baseline and at Month 1, 3, 6, 12, 18 and 24 for the FAS, for EVP-compliant subjects and for completers.

	Baseline	Month 1	Month 3	Month 6	Month 12	Month 18	Month 24
Vital signs	n = 206	n = 184	n = 156	n = 141	n = 114	n = 104	n = 102
	n = 110	*n = 107*	*n = 96*	*n = 90*	*n = 78*	*n = 72*	*n = 71*
	n = 102	n = 102	n = 101	n = 102	n = 102	n = 102	n = 102
Sitting systolic blood pressure (mmHg)	121.5 (12.2)	120.7 (12.6)	119.4 (13.1)	120.5 (11.8)	123.2 (12.3)	122.5 (10.9)	122.4 (11.1)
	121.0 (11.6)	*120.8 (12.7)*	*119.0 (13.7)*	*120.6 (11.4)*	*123.6 (11.8)*	*122.7 (10.8)*	*122.2 (10.4)*
	121 (11.5)	120.6 (13.5)	119.2 (13.4)	121.0 (11.9)	123.2 (12.3)	122.4 (10.9)	122.4 (11.1)
Sitting diastolic blood pressure (mmHg)	72.0 (9.0)	72.2 (9.3)	72.3 (9.6)	72.3 (8.3)	76.1 (9.7)	75.5 (9.2)	75.8 (9.5)
	72.4 (8.4)	*72.4 (9.2)*	*72.3 (9.2)*	*72.2 (8.1)*	*76.8 (8.9)*	*76.9 (8.8)*	*77.2 (9.7)*
	72.0 (8.8)	72.2 (9.4)	72.3 (9.3)	72.1 (8.4)	76.1 (9.2)	75.7 (9.2)	75.8 (9.5)
Sitting pulse rate (bpm)	72.3 (10.8)	71.4 (9.7)	70.5 (9.9)	71.6 (9.9)	71.1 (11.1)	69.1 (10.9)	70.4 (11.4)
	73.2 (11.0)	*70.5 (9.9)*	*70.0 (9.5)*	*71.3 (8.8)*	*71.6 (12.0)*	*68.7 (11.2)*	*71.3 (11.9)*
	72.4 (12.2)	70.7 (10.4)	70.6 (10.5)	71.6 (10.1)	70.4 (10.9)	69.1 (11.0)	70.4 (11.4)
ECG parameters	n = 206	n = 182	n = 156	n = 141	n = 114	n = 104	n = 102
	n = 110	*n = 105*	*n = 96*	*n = 90*	*n = 78*	*n = 72*	*n = 71*
	n = 102	n = 102	n = 101	n = 102	n = 102	n = 102	n = 102
PR Interval (ms)	152.0 (20.9)	151.8 (20.2)	150.3 (19.7)	151.0 (18.9)	151.1 (18.8)	152.4 (20.0)	152.3 (18.7)
	151.0 (20.2)	*151.2 (19.4)*	*149.2 (19.1)*	*151.2 (17.8)*	*152.3 (19.7)*	*153.2 (21.5)*	*154.1 (19.9)*
	151.6 (20.8)	152.5 (19.4)	151.2 (19.6)	151.9 (19.1)	152.5 (18.7)	152.9 (19.8)	152.3 (18.7)
QRS Duration (ms)	88.2 (9.9)	88.8 (9.7)	89.8 (10.2)	87.5 (10.2)	90.4 (10.6)	89.0 (10.4)	90.4 (10.1)
	87.5 (10.3)	*88.9 (9.6)*	*90.1 (10.8)*	*88.4 (11.0)*	*91.9 (10.7)*	*90.4 (11.0)*	*91.2 (10.1)*
	86.9 (10.3)	88.4 (10.3)	89.9 (10.7)	88.2 (10.6)	90.7 (10.6)	89.1 (10.4)	90.4 (10.1)
QTcB Interval (ms)	409.3 (19.2)	406.9 (18.1)	406.9 (19.5)	404.7 (17.7)	404.8 (20.9)	402.5 (21.1)	409.2 (19.3)
	406.9 (17.7)	*405.7 (17.3)*	*406.1 (18.9)*	*403.1 (17.4)*	*404.6 (20.4)*	*401.6 (20.7)*	*408.6 (19.7)*
	407.7 (19.7)	405.8 (19.5)	407.0 (20.2)	404.9 (18.6)	405.0 (21.5)	402.8 (20.9)	409.2 (19.3)
QTcF Interval (ms)	403.1 (17.1)	401.7 (16.0)	402.7 (16.9)	400.4 (15.9)	399.1 (17.8)	398.4 (17.3)	402.3 (16.9)
	400.6 (15.4)	*400.8 (14.8)*	*402.3 (16.4)*	*398.5 (16.5)*	*397.8 (17.1)*	*396.9 (17.2)*	*400.4 (16.4)*
	402.0 (16.9)	401.2 (16.0)	402.9 (17.5)	400.6 (16.1)	399.9 (18.1)	398.5 (17.2)	402.3 (16.9)
Lung function tests	n = 206	n = 184	n = 156	n = 141	n = 114	n = 104	n = 102
	n = 110	*n = 107*	*n = 96*	*n = 90*	*n = 78*	*n = 72*	*n = 71*
	n = 102	n = 102	n = 101	n = 102	n = 102	n = 102	n = 102
FVC (L)	4.552 (1.031)	4.519 (0.991)	4.499 (1.268)	**4.386 (0.985)**	**4.431 (0.988)**	**4.444 (1.001)**	**4.369 (1.007)**
	4.679 (1.010)	*4.625 (0.969)*	*4.606 (0.962)*	*4.571 (0.988)*	*4.559 (0.969)*	*4.573 (0.991)*	*4.511 (0.981)*
	4.546 (1.048)	4.524 (0.993)	4.476 (0.995)	4.451 (1.023)	4.465 (1.008)	4.467 (0.997)	4.369 (1.007)
FEV_1 (L)	3.506 (0.809)	3.486 (0.797)	**3.415 (0.759)**	**3.372 (0.748)**	**3.341 (0.748)**	**3.359 (0.777)**	**3.316 (0.784)**
	3.603 (0.790)	*3.562 (0.804)*	*3.544 (0.748)*	*3.492 (0.739)*	*3.417 (0.735)*	*3.438 (0.765)*	*3.406 (0.773)*
	3.474 (0.834)	3.454 (0.796)	3.404 (0.773)	3.392 (0.783)	3.349 (0.772)	3.372 (0.777)	3.316 (0.784)
FEF_{25-75} (L/sec)	3.090 (1.082)	3.066 (1.094)	**3.003 (1.037)**	**2.956 (0.989)**	**2.795 (1.020)**	**2.841 (1.090)**	**2.835 (1.066)**
	3.182 (1.146)	*3.092 (1.153)*	*3.064 (1.091)*	*2.980 (0.998)*	*2.809 (1.027)*	*2.865 (1.095)*	*2.873 (1.057)*
	3.021 (1.141)	2.966 (1.126)	2.928 (1.088)	2.928 (1.036)	2.770 (1.056)	2.844 (1.101)	2.835 (1.066)
PEF (L/min)	509.34 (120.08)	506.64 (118.93)	497.44 (111.20)	494.02 (112.26)	**490.54 (102.92)**	503.43 (108.04)	**496.38 (108.16)**
	513.02 (114.62)	*511.98 (116.12)*	*511.69 (114.19)*	*510.42 (112.67)*	*495.46 (107.06)*	*514.32 (107.37)*	*507.54 (110.38)*
	512.60 (117.81)	507.53 (108.07)	499.13 (109.47)	498.08 (109.44)	495.30 (106.38)	505.17 (108.29)	496.38 (108.16)

Note: Data for the EVP-compliant subgroup is in grey italic. Data for completers is in grey. A RMANCOVA model was fitted to the lung function test parameters, for the FAS. Values for which the change from baseline was statistically significantly different from zero are highlighted in bold (p-value < .05). n = number of observations.; *Abbreviations*: bpm: beats per minute; FAS: Full Analysis Set; FEF_{25-75}: forced expiratory flow 25–75%; FEV_1: forced expiratory volume in one second; FVC: forced vital capacity; QTcB: QT interval corrected for heart rate using Bazett's formula; QTcF: QT interval corrected for heart rate using Fridericia's formula; PEF: peak expiratory flow; SD: standard deviation.

product switch, the frequency of AEs increased in all subject groups, but to a much greater extent in pre-CC subjects than in pre-EVP subjects (26.9% of pre-EVP subjects reported AEs at baseline (Cravo et al., 2016), and 30.6% at Month 1, whereas for pre-CC subjects, the frequency of AEs was 13.8% at baseline and 45.2% at Month 1). Regarding pre-CC subjects, this is consistent with what was observed in our previous study for subjects who switched to the EVP. Regarding pre-EVP subjects, it suggests that even if they were used to using another EVP, switching product within the EVP category also requires an acclimation time.

The overall frequency of AEs was higher in EVP-compliant subjects (81.8%) and in completers (92.2%), than in the whole study population (76.1%). However, the frequency of AEs reported at each study visit from Month 1 to approximately Month 12 was lower in EVP-compliant subjects and in completers than in all subjects (Fig. 4). In general, completers and EVP-compliant subjects may have better tolerated the product after the switch, hence were more compliant and therefore more inclined to stay on study, or alternatively may have been more motivated to reduce or cease tobacco consumption and continue with the EVP product. Indeed during the first study months, completers used more EVP cartomisers than all subjects, and EVP-compliant subjects rapidly decreased their CC consumption (Fig. 3).

No clinically relevant, product-related findings were observed for the other safety parameters, namely vital signs, ECG and lung function tests. Findings with regards to blood pressure and heart rate confirm those of our previous 12-week study (Cravo et al., 2016), and of another 12-month study with another EVP (Caponnetto et al., 2013; Farsalinos et al., 2016), where no changes were observed after product switch in CC smokers who had normal baseline blood pressure.

Regarding lung function, small, statistically significant decreases from baseline to Month 24 in all four spirometry parameters were observed. These decreases were not judged to be clinically significant. Small but significant decreases in FVC, FEV_1 and FEF_{25-75} were also observed in our previous 12-week study, with decreases being of greater amplitude in subjects who had continued smoking CCs (Cravo et al., 2016). Lung function is maximal at age 20–25 years, and starts declining after age 35 years, at an estimated rate of 25–30 ml/year for FEV_1 (Sharma and Goodwin, 2006). As the mean (± SD) age of our study population was 36.6 (± 10.2) years, the observed declines may at least partly be due to aging of subjects during the two years of the study.

The decline in lung function over time is known to be more pronounced in smokers, even in so-called healthy smokers, than in non-smokers or ex-smokers, and it slows after smoking cessation (Willemse

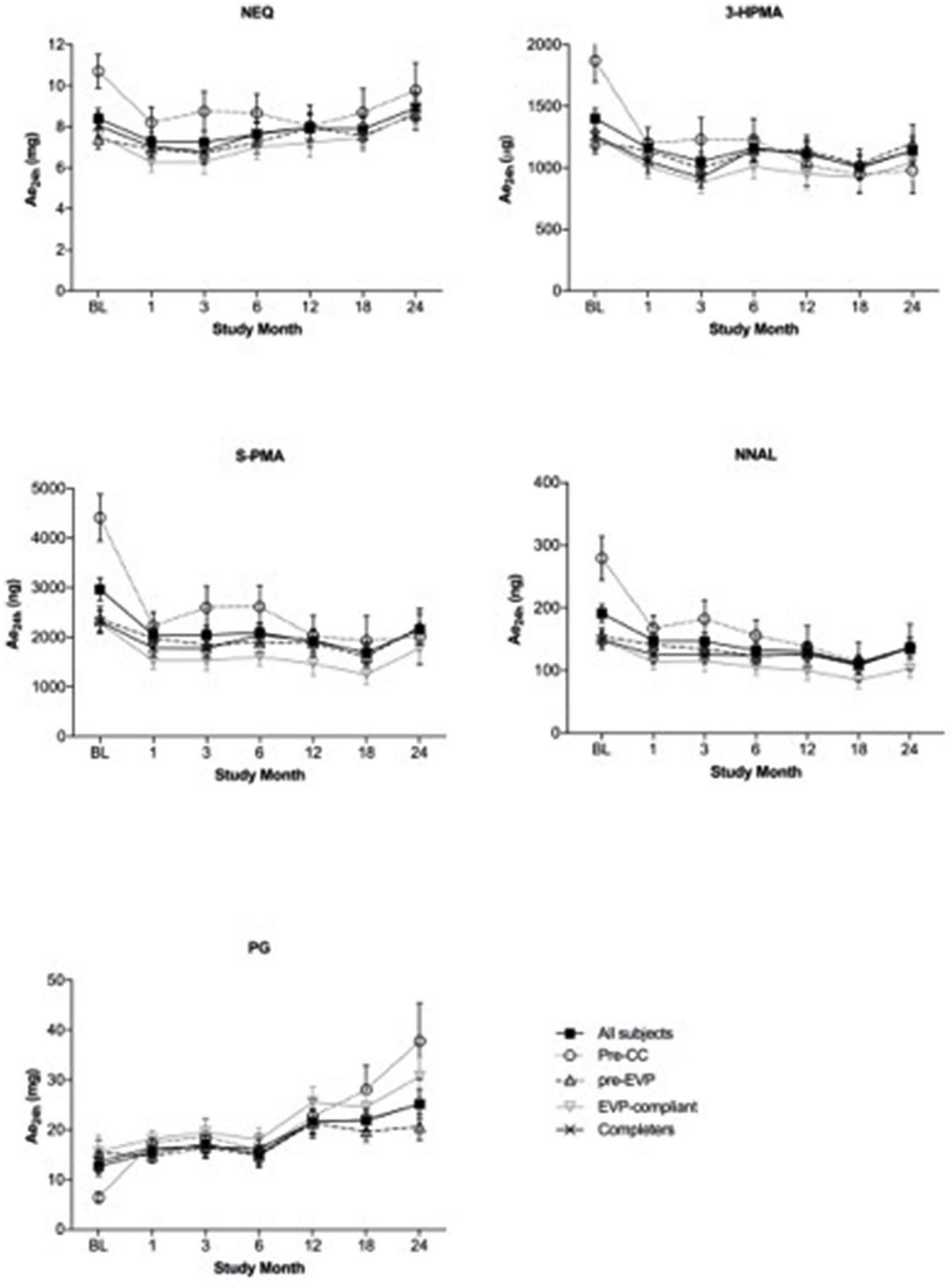

Fig. 5. Mean (± SEM) levels of selected biomarkers of exposure excreted in urine in 24 h (Ae_{24h}). Data are shown for all subjects, for EVP-compliant subjects and for completers, as well as for subjects who had used the EVP (pre-EVP) and those who had used the CC (pre-CC) in the previous clinical study (Cravo et al., 2016).; *Abbreviations*: CC: conventional cigarette; EVP: e-vapour product; 3-HPMA: 3-hydroxypropyl mercapturic acid; NNAL: 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol; NEQ: nicotine equivalents; PG: propylene glycol; SEM: standard error of the mean; S-PMA: S-phenylmercapturic acid.

et al., 2004). In the present study, no group of subjects continuing to smoke CCs was included, therefore a comparison with lung function evolution in subjects who would have continued smoking CCs is not possible within this study. However, in our study, EVP-compliant subjects, who did not use more cartomisers but fewer CCs than the whole study population, showed similar or lower declines in lung function parameters than the overall study population, confirming the positive effect of smoking reduction, even if accompanied by EVP use.

Our results differ from those obtained by Cibella et al. (2016), who reported no changes in FVC and FEV_1, and a small but significant

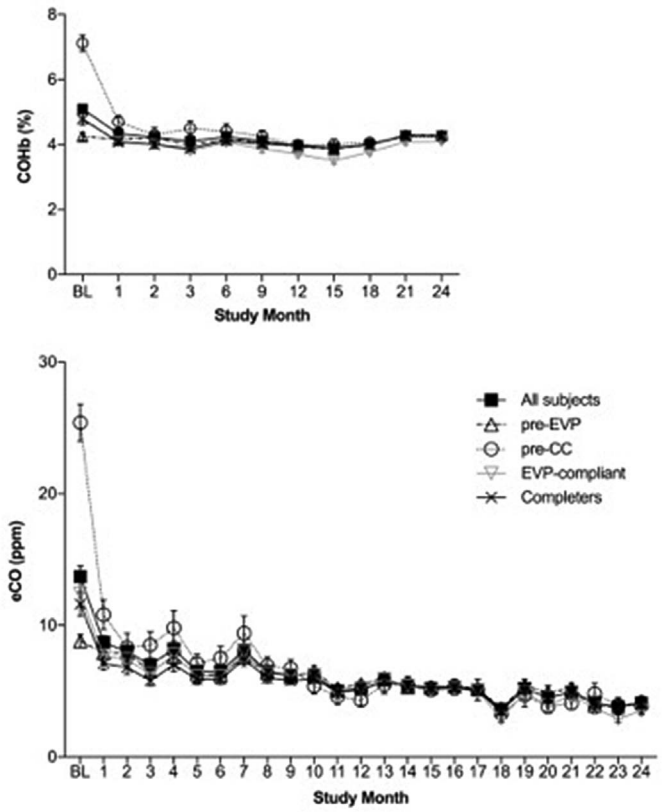

Fig. 6. Mean (± SEM) levels of biomarkers of exposure to carbon monoxide. Data are shown for all subjects, for EVP-compliant subjects and for completers, as well as for subjects who had used the EVP (pre-EVP) and those who had used the CC (pre-CC) in the previous clinical study (Cravo et al., 2016).; *Abbreviations*: CC: conventional cigarette; COHb: carboxyhaemoglobin; eCO: exhaled carbon monoxide; EVP: e-vapour product; ppm: parts per million.

increase in FEF_{25-75}, in smokers (mean age 42.2 ± 12.6 years) switching to exclusive EVP use for one year. Changes in lung function are usually observed over a follow-up of several years, up to several decades (Willemse et al., 2004). The changes observed in our study may thus, at least partly, be due to other parameters such as subjects having been over motivated to perform at the baseline lung function measurements. Currently, little long-term data is available on the effect of EVPs on lung function in order to draw conclusions. Additional, longer term data would be needed, in order to discriminate between a true product effect and a natural age-related decrease in lung function.

In our study, no clinically relevant changes were observed in biomarkers of biological effect. Regarding WBC (a marker of inflammation), data from others have shown that WBC decreases within some days after complete smoking cessation, that smokers have an average of approximately 20% higher WBC than non-smokers (19% in one study and 23.9% in another), and that the level of WBC is related to the number of CPD consumed (Bain et al., 1992; Frost-Pineda et al., 2011; Ludicke et al., 2015). In our study, there was a small trend for WBC to decrease (by 4.2% for all subjects, and by 6.2% for EVP-compliant subjects, at Month 24), which indicates that some degree of WBC decrease would occur and be maintained in the long term with sustained EVP use. A decrease in WBC by 6.6% was also observed in CCs smokers who switched to the EVP in our previous study (Cravo et al., 2016). In both our studies, the expected decrease in WBC of approximately 20% was not reached, likely due to continued CC smoking by subjects. Moreover, Frost-Pineda and colleagues highlighted that other factors such as BMI, race, age and gender, also significantly influence WBC, with BMI being the most important factor (Frost-Pineda et al., 2011). In a second model including urine NEQ, urine NEQ was the most important factor explaining WBC. The subjects in our study maintained their urine NEQ to levels within 75% of their baseline levels (including the pre-CC sub-group; Table S1), which may have been sufficient to prevent further decreases in WBC.

Regarding haemoglobin (a marker of haematology) and HDL and LDL cholesterol (markers of lipid metabolism), no clear and consistent trends were observed, with no clear differences between the whole study population, EVP-compliant subjects and completers. Smoking has been shown to influence the levels of both HDL and LDL cholesterol, with smokers having higher levels of LDL cholesterol, and lower levels of HDL cholesterol, than non-smokers or former smokers (Forey et al., 2013; Frost-Pineda et al., 2011; Ludicke et al., 2015). We would thus have expected to observe a decrease in LDL cholesterol and an increase in HDL cholesterol after the product switch in our subjects, as the CPD decreased to below 1 (Fig. 2). Significant trends may however be difficult to highlight, as similarly to WBC, cholesterol levels are also influenced by many other factors. The main factor explaining HDL cholesterol was BMI, and the main factor influencing LDL cholesterol was age, in models developed by Frost-Pineda and colleagues (Frost-Pineda et al., 2011). In our study population, the ranges of BMI and age was quite large (subjects were aged 22–65 years, with a BMI of 18–35 kg/m^2), which may have hidden an effect of CPD reduction. Moreover, Lowe and colleagues found a significant difference in LDL cholesterol only between non-smokers and smokers of over 20 CPDs. No difference was observed between non-smokers and smokers of below 10 CPDs, and no differences were observed in the level of HDL cholesterol between non-smokers and smokers of over 20 CPD (Lowe et al., 2009). The baseline mean CPD consumption in our study was only 2.5 (6.8 in the pre-CC sub-group and 0.5 in the pre-EVP sub-group), so that the decreased CPD in our study may not have been sufficient to trigger a change in cholesterol levels.

Body weight remained stable during the two years of Puritane™ use. This important result confirms and extends earlier findings from Russo and colleagues, who showed that CC smokers who decreased their CC consumption when switching to an EVP ("reducers") did not gain weight, for up to 52 weeks (Russo et al., 2016). In our study, the mean CC consumption indeed decreased substantially up to a few months after the switch, and subsequently remained below 1 CPD until EoS, without consequences on body weight.

The cartomiser usage also steadily decreased during the first 6–7 months of the study, and subsequently remained stable up to EoS, with however a slight increase from Month 23 to Month 24. A decrease in consumption (measured as cartomisers/day) during the first few months after switching to an EVP was not observed in our previous study, and was observed in a study by Polosa et al. only in subjects who did not significantly reduce their CC consumption (Polosa et al., 2011). In the current study, subjects judged as being compliant to Puritane™ followed the same product usage trend, and used a similar number of cartomisers per day to the whole study population. The observed decrease in cartomisers per day may thus be due to subjects changing their puffing behaviour and taking fewer puffs, or simply to a desire to consume less. Of note, the consumption of CCs decreased by more than 50% during the first 3 months of Puritane™ use, and stayed below one CPD from Month 4. This indicates that subjects did not compensate for the decreased consumption of cartomisers with CC smoking, and were satisfied with Puritane™. Indeed, smoking desire steadily decreased in all subjects throughout the study. Our results on CC consumption are in agreement with Polosa et al. (2011), who measured a decrease in CC consumption following four weeks of EVP use, although not as strong as the decrease seen in Puritane™ users. In our previous study (Cravo et al., 2016), subjects who switched to the EVP also decreased their CC consumption from baseline to Week 1, however, a further decrease was observed only in heavy smokers (with a history of 21–30 CPDs). Some care should be applied when interpreting decreases in CC consumption and smoking desire, as subjects with a higher cigarette consumption and being least satisfied with the study product may have been more

Table 5
Levels of biomarkers of biological effect at baseline (mean absolute values) and mean changes from baseline at Month 1, 3, 6, 12, 18 and 24.

	All subjects (n = 206)				EVP-compliant (n = 110)				Completers (n = 102)			
	n	Mean change	% change	95% CI for the mean change	n	Mean change	% change	95% CI for the mean change	n	Mean change	% change	95% CI for the mean change
Haemoglobin (g/L)												
Baseline	206	147	n/a	146, 149	110	148	n/a	146, 150	102	147	n/a	145, 150
Month 1	184	−1.48	−1.0	−2.48, −0.49	107	−2.70	−1.8	−3.96, −1.44	102	−2.26	−1.5	−3.59, −0.94
Month 3	156	−2.12	−1.4	−3.26, −0.98	96	−2.49	−1.7	−3.98, −1.00	101	−2.24	−1.5	−3.74, −0.74
Month 6	141	0.70	0.5	−0.52, 1.91	90	0.02	0.0	−1.52, 1.56	102	0.00	0.0	−1.43, 1.43
Month 12	114	−0.19	−0.1	−1.63, 1.24	78	−0.83	−0.6	−2.61, 0.95	102	−0.304	−0.2	−1.85, 1.25
Month 18	103	−1.65	−1.1	−3.02, −0.2	71	−1.87	−1.3	−3.59, −0.15	101	−1.66	−1.1	−3.05, −0.27
Month 24	102	−1.63	−1.1	−3.26, 0.0	71	−2.79	−1.9	−4.61, −0.97	102	−1.63	−1.1	−3.26, 0.01
WBC (G/L)												
Baseline	206	7.30	n/a	7.05, 7.55	110	7.17	n/a	6.83, 7.50	102	7.2	n/a	6.85, 7.56
Month 1	184	0.13	1.8	−0.10, 0.36	107	0.12	1.7	−0.19, 0.43	102	0.08	1.1	−0.22, 0.37
Month 3	156	−0.11	−1.5	−0.34, 0.12	96	−0.20	−2.8	−0.48, 0.08	101	−0.17	−2.4	−0.44, 0.11
Month 6	141	0.00	0.0	−0.28, 0.29	90	−0.09	−1.3	−0.46, 0.27	102	−0.07	−1.0	−0.41, 0.26
Month 12	114	−0.07	−1.0	−0.36, 0.22	78	−0.10	−1.4	−0.44, 0.24	102	−0.17	−2.4	−0.47, 0.12
Month 18	103	−0.28	−3.8	−0.63, 0.07	71	−0.31	−4.3	−0.77, 0.16	101	−0.26	−3.6	−0.62, 0.09
Month 24	102	−0.31	−4.2	−0.62, −0.00	71	−0.45	−6.2	−0.83, −0.06	102	−0.31	−4.3	−0.62, −0.00
HDL cholesterol (mmol/L)												
Baseline	206	1.41	n/a	1.35, 1.46	110	1.40	n/a	1.32, 1.48	102	1.40	n/a	1.33, 1.48
Month 1	184	−0.01	−0.7	−0.04, 0.02	107	−0.01	−0.4	−0.04, 0.03	102	0.00	0.0	−0.04, 0.04
Month 3	156	−0.04	−2.7	−0.07, −0.01	96	0.00	0.0	−0.04, 0.04	101	−0.03	−2.1	−0.07, 0.01
Month 6	141	−0.03	−2.1	−0.07, 0.01	90	−0.00	−0.3	−0.05, 0.04	102	−0.03	−2.1	−0.07, 0.01
Month 12	114	−0.06	−4.0	−0.10, −0.01	78	−0.06	−4.4	−0.12, −0.01	102	−0.05	−3.6	−0.09, −0.01
Month 18	104	−0.02	−1.2	−0.06, 0.03	72	−0.01	−0.8	−0.07, 0.04	102	−0.02	−1.4	−0.06, 0.03
Month 24	102	−0.03	−2.1	−0.08, 0.02	71	0.00	0.0	−0.06, 0.06	102	−0.03	−2.1	−0.08, 0.02
LDL cholesterol (mmol/L)												
Baseline	206	3.10	n/a	2.96, 3.23	110	2.96	n/a	2.77, 3.15	102	3.00	n/a	2.81, 3.20
Month 1	184	−0.03	−0.8	−0.09, 0.03	107	−0.06	−1.9	−0.14, 0.03	102	0.04	1.3	−0.05, 0.12
Month 3	156	−0.00	−0.1	−0.08, 0.07	96	0.01	0.3	−0.09, 0.12	101	0.03	1.0	−0.06, 0.12
Month 6	141	0.12	3.9	0.04, 0.21	90	0.07	2.4	−0.03, 0.17	102	0.12	4.0	0.02, 0.22
Month 12	114	0.16	5.1	0.06, 0.26	78	0.15	5.2	0.03, 0.28	102	0.15	5.0	0.04, 0.26
Month 18	104	0.14	4.4	0.02, 0.25	72	0.11	3.6	−0.04, 0.25	102	0.14	4.7	0.03, 0.26
Month 24	102	0.12	3.9	−0.00, 0.24	71	0.10	3.4	−0.05, 0.25	102	0.12	4.0	−0.00, 0.24

Note: Actual values are reported for Baseline.

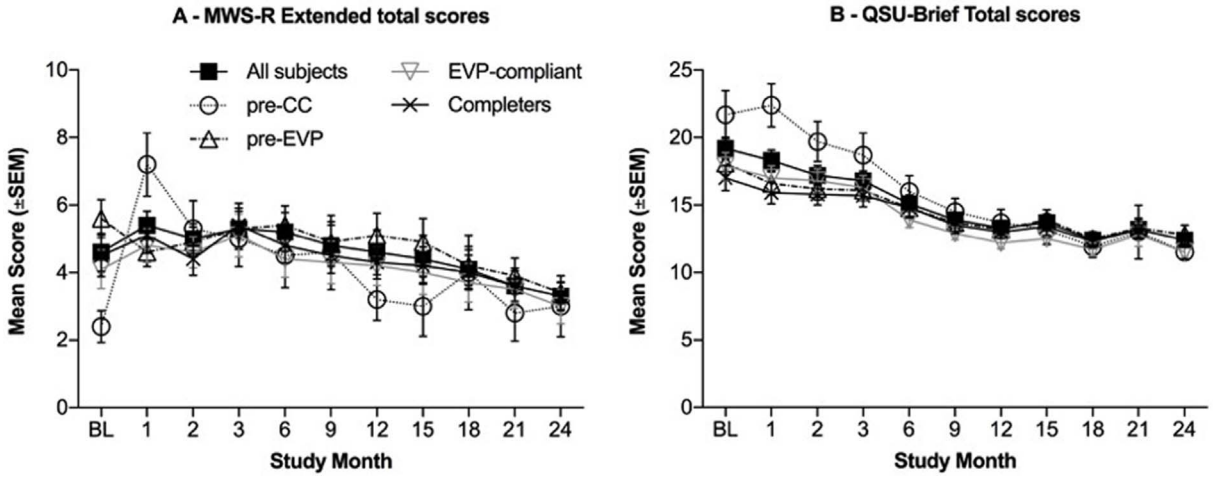

Fig. 7. Subjective effects based on questionnaire data. (**A**) Revised Minnesota Withdrawal Scale (MWS-R) mean (± SEM) extended total scores and (**B**) Brief Questionnaire of Smoking Urges (QSU-Brief) mean (± SEM) total scores. Data are shown for all subjects and EVP-compliant subjects, as well as for subjects who had used the EVP (pre-EVP) and those who had used the CC (pre-CC) in the previous clinical study (Cravo et al., 2016).; *Abbreviations*: BL: baseline; SEM: standard error of the mean.

likely to discontinue from the study during its course. Indeed, smoking cessation rates have been shown to be inversely correlated with the number of CCs smoked daily (Hymowitz et al., 1997).

In pre-CC subjects, the switch to Puritane™ coincided with an increase in withdrawal symptoms at Month 1, which then subsided. This trend was also observed in our previous study (Cravo et al., 2016). Urinary nicotine levels in pre-CC subjects decreased in parallel to the increase in withdrawal symptoms. From Month 1, both the urine nicotine levels and the withdrawal symptoms stabilised. There was even a small tendency for urinary nicotine levels to increase during the course of the study, despite subjects not using a higher number of cartomisers per day. Subjects may have adapted their EVP use behaviour, becoming more familiar with use of the EVP.

As expected, exposure to CO, acrolein, benzene and NNK rapidly decreased after product switch in pre-CC subjects, as shown by their respective measured biomarkers eCO and COHb, 3-HPMA, S-PMA and

NNAL. These results are consistent with data from others, showing decreases in various BoE to HPHCs in smokers switching to EVP use (Goniewicz et al., 2017; McRobbie et al., 2015; O'Connell et al., 2016; Pulvers et al., 2016; Shahab et al., 2017). The extent of decrease in BoE varies depending on the biomarker, and on factors such as the study design (controlled, observational or cross-sectional), the measurement timepoints and the baseline CC consumption. At Month 1, the extent of decrease in urinary BoE from baseline observed in pre-CC subjects was in the same order of magnitude (approximately 17–50%) to the decrease seen in smokers at Week 4 after switching to an EVP, in a study from Pulvers and colleagues (Pulvers et al., 2016). The baseline CPD consumption was similar in pre-CC subjects (6.8 CPD) and in subjects enrolled by Pulvers and colleagues (8.8 CPD), and in both populations, CC consumption had decreased by approximately 50% after four weeks of EVP use.

To our knowledge, our study is the only one so far that monitored urinary BoE over 2 years of EVP use in a real-life setting, showing a sustained reduced exposure to HPHCs, with sustained nicotine levels close to baseline. Exposure to HPHCs had a tendency to increase from Month 23 to Month 24, which is consistent with the observed CC consumption towards EoS. This increase is likely to be a sign of compliance loosening when the end of the study approaches. In EVP-compliant subjects, exposure to HPHCs decreased from baseline to Month 1, and stayed slightly lower than in the whole population throughout the study, which is also consistent with CC consumption data in that group of subjects.

As opposed to exposure to HPHCs that stayed stable, exposure to PG increased during the study, in particular from Month 6 to EoS. This is surprising as EVP usage stayed stable from Month 6 to Month 23, with only a slight increase from Month 23 to EoS. It has been shown that an average 91.7% of the PG present in EVP e-liquids is systemically retained when using an EVP (St Helen et al., 2016). However, PG is a small highly water-soluble molecule, with a relatively short serum half-life in humans (approximately 1–4 h after oral uptake) and rapid total body clearance ("NTP-CERHR Monograph on the Potential Human Reproductive and Developmental Effects of Propylene Glycol. National Toxicology Program. NIH Publication No. 04–4482.," 2004). Although the build-up of toxicologically relevant doses of PG would be unlikely, the exposure to PG from EVP use warrants further research. Currently, we are not aware of any other study that presents data on PG exposure from EVP use over this duration.

In summary, using Puritane™ for up to two years, in a population of CC smokers, was only associated with transient AEs such as headache, cough and sore throat. Such effects are commonly observed in smokers switching to EVPs or to NRT products. No clinically relevant findings were observed, which indicates that Puritane™ product aerosol is well tolerated when used by CC smokers for up to two years. This is consistent with the findings from the recent 2016 Cochrane Review that found no serious side effects in smokers who used EVPs short-to mid-term (up to 2 years) (Hartmann-Boyce et al., 2016). Transient nicotine withdrawal symptoms were only experienced in subjects who were using CCs straight before switching to Puritane™. Biomarkers of exposure data confirm the CC consumption data, with prolonged reduced exposure to HPHCs found in CC smoke. The reduction of CC consumption was not compensated with an elevated consumption of cartomisers, and no increase in mean body weight was observed.

The e-liquid used in our study was available in two different flavours: tobacco and menthol. The variety of commercially available e-liquid flavours is increasing rapidly: in January 2014, a study identified 7764 unique flavour names available online, with 242 new flavours being added per month (Zhu et al., 2014). There are concerns in the public health community that some flavourings may add to the toxicity of e-cigarettes. Studies have found significant variations in the toxicity of different flavoured e-liquids and their resulting aerosols when assessed in *in vitro assays* (Behar et al., 2017; Leslie et al., 2017). Our findings extrapolated to other flavours should therefore be treated with caution.

In conclusion, the use of the EVP for up to 2 years in this study appears to be an acceptable alternative for smokers, with the advantage of reducing the exposure to potentially harmful smoke constituents.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.yrtph.2017.12.010.

Transparency document

Transparency document related to this article can be found online at http://dx.doi.org/10.1016/j.yrtph.2017.12.010.

References

Bain, B.J., Rothwell, M., Feher, M.D., Robinson, R., Brown, J., Sever, P.S., 1992. Acute changes in haematological parameters on cessation of smoking. J. Roy. Soc. Med. 85 (2), 80–82.

Behar, R.Z., Wang, Y., Talbot, P., 2017. Comparing the cytotoxicity of electronic cigarette fluids, aerosols and solvents. Tobac. Contr. http://dx.doi.org/10.1136/tobaccocontrol-2016-053472.

Bullen, C., Howe, C., Laugesen, M., McRobbie, H., Parag, V., Williman, J., Walker, N., 2013. Electronic cigarettes for smoking cessation: a randomised controlled trial. Lancet 382 (9905), 1629–1637. http://dx.doi.org/10.1016/S0140-6736(13)61842-5.

Callahan-Lyon, P., 2014. Electronic cigarettes: human health effects. Tobac. Contr. 23 (Suppl. 2). http://dx.doi.org/10.1136/tobaccocontrol-2013-051470. ii36–40.

Caponnetto, P., Campagna, D., Cibella, F., Morjaria, J.B., Caruso, M., Russo, C., Polosa, R., 2013. EffiCiency and Safety of an eLectronic cigAreTte (ECLAT) as tobacco cigarettes substitute: a prospective 12-month randomized control design study. PLoS One 8 (6), e66317. http://dx.doi.org/10.1371/journal.pone.0066317.

Chen, I.L., 2013. FDA summary of adverse events on electronic cigarettes. Nicotine Tob. Res. 15 (2), 615–616. http://dx.doi.org/10.1093/ntr/nts145.

Cibella, F., Campagna, D., Caponnetto, P., Amaradio, M.D., Caruso, M., Russo, C., ... Polosa, R., 2016. Lung function and respiratory symptoms in a randomized smoking cessation trial of electronic cigarettes. Clin. Sci. http://dx.doi.org/10.1042/CS20160268.

Cox, L.S., Tiffany, S.T., Christen, A.G., 2001. Evaluation of the brief questionnaire of smoking urges (QSU-brief) in laboratory and clinical settings. Nicotine Tob. Res. 3 (1), 7–16. http://dx.doi.org/10.1080/14622200020032051.

Cravo, A.S., Bush, J., Sharma, G., Savioz, R., Craige, S., Walele, T., 2016. A randomized, parallel group study to evaluate the safety profile of an electronic vapour product over 12 weeks. Regul. Toxicol. Pharmacol. 81 (Suppl. 1), S1–S14. http://dx.doi.org/10.1016/j.yrtph.2016.10.003.

D'Ruiz, C.D., Graff, D.W., Yan, X.S., 2015. Nicotine delivery, tolerability and reduction of smoking urge in smokers following short-term use of one brand of electronic cigarettes. BMC Publ. Health 15, 991. http://dx.doi.org/10.1186/s12889-015-2349-2.

D'Ruiz, C.D., O'Connell, G., 2016. Measurement of cardiovascular and pulmonary function endpoints and other physiological effects following partial or complete substitution of cigarettes with electronic cigarettes in adult smokers. Regul. Toxicol. Pharmacol. 87, 36–53. http://dx.doi.org/10.1016/j.yrtph.2017.05.002.

Dollery, C.D., Bankowski, Z., 1983. Safety Requirements for the First Use of New Drugs and Diagnostic Agents in Man. ISBN 9290360125. CIOMS.

Farsalinos, K., Cibella, F., Caponnetto, P., Campagna, D., Morjaria, J.B., Battaglia, E., ... Polosa, R., 2016. Effect of continuous smoking reduction and abstinence on blood pressure and heart rate in smokers switching to electronic cigarettes. Intern Emerg Med 11 (1), 85–94. http://dx.doi.org/10.1007/s11739-015-1361-y.

Farsalinos, K.E., Tsiapras, D., Kyrzopoulos, S., Savvopoulou, M., Voudris, V., 2014. Acute effects of using an electronic nicotine-delivery device (electronic cigarette) on myocardial function: comparison with the effects of regular cigarettes. BMC Cardiovasc. Disord. 14, 78. http://dx.doi.org/10.1186/1471-2261-14-78.

Ferguson, S.G., Shiffman, S., Gitchell, J.G., 2011. Nicotine replacement therapies: patient safety and persistence. Patient Relat. Outcome Meas. 2, 111–117. http://dx.doi.org/10.2147/PROM.S11545.

Flouris, A.D., Chorti, M.S., Poulianiti, K.P., Jamurtas, A.Z., Kostikas, K., Tzatzarakis, M.N., ... Koutedakis, Y., 2013. Acute impact of active and passive electronic cigarette smoking on serum cotinine and lung function. Inhal. Toxicol. 25 (2), 91–101. http://dx.doi.org/10.3109/08958378.2012.758197.

Flouris, A.D., Poulianiti, K.P., Chorti, M.S., Jamurtas, A.Z., Kouretas, D., Owolabi, E.O., ... Koutedakis, Y., 2012. Acute effects of electronic and tobacco cigarette smoking on complete blood count. Food Chem. Toxicol. 50 (10), 3600–3603. http://dx.doi.org/10.1016/j.fct.2012.07.025.

Forey, B.A., Fry, J.S., Lee, P.N., Thornton, A.J., Coombs, K.J., 2013. The effect of quitting smoking on HDL-cholesterol - a review based on within-subject changes. Biomater. Res. 1 (1), 26. http://dx.doi.org/10.1186/2050-7771-1-26.

Frost-Pineda, K., Liang, Q., Liu, J., Rimmer, L., Jin, Y., Feng, S., ... Sarkar, M., 2011. Biomarkers of potential harm among adult smokers and nonsmokers in the total exposure study. Nicotine Tob. Res. 13 (3), 182–193. http://dx.doi.org/10.1093/ntr/ntq235.

Goniewicz, M.L., Gawron, M., Smith, D.M., Peng, M., Jacob 3rd, P., Benowitz, N.L., 2017. Exposure to nicotine and selected toxicants in cigarette smokers who switched to electronic cigarettes: a longitudinal within-subjects observational study. Nicotine Tob. Res. 19 (2), 160–167. http://dx.doi.org/10.1093/ntr/ntw160.

Hajek, P., Goniewicz, M.L., Phillips, A., Myers Smith, K., West, O., McRobbie, H., 2014. Nicotine intake from electronic cigarettes on initial use and after 4 Weeks of regular use. Nicotine Tob. Res. http://dx.doi.org/10.1093/ntr/ntu153.

Hartmann-Boyce, J., McRobbie, H., Bullen, C., Begh, R., Stead, L.F., Hajek, P., 2016. Electronic cigarettes for smoking cessation. Cochrane Database Syst. Rev. 9, CD010216. http://dx.doi.org/10.1002/14651858.CD010216.pub3.

Hjalmarson, A., Nilsson, F., Sjostrom, L., Wiklund, O., 1997. The nicotine inhaler in smoking cessation. Arch. Intern. Med. 157 (15), 1721–1728.

Hughes, J.R., 2007. Measurement of the effects of abstinence from tobacco: a qualitative review. Psychol. Addict. Behav. 21 (2), 127–137. http://dx.doi.org/10.1037/0893-164X.21.2.127.

Hymowitz, N., Cummings, K.M., Hyland, A., Lynn, W.R., Pechacek, T.F., Hartwell, T.D., 1997. Predictors of smoking cessation in a cohort of adult smokers followed for five years. Tobac. Contr. 6 (Suppl. 2), S57–S62.

ICH, 1994. International Conference on Harmonisation. ICH Harmonised Tripartite Guideline ICH E1: the Extent of Population Exposure to Assess Clinical Safety for Drugs Intended for Long-term Treatment of Non-life-threatening Conditions.

Leslie, L.J., Vasanthi, B., Jackson, P., Mabiala Ma Muanda, J.A., Pallett, R., Stillman, C.P., J, M.L., 2017. A comparative study of electronic cigarette vapor extracts on airway-related cell lines in vitro. Inhal. Toxicol. 29 (3), 126–136.

Lowe, F.J., Gregg, E.O., McEwan, M., 2009. Evaluation of biomarkers of exposure and potential harm in smokers, former smokers and never-smokers. Clin. Chem. Lab. Med. 47 (3), 311–320. http://dx.doi.org/10.1515/CCLM.2009.069.

Ludicke, F., Magnette, J., Baker, G., Weitkunat, R., 2015. A Japanese cross-sectional multicentre study of biomarkers associated with cardiovascular disease in smokers and non-smokers. Biomarkers 20 (6–7), 411–421. http://dx.doi.org/10.3109/1354750X.2015.1096303.

Manzoli, L., Flacco, M.E., Ferrante, M., La Vecchia, C., Siliquini, R., Ricciardi, W., ... Group, I.W., 2016. Cohort study of electronic cigarette use: effectiveness and safety at 24 months. Tobac. Contr. http://dx.doi.org/10.1136/tobaccocontrol-2015-052822.

McNeill, A., Brose, L.S., Calder, R., Hitchman, S.C., Hajek, P., McRobbie, H., 2015. E-cigarettes: an Evidence Update. A Report Commissioned by Public Health England. Public Health England, London.

McRobbie, H., Bullen, C., Hartmann-Boyce, J., Hajek, P., 2014. Electronic cigarettes for smoking cessation and reduction. Cochrane Database Syst. Rev.(12), CD010216. http://dx.doi.org/10.1002/14651858.CD010216.pub2.

McRobbie, H., Phillips, A., Goniewicz, M.L., Smith, K.M., Knight-West, O., Przulj, D., Hajek, P., 2015. Effects of switching to electronic cigarettes with and without concurrent smoking on exposure to nicotine, carbon monoxide, and acrolein. Canc. Prev. Res. 8 (9), 873–878. http://dx.doi.org/10.1158/1940-6207.CAPR-15-0058.

Murray, R.P., Bailey, W.C., Daniels, K., Bjornson, W.M., Kurnow, K., Connett, J.E., ... Kiley, J.P., 1996. Safety of nicotine polacrilex gum used by 3,094 participants in the Lung Health Study. Lung Health Study Research Group. Chest 109 (2), 438–445.

Nelson, V.A., Goniewicz, M.L., Beard, E., Brown, J., Sheals, K., West, R., Shahab, L., 2015. Comparison of the characteristics of long-term users of electronic cigarettes versus nicotine replacement therapy: a cross-sectional survey of English ex-smokers and current smokers. Drug Alcohol Depend. 153, 300–305. http://dx.doi.org/10.1016/j.drugalcdep.2015.05.005.

Nides, M.A., Leischow, S.J., Bhatter, M., Simmons, M., 2014. Nicotine blood levels and short-term smoking reduction with an electronic nicotine delivery system. Am. J. Health Behav. 38 (2), 265–274. http://dx.doi.org/10.5993/AJHB.38.2.12.

NTP-CERHR Monograph on the Potential Human Reproductive and Developmental Effects of Propylene Glycol, 2004. National Toxicology Program. NIH Publication No. 04–4482. Retrieved from. https://ntp.niehs.nih.gov/ntp/ohat/egpg/propylene/pg_monograph.pdf.

O'Connell, G., Graff, D.W., D'Ruiz, C.D., 2016. Reductions in biomarkers of exposure (BoE) to harmful or potentially harmful constituents (HPHCs) following partial or complete substitution of cigarettes with electronic cigarettes in adult smokers. Toxicol. Mech. Meth. 26 (6), 443–454. http://dx.doi.org/10.1080/15376516.2016.1196282.

Ossip, D.J., Abrams, S.M., Mahoney, M.C., Sall, D., Cummings, K.M., 2009. Adverse effects with use of nicotine replacement therapy among quitline clients. Nicotine Tob. Res. 11 (4), 408–417. http://dx.doi.org/10.1093/ntr/ntp005.

Polosa, R., Caponnetto, P., Maglia, M., Morjaria, J.B., Russo, C., 2014a. Success rates with nicotine personal vaporizers: a prospective 6-month pilot study of smokers not intending to quit. BMC Publ. Health 14, 1159. http://dx.doi.org/10.1186/1471-2458-14-1159.

Polosa, R., Caponnetto, P., Morjaria, J.B., Papale, G., Campagna, D., Russo, C., 2011. Effect of an electronic nicotine delivery device (e-Cigarette) on smoking reduction and cessation: a prospective 6-month pilot study. BMC Publ. Health 11, 786. http://dx.doi.org/10.1186/1471-2458-11-786.

Polosa, R., Morjaria, J.B., Caponnetto, P., Campagna, D., Russo, C., Alamo, A., ... Fisichella, A., 2014b. Effectiveness and tolerability of electronic cigarette in real-life: a 24-month prospective observational study. Intern Emerg Med 9 (5), 537–546. http://dx.doi.org/10.1007/s11739-013-0977-z.

Pulvers, K., Emami, A.S., Nollen, N.L., Romero, D.R., Strong, D.R., Benowitz, N.L., Ahluwalia, J.S., 2016. Tobacco consumption and toxicant exposure of cigarette smokers using electronic cigarettes. Nicotine Tob. Res. http://dx.doi.org/10.1093/ntr/ntw333.

RCP, 2016. The Royal College of Physicians. Nicotine without Smoke: Tobacco Harm Reduction. RCP, London978-1-86016-600-6.

Russo, C., Cibella, F., Caponnetto, P., Campagna, D., Maglia, M., Frazzetto, E., ... Polosa, R., 2016. Evaluation of post cessation weight gain in a 1-year randomized smoking cessation trial of electronic cigarettes. Sci. Rep. 6, 18763. http://dx.doi.org/10.1038/srep18763.

Shahab, L., Goniewicz, M.L., Blount, B.C., Brown, J., McNeill, A., Alwis, K.U., ... West, R., 2017. Nicotine, carcinogen, and toxin exposure in long-term e-cigarette and nicotine replacement therapy users: a cross-sectional study. Ann. Intern. Med. 166 (6), 390–400. http://dx.doi.org/10.7326/M16-1107.

Sharma, G., Goodwin, J., 2006. Effect of aging on respiratory system physiology and immunology. Clin. Interv. Aging 1 (3), 253–260.

St Helen, G., Havel, C., Dempsey, D.A., Jacob 3rd, P., Benowitz, N.L., 2016. Nicotine delivery, retention and pharmacokinetics from various electronic cigarettes. Addiction 111 (3), 535–544. http://dx.doi.org/10.1111/add.13183.

Vansickel, A.R., Cobb, C.O., Weaver, M.F., Eissenberg, T.E., 2010. A clinical laboratory model for evaluating the acute effects of electronic "cigarettes": nicotine delivery profile and cardiovascular and subjective effects. Canc. Epidemiol. Biomarkers Prev. 19 (8), 1945–1953. http://dx.doi.org/10.1158/1055-9965.EPI-10-0288.

Vansickel, A.R., Eissenberg, T., 2013. Electronic cigarettes: effective nicotine delivery after acute administration. Nicotine Tob. Res. 15 (1), 267–270. http://dx.doi.org/10.1093/ntr/ntr316.

Vardavas, C.I., Anagnostopoulos, N., Kougias, M., Evangelopoulou, V., Connolly, G.N., Behrakis, P.K., 2012. Short-term pulmonary effects of using an electronic cigarette: impact on respiratory flow resistance, impedance, and exhaled nitric oxide. Chest 141 (6), 1400–1406. http://dx.doi.org/10.1378/chest.11-2443.

Walele, T., Sharma, G., Savioz, R., Martin, C., Williams, J., 2016a. A randomised, crossover study on an electronic vapour product, a nicotine inhalator and a conventional cigarette. Part A: Pharmacokinetics. Regul. Toxicol. Pharmacol. 74, 187–192. http://dx.doi.org/10.1016/j.yrtph.2015.12.003.

Walele, T., Sharma, G., Savioz, R., Martin, C., Williams, J., 2016b. A randomised, crossover study on an electronic vapour product, a nicotine inhalator and a conventional cigarette. Part B: safety and subjective effects. Regul. Toxicol. Pharmacol. 74, 193–199. http://dx.doi.org/10.1016/j.yrtph.2015.12.004.

Willemse, B.W., Postma, D.S., Timens, W., ten Hacken, N.H., 2004. The impact of smoking cessation on respiratory symptoms, lung function, airway hyperresponsiveness and inflammation. Eur. Respir. J. 23 (3), 464–476.

Zhu, S.H., Sun, J.Y., Bonnevie, E., Cummins, S.E., Gamst, A., Yin, L., Lee, M., 2014. Four hundred and sixty brands of e-cigarettes and counting: implications for product regulation. Tobac. Contr. 23 (Suppl. 3). http://dx.doi.org/10.1136/tobaccocontrol-2014-051670. iii3–9.