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A randomised, parallel group study to evaluate the safety profile of an electronic vapour product over 12 weeks

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ABSTRACT

A randomised, parallel group clinical study was performed to evaluate the safety profile of an e-vapour product (EVP; 2.0% nicotine) in smokers of conventional cigarettes (CCs) switching to use the EVP for 12 weeks. During the study, no clinically significant product-related findings were observed in terms of vital signs, electrocardiogram, lung function tests and standard clinical laboratory parameters. Adverse events (AEs) reported by EVP subjects were more frequent during the first week after switching to the EVP. The frequency of AEs reduced thereafter and out of a total of 1515 reported AEs, 495 were judged as being related to nicotine withdrawal symptoms. The most frequently stated AEs were headache, sore throat, desire to smoke and cough reported by 47.4, 27.8, 27.5 and 17.0% of subjects, respectively. Only 6% of AEs were judged as probably or definitely related to the EVP. Additional observations in EVP subjects included a decrease in the level of urine nicotine equivalents by up to 33.8%, and decreases in the level of three biomarkers of exposure to toxicants known to be present in CC smoke (benzene, acrolein and 4-[methylnitrosamino]-1-[3-pyridyl]-1-butanone). The decrease in nicotine equivalents coincided with an increase in nicotine withdrawal symptoms, measured by a questionnaire, which subsided after two weeks. The data presented here shows the potential EVPs may offer smokers looking for an alternative to CCs.

1. Introduction

The first electronic vapour products (EVPs), such as e-cigarettes, were launched more than a decade ago, and since then their market has constantly increased and diversified (Zhu et al., 2014).

EVPs may be used by smokers of conventional cigarettes (CCs) as a means to reduce, replace or stop smoking (Berg et al., 2015; Dockrell et al., 2013); however, there is ongoing debate with regards to their efficacy, long-term safety and how such products should be regulated. EVPs are battery-powered devices that deliver an aerosol (popularly referred to as "vapour") to users from an e-liquid. E-liquids typically contain glycerol and propylene glycol (PG) in varying proportions from which the aerosol is generated and may contain nicotine and various flavours. Most EVPs do not contain tobacco, do not require combustion, or generate side-stream smoke. To date, there is data available in the published literature on the chemical composition of e-liquids and aerosols (Etter et al., 2013; Farsalinos et al., 2015a; Goniewicz et al., 2014; Tayyarah and Long, 2014), the efficacy of EVPs to deliver nicotine to users (D'Ruiz et al., 2015; Etter, 2016; Farsalinos et al., 2014, 2015b; Hajek et al., 2014), and on the health and subjective effects of EVPs when used in the short-term (Dicpinigaitis et al., 2016; Farsalinos, 2012; Flouris et al., 2012; van Staden et al., 2013; Vansickel et al., 2010; Vardavas et al., 2012).

Studies on the chemistry of the aerosol generated by EVPs have shown that it tends to contain fewer and lower amounts of selected harmful and potentially harmful constituents (HPHCs) than those typically found in CC smoke. For example, many carbonyls, tobacco

Abbreviations: AE, adverse event; BoBE, biomarker of biological effect; BoE, biomarker of exposure; BP, blood pressure; CC, conventional cigarettes; CI, confidence interval; COHb, carboxyhaemoglobin eCO, exhaled carbon monoxide; CPD, cigarettes per day; ECG, electrocardiogram; EoS, end of study; EVP, electronic vapour product; PG, propylene glycol; NEQs, Nicotine equivalents.

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specific nitrosamines (TSNAs), phenols and volatiles known to be present in CC smoke were not detectable in the machine-generated aerosol of several different EVPs (Tayyarah and Long, 2014). A number of studies have demonstrated that this also results in reduced exposure to these toxicants in users. For example, the urine collected from EVP users has been shown to contain significantly lower amounts of selected toxicants and carcinogenic metabolites than the urine of CC smokers (Hecht et al., 2015). Moreover, both exclusive (EVP only) and dual (concomitant use of CCs and EVPs) users have been shown to have reduced levels of the primary metabolite of acrolein in urine, four weeks after switching from exclusive CC use (McRobbie et al., 2015).

To date, evidence regarding the safety of mid- and long-term EVP use is available from two randomised trials (one of which is published in two separate articles) (Bullen et al., 2013; Caponnetto et al., 2013; Farsalinos et al., 2016) and from three prospective cohort studies (Manzoli et al., 2016; Polosa et al., 2011, 2014) conducted in Italy and in New Zealand. These studies followed participants using EVPs for 6, 12 or 24 months, and identified few, if any, persistent adverse health effects related to EVP use. The main focus of those studies was on smoking cessation or reduction, and none of them assessed exposure to potential toxicants in parallel with safety.

We recently conducted an evaluation of a closed system EVP prototype, which included a nicotine pharmacokinetic (PK) study, and an assessment of safety and subjective effects in healthy, established smokers of CCs, using the product over a short-term period (5-day study) (Walele et al., 2016a, 2016b). In the present study, we evaluated the safety and subjective effects when the same closed system EVP prototype was used by established smokers of CCs for 12 weeks. The safety evaluation included monitoring of adverse events (AEs), vital signs, electrocardiogram (ECG) parameters, lung function tests and clinical laboratory parameters. In particular, parameters reported to change following CC smoking (Frost-Pineda et al., 2011; Lowe et al., 2009; Ludicke et al., 2015), such as the blood level of white blood cells and high-density lipoprotein cholesterol, were also evaluated and referred to as biomarkers of biological effect (BoBE). We also assessed whether EVP use was associated with reduced exposure to selected HPHCs, by measuring the level of biomarkers of exposure (BoE) to these HPHCs in urine, blood and exhaled breath.

2. Material and methods

2.1. Study design

The study was designed as an open-label, randomised, parallel group, clinical trial conducted in two centres in the UK (Covance Clinical Research Unit Ltd, Leeds and Simbec Research Ltd, Wales). A total of 420 adult smokers of CCs were planned to be enrolled (210 subjects per centre, recruited from the local population). The study was performed in ambulatory settings, however, a subgroup of 40 subjects at the Covance centre (referred to as Cohort 2) stayed in confinement for the first study week. Subjects requiring only the ambulatory visits were labelled Cohort 1. The confinement component was included in order to monitor and evaluate study outcomes in subjects using exclusively the allocated products. Subjects in both cohorts were randomised in a 3:1 ratio to either switching to using an EVP prototype or continuing to smoke their own CC brand for a total of 12 weeks.

All relevant study documents were approved by the South East Wales Research Ethics Committee on 13 December 2013. The study is registered at the US National Institutes of Health (ClinicalTrials.gov) #NCT02029196. All procedures were performed in accordance 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 prior to participating in the study.

2.2. Study population

In order to be included in the study, subjects of either gender had to be between 21 and 65 years of age, with a body mass index in the range of 18–35 kg/m^2, to have smoked 5–30 cigarettes per day (CPD) for at least one year (self-reported) and to be in good health (as determined by a medical history, a physical examination, a 12-lead electrocardiogram [ECG], lung function tests and clinical laboratory evaluations). Subjects had to be established smokers, as determined by urinary cotinine levels (a score of 3 and above on a NicAlert™ test strip [Nymox Pharmaceutical Corporation] was considered positive), and exhaled carbon monoxide (eCO) levels (measured through Bedfont Micro + Smokerlyzer; a readout greater than 6 ppm was considered positive). Subjects who had taken or received any form of nicotine replacement therapy (NRT), snuff, or chewing tobacco within 14 days of the pre-study screening visit, or intended to use it during the study, were excluded. Subjects were also excluded if they were trying to stop smoking or were considering quitting, if they had a clinically significant illness such as bronchitis or a history of any clinically significant disorders such as cardiovascular, neurological or respiratory disorders, and also if they had a history of drug or alcohol abuse within two years prior to the study start. Female subjects who were of childbearing potential and who were not willing to use an acceptable contraceptive method for the duration of the study were also excluded. Prior or concomitant use of EVPs was not an exclusion criteria.

2.3. Products used in this study

The EVP prototype used in this study was developed by Fontem Ventures B.V. (Amsterdam, the Netherlands); an illustration is available in Walele et al. (2016a). It consisted of a rechargeable battery (voltage range of 3.0–4.2 V), an atomiser and a capsule (small cartridge) containing e-liquid. The capsules were replaceable and the battery and atomiser were reusable. The wick consisted in a fiberglass string, and the heating coil was a nichrome resistance wire. The base components of the e-liquids used were PG (70–75% w/w), glycerol (18–20% w/w) and water (5% w/w). Subjects randomised to the EVP arm could choose between two different e-liquids, which differed solely in their flavour: a menthol-flavoured e-liquid with 2.0% nicotine (2.7 mg nicotine/capsule) and a tobacco-flavoured e-liquid with 2.0% nicotine (2.7 mg nicotine/capsule). Each capsule was expected to provide 40 to 60 puffs, depending on the user's puffing behaviour.

Subjects randomised to the CC arm used their own usual CC brand (representative of the UK market; mean ISO nicotine yield 0.81 mg and mean ISO tar yield 9.2 mg).

2.4. Study schedule and procedures

Subjects signed the informed consent form and were allowed to familiarise themselves with the EVP at the screening visit (subjects could see and try the EVP). Enrolled subjects then returned to the study site for a second visit within the two weeks prior to the study start to select which EVP flavour they preferred should they be randomised to the EVP, and to collect product use diary cards and urine collection containers. Cohort 1 subjects came to the site for a baseline visit (Day -1) and then at Weeks 1, 2, 4, 6, 8, 10 and 12 (end of study - EoS). Cohort 2 subjects were admitted to the centre (Covance, Leeds) on Day -2, and stayed in confinement until Day 6.

Afterwards, they continued the study in ambulatory settings, and reported to the centre as subjects in Cohort 1.

Baseline procedures were conducted on Day -2 and Day -1 for Cohort 2 subjects, and on Day -1 for Cohort 1 subjects. Baseline procedures included:

- confirmation of eligibility criteria and verification of smoking status by measuring the urinary cotinine levels in a spot urine sample;
- eCO levels and blood carboxyhaemoglobin (COHb) levels;
- body weight and physical examination;
- safety assessment, which included vital signs, a lung function test and a 12-lead ECG;
- blood and urine sampling for haematology, clinical chemistry and urinalysis parameters (Table 1);
- blood sampling for determination of trans-3′-hydroxycotinine/cotinine ratio;
- 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), to measure smoking desire (Cox et al., 2001);
- randomisation of subjects to the EVP or CC arm.

At baseline, subjects randomised to the EVP arm attended a face-to-face session where they were trained on how to use the EVP properly. At baseline, EVP subjects were also provided with an EVP device and with sufficient capsules to last for the duration of the study. They were asked to start using the EVP from Day 1.

Vital signs, 12-lead ECG, lung function, clinical chemistry and clinical haematology were checked on Day 6 (Cohort 2 subjects only) and during Weeks 2, 4, 8 and 12 (all subjects). Body weight was measured on Weeks 4, 8 and 12 for all subjects. Spot urine samples were taken for urinalysis on Day 6 (Cohort 2 only) and in Week 12 (all subjects). Subjects collected and returned 24-h urine samples (acquired the day before the visit and kept in the fridge or in a cool bag) for biomarker analysis on Days 1–6 (Cohort 2) and in Weeks 4, 8 and 12 (all subjects). eCO and blood COHb were analysed at every study visit for both cohorts. AEs were also monitored at every study visit. Subjects answered both the MWS-R and QSU-Brief questionnaires on Days 1, 3 and 5 (Cohort 2 only) and in Weeks 1, 2, 4, 6, 8, 10 and 12 (all subjects). All subjects were requested to record the number of CCs smoked and capsules used throughout the study in diary cards. Diary cards, as well as used capsules, were collected at each visit and subjects were reminded to be compliant to their assigned product.

Fasting was not required for any study visit, except a 6-h fast prior to the screening visit. Subjects were not required to be abstinent from smoking or using the EVP prior to any study visit. Subjects were not allowed to use alcohol 48 h prior to the baseline visit, and from waking time on the other study visit days. On Week

Table 1
Study outcome measures.

Outcome measure	Details of measured parameters
Primary outcomes (safety)	
Adverse events (AEs)	Monitoring of AEs along with severity grades and relationship to product were assessed throughout the study. Subjects could report AEs on their diary cards, and were subject to open questioning on how they felt, at each visit. Moderate and severe symptoms reported on the MWS-R questionnaire were also listed as AEs (with the same severity). All AEs were coded using the Medical Dictionary for Regulatory Activities (MedDRA), version 16.1, 2013. AEs judged by the investigator as being related to nicotine withdrawal, with an onset within the two first study weeks, were identified.
Vital signs	Sitting systolic and diastolic blood pressure, pulse rate and oral temperature, after the subject has been resting for at least five minutes.
12-lead ECG	10-s strips, after the subject has been resting for at least five minutes: heart rate (60/R-R duration), PR interval, QT interval, QTcB, QTcF, QRS duration. A physician performed a clinical assessment of each 12-lead ECG, and categorised them as normal, abnormal-not clinically significant (NCS) or abnormal-clinically significant (CS).
Lung function tests	Sitting spirometry to measure forced vital capacity (FVC), forced expiratory flow 25%–75% (FEF_{25-75}), peak expiratory flow (PEF) and forced expiratory volume in one second (FEV_1). The best of three acceptable attempts is taken as the true measure. Acceptable repeatability is determined according to the American Thoracic Society and European Respiratory Society (ATS/ERS) guidelines (Miller et al., 2005).
Haematology	White blood cell count (WBC), red blood cell count (RBC), haemoglobin, haematocrit (PCV), mean cell volume (MCV), mean cell haemoglobin (MCH), mean cell haemoglobin concentration (MCHC), platelet count, differential WBC.
Clinical biochemistry	Blood levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase, gamma-glutamyl transferase (GGT), sodium, potassium, chloride, calcium, inorganic phosphate, glucose, urea nitrogen (BUN), total bilirubin, creatinine, total protein, albumin, cholesterol (HDL, LDL, and total).
Urinalysis	pH, protein, glucose, ketones, urobilinogen, blood and specific gravity
Secondary outcomes	
Biomarkers of exposure in urine	Amount excreted in 24-h urine (Ae_{24h}) for: nicotine equivalents (NEQs: nicotine, cotinine, nicotine-N-glucuronide, cotinine-N-glucuronide, trans 3'-hydroxycotinine and trans 3'-hydroxycotinine glucuronide); S-PMA; 3-HPMA; PG; total NNAL (NNAL + NNAL-glucuronide).
Other biomarkers of exposure	Exhaled CO, blood COHb
Biomarkers of biological effect	Haemoglobin, PCV, RBC, WBC and cholesterol (LDL, HDL and total)
MWS-R scores	The questionnaire was modified to include only the 15 questions of subject's part. The core total scores (sum of the first nine questions on behaviour) and the extended total scores (sum of all 15 questions) were calculated. Symptoms (*e.g.* angry, irritable, frustrated, depressed, restless, insomnia) were rated from 0 (none) to 4 (severe). Extended total scores may range from 0 to a maximum of 60.
QSU-brief scores	Ten statements such as "I have a desire for a cigarette right now", were rated by a number ranging from 1 (strongly disagree) to 7 (strongly agree). Factor 1 scores (sum of questions 1, 3, 6, 7, and 10 for desire and intention to smoke), Factor 2 scores (sum of questions 2, 4, 5, 8, and 9, for anticipation of relief from negative effects with urgent desire to smoke) and total scores (sum of all questions) were calculated. Total scores may range from 0 to a maximum of 70.

Abbreviations: CO: carbon monoxide; COHb: carboxyhaemoglobin; MWS-R: revised Minnesota Nicotine Withdrawal Scale; QSU-Brief: Brief Questionnaire of Smoking Urges; QTcB: QT interval corrected for heart rate using Bazett's formula; QTcF: QT interval corrected for heart rate using Fridericia's formula; HDL: high-density lipoprotein; LDL: low-density lipoprotein; NNAL: 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (biomarker of exposure for the tobacco nitrosamine 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone, or NNK); PG: propylene glycol; S-PMA: s-phenyl mercapturic acid (biomarker of exposure for benzene); 3-HPMA: 3-hydroxypropyl mercapturic acid (biomarker of exposure for acrolein).

12, a physical examination was performed in addition to the other procedures mentioned above. After all procedures were completed, subjects were provided full verbal smoking cessation advice by the investigator.

2.5. Study outcomes

The primary outcomes measured in this study were the safety parameters, which included vital signs, AEs, ECGs, lung function tests and clinical laboratory parameters. The secondary outcomes included a determination of the level of selected BoE in urine (HPHCs typically found in CC smoke, and for which a BoE in urine has been identified), the level of selected BoBE in blood, and smoking desire and withdrawal symptoms measured by questionnaires. Primary and secondary outcome measures are detailed in Table 1.

2.6. Sample size and assignment to study arm

The sample size for the EVP arm was determined according to the Council for International Organizations of Medical Sciences (CIOMS) guideline indicating that in order to have a power of 95% to detect AEs which occur in the study population at a frequency of 1% (common AEs), an exposed population of 300 subjects would be necessary (Dollery and Bankowski, 1983). A total of 100 subjects were planned to be enrolled into the CC study arm as a control. To allow for an anticipated dropout rate of 5%, a total of 420 subjects were planned to be enrolled.

Randomisation was performed using an Interactive Web Response System (IWRS; Almac Clinical Technologies). Age was selected as a stratification factor (21–39 years or ≥40 years), as frequency and intensity of AEs can be affected by age. The stratified randomisation ensured balanced allocation of both age groups to the two study arms. In addition, the first 40 subjects that agreed to a week-long confinement period were assigned to Cohort 2.

In general, data was stratified by study arm, *i.e.* EVP vs CC. The following additional stratification criteria were used where deemed appropriate: age (21–39 vs ≥ 40 years), sex, baseline smoking history (low: 5–10 vs medium: 11–20 vs high: 21–30 self-reported CPD at baseline) and EVP compliance (EVP-compliant vs less EVP-compliant). EVP compliance was defined as being compliant for 80% or more of the study days. A subject was deemed compliant on any study day if the number of CCs smoked reported on the subject's diary card was equal to zero for that day. On study visit days, a subject was judged compliant if, in addition to having not reported any CCs smoked, the eCO level was ≤8 ppm. Use of CCs in the EVP arm would not generally lead to termination although subjects were reminded to use only the EVP.

2.7. Bioanalytical methods

2.7.1. Primary outcomes

Haematology samples were analysed using the Siemens Advia 2120® or Siemens Advia 120®. Clinical biochemistry samples were analysed using the Roche Modular Analytics System®. Urinalysis parameters were measured using the Siemens Clinitek 500 analyser.

2.7.2. Secondary outcomes

COHb in whole blood samples was assessed with the Roche Cobas B221 Blood Gas Analyser System using a spectrophotometric method (Roche, 2009).

The BoE analysis in 24-h urine samples and urine spot samples was performed by Covance Laboratories, Harrogate, UK, using validated procedures.

For the quantification of nicotine equivalents (NEQ), s-phenyl mercapturic acid (S-PMA), 3-hydroxypropyl mercapturic acid (3-HPMA) and total 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL), urine samples were prepared by solid phase extraction, followed by liquid chromatography coupled with tandem mass spectrometric detection (LC-MS/MS). For the quantification of NEQ and total NNAL, an enzymatic deconjugation was performed prior to the extraction, to liberate the glucuronide forms.

For the analysis of PG, samples underwent derivatisation followed by liquid-liquid extraction. The centrifuged eluates were quantified by liquid chromatography with tandem mass spectrometry (LC-MS/MS).

2.8. Statistical analyses

BP, pulse rate, temperature, ECG parameters, clinical laboratory parameters and questionnaire scores were summarised using descriptive statistics, including 95% confidence intervals (CIs) for ECG parameters and questionnaire scores. A Poisson regression analysis was performed on the incidence rates of AEs occurring from Day 1 onwards. The incidence rate is defined as the frequency with which an AE occurs per subject over the study treatment period. Estimates of the incidence rate intensity per product and 95% CIs of these estimates were calculated based on the regression analysis. Exploratory inferential statistical analyses were performed on lung function test parameters, BoE and BoBE. A repeated measures analysis of covariance (RMANCOVA) model was used to compare changes from baseline in these parameters between the two groups. The model included terms for baseline measurement, sex, age classification, study arm, timepoint, and the interaction between product and timepoint. No adjustments were made for multiple comparisons. An exploratory analysis, investigating whether mean levels of PG in urine was related to the mean number of capsules used was explored through a scatter plot and regression analysis. Statistical significance was set at $\alpha = 0.05$ for all analyses and all statistical tests were conducted using SAS® version 9.3.

3. Results

3.1. Subjects

Out of the 420 planned subjects, a total of 419 were enrolled onto the study and randomised in a 3:1 ratio to the EVP or CC arm. Eleven subjects out of the 419 were excluded prior to any product use. The remaining 408 (Full Analysis Set or FAS) used the study product at least once. Of these 408 subjects, twenty in the EVP arm and one in the CC arm were withdrawn from the study, leading to a total of 387 subjects (94.9% of the FAS) having completed the study (Fig. 1). In the EVP arm, one subject was withdrawn due to non-study related death and two due to an AE (see Section 3.3.1 on AEs).

Subjects' characteristics at screening for the FAS are presented in Table 2, and were similar in both study arms. The majority of subjects reported using 11–20 CPD, and had moderate nicotine dependence based on FTND scores. At baseline, subjects in both study arms had similar CYP2A6 enzyme activities, as shown by mean (95% CI) trans-3'hydroxycotinine to cotinine plasma concentration ratios of 0.297 (0.281–0.314) in the EVP arm and 0.308 (0.279–0.336) in the CC arm.

3.2. Product use and compliance

Subjects in the EVP arm were asked to record the total number of capsules that they started each day, on a diary card. EVP subjects were reminded not to use CCs, but if they did, were also asked to record the number of CC smoked per day.

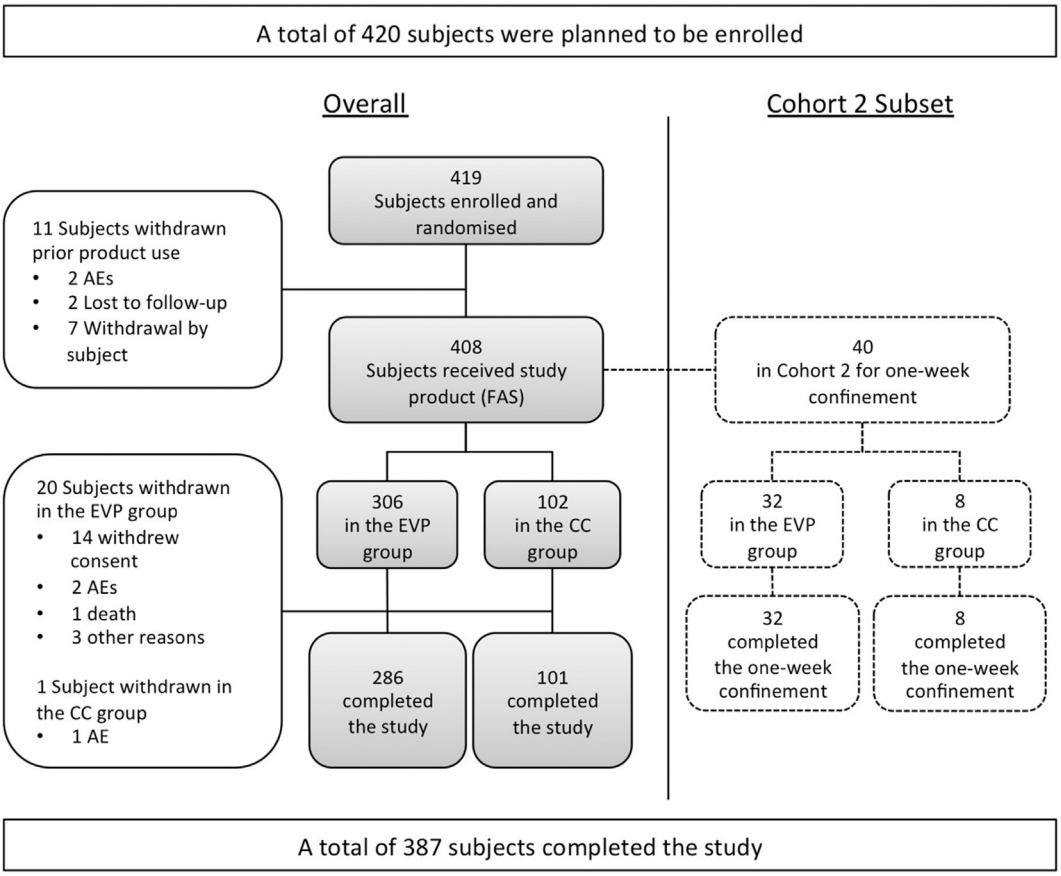

Fig. 1. Study subjects' flow.

Table 2
Subjects' screening characteristics (Full Analysis Set).

	Statistic	All subjects		Cohort 2	
		EVP (N = 306)	CC (N = 102)	EVP (N = 32)	CC (N = 8)
Age (years)					
	Mean ± SD	34.1 ± 10.6	35.1 ± 10.6	34.7 ± 12.2	40.6 ± 15.4
Sex					
Males	n (%)	168 (54.9%)	58 (56.9%)	22 (68.8%)	6 (75.0%)
Females	n (%)	138 (45.1%)	44 (43.1%)	10 (31.3%)	2 (25.0%)
BMI (kg/m^2)					
	Mean ± SD	25.8 ± 3.9	25.3 ± 3.7	25.0 ± 3.1	23.6 ± 4.1
Body weight (kg)					
	Mean ± SD	75.6 ± 13.7	73.9 ± 13.6	75.4 ± 11.5	71.9 ± 14.8
eCO (ppm)					
	Mean ± SD	15.8 ± 6.3	16.7 ± 7.3	15.0 ± 5.4	15.1 ± 4.0
Daily cigarette use history					
5–10 CPD	n (%)	109 (35.6%)	32 (31.4%)	12 (37.5%)	1 (12.5%)
11–20 CPD	n (%)	172 (56.2%)	63 (61.8%)	17 (53.1%)	7 (87.5%)
21–30 CPD	n (%)	25 (8.2%)	7 (6.9%)	3 (9.4%)	0 (0%)
FTND classification					
Mild	n (%)	91 (29.7%)	30 (29.4%)	13 (40.6%)	2 (25.0%)
Moderate	n (%)	173 (56.5%)	55 (53.9%)	14 (43.8%)	6 (75.0%)
Severe	n (%)	42 (13.7%)	17 (16.7%)	5 (15.6%)	0 (0%)
ISO nicotine yield of CCs smoked (mg)					
	Mean ± SD	0.81 ± 0.13	0.81 ± 0.14	0.81 ± 0.15	0.73 ± 0.18
ISO tar yield of CCs smoked (mg)					
	Mean ± SD	9.2 ± 1.5	9.2 ± 1.5	9.0 ± 1.6	8.3 ± 2.0

Abbreviations: BMI: body mass index; CC: conventional cigarette; CPD: cigarettes per day; eCO: exhaled carbon monoxide as measured by the Smokerlyzer device; EVP: e-vapour product; FTND: Fagerstroem Test of Nicotine Dependence (mild dependence is defined as a score of 0–3, moderate dependence as a score of 4–6 and severe, 7–10); ISO: International Organisation for Standardisation; N: number of subjects; SD: standard deviation.

Overall, subjects in the EVP arm used a mean (±SEM) of 3.29 (±0.11) to 4.15 (±0.14) capsules per day over the study weeks. Capsule use reflected the CC smoking history, with higher CPD consumption at baseline being associated with higher capsule use

per day (Fig. 2A).

Capsule use per day during the one-week confinement (Cohort 2) was approximately three times lower than the average use reported by the entire EVP population at the ambulatory visits. Cohort 2 subjects in the EVP group used a mean (±SEM) of 1.07 (±0.13) capsules per day during the first study week.

A total of 123 subjects (40.2%) were classified as "EVP-compliant" (see Section 2.6.), and 183 (59.8%) were classified as "less-EVP-compliant". The proportion of EVP-compliant subjects was highest in the subgroup of subjects with lowest CPD consumption at baseline, and decreased with increasing CPD at baseline. EVP-compliant subjects did not use more EVP capsules than less-compliant subjects (the mean daily capsule use was between 3.16 and 4.16 for EVP-compliant subjects, compared with 3.39–4.15 for less EVP-compliant subjects).

The mean self-reported consumption of CCs in the EVP group was stable over the course of the study, with means (±SEM) ranging from 1.43 (±0.11) to 1.86 (±0.14) CPD at each study week (Fig. 2B). The consumption of CCs by subjects having low and medium CPD at baseline also remained below 2 CPD. The mean CC consumption was higher and more variable in the subgroup with high CPD at baseline (1.64–3.31 CPD over the 12 study weeks). Cohort 2 EVP subjects reported using a mean (±SEM) of 0.49 (±0.08) CPD during the confinement period. No trend was observed in Cohort 2 in the number of CCs smoked during confinement, when stratifying subgroups by CPD at baseline.

Subjects in the CC arm were asked to record the total number of CCs that they smoked on each study day, on diary cards. The mean (±SEM) daily CC use in the CC group over the 12 study weeks ranged from 12.33 (±0.44) to 14.1 (±0.49) CPD (Fig. 2C). While in confinement, Cohort 2 subjects reported fewer CCs smoked per day than the entire CC arm population, with a mean of 3.86 (±0.53) CPD. None of the subjects randomised to the CC arm in Cohort 2 had a high CPD consumption at baseline.

3.3. Safety outcomes

3.3.1. Adverse events

In the EVP group, 271 subjects (88.6%) reported a total of 1515 AEs, and in the CC group, 80 subjects (78.4%) reported a total of 225 AEs. The least square mean (LSM) and 95% CI for the AE incidence rate was at 1.60 (1.55, 1.65) for the EVP group, compared with a rate of 0.79 (0.66, 0.92) for the CC group. Table 3 shows the number and percentage of AEs by severity and by relationship with study product. The proportion of mild, moderate and severe AEs was similar in both groups, with the majority of reported AEs being of moderate severity. None of the AEs in the CC group were judged by the investigator as being related to the product, whereas in the EVP group, 28.2% of AEs were not suspected to be related to the product; 16.2% were unlikely to be related to the product; 49.6% were possibly related to the product; 4.7% were probably related and only 1.3% were judged as almost definitely related to the product.

Headache, sore throat, desire to smoke, cough, increased appetite, nasopharyngitis and irritability were very common AEs (with a frequency, or percentage of subjects reporting the AE, ≥10%) in the EVP group (Table 4). All common AEs (frequency ≥1%) that

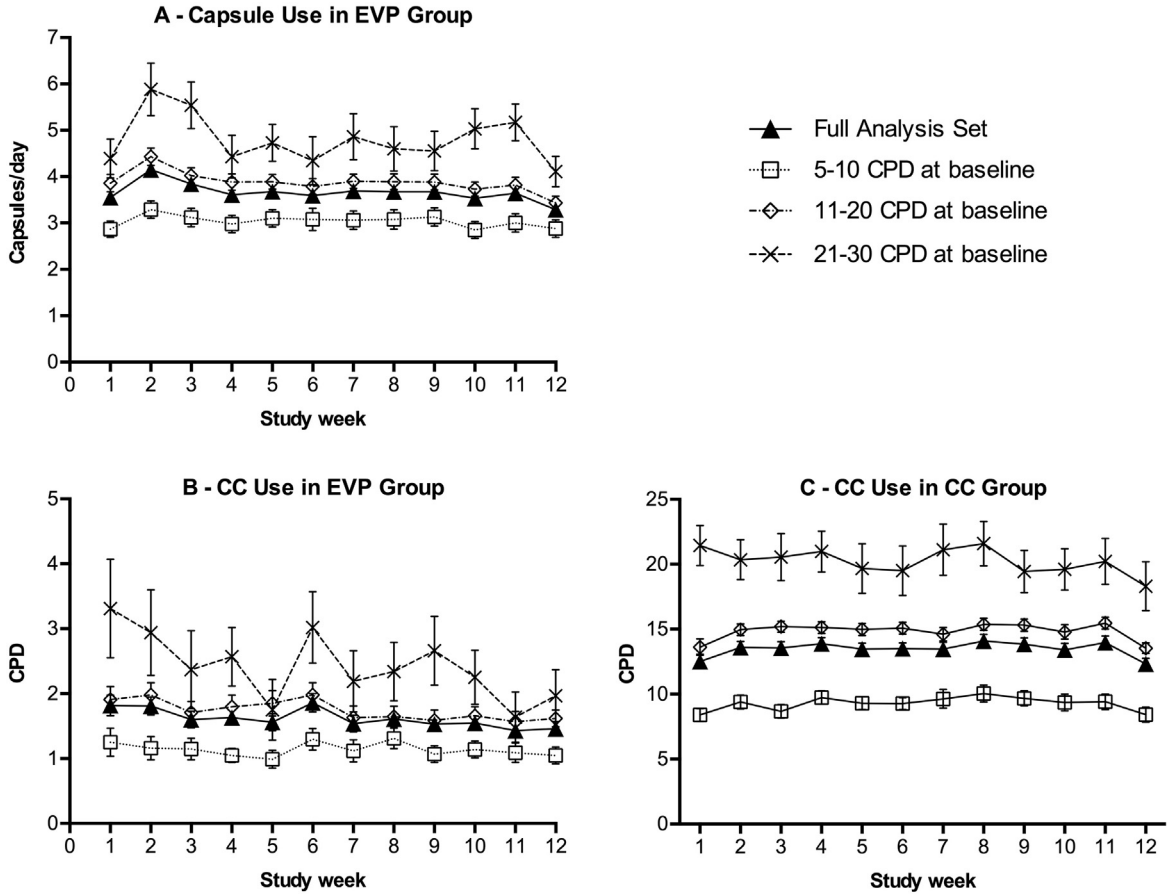

Fig. 2. Product consumption by study week. (A) Mean (±SEM) capsules started per day in the EVP group, (B) mean (±SEM) CCs smoked per day in the EVP group and (C) mean (±SEM) CCs smoked per day in the CC group, based on self-reported product use on subjects' diary cards. Data is shown for the Full Analysis Set, as well as per baseline cigarette consumption. Abbreviations: CC: conventional cigarette; CPD: cigarettes per day; EVP: e-vapour product.

Table 3
Number of AEs (%), by severity and by relationship to product.

	EVP (N = 306)	CC (N = 102)
Total	1515 (100%)	225 (100%)
SAEs (including deaths)	5 (0.3%)	0
AEs leading to study withdrawal	2 (0.1%)	0
AEs by severity		
Mild	449 (29.6%)	64 (28.4%)
Moderate	827 (54.6%)	129 (57.3%)
Severe	239 (15.8%)	32 (14.2%)
AEs by relationship to product		
Almost definitely related	19 (1.3%)	0
Probably related	71 (4.7%)	0
Possibly related	752 (49.6%)	0
Unlikely to be related	246 (16.2%)	0
Not related	427 (28.2%)	225 (100%)

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

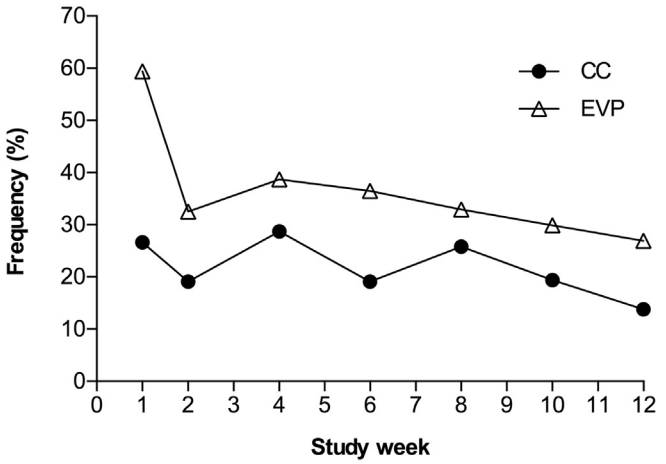

Fig. 3. Overall frequency of AEs (percentage of subjects reporting AEs) in the EVP group and in the CC group, by study week.

occurred in the EVP group, such as nausea (8.8%), anger (7.5%) and disturbance in attention (7.2%) are presented in Supplementary Table S1. In the CC group, the very common AEs were headache (33.3%) and desire to smoke (12.7%), and common AEs included sore throat (8.8%), cough (7.8%), nasopharyngitis (7.8%), upper respiratory tract infection (7.8%), seasonal allergy (5.9%) and hypersensitivity (5.9%). The overall frequency of AEs was stable throughout the study in the CC group between 13.8 and 28.7%, with a tendency to decrease at EoS. In the EVP group, the frequency peaked at Week 1 with a value of 59.4%, and steadily decreased from Week 4, to reach 26.9% at EoS (Fig. 3).

Over the course of the study, five subjects, all assigned to the EVP, experienced a total of five serious AEs (SAEs), none of which were suspected to be related to the product, or resulted in the withdrawal of the subject from the study: a puncture wound to the hand of moderate severity, an acute pancreatitis of moderate severity, a severe headache, a lobar pneumonia of moderate severity and one death due to severe cardiac arrhythmia.

One subject in the EVP arm was withdrawn from the study on Day 47 due to a pregnancy and one subject in the EVP arm was withdrawn as a precautionary measure on Day 33 due to mild anaemia detected at Week 2 (3.53×10^{12}/L) and on Day 33 (3.68×10^{12}/L). As per the investigator, the anaemia was not considered to be related to EVP use, as red blood cell count was below the reference range ($3.99–5.14 \times 10^{12}$/L) already at screening (3.60×10^{12}/L). The red blood cell count was normal at Baseline.

Out of the 1515 AEs reported by subjects using the EVP, 495 (32.7%) AEs, reported by 256 subjects (83.7%), were considered to be associated with nicotine withdrawal symptoms. After exclusion of the 495 AEs related to nicotine withdrawal (all in the EVP group), the incidence rate (LSM [95% CI]) of AEs was still higher in the EVP group (1.20 [1.14, 1.27]) than in the CC group (0.79 [0.66, 0.92]).

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

There were no clinically significant findings or changes from baseline in sitting BP, sitting pulse rate, body temperature or 12-lead ECG parameters at any study visit, with the following exceptions: one subject in the EVP group experienced frequent ventricular ectopic beats from Study Day 28, and several subjects in both groups experienced occurrences of increased heart rate-corrected QT intervals compared with baseline (24 subjects in EVP group and 7 subjects in the CC group had occurrences of increased QTcB > 30 ms and 17 subjects in EVP group and 3 subjects in the CC group had occurrences of increased QTcF > 30 ms). The ventricular ectopic beats resolved without corrective treatment and were not of sufficient concern to withdraw the subject from the study. None of the changes from baseline in QTcB and QTcF values were above 60 ms, and none of the QTcB or QTcF absolute values were above the threshold of 480 ms, at any visit, for both groups.

No clinically significant changes in clinical laboratory parameters, with an onset after the first product use, were observed. Urinalysis revealed a urinary tract infection in three subjects (one in CC arm and two in EVP arm), none of which was considered to be

Table 4
Very common AEs (frequency of ≥10%) in the EVP group, by system organ class, and frequency of these AEs in the CC group.

	EVP (N = 306)			CC (N = 102)		
	Number of subjects	% of subjects	Number of AEs	Number of subjects	% of subjects	Number of AEs
Respiratory, thoracic and mediastinal disorders						
Sore Throat	85	27.8%	119	9	8.8%	9
Cough	52	17.0%	62	8	7.8%	9
Nervous system disorders						
Headache	145	47.4%	372	34	33.3%	72
Infection and infestation						
Nasopharyngitis	34	11.1%	36	8	7.8%	9
Psychiatric disorders						
Desire to smoke	84	27.5%	86	13	12.7%	13
General disorders and administration site conditions						
Irritability	33	10.8%	36	1	1.0%	1
Metabolism and nutrition disorders						
Increased appetite	43	14.1%	43	1	1.0%	1

Abbreviations: AE: adverse event; N: total number of subjects.

related to the study product or led to the withdrawal of the subject from the study.

Body weight remained stable throughout the study in both study groups. In the EVP group, body weight ranged from 47.7 to 115.3 kg for a mean (±SD) of 75.8 kg (±14.0) at baseline, and ranged from 45.8 to 115.3 kg for a mean (±SD) of 76.1 kg (±13.81) at EoS. In the CC group, mean (±SD) body weight was at 74.0 kg (±13.53) at baseline and at 74.1 kg (±13.5) at EoS.

3.3.3. Lung function tests

Lung function test parameters at baseline and the changes from baseline at Week 2, 4, 8 and 12 are shown in Supplementary Table S2, along with the RMANCOVA analysis results. No clinically significant changes from baseline were observed in any lung function test parameter, at any study visit. Forced vital capacity (FVC), forced expiratory volume in one second (FEV_1) and forced expiratory flow 25%–75% (FEF_{25-75}) appeared to decrease during the course of the study by a maximum of 2.5, 2.4 and 1.5% in the EVP group, respectively, and by a maximum of 3.2, 3.0 and 5.8% in the CC group. The RMANCOVA analysis indicated that the decrease was more pronounced in the CC group compared with the EVP group for FEV_1 at Week 8, and for FEF_{25-75} at Week 8 and 12. On the contrary, peak expiratory flow (PEF) appeared to increase during the course of the study by a maximum of 2.5% in the EVP group and a maximum of 3.8% in the CC group. These observed changes in PEF were not different between the two study groups.

The observed increase in PEF was more pronounced in subjects categorised as less EVP-compliant than those categorised as EVP-compliant (data not shown). EVP compliance had no influence on the observed decreases in the other three parameters.

3.4. Biomarkers of exposure

Fig. 4 shows the mean amounts of NEQ, 3-HPMA, S-PMA, total NNAL and PG excreted in urine in 24 h (Ae_{24h}), during the course of the study, for the FAS as well as for Cohort 2 and EVP-compliant sub-groups. Table 5 shows the changes from baseline in the Ae_{24h} for the FAS, along with the results of the RMANCOVA analysis. In the EVP group, the Ae_{24h} of NEQ, 3-HPMA, S-PMA, total NNAL were lower than baseline at all post-baseline timepoints, and as per the RMANCOVA analysis, these changes from baseline were more pronounced in EVP subjects compared with CC subjects. Four weeks after switching, Ae_{24h} for NEQ, 3-HPMA, S-PMA and total NNAL were lower than at baseline by 33.8, 34.5, 54.5 and 43.5%, respectively (Table 5). EVP-compliant subjects displayed even lower levels of these biomarkers than the EVP FAS, at all post-baseline timepoints (Fig. 4). The Ae_{24h} for NEQ, 3-HPMA, S-PMA and total NNAL in EVP subjects appeared to increase from Week 4 to EoS, however, at EoS, levels were still lower than at baseline by 25.5, 29.1, 35.1 and 30.9%, respectively.

Cohort 2 EVP subjects, who were monitored for exclusive use of the EVP for the first week of the study, had lower levels of each of the four BoE than Cohort 2 CC subjects, during the whole first week. The decreases were evident from Day 2 (Fig. 4). At baseline, Cohort 2 subjects from both groups displayed lower levels of NEQ, 3-HPMA, S-PMA and total NNAL than the FAS. The level of these BoE increased after the confinement week, to reach similar levels as the FAS at Week 4, 8 and 12.

Regarding PG, the Ae_{24h} in CC subjects was similar to baseline during the whole study. By contrast, the urine level of PG increased in EVP subjects from Day 2 (Cohort 2), and continued to increase at Week 4, where it was higher than baseline by 182.5% (FAS). The PG increase was proportional to the number of EVP capsules used (data not shown). From Week 4 to EoS, PG appeared to decrease in the EVP group, but was still higher than baseline by 119.2%. As per the RMANCOVA analysis, the PG level was significantly higher in EVP subjects compared with CC subjects at all post-baseline timepoints (Table 5).

As with the four BoE measured in urine, blood COHb and eCO rapidly decreased in subjects switching to use the EVP. At baseline, eCO was at 20.3 ppm (±8.4) in EVP subjects and at 21.3 ppm (±9.2) in CC subjects. In the EVP group, eCO levels dropped to 7.4 ppm at Week 1 and were between 7.6 and 9.0 ppm from Week 2 to EoS (eCO levels in non-smokers are typically ≤6 ppm (Bedfront, 2016)). In the CC group, eCO remained at levels close to baseline during the whole study (21.3–23.3 ppm).

At baseline, COHb was at a mean level (95% CI) of 6.79% (6.60, 6.98) and decreased to 3.61% (3.50, 3.72) on Day 2 (in Cohort 2 EVP subjects). COHb was subsequently measured in EVP subjects at mean levels ranging from 4.06% (3.92, 4.19) to 4.37% (4.22, 4.52) from Week 1 to EoS (COHb levels in non-smokers are typically <2.0% (Buchelli Ramirez et al., 2014)). The mean COHb level in CC subjects remained between 6.70% (6.35, 7.05) and 6.94% (6.55, 7.32) throughout the study. EVP-compliant subjects had similar COHb levels to the whole EVP population.

3.5. Biomarkers of biological effect

The mean levels of haemoglobin, white blood cell count (WBC), low-density lipoprotein (LDL) and high-density lipoprotein (HDL) cholesterol at baseline and the changes from baseline at Week 2, 4, 8 and 12 are shown in Supplementary Table S3, along with the RMANCOVA analysis results. None of the observed changes from baseline in those parameters were judged as being clinically significant.

Mean haemoglobin levels appeared to be lower than at baseline during the course of the study by a maximum of 2.9% in the EVP group and 2.1% in the CC group. The changes from baseline in haemoglobin levels were not different in EVP subjects compared with CC subjects (Table S3). Similar patterns to haemoglobin were observed for haematocrit (PVC) and red blood cell count (RBC; data not shown).

Mean WBC appeared to be lower than at baseline during the study in the EVP group, by a maximum of 6.6%, whereas in the CC group, no consistent changes were observed. The RMANCOVA analysis indicated that at Week 8, the mean WBC was significantly lower in the EVP group compared with the CC group (Table S3). EVP-compliant subjects displayed greater decreases from baseline in mean WBC than less EVP-compliant subjects (not shown).

Regarding cholesterol, the mean level of HDL cholesterol remained stable throughout the study in the EVP group, whereas in the CC group, it appeared to decrease during the study by a maximum of 3.5%. The RMANCOVA analysis indicated that at Week 4 and 8, the observed changes were significantly greater in the CC group compared with the EVP group (Table S3). Mean LDL cholesterol levels appeared to be lower than at baseline in both groups during the study. However, the decreases were small (maximum 3.0% in the EVP group and maximum 1.2% in the CC group), and according to the RMANCOVA analysis, they were not different between the two study groups. Total cholesterol changes (data not shown) were similar to those observed for LDL cholesterol, and none of the changes that were observed during the study were remarkably different between the two study groups. EVP compliance did not appear to influence the changes of cholesterol levels in any direction (data not shown).

3.6. Subjective effects

At baseline, mean MWS-R extended total scores were similar for both study groups (4.3 for EVP subjects and 4.8 for CC subjects). For

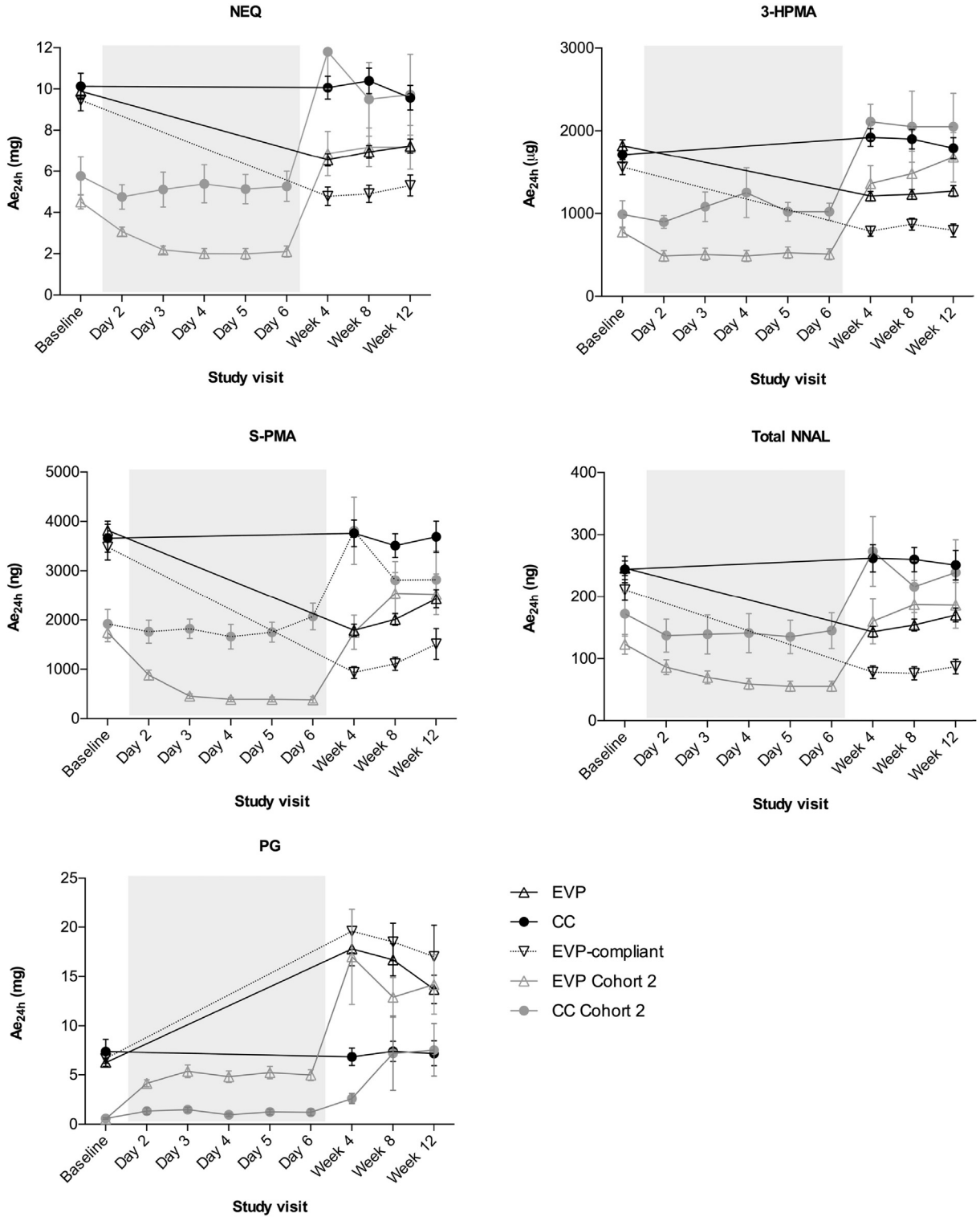

Fig. 4. Mean (±SEM) amounts of selected biomarkers of exposure excreted in urine in 24 h (Ae24h) for all subjects and for Cohort 2 subjects (grey area). Abbreviations: CC: conventional cigarette; EVP: e-vapour product; 3-HPMA: 3-hydroxypropyl mercapturic acid; NNAL: 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol; NEQ: nicotine equivalents; PG: propylene glycol; SEM: standard error of the mean; S-PMA: s-phenyl mercapturic acid.

CC subjects, MWS-R scores varied little during the study. For EVP subjects, scores increased to a mean (±SEM) of 10.2 (±0.5) at Week 1 (Fig. 5A), indicating that EVP subjects experienced more pronounced withdrawal symptoms at Week 1, than CC subjects. At Week 1, MWS-R scores in EVP subjects were higher than at baseline by 139.5%. The scores for EVP subjects subsequently decreased steadily along study weeks to reach 5.8 (±0.4) at EoS (higher than at baseline by 39.5%). The MWS-R core total scores showed similar trends to the extended total scores (not shown).

QSU-Brief total scores were similar for the two groups at

Table 5
Changes from baseline in the amount of biomarkers of exposure excreted in 24 h in urine (Ae24h) at Week 4, 8 and 12, and between-group comparisons of the changes from baseline, for the FAS.

	Changes from baseline								RMANCOVA analysis of the changes from baseline			
	EVP				CC				EVP	CC	Difference (EVP - CC)	
	n	Mean	% change	95% CI for the mean	n	Mean	% change	95% CI for the mean	LSM	LSM	LSM difference	95% CI for the LSM difference
NEQ (mg)												
Baseline	305	9.9	n/a	9.2, 10.6	102	10.1	n/a	8.9, 11.4	n/a	n/a	n/a	n/a
Week 4	295	−3.4	−33.3	−4.0, −2.7	101	−0.1	−1.0	−1.1, 0.9	−3.1	0.3	−3.4	**−4.5, -2.4**
Week 8	282	−2.9	−29.3	−3.6, −2.3	100	0.3	3.0	−0.7, 1.2	−2.7	0.7	−3.4	**−4.4, -2.4**
Week 12	284	−2.5	−25.3	−3.2, −1.9	100	−0.6	−5.9	−1.7, 0.6	−2.4	−0.2	−2.2	**−3.2, -1.1**
3-HPMA (ug)												
Baseline	305	1820	n/a	1680, 1950	102	1710	n/a	1510, 1900	n/a	n/a	n/a	n/a
Week 4	295	−628	−34.5	−762, −495	101	215	12.6	49, 381	−554	217	−771	**−984, -558**
Week 8	282	−590	−32.4	−737, −442	100	192	11.2	7, 378	−527	191	−719	**−933, -505**
Week 12	284	−530	−29.1	−678, −382	100	96	5.6	−128, 320	−471	88	−559	**−773, -345**
S-PMA (ng)												
Baseline	305	3820	n/a	3450, 4190	102	3660	n/a	3090, 4220	n/a	n/a	n/a	n/a
Week 4	295	−2080	−54.5	−2420, −1740	101	114	3.1	−267, 494	−1988	103	−2091	**−2585, -1597**
Week 8	282	−1800	−47.1	−2130, −1470	100	−144	−3.9	−509, 220	−1754	−166	−1588	**−2085, -1091**
Week 12	284	−1340	−35.1	−1740, −943	100	31	0.8	−449, 512	−1307	19	−1325	**−1822, -829**
Total NNAL (ng)												
Baseline	305	246	n/a	222, 269	102	244	n/a	202, 286	n/a	n/a	n/a	n/a
Week 4	295	−106	−43.1	−127, −85	101	18	7.4	−6, 43	−102	20	−122	**−155, -90**
Week 8	282	−95	−38.5	−117, −72	100	15	6.1	−9, 40	−93	17	−110	**−142, -77**
Week 12	284	−76	−30.9	−98, −54	100	6	2.5	−26, 38	−75	8	−83	**−116, -51**
PG (mg)												
Baseline	305	6.3	n/a	5.4, 7.2	102	7.4	n/a	5.0, 9.8	n/a	n/a	n/a	n/a
Week 4	295	11.5	182.5	8.1, 15	101	−0.5	−6.8	−2.5, 1.4	11.1	−0.3	11.4	**6.0, 16.7**
Week 8	282	10.5	166.7	7.2, 13.8	100	−0.03	−0.4	−2.7, 2.7	9.8	0.3	9.5	**4.1, 14.9**
Week 12	284	7.5	119.2	4.6, 10.4	100	−0.2	−2.7	−2.7, 2.3	6.8	0.1	6.8	**1.4, 12.2**

Note: The absolute value is given for Baseline. The LSM of the changes from baseline for each product at each timepoint was calculated, along with the LSMs difference (EVP-CC) and the 95% CI for the difference, using a RMANCOVA model. Cases where the 95% CI for the difference does not include 0.0 are highlighted in bold.
Abbreviations: CC: conventional cigarette; CI: confidence interval; EVP: e-vapour product; LSM: least square mean; 3-HPMA: 3-hydroxypropyl mercapturic acid; NNAL: 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol; NEQ: nicotine equivalents; PG: propylene glycol; S-PMA: s-phenyl mercapturic acid.

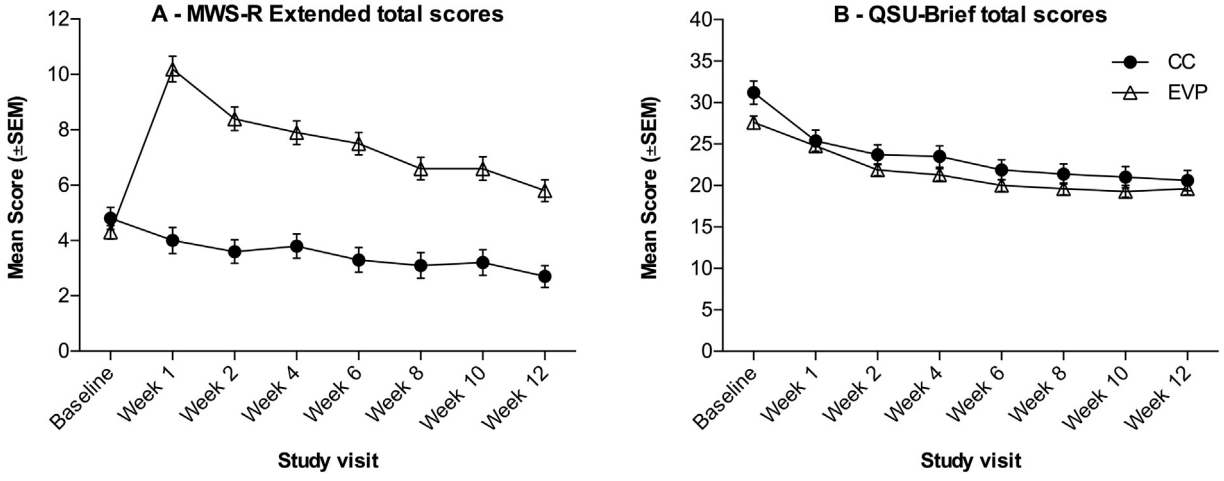

Fig. 5. (A) Revised Minnesota Nicotine Withdrawal Scale (MWS-R) mean (±SEM) extended total scores and (B) Brief Questionnaire of Smoking Urges (QSU-Brief) mean (±SEM) total scores at each study visit. The y-axes do not reflect the whole score ranges. Abbreviations: CC: conventional cigarette; EVP: e-vapour product; SEM: standard error of the mean.

baseline and from Week 1 to EoS (mean of 27.6 for EVP subjects and 31.2 for CC subjects). Scores appeared to steadily decrease throughout the study for both study groups (Fig. 5B), indicating a decrease in desire to smoke during the course of the study. At EoS, scores were lower than at baseline by 28.6% for EVP subjects and by 33.3% for CC subjects. QSU-Brief Factor 1 and Factor 2 scores showed similar trends to the total scores (not shown).

Subjective effects for Cohort 2 subjects are shown in Supplementary Fig. S4. During the first study week in confinement, QSU-Brief total scores and MWS-R extended total scores reported by Cohort 2 subjects using CCs appeared to decrease compared with baseline, whereas they appeared to slightly increase for subjects using the EVP. On Day 5, the mean (±SEM) MWS-R score was 6.8 (±1.2), and QSU-Brief score was 26.70 (±2.90) for Cohort 2 EVP subjects. When EVP subjects got out of confinement, the mean MWS-R score decreased to 2.7 (±0.5) at Week 1, and the mean QSU-Brief score decreased to 18.20 (±1.80). Both scores for Cohort 2 EVP subjects subsequently followed the same trend as Cohort 1 EVP subjects, MWS-R score being however lower for Cohort 2 than for Cohort 1 subjects.

4. Discussion

This study was designed as follow-up to a previous investigation that evaluated nicotine PK, the short-term safety profile and subjective effects of an EVP used by smokers for four days in clinical confinement (Walele et al., 2016a, 2016b). The previous study showed that the EVP had a similar PK and short-term safety profile to a licensed nicotine inhalator. For the current study, the primary objective was to evaluate the safety profile of smokers switching to use the same EVP device for a period of 12 weeks. This evaluation also included the determination of the level of selected BoE in urine, the level of selected BoBE in blood, smoking desire and withdrawal symptoms measured by questionnaires. For comparison purposes, a group of subjects who continued using their usual CC brand for 12 weeks was also included.

During the course of the study, there were no withdrawals due to product-related AEs and only 1.3% of all AEs reported by subjects using the EVP were judged by the Principal Investigator as almost definitely related to the product. Subjects using the EVP reported AEs primarily within the first week after product switch and the frequency of AEs steadily subsided from the second week of product use. Moreover, AEs judged by the Principal Investigator as being related to nicotine withdrawal were identified, and accounted for a third of all AEs in the EVP group. Overall, there were no clinically significant findings or changes from baseline for any of the clinical laboratory evaluations, vital signs, lung function tests or ECG parameters during the course of this study.

The most common AE in both study groups was headache (reported by 33.3% of subjects in the CC group and by 47.4% of subjects in the EVP group). The other common AEs, reported by $\geq$10% of subjects, in the EVP group, in order of frequency were: sore throat, desire to smoke, cough, increased appetite, nasopharyngitis and irritability. Of these, desire to smoke, sore throat, cough and nasopharyngitis were also commonly reported by subjects who continued smoking CCs (refer to Table S1 for a complete list of common AEs). Such AEs may be expected in subjects using a new type of inhaled product, with frequency decreasing towards EoS possibly due to an acclimation effect. Headache and other AEs related to the respiratory and gastrointestinal systems as reported here are commonly experienced in CC smokers switching to use EVPs (including in real-life settings) (Bullen et al., 2010; Cantrell, 2014; Chen, 2013; D'Ruiz et al., 2015; Polosa et al., 2011, 2014) or nicotine replacement therapies in the form of nicotine inhalers or sprays (Bolliger et al., 2000; Kralikova et al., 2009; Moyses et al., 2015; Tonnesen et al., 2012). None of the five SAEs experienced by EVP subjects during the course of the study were judged by the investigator as being related to the EVP. One subject in the EVP group died from severe cardiac arrhythmia on Study Day 42, and was confirmed post-mortem. ECG and vital signs were considered normal on Day -1, Day 14 and Day 28. The investigator used The European Society of Cardiology's online Heartscore tool to assess the subject's total cardiovascular disease (CVD) mortality risk on study Day -1 (ESC, 2016). Based on the subject's age (45 years), gender (male), cholesterol levels (total at 4 mmol/L and HDL cholesterol at 1.1 mmol/L), systolic BP (124 mmHg), and smoking habit (11–20 CPD), the subject had a total CVD risk of 1% (10-year risk mortality). As per the investigator, the death was not related to EVP use.

Body weight was not affected by EVP use (despite increased appetite being reported by 14.1% of subjects in the EVP group, compared with 1.0% in the CC group). In another study where smokers of CCs switched to an EVP and were followed-up for 52 weeks, body weight gain at 12 and 24 weeks was small (2.4 kg and 2.9 kg), but significantly higher than at baseline only in subjects who completely quit CCs. Interestingly, 12 months after the switch to EVP, weight gain was reversed, and body weight was no longer different from baseline. Body weight remained stable in subjects who reduced, but did not completely stop their consumption of CCs (Russo et al., 2016). It thus appears that EVPs at least partially prevent the body weight gains typically associated with smoking cessation.

With regards to lung function, the observed changes in spirometry parameters (FVC, FEV_1, FEF_{25-75} and PEF) were not of clinical relevance. None of the FEV_1 values were below the threshold of 80% of the predicted value. Moreover, those changes were more pronounced in the CC group than in the EVP group. In the light of these findings, it is possible that subjects modified their breathing pattern for spirometry tests during the study. Unpublished data from Polosa et al., suggests that improvements in FEF_{25-75} are observed in smokers of CCs after three months from switching to an EVP (Polosa, 2015). In order to adequately discriminate between a true and false product related effect, longer studies with a greater number of subjects would be required. Moreover, more sensitive lung function testing methodologies than spirometry could be used. In short-term studies, significant acute pulmonary effects after EVP use were detected by impulse oscillometry, while spirometry did not reveal any changes (Flouris et al., 2013; Vardavas et al., 2012).

Current evidence from published literature indicates that CC smokers have significantly higher blood levels of WBC and LDL cholesterol and lower blood levels of HDL cholesterol than non-smokers (Frost-Pineda et al., 2011; Lowe et al., 2009; Ludicke et al., 2015). HDL cholesterol has been shown to increase after smoking cessation (Maeda et al., 2003), whereas haemoglobin, WBC and RBC are shown to decrease (Bain et al., 1992). An increase in HDL levels has purportedly been associated with decreased cardiovascular disease risks (Kapur et al., 2008). In our study, no consistent changes were observed in these BoBE, therefore no firm conclusions could be drawn. However, HDL cholesterol levels appeared to decrease in CC subjects, whereas in EVP subjects they appeared to remain stable. EVP users also appeared to have greater decreases in WBC, haemoglobin and LDL cholesterol than CC smokers, which is consistent with changes observed during smoking cessation.

Regarding nicotine exposure, the level of NEQs in the urine of CC subjects remained stable during the whole study, as expected. NEQs in urine decreased rapidly in EVP subjects (from Day 2 in Cohort 2), and were significantly lower than the CC group at Week 4, 8 and 12. NEQ levels were even lower for the EVP-compliant subgroup, with an approximate 2-fold decrease compared with baseline. In our previous PK study, the mean maximum plasma nicotine concentration (C_{max}) in CC smokers switching to the EVP with 2% nicotine containing e-liquids was 2.5 ng/ml for unflavoured e-liquids and 3.6 ng/ml for flavoured e-liquids. This was reached in 7–10 min, compared with a C_{max} of 21.2 ng/ml reached in 3 min for CCs (Walele et al., 2016a). A decrease in the level of NEQs in the urine of smokers switching to the EVP was therefore expected in a study population consisting of established smokers of CCs with very limited prior experience with EVPs (at screening, only three out of all subjects declared using EVPs). Such a decrease was also observed by Hecht et al. (2015) in exclusive EVP users who had switched for a period between 2 and 36 months; the reported urine nicotine level was 1.5-fold lower in these EVP users compared with regular smokers of CCs.

In EVP subjects, the observed decrease in urine NEQ after product switch coincided with an increase in nicotine withdrawal symptoms (by 139.5% at Week 1), as measured by the MWS-R questionnaire. This was expected in CC smokers switching to a product that was shown to deliver less nicotine to plasma than CCs (Walele et al., 2016a). After two weeks, the symptoms subsided,

which is also commonly observed in smokers completely quitting tobacco use indicating a resolution of withdrawal effects. At EoS, the mean MWS-R score for EVP subjects was still higher than at baseline, but only by 39.5%. In a longer-term published study, product satisfaction (measured by a questionnaire on perception and liking of the product) was assessed as moderate in smokers switching to an EVP for 12–52 weeks, however, nicotine withdrawal related side effects were not frequently reported (Caponnetto et al., 2013). In that study, both product satisfaction and withdrawal side effects were similar in subjects using an EVP with and without nicotine indicating that withdrawal effects are complex symptoms most probably associated with additional factors besides nicotine. In our previous investigation, nicotine withdrawal symptoms were also reduced to a similar extent with and without nicotine, when the EVP was used by CC smokers after overnight smoking abstinence (Walele et al., 2016b).

Despite 27.5% of subjects in the EVP group reporting desire to smoke compared with 12.7% in the CC group, no differences were observed in QSU-Brief scores between the two study groups. This indicates that smokers of CC switching to use the EVP in real-life settings do not experience a higher degree of smoking urges than smokers of CCs continuing to smoke their usual CC brand. During the confinement period, Cohort 2 CC subjects reported lower QSU-Brief scores than EVP subjects. In confinement, EVP subjects were told to refrain from smoking CCs, whereas during the rest of the study, designed to reflect real-life settings, EVP subjects were free to smoke a CC if they experienced moderate or severe desire to smoke. In addition, desire to smoke effects, similarly to other reported AEs, may appear early after product switch, and subside from the second week of EVP use, due to increasing product acceptance. At baseline, QSU-Brief scores were higher than at any other study visits for both study groups. Another confounding factor relates to the possibility of subjects overestimating their desire to smoke when filling the QSU-Brief questionnaire for the first time, despite not being abstinent from smoking.

Unsurprisingly, PG exposure, as shown by urine excretion levels, increased rapidly and was significantly higher in EVP subjects than in CC subjects at all timepoints. Although PG is used as a humectant in CCs, levels are greater in EVP e-liquids due to its use as an excipient. The levels of the other investigated urine BoE, *i.e.* 3-HPMA, S-PMA and total NNAL were observed to decrease in subjects switching to the EVP (by up to 54.5%), with a similar pattern for subgroups to that observed for NEQs. Such decreases may be expected knowing that closed system EVPs similar to the one tested here, generate several lower orders of magnitude of volatiles (including benzene), carbonyls (including acrolein) and TSNAs per puff of aerosol than CCs (Goniewicz et al., 2014; Lauterbach et al., 2012; Tayyarah and Long, 2014). When looking at study groups in general, it clearly appears that the levels of these BoE decrease with decreasing CC consumption. CC smokers had the highest levels, followed by the EVP FAS (who reported smoking means of 1–2 CPD throughout the study) and followed by EVP-compliant subjects who were abstinent from CCs for over 80% of study days. This observation is in line with findings from others, that urine BoE from toxicants present in CC smoke, including 3-HPMA, S-PMA and total NNAL, decrease with decreasing CPD in a dose-dependent manner (O'Connell et al., 2016; Theophilus et al., 2015). Our results are also in broad agreement with findings from Hecht et al. (2015) and McRobbie et al. (2015). The former found that exclusive EVP users had approximately 65-fold, 5-fold and 10-fold lower urine levels of total NNAL, 3-HPMA and S-PMA, respectively, than CC smokers (Hecht et al., 2015), and the latter found that CC smokers switching to using an EVP in a dual mode had 2.5-fold less 3-HPMA in urine than at baseline (McRobbie et al., 2015). In a short-term confinement study by O'Connell et al. (2016), smokers of CCs switching to exclusive EVP use reported greater than 80%, 90% and 60% decreases of 3-HPMA, S-PMA and NNAL respectively in urine compared with baseline, which was very similar to decreases observed in smokers completely quitting tobacco products. For comparison, Cohort 2 subjects in our study reported a 32%, 78% and 55% reduction in these three BoE, respectively, after five days in confinement. These reductions are not as great as those observed in the O'Connell et al. study, mainly because Cohort 2 subjects displayed lower levels of these urine BoEs at baseline. The baseline levels of all five investigated BoE were lower for Cohort 2 subjects (both arms) than for Cohort 1 subjects. This may be due to the small number of subjects in Cohort 2, of which very few (only three) had a high baseline CPD consumption (21–30 CPD). The magnitude of the decrease in exposure to toxicants could be even greater in subjects with a high CC consumption history, switching to use exclusively the EVP. It should also be noted that from Day -2 until the end of confinement, Cohort 2 subjects received a diet without any grilled or smoked meat.

When Cohort 2 subjects got out of confinement, the level of BoE increased in both study groups to levels similar to Cohort 1 subjects, which was most probably due to concomitant use of CCs in the EVP group, and an increase of CC consumption in the CC group. Moreover, when Cohort 2 EVP subjects got out of confinement, a decrease in MWS-R and QSU-Brief scores was observed, which also indicates that subjects may have consumed CCs. Towards EoS, the level of NEQs, 3-HPMA, S-PMA and total NNAL in urine appeared to increase in EVP subjects, which could be explained by EVP subjects smoking more CCs than reported on their diary cards when approaching the end of the study.

Our results, when taken together with other studies reported in the literature, suggest that for acrolein, benzene and NNK, reduced emissions in the aerosol translate into reduced exposure in smokers switching to EVPs, and that this effect can be observed in a short period of time after the switch. In our study, the level of these three BoE showed similar trends to the level of NEQ throughout the study and for all study groups. This study does not inform, however, on the magnitude of exposure to different chemicals in experienced EVP users, who were shown to intake as much nicotine as CC smokers (Etter, 2016). Additional studies should be performed in order to assess if, and how exposure to such chemicals correlates with exposure to nicotine in EVP users.

For future work, a wider selection of measured BoEs should be addressed, alongside formaldehyde and acetaldehyde quantification or any related biomarker. Although these were initially included in our analysis plan, due to a sample instability issue, these BoE could not be quantified. In addition, this study was designed to detect AEs occurring at a frequency of 1%, and not to detect differences between the two arms. Therefore, any observed differences between groups should be interpreted with caution. Finally, a longer study period than the 12-week exposure performed here would provide a better insight at detecting AEs that increase in frequency or severity with time (ICH, 1994).

5. Conclusions

In this study, we have demonstrated that no clinically relevant, product-related safety findings were observed for smokers of CCs switching to an EVP for 12 weeks under real-life settings. AEs reported by subjects switching to the EVP occurred primarily within the first week after switching, and only 1.3% of all AEs reported were considered to be almost definitely related to the product. Up to a third of the all reported AEs in the EVP group were related to nicotine withdrawal symptoms, which were observed to decrease after the first two weeks from product switch. EVP use was associated with significant decreases in exposure to nicotine and other

chemicals such as benzene and acrolein, typically found in CC smoke. Changes were also observed in the level of WBC, haemoglobin, RBC and LDL cholesterol, which although minor, were consistent with those observed after smoking cessation. The data presented in this study shows the potential that EVPs may offer to smokers looking for an alternative to CCs.

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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.2016.10.003.

Transparency document

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