Electronic Cigarettes: Puffing Topography and Self-titration – the Importance of Nicotine Concentration, User Experience and Device Characteristics

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Abstract

Background: Although well documented in tobacco smokers, there is little empirical evidence on self-titration (i.e. adjusting puffing patterns to obtain desired blood nicotine levels) in e-cigarette users. The primary aim was to explore the inter-relationships between e-cigarette puffing topography, nicotine concentrations and e-cigarette characteristics. Other aims were to identify the possible predictors of smoking cessation in smokers attempting to quit using an e-cigarette.

Methods: **Study 1** (N = 12 experienced e-cigarette users) employed a within-participants design and measured how puffing topography differs between high (24 mg/mL) and low (6 mg/mL) nicotine concentrations in 2 separate ad lib vaping sessions. **Study 2** used a mixed-participants design in which e-cigarette naïve smokers (N = 70; 62.9% female) were randomly allocated to a cigalike (18mg/mL) or a tank containing either 18 or 6 mg/mL nicotine concentrations to obtain profiles of puffing topography characteristics associated with each of the aforementioned conditions and how these evolve over a 2-week period (in 3 separate 20-minute ad lib vaping sessions). Puff duration, number and inter-puff intervals (IPI) were recorded along with exhaled carbon monoxide (CO), cigarette dependence, craving and withdrawal, and subjective effects at each session. Participants were given the e-cigarette to take home and asked to record cigarettes smoked per day (CPD) and subjective symptoms. In **Study 3**, Study 2 participants were followed up at 1, 3 and 6 months. Logistic regression analyses explored whether device type, nicotine concentrations, craving reduction, mean puff duration, cigarette dependence and motivation to quit could predict cessation.

Results: **Study 1**: Liquid consumption and puff number were higher and puff duration longer in the low nicotine concentrations condition (all ps < 0.01). There were no

statistically significant differences between conditions in self-reported craving, withdrawal symptoms, satisfaction, hit or adverse effects. Nicotine plasma levels was significantly higher in the high nicotine concentration condition. **Study 2**: Puff duration significantly increased one week post e-cigarette initiation whilst puff numbers and IPI decreased. Cigalikes were associated with longer puff duration, shorter IPI and greater number of puffs; the use of Tank 18 led to longer IPI and shorter puff duration but this was not statistically significant when compared to Tank 6. The Tank 18 and cigalikes were more efficient in reducing craving compared to the Tank 6 at baseline. Participants rated both Tanks (18 and 6 mg/mL) as more satisfying at baseline and Time 1 compared with the cigalike. CPD, CO and nicotine dependence reduced significantly at week 1 then plateaued between week 1 and 2, but did not differ between conditions. **Study 3**: Lower cigarette dependence, greater craving reduction with e-cigarette use reported at baseline and reports of tank use at follow up were significant predictors of cessation at 1, 3 and 6 months respectively. Nicotine concentrations, mean puff duration and motivation to quit at baseline were not significant predictors.

Conclusion: Consistent with the self-titration theory, e-cigarette users engaged in compensatory puffing with lower (6 mg/mL) nicotine concentration liquids, nearly doubling their consumption. Although self-titration was incomplete with significantly higher plasma nicotine levels in the high condition, compensatory puffing was sufficient to reduce craving and withdrawal discomfort. The rapid adjustment in puffing topography and the more erratic puffing regimen in the cigalike condition both lend support to the self-titration theory. Whilst higher nicotine concentrations are more effective in reducing craving, tank systems are preferable in achieving satisfaction. This suggests an important role for device type in providing satisfaction and equally important role for nicotine concentrations to reduce craving. Delineating the

mechanisms by which device types play a role in providing satisfaction and its subtle difference with craving should be one area to concentrate for future research. Those with lower cigarette dependent scores were more likely to quit at 1 month, whilst those who reported greater craving reduction following e-cigarette use in the lab were more likely to have quit at 3 months; the predictive utility of measures of craving reduction at first use can be fostered to inform smoking cessation programmes. The odds of quitting at 6 months were higher for those using a tank device at the time of follow-up compared to those using a cigalike, which is in line with previous studies suggesting that tank systems are associated with greater likelihood of successful cessation. Key findings suggest that e-cigarettes have good potential in helping reduce tobacco smoking.

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List of Acronyms and Abbreviations

ACC	Anterior Cingulate Cortex
Ach	Acetylcholine
ANOVA	Analysis of Variance
ANDS	Alternative Nicotine Delivery Systems
ANS	Autonomic Nervous System
AUC	Area under the curve
BVC	Bottom Vertical Coil
CO	Carbon monoxide
CPD	Cigarette smoked Per Day
C_{max}	Peak nicotine plasma concentrations
CNS	Central Nervous System
CReSS	Clinical Research Support System
CVD	Cardio Vascular Disease
CYP and CYP2A6	Cytochrome P450-dependent monooxygenases
DA	Dopamine
DSM-IV	Diagnostic and Statistical Manual of Mental Disorder
E-cigarette	Electronic cigarette
fMRI	Functional Magnetic Resonance Imaging
FTCD	Fagerström Test for Cigarette Dependence
GABA	Gamma-aminobutyric acid (γ-aminobutyric acid)
HPHC	Harmful and Potentially Harmful Constituents
HSD	Honestly Significant Difference
IPI	Inter-puff Interval
KO	Knock-out
LoQ	Limit of Quantification
MAO	Monoamine Oxidase
MAOI	Monoamine Oxidase Inhibitor
min	minute
mg	milligram
mL	millilitre
MPSS	Mood and Physical Syndrome Scale
MTSS	Motivation to Stop Scale
NA	Noradrenaline
NAcc	Nucleus Accumbens
NAChR	Nicotinic Acetylcholine Receptors
ng	nanogram
NMJ	Neuromuscular Junction
NRT	Nicotine Replacement Therapy product
OFC	Orbito-Frontal Cortex
OH-cot/cotinine	ratio of 3-hydroxycotinine to cotinine
PAH	Polynuclear Aromatic Hydrocarbon
PET	Positron Topography
PG	Propylene Glycol
PK	Pharmacokinetic
PNS	Peripheral Nervous System
ppm	Concentrations in part per million

RCT	Randomised Controlled Trial
T_{max}	Time to the maximum nicotine plasma concentration
TSNAs	Tobacco Specific N-nitrosamines
VG	Vegetable Glycerin
VTA	Ventral Tegmental Area

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Author's contribution

The original idea for Study 1 was conceived by Dr Lynne Dawkins & Professor Olivia Corcoran; the design and methodology were fully operationalised by Dr Dawkins, Professor Corcoran and the author of this thesis. The author has contributed substantially to the operationalisation of Study 1 and the pilot study. All subsequent studies, Study 2 and 3 were conceived by the author with input from Dr Dawkins and the supervision of Dr Soar. Data collection and analyses for the pilot study, 2 and 3 were conducted solely by the author, whilst Study 1 required the presence of 2 other researchers Dr Dawkins and Professor Corcoran. The plasma extraction was conducted by the author under Professor Corcoran's supervision. To facilitate the inter-intra-reliability measurement for the frame-by-frame time analyses, both Dr Anita Polton and the author analysed all the video recordings for the pilot study; whilst for Study 2, Miss Corrine Lacey analysed approximately 20% of the video recordings and the remaining 80% were analysed by the author. The author takes full responsibility for and ownership of the interpretation of the data in its entirety.

At the time of writing, two publications and various conference proceedings have derived from the current thesis for which the author is the second (Dawkins, Kimber, Doig, Feyerabend & Corcoran, 2016; Kósmider, Kimber, Kurek, Corcoran & Dawkins, 2017) and first author (see appendix 24 for listed conferences).

CHAPTER I

THE BIOLOGICAL VERSUS THE PSYCHO-BEHAVIOURAL MODEL OF SMOKING ADDICTION: AN INTRODUCTION TO E-CIGARETTES

Brief History of Tobacco Use

As a genus of the Solanaceae family, the tobacco plant contains more than 50 species; of these, Nicotiana Tabacum, the principal source of tobacco, first originated in South and Central America, prior to its introduction into Europe (Stewart, 1967). Chewed, snuffed, burnt or consumed as a beverage, the custom of using tobacco leaves and inhaling the smoke, stems from religious ceremonies by the Mayans who attributed healing powers to the plant (Doll, 1999) and the Aztecs who credited it for its godly puissance (Elferink, 1983). Early accounts of the medicinal uses of tobacco and its therapeutic applications to cure a number of ailments from ulcerated abscesses, earaches, constipation to healing wounds and burns, were documented in the 1500s and beyond (Hajdu & Vadmal, 2010; Stewart, 1967). Although in the early 1600s, concerns were raised vis-à-vis its widespread and indiscriminate use, these were not sufficient to warrant its removal from pharmacopoeias (Charlton, 2004). However, at the end of the fifteenth century, the medicinal use of tobacco was soon replaced by the ritual of smoking for pleasure which spread to the Caribbean Islands ports leading to its further propagation into Europe and the East by the Spaniards and the Portuguese. Subsequently, Jean Nicot de Villemain a French ambassador to Lisbon, convinced that the plant held medicinal properties, introduced it to the French court in 1560 and to Portugal, whilst Sir Walter Raleigh propagated pipe smoking in England in 1578 (Corti, 1931 cited in Doll, 1999).

Following the original work of a French chemist Vauquelin, a colourless and viscous alkaloid, nicotine (named after the French ambassador Jean Nicot), was first isolated from the tobacco plant in 1828 by Posselt and Reimann. This discovery furthered the understanding of the pharmacological potency of tobacco and the realisation of the potential dose-dependent toxicity of nicotine (Stewart, 1967).

Cigarette smoking superseded pipe tobacco, cigars and snuff to only become popularised following the invention of the cigarette-rolling machine in 1880 which allowed their commercialisation (Dani & Balfour, 2011). The first tobacco companies namely Philip Morris & Co, Imperial Tobacco in the UK, and in the US, American Tobacco Company, R.J. Reynolds, Ligget & Myers Tobacco Company, Lorillard, and British-American Tobacco started to emerge. Tobacco became a protected crop and free cigarettes were included in soldiers' rations. By the end of World War II, cigarette smoking saw such a dramatic increase that its prevalence amongst adult males reached 80% of the UK population (Doll, 1999). By then, the realisation of the adverse health effects of tobacco smoking started to emerge (Doll, 1999). Through correlational and epidemiological studies the link between tobacco smoking and cancer was becoming apparent. The large predominance of smokers amongst lung cancer patients (Doll & Hill, 1950; Hoffman, 1931) and alarming mortality rates caused by coronary disease and various cancers amongst smokers (Hammond & Horn, 1958) came to light, despite significant efforts by the tobacco industry to suppress adverse media coverage and influence public perceptions (Brandt, 1990; TIRC, 1954). Public awareness and the growing consensus vis-á-vis the harmful effects of smoking peaked at the beginning of the 1960s with the Royal College of Physicians report in 1962 followed by the first US surgeon General report in 1964, both suggesting a causal link between smoking and lung cancer, other lung and heart diseases and calling for the implementation of public

health measures to reduce smoking rates (Royal College of Physicians of London, 1962; Stanhope et al., 1964); this would eventually lead to a ban on cigarette advertisements in 1965 and 1970 in the UK and the US respectively, and the introduction of health warnings albeit timid, on packs of cigarettes (Action on Smoking and Health, 2015; Brandt, 1990). This was followed by the foundation of anti-tobacco groups such as ASH (Action on Smoking and Health) by a lawyer called John Banzhaf III who following a court hearing secured free air time on radio networks for the advertisement of anti-smoking messages (Brandt, 1990). This period marked the beginning of a shift toward a broad consensus about the health risks associated with smoking.

Harms and costs of smoking

Today, there is greater recognition that tobacco smoking is the leading cause of preventable disabilities and mortalities. The differences in mortality rates in continuing smokers are consistently greater compared to ex- and non- smokers (Doll, Peto, Boreham, & Sutherland, 2004). Smoking has been responsible for the premature death of approximately 6 million people worldwide every year, including 80% in lower income countries and 600,000 non-smokers from exposure to environmental tobacco smoke (The Tobacco Atlas, 2010). Should the current trends continue, it is forecasted that the death toll may rise to one billion in the twenty-first century (Cancer Research UK, 2014). Despite its known harmful effects, the current smoking prevalence remains at approximately 7.6 million which represents 15.6% of the UK population (Office for National Statistics, 2017). Although this represents a significant and encouraging decline, with smoking rates continuing to increase in low and middle income countries, the global death toll is predicted to increase (Cancer Research UK, 2014). In the UK, smoking is responsible for around 100,300 deaths annually, and, in England alone, an approximate 474,000 hospital admissions between 2015 and 2016 (Office for National

Statistics, 2017). In England, smoking is estimated to cost an approximate £11 billion annually. However, this figure is likely to be an underestimation due to a wide range of long term illnesses which impact on the social care system combined with the costs related to policing the illicit tobacco trade (Department of Health, 2017).

Smoking is the leading cause of cardiovascular disease, Chronic and Obstructive Pulmonary Disease (COPD) and deaths related to cancer, and the extent of its adverse direct and indirect (environmental tobacco smoke) health consequences are extensively documented. Smoking causes harms through multiple and complex mechanisms. By exposing target sites within the body to more than 7 000 chemicals from which many are known human carcinogens (Pryor, Stone, Zang, & Bermúdez, 1998), it increases the risks of developing pneumonia, Cardiovascular Disease (CVD) (Ambrose & Barua, 2004) such as emphysema, chronic bronchitis, and exacerbates asthma symptoms (Center for Disease Control and Prevention, 2010). While smoking is more often associated with lung cancer, there is mounting evidence which suggests that exposure to tobacco smoke may lead to various other cancers, including but not exhaustive, cancer of the larynx, oesophagus, mouth, kidneys, cervix, bladder and so on (United States Department of Health and Human Services, 2014). By convention, tobacco smoke is characterised by 2 phases, the tar (or particulate) which is long-lasting and the gaseous phase which is short lived (Pryor et al., 1998). Although, nicotine is present in both phases (Hoffmann & Hoffmann, 1997), epidemiological data suggest no causal link between nicotine and cancer (Shields, 2011); it is the tar and exposure to carcinogens in the smoke that are associated with the harms of smoking (Center for Disease Control and Prevention, 2010). To enable the establishment of toxicological profiles and the assessment of risks related to the exposure of tobacco smoke, its most potent constituents have been identified. Tobacco smoke comprises a highly complex mixture

of i) chemical compounds for example, tobacco-specific N-nitrosamines (TSNAs) and polynuclear aromatic hydrocarbons (PAHs) (Hoffmann & Hoffmann, 1997), ii) chemicals specific to the tobacco leaves and iii) non-tobacco specific organic compounds which are the products of the combustion process (such as aldehydes) (Fowles & Dybing, 2003; Jefferson Fowles, Bates, & Noiton, 2000). Carbon monoxide (CO), nitrogen oxides and other gaseous ingredients play an important role in CVD (Kjeldsen, Thomsen, & Astrup, 1974). Amongst the harmful compounds present in the tobacco smoke, 1,3-butadiene represents the greatest risk of developing cancer (Fowles & Dybing, 2003) whilst cyanide, arsenic, and the cresols increase the risk of CVD (Smith & Fisher, 2001). Of equal concern, other metals, TSNAs, and PAHs have been identified as harmful chemicals in tobacco smoke while acrolein and acetaldehyde are known respiratory irritants (Talhout et al., 2011).

Pharmacological and non-pharmacological factors, and nicotine delivery

Many lines of evidence suggest nicotine to be the primary addictive component of tobacco smoking, and the main contributor of continued use despite the known harmful consequences (Balfour, Wright, Benwell, & Birrell, 2000; Dani & De Biasi, 2001; Dani & Heinemann, 1996; Gaetano Di Chiara, 2000). In fact, despite the well-known harmful effects of smoking and early suggestions that smoking cessation significantly reduces the risks of smoking-related death and disease (Hammond & Horn, 1958), and despite the fact that annually, a third of smokers in the UK make a quit attempt, the rate of long term successful attempts remain quite low (Royal College of Physicians & Group, 2016). The large discrepancy between desire to quit and cessation success is testimony of the addictive nature of tobacco use. Failed attempts to market nicotine free cigarettes (United States Department of Health and Human Services, 2014) further supports the concept that nicotine is the primary reinforcer of smoking

behaviours due to its addictive properties. The notion that nicotine dependence drives smoking behaviour is based upon the premise of drug-seeking and -taking behaviours (Dani & Balfour, 2011). Many definitions of addiction have been proposed, thus for the purpose of clarity, the term 'addiction' as referred to throughout is based on Professor Robert West and Dr Jamie Brown's Theory of Addiction and is quoted verbatim:

> "Addiction is a chronic condition involving a repeated powerful motivation to engage in a rewarding behaviour, acquired as a result of engaging in that behaviour that has significant potential for unintended harm. Someone is addicted to something to the extent that they experience this repeated powerful motivation" (West & Brown, 2013, pp.15-18).

Although some argue for a distinction between 'addiction' and 'dependence' (West & Brown, 2013), since they both describe similar neurochemical and psychological processes and involve characteristics of loss of control over drug self-administration (US Surgeon General, 1988), these terms are used interchangeably in the current thesis.

Nicotine: Principal mechanisms of actions in the brain

In conjunction with its addictive properties, the mechanisms underpinning the interactions of nicotine with brain receptors have been a focus of research with the central assumption that the binding of nicotine to specific brain receptors is the key mediator of tobacco dependence and positive reinforcement (Leshner & Koob, 2015). The resemblance of nicotine molecules to the natural neurotransmitter, acetylcholine (ACh) allows it to bind easily to and activate nicotinic ACh receptor (nAChR) subunits in the extracellular cleft allowing a flow of ions (Dani & De Biasi, 2001). These receptor subunits are ligand-gated ion channels and are ubiquitous in the central nervous system (CNS), autonomic division of the peripheral nervous system (PNS) (McKay, Placzek, & Dani, 2007) and at the neuromuscular junction (NMJ) (Millar & Gotti, 2009;

Millar & Harkness, 2008) accounting for the complexity and multiplicity of the effects of nicotine. In the autonomic division of the peripheral nervous system (PNS), nicotine raises heart rate and blood pressure whilst causing skin blood vessels constriction and releasing adrenaline from the medulla adrenal glands which mediate physiological and biochemical stress responses (Al-Safi, 2005; Grassi, Seravalle, Calhoun, Giannattasio, Marabini, & Del Bo, Mansia, 1994). Neuronal nAChR subunits vary in binding characteristics, their heteromeric (that is, α (2-7) β (2-4) and homomeric (for example α7) types can form various combinations and differ in pharmacological and biophysical properties (Wonnacott, Sidhpura, & Balfour, 2005), which in turn differentially influence their affinity and potency of agonists and antagonists (Grilli, Parodi, Raiteri, & Marchi, 2005). These nAchR undergo three conformational states, i) open (or active), ii) closed (or non-conducting) and iii) desensitised in which receptors are rendered unresponsive following a prolonged and repetitive stimulation (Quick & Lester, 2002). Agonists binding to the nAChR subunits occur via both stimulation and deactivation of these receptors and a complex mechanism of desensitisation. Nicotinic AChRs' permeability to Na^{2+} and Ca^{2+} through local depolarisation allow the modulation of neurotransmitters release (Fucile, 2004). The opening of the ion channel creates a change at the conducting pathway passing through the membrane through reversal potential and allowing a flux of positively charged sodium and potassium ions (Dani & De Biasi, 2001). A further change of the state of the receptors causes the ion channel to close rendering the receptors in a resting desensitised phase. Desensitisation therefore corresponds to the gradual closure of these receptors due to continuous exposure to nicotinic agonists. Animal studies suggest that desensitisation is produced by repeated activation of the nAChR, and repetitive or prolonged exposure to nicotine leads to an increased number of nicotinic receptor binding sites known as upregulation

(Buisson & Bertrand, 2001; Gentry & Lukas, 2002); this is also evidenced in post-mortem studies of brains of human smokers (Benwell, Balfour, & Anderson, 1988; Breese et al., 1997). Other animal studies suggest that chronic nicotine injections cause a proportion of low affinity nAChRs to convert into high affinity receptors (Romanelli, Öhman, Adem, & Nordberg, 1988). In contrast, an excess of nicotine may lead to down-regulation or to a loss of nAChR functional activity. For instance, a study found that in rats (weighing 200-250g), chronic infusion of 1 mg/kg/h of nicotine over a period of 10 days dramatically affected the function of nAChR subtypes in the CNS, with a down-regulation of nAChR in the hippocampal synaptosomes (Grilli et al., 2005). Thus, the binding of nicotine, mediation and modulation of these nAChR release are dose- and time-dependent (Grilli et al., 2005; Ulrich, Hargreaves, & Flores, 1997), which has significant implications for the role played by nicotine in tobacco tolerance and dependence. In fact, studies have found that levels of nicotine present in smokers' blood are able to activate and desensitise multiple nAChRs and that whilst initial applications increase activities of DA neurons, prolonged exposure will cause desensitisation of these nAChRs which may explain smokers' reported biases for their first tobacco cigarette in the morning over later cigarettes of the day (Pidoplichko, DeBiasi, Williams, & Dani, 1997).

Of all the neuronal nAChR subtypes, the subunit with the highest affinity for nicotine is the α4β2 and has therefore been of key interest in this area of research. Indeed, whilst genetically modified mice lacking β2 receptors ('knockout' (KO) mice) fail to engage in nicotine-seeking behaviour, mice which have been engineered to have hypersensitive α4 subunits, display nicotine seeking behaviour (Maskos et al., 2005). Although most preclinical studies suggest that β2 receptors activation is necessary for the reward pathway and nicotine reinforcement (Picciotto et al., 1998); by selectively

activate α4 nicotinic acetylcholine receptors with low doses of agonist, it is possible to induce nicotine reward, tolerance, and sensitization (Tapper et al., 2004). Administration of low nicotine doses (usually insufficient to activate other nAChRs) into selectively activated α4 receptors have shown to induce nicotine related reward in mice (Tapper et al., 2004). Likewise, in other studies, in which the α4 and β2 subunits were selectively re-expressed in the ventral tegmental area (VTA) exclusively, KO mice also displayed prompt nicotine self-administration (Pons et al., 2008); while others demonstrated that attenuating the α4 nAChR subunit function in the ventral mid-brain can lead to an increase in nicotine intake (Peng et al., 2017). Data obtained from human smokers also provide convincing evidence for the importance of the α4β2. A study utilising Positron Emission Tomography (PET) scanning found that the smoking of a tobacco cigarette increases the occupancy of nicotinic receptor subunits α4β2 (Brody et al., 2006), remarkably, a subsequent study found that in habitual smokers, the smoking of a denicotinised cigarette can also lead to an increase in the aforementioned receptors albeit to a much lesser extent (Brody et al., 2009). This suggests that factors other than nicotine such as psychomotor or other ingredients in the smoke my also affect these receptor subunits, which questions the assumption of nicotine as the sole component to the pharmacological effects of smoking.

Other nAChR subunits with high affinity to nicotine have been identified. The α6β2 nAChR subunits are widely distributed in the mesolimbic DA system but restricted mainly to the ventral tegmental (VTA) and Substantia Nigra pars compacta DA neurons (Pons et al., 2008) and have generated great interest in nicotine research. Using transgenic mice in which the α6 nAChR subunits were engineered to be hypersensitive to ACh and nicotine, a study found that selectively activating the α6 nAChR leads to stimulation of action potential firing of DA neurons in the VTA and

enhances synaptic plasticity, which is critical in the development of nicotine dependence (Berry, Engle, Mcintosh, & Drenan, 2016). This is in line with a separate study in which the α6 nAChR subunits were re-expressed in the VTA of KO-mice; these mice displayed acute self-administration of nicotine versus saline (Pons et al., 2008), suggesting an important role of the α6 nAChR subunits in the VTA in nicotine self-administration behaviour. Others found that nicotine can stimulate the α6 nAChR subunit at lower concentrations than is required for nicotine induced activation, which may partly explain how low doses of nicotine may reinforce smoking (Salminen et al., 2007). The ability of nicotine to stimulate the α6 at low concentrations may be explained by the formation of α4α6β2 nAChR subunits in the midbrain dopamine (DA) areas (McKay et al., 2007).

The actions at the neuronal level are extremely complex and dynamic, and, given that receptor subtypes differ in their ability to respond to nicotine in both activation and desensitisation phases (Olale, Gerzanich, Kuryatov, Wang, & Lindstrom, 1997), these actions strongly relate to the pattern and level of nicotine delivery (Rose, Behm, Westman, & Coleman, 1999).

On the other hand, receptor sub-types with low affinity, for instance the α7 receptor subunit, by virtue of its inverse relationship with nicotine may offer a promising route for smoking cessation medicine. For instance, selectively stimulating this receptor may diminish nicotine reward and ultimately the motivation to self-administrate nicotine (Brunzell, Mcintosh, & Papke, 2014).

Altogether, research studies suggest that nicotine can regulate and activate several nAChRs subtypes, releasing ACh at the presynaptic axon terminals. Several lines of evidence suggest that chronic exposure of nicotine differentially affects the function of these release-regulating nAChR subtypes and these nAChR subunits have high

importance for the self-administration of nicotine (Pons et al., 2008). Although other receptors are expressed in the CNS (amongst others α2 and α3), in this section, the focus has been on primary receptors that are expressed in great quantity and interact with DA neurons in the VTA and nucleus accumbens (NAcc) since dopamine appears to hold a central role in mediating addiction.

Nicotine as a positive reinforcer: Mesolimbic dopamine reward pathways

The areas of the brain that have been identified to act as mediators of nicotine discrimination are the prefrontal cortex, the ventral striatum and the hippocampus (Ando et al. 1993; Miyata et al. 1999; 2002; Rosecrans and Meltzer 1981). The complex mechanisms of action and effects exerted by nicotine is underpinned by its ability to promote the release of multiple neurotransmitters, which produce multiple effects on the CNS and PNS varying from mood modulation and arousal to pleasure (Benowitz, 2008). Because nicotine alters the concentrations of multiple neurotransmitters, its administration triggers many physiological responses including acute increased heart rate and blood pressure, vasoconstriction in the skin (Grassi et al., 1994) and coronary arteries (Balfour et al., 2000). Aside from the release of noradrenaline (NA) (Clarke & Reuben, 1996) and glutamate (McGehee et al., 1995) at pre- and post-synaptic levels and GABA (Yang, Criswell, & Breese, 1996), nAChRs chiefly promote the release of ACh (Reid, Lloyd, & Rao, 1999) and DA (Wonnacott et al., 2005) and, the core pathway which has been advanced to explain the actions and effects of nicotine associated with the reinforcement of nicotine addiction is the mesolimbic dopamine (DA) reward pathway (Leshner & Koob, 2015; Tanner, Chenoweth, & Tyndale, 2015). It is proposed that nicotine is able to stimulate dopamine (DA) secreting cells in the NAcc by attaching to ACh receptors in the ventral

tegmental area (VTA) of the mid-brain which project to the limbic structures. These projections may involve the locomotor excitatory properties of nicotine and its ability to reinforce self-administration (Corrigall, Coen, & Adamson, 1994; Di Chiara, 2000; Leshner & Koob, 2015). Studies have found that animals are capable of learning to self-stimulate electrodes implanted in the DA reward pathways (Bozarth, Pudiak, & KuoLee, 1998); however, pre-treatment with nicotine to enhance DA overflow in the NAcc was found to reduce the threshold previously required to evoke a reward response; this suggests nicotine long-term exposure causes desensitisation of the nicotinic receptors in the mesolimbic DA system (Pidoplichko et al., 1997). Located at the terminal field of the mesolimbic DA system, the NAcc is comprised of two major anatomically distinct subdivisions called the shell and the core (Zham & Brog, 1992) which seemingly play complementary roles central to nicotine dependence (Balfour, 2004). Much of the evidence available on the reinforcing properties of nicotine can be found in studies in which the delivery of nicotine is paired with a stimulus that is engineered to become a conditioned stimulus. For example, animal studies found that responses to a visual conditioned stimulus were enhanced by increases in DA overflow in the NAcc (Donny et al., 2003). In rats, Donny (1999) has also reported robust nicotine self-administration when lever pressing for nicotine was paired with a light cue (Harvey et al., 2004). Functional Magnetic Resonance Imaging (fMRI) studies looking at the effects of smoking related cues, also highlight how the mesolimbic system is implicated in nicotine discrimination. In a study in which abstinent habitual smokers matched to control non-smokers were shown smoking-related and neutral images, pictures of cigarette smoking evoked greater activation in the ventral striatum (including the NAcc) in smokers compared to non-smokers (David et al., 2007), suggesting that these subcortical areas of the brain are important in nicotine craving and reinforcement.

These findings indicate that stimuli associated with the delivery of nicotine may be very important for the effectiveness of nicotine as a reinforcer.

Earlier studies found that local nicotine injections lead to significant increases of DA release in the striatum and in the NAcc but have modest effects in the frontal cortex (Marshall, Redfern, & Wonnacott, 1997). Interestingly, others found that whilst infusions of the nicotine antagonist dihydro-ß-erythroidine in the VTA reduce nicotine self-administration, the same dosage in the NAcc produces no effect compared to operant responding for cocaine self-administration, food or locomotor activity (Corrigall et al., 1994). Shedding light on the complex structure of the NAcc, later studies demonstrated that acute nicotine injections are able to evoke stimulation of DA overflow in the accumbal shell (Cadoni & Di Chiara, 2000) (Benwell & Balfour, 1992; Iyaniwura, Wrigt, & Balfour, 2001), but not in the accumbal core, unless experimental animals had been subject to sensitisation by receiving daily injections prior to the experiment (Iyaniwura, Wright, & Balfour, 2001). Increases in DA overflow may therefore play an important role in the development of habit forming associated with reward (Di Chiara, 2000a,b, 2002). Nonetheless, repetitive injections in the media shell diminish DA response (Cadoni & Di Chiara, 2000). Thus, the accumbal DA responses are contingent upon the delivery of the drug (whether chronic or repeated exposure), and these sensitised responses are dependent upon DA neurons which project to the accumbal shell (Balfour, 2004). In contrast, extracellular DA in the core subdivision of the NAcc serves to enhance the effects of conditioned stimuli on drug seeking behaviours. This is supported by a study in which lesions to the NAcc core but not the shell led to the attenuation of the effects of the conditioned cue on responding to food reward (Hall, Parkinson, Connor, Dickinson, & Everitt, 2001). Similarly, blockade of DA receptors have shown to inhibit nicotine self-administration in animals (Corrigall &

Coen, 1991) including using nicotine antagonists such as mecamylamine in the striatum, the NAcc and the frontal cortex (Ji, Lape, & Dani, 2001; Marshall et al., 1997). Likewise, re-expressing nAChR DA neurons in the VTA of KO mice have shown to evoke DA release and successfully reinstate nicotine self-administration (Maskos et al., 2005). Therefore, continued and increased levels of DA overflow in the accumbal medial shell causes the rewarding value of the behaviour or stimuli (nicotine/smoking) to increase, and renders the behaviour itself (act of smoking) to become pleasurable whilst promoting an association between the stimuli or behaviour and reward. Meanwhile, in the accumbal core, increased extracellular DA levels enhance responding for a conditioned cue which has been previously associated with the delivery of nicotine (Balfour, 2004). Taken together, the evidence presented here indicates that the increased DA overflow in both the accumbal core and shell, is instrumental in the rewarding properties of nicotine.

Evidence for nicotine discrimination

The role of nicotine as the primary reinforcer promoting the maintenance of smoking is well established. A large number of studies support the notion that nicotine has the ability to elicit behaviours that are characteristics of addictive drug use by promoting self-administration, enhancing locomotor activity and reward from brain stimulation (Clarke, 1990; Corrigall & Coen, 1991; Corrigall et al., 1994) and reinforcing place preference (Gaetano Di Chiara, 2000), including studies of human smokers in which nicotine is delivered in forms other than tobacco smoke (Harvey et al., 2004). Key to the reinforcing properties of nicotine is the ability to perceive the effects of the drug following administration and discriminate the drug.

Behavioural drug discrimination procedures in animals are very useful as dosage of the drug can be administered in a careful and controlled manner. This enables reliable matching of differential responses with each dose administered. Contrary to experimental drug discrimination studies in animals, self-report effects in humans provide in-depth qualitative knowledge of the effects experienced following drug administration. Both procedures have been useful in furthering our understanding of the psycho-behavioural effects of nicotine. Nicotine is able to produce interoceptive stimulus effects, that is physiological and autonomic responses (e.g. hedonic sensations) elicited by external stimuli, thought to be positively reinforcing (Perkins, Kunkle, Michael, Karelitz, & Donny, 2016). For instance, animals can be trained to reliably discriminate nicotine from saline systematically by pressing a lever to obtain food reward or to avoid punishment (e.g. an electric shock) (Solinas, Panlilio, Justinova, Yasar, & Goldberg, 2006) and, treatments with nicotine antagonists extinguish their ability to discriminate nicotine from saline (Morrison & Stephenson, 1969). In another study exploring the effects of varying treatment time and the onset of the effects of various doses of nicotine, higher nicotine (0.4 versus 0.2 mg/kg^{-1}) doses were preferentially selected by the rats and the effects of this higher dose were visible as early as 5 min following subcutaneous injections and lasted up to 60 minutes. Others suggest that nicotine discriminative functions can be long lasting and endure several weeks following discrimination training, but are influenced by motivation (Troisi, Bryant, & Kane, 2012). These findings clearly indicate that nicotine can serve as a reinforcer. However, some have expressed reservations about the reinforcement properties of nicotine to maintain smoking by pointing to the inconsistencies in study findings in both humans and animals (Dar & Frenk, 2004). Among these inconsistencies are the ability of the nicotine antagonist mecamylamine to induce

withdrawal symptoms in rodents but not in human smokers (Rose, Behm, & Westman, 2001). This suggests that the effects of nicotine on human smokers differ greatly from those on rodents. In addition to the inconsistent findings concerns have been raised vis-à-vis the use of food-deprived and trained animals and experimental conditions employed in past studies (Dar & Frenk, 2002). It is worth noting however that differing methodologies including the use of varied doses of nicotine between studies are likely to generate different results, specifically the use of different strains of rat species which likely to differ in sensitivities. Indeed, the experimental conditions employed in drug discrimination paradigm studies are of crucial importance and, inadequate pace and duration of nicotine injections and the use of nicotine dependent primed rodents such as those used in past studies may have rendered some past results erroneous (Le Foll, Wertheim, & Goldberg, 2007). Subsequently, later studies have employed naïve-experimental primates as opposed to rodents to assess the reinforcing properties of nicotine. In one study, five naïve-experimental monkeys were trained to respond to a fixed- or a progressive-ratio schedule which was paired with a stimulus light and required numerous presses on an 'active' or an 'inactive' lever to obtain a nicotine drug reward (Le Foll et al., 2007). The monkeys displayed clear preference for responding on the 'active' lever thereby self-administrating nicotine even in instances when 600 presses were required per injection, reiterating that nicotine is a robust reinforcer (Le Foll et al., 2007). This bias for the 'active' lever presser was not present at the initial sessions but developed only after repeated sessions, which echoes the theory of sensitisation discussed previously. Experiments in human smokers also provide strong evidence that nicotine acts as a reinforcer and promotes drug-seeking and -taking behaviours. For example, in a study, using intravenous self-administration, regular smokers were given the choice to pull on two levers which linked to the delivery of

either nicotine or saline both paired with a stimulus light (Harvey et al., 2004). Lever-pull responses corresponding to nicotine injections were significantly greater than those for saline including when the response requirement for nicotine injections increased. Interestingly, responses to the nicotine lever positively correlated with nicotine doses. Nicotine lever-pull responses increased in conjunction to the response requirement for nicotine injections (Harvey et al., 2004), suggesting that smokers adjusted their responses in order to maintain constant blood nicotine levels. Indeed, studies using dose-response paradigms also suggest that dependent smokers are able to discriminate nicotine and prefer higher doses of nicotine. In a counterbalanced double-blind experiment, 10 abstinent human smokers sampled 3 assigned doses of nicotine intravenously (0.7, 0.4, 0.1 mg/5mL of nicotine) and a matching dose of sodium chlorine as the placebo condition in 4 separate experimental sessions of 90 minutes each (Sofuoglu, Yoo, Hill, & Mooney, 2008). Following sampling, participants were instructed to choose a condition on six instances. Participants demonstrated clear preference for the highest doses over the 0.1 mg/5mL and the placebo condition, and these higher doses were associated with self-reports of 'drug liking' and 'high' (Sofuoglu et al., 2008). Likewise, the potency of the actions of nicotine on the CNS to reinforce smoking behaviours has also been documented in earlier studies in which the administration of the nicotine antagonist mecamylamine produced a 30% increase in smoking, decrease in blood pressure, impairment in cognitive tasks and reports of dysphoria (Stolerman, Goldfarb, Fink, & Jarvik, 1973). Altogether these findings provide clear evidence that even when removed from its conventional mode of delivery (that is smoking), nicotine produces effects that are analogous of those seen in other potent drugs of abuse (such as cocaine or amphetamine) which reiterates that nicotine itself is a robust reinforcer.

Nicotine discrimination paradigms play an important role in understanding the abuse liability of nicotine that is relevant to the development of nicotine agonists and antagonists for smoking cessation medications. The reinforcing properties of nicotine is also evidenced in smoking cessation studies demonstrating the efficacy of nicotine containing products over placebo (Fiore, Smith, Jorenby, & Baker, 1993). Furthermore, historically, tobacco cigarettes from which the nicotine content has been considerably reduced or removed have fallen short of receiving public acceptance in comparison to other forms of nicotine products delivering larger amount of nicotine (Everitt, 1996).

The important role of nicotine in reinforcing smoking is apparent when considering the heavy investments made by tobacco companies to further their understanding of the role of nicotine in shaping smoking behaviours. In the past, many tactics were used to maximise the pharmacological and physiological effects (including sensory effects) of smoking thereby enhancing satisfaction to maintain addiction (Carpenter, Wayne, & Connolly, 2007). Such tactics included manipulating nicotine dosage and continuously modifying cigarettes (the use of additives and ventilation holes). For example, the 1980s saw the development of tobacco cigarettes which were manufactured from air-cured Burley tobacco blend as a means to enhance their nicotine content two-fold of that of conventional cigarettes and alter their nicotine to tar ratio (Chakraborty, 1985 in Wayne & Carpenter, 2009). Furthermore, specific chemicals were also added to alter the harshness of the product whilst enhancing the nicotine yield to maximise sensory effects (Keithly, Ferris Wayne, Cullen, & Connolly, 2005). These remarks are consistent with the notion that cigarette manufacturers have long understood that nicotine is chiefly responsible for the continuation of smoking.

Hitherto, the discussion has focused on the positive reinforcement properties of nicotine in promoting smoking by i) presenting empirical evidence that experimental

animals and human smokers are able to perceive and discriminate doses of the drug, and ii) briefly discussing how nicotine containing tobacco cigarettes have been engineered to promote the continuation of smoking. Although many theories have been proposed to explain nicotine dependence, in the following two sections the discussion will focus on the most prominent theories; theories that describe nicotine as a positive reinforcer, those which describe nicotine as a negative reinforcer and the contribution of non-nicotine cues that contribute to reinforcing smoking behaviours.

Nicotine as a Negative Reinforcer: Dependence, Craving and Withdrawal symptoms

In addition to the positive physiological and autonomic responses (e.g. hedonic sensations) described above, continued smoking may be driven by the avoidance of negative psychological and physiological effects that are contingent upon smoking abstinence. Prior to turning to a discussion on the negative reinforcement theory, definitions of the key concepts relevant here will be offered. For the purposes of this thesis, craving refers to the experience of a powerful motivation to engage in a specific behaviour; in this thesis craving may also be referred to as 'urge' or 'desire'. Early studies to document drug dependence were based on the observations that following the development of tolerance of a drug, falling levels of that drug in the blood systematically induce a withdrawal state (Wikler, 1980), thus drug dependence can be defined as a process wherein regular intake of a psychoactive substance results in a cycle of increased tolerance characterised by neuroadaptations, which in turn causes an increase in the motivation to self-administer. The central tenet of this early theory posited that drug intake is primarily driven by avoidance of aversive states such as withdrawal which can be evoked by associative cues. It was further explicated that environmental cues which were initially neutral, develop reinforcing properties to

become conditioned cues, subsequently, exposure to these conditioned cues will induce cravings and withdrawal in turn increasing the likelihood of relapse, even after a long period of abstinence (Wikler, 1948). That smoking abstinence is typically followed by a range of negative signs and symptoms is widely documented (Hughes et al. 1991; Hughes and Hatsukami 1986). Indeed, cessation of nicotine use (Hughes & Hatsukami, 1986) or reducing the nicotine content in tobacco cigarettes typically induce a state of withdrawal in regular smokers, whilst reinstating nicotine intake (for example with nicotine replacement therapy) tend to attenuate the intensity of these symptoms (West, Russell, Jarvis, & Feyerabend, 1984). Because withdrawal ensues upon cessation and withdrawal state can be reversed by smoking, the smoker learns to avoid the discomfort by continuing to smoke. Likewise, reduction in dosage will successfully reinforce drug taking behaviours even in the absence of positive subjective effects (Lamb et al., 1991; Panlilio et al., 2005). Interestingly, the negative correlation between blood nicotine levels and craving may indicate that smoking behaviour is driven not just by a need to avoid the discomfort of withdrawal but equally to satisfy craving (Jarvik et al., 2000).

A state of withdrawal may comprise a wide range of affective, somatic and cognitive symptoms including depressed mood, cravings for tobacco, anhedonia, anxiety, irritability, drowsiness, nausea, increased hunger, headache, inability to concentrate amongst others (West & Hajek, 2004). Since nicotine administration alleviates these symptoms, it is reasonable to assume that withdrawal is a significant motivational drive for the perpetuation of nicotine dependence and continued smoking behaviours. Moreover, studies suggest that although withdrawal peaks as early as two days following cessation, some symptoms can last up to six months (Le Foll & Goldberg, 2005) increasing the risk of relapse and providing evidence that nicotine dependence may be negatively reinforced.

Drawing from the central assumption of negative reinforcement, later models added more emphasis to the associative processes involved, arguing that smoking behaviours are reinforced not by withdrawal-related stimuli as advanced by Wikler, rather by cues that were previously paired with administration (Siegel, 1983). This concords with a later theory, the opponent-process theory which posits that smoking behaviour is negatively reinforced via an intrinsic homeostasis mechanism (Solomon, 1997). This was described as compensatory responses that are elicited by cues in order to counteract the appetitive effects of the drug. For example, encountering a conditioned cue may lead to an increase in positive mood, resulting in turn in an increase in negative mood in anticipation as an attempt to compensate and reinstate homeostasis; and, because this behavioural mechanism is *unopposed*, withdrawal ensues (Solomon, 1977).

Though there is substantial evidence suggesting that avoidance of aversive withdrawal symptoms is the primary driver of smoking, criticisms vis-à-vis negative reinforcement accounts would eventually render it to fall in disfavour. Firstly, the suggestion that drugs can evoke responses in the absence of withdrawal; for instance, that in many cases relapse occurs even after withdrawal has subsided (Robinson & Berridge, 1993), concords with self-reports of street addicts (McAuliffe, 1982). Secondly, a large body of evidence which points to the importance of, and the role played by, smoking related cues. Some animal studies have found that exposure to drug cues is sufficient to reinstate self-administration even following a period of extinction in which the drug was no longer available (Stewart, 1984); this challenges the key assumption that avoiding withdrawal is the chief motivator for smoking behaviours.

Although there is convincing empirical evidence to attribute the pharmacological reinforcement effects of nicotine to smoking maintenance, several

lines of evidence suggest that there may be other candidates that contribute to the reinforcing effects of smoking. For example, combined with nicotine, monoamine oxidase inhibitors (MAOIs) are able to exert reinforcing effects (Villégier et al., 2006; Villégier, Blanc, Glowinski, & Tassin, 2003).

Following the discovery of the important role of MAOs in the regulation of various monoamine neurotransmitters including DA and serotonin, MAOIs have been implicated in the regulation of mood and anti-depressant properties of smoking (Glover et al, 1980 in Fowler et al, 1996). Since monoamine oxidase (MAO) enzymes are involved in the oxidation of serotonin (5-HT), MAOIs promote the prolongation of these neurotransmitters release, potentially through inhibiting their oxidation (Lewis, Miller, & Lea, 2007). One likely explanation might be that smokers have significantly reduced brain MAO levels compared to non-smokers (Fowler et al., 1996) thus smoking may be the manifestation of a self-medication mechanism that helps regulate negative affective states (Cohen, McCarthy, Brown, & Myers, 2002). Moreover, animal experiments suggest that inhibiting the subtype MAO-A with an antidepressant drug, results in increased extracellular release of serotonin (Celada, Bel, & Artigas, 1994). One possible mechanism by which MAOIs act with nicotine may be through prolonging the release of DA neurotransmitters thereby potentiating the reinforcing effects of nicotine (Glover et al, 1980 in Fowler et al, 1996). This is supported by studies which found that pre-treatment with tranylcypromine (a MAOI) produced robust nicotine self-administration, but this was not the case in tranylcypromine-naïve rats (Villégier et al., 2006). Rats are also able to re-establish behavioural sensitization following concurrent injections of MAOIs and nicotine (Villégier et al., 2003). Thus, it appears that MAOI combined with nicotine has reinforcing properties.

Non-nicotine stimuli as reinforcers: Incentive models

Thus far, the discussion suggests that it is likely that smokers continue to engage in smoking behaviours primarily to experience the rewarding and pleasurable effects of nicotine or to avoid the aversive effects experienced upon abrupt cessation. However, although the evidence to support the positive and negative reinforcement models is robust, both models fail to fully account for the continuation of, or relapse to, smoking i) when these subjective pleasurable effects are minimal and ii) in the absence of withdrawal (Robinson & Berridge, 1993). Amongst its multiple mechanisms of actions, through its conditioning properties, nicotine is able to enhance the rewarding value of smoking cues (Donny et al., 2003). Aside from components of the smoke, smoking addiction is also context-dependent pointing more specifically at the sensory and motor cues associated with smoking (Balfour, 2004; Caggiula et al., 2002a; Donny et al., 2003; Palmatier et al., 2006; Rose, Behm, & Levin, 1993). Indeed, it is argued that smokers' liking for the sensory attributes of tobacco smoke such as the smell, taste, olfactory and respiratory tract sensations, and the tactile aspects of smoking are major reinforcers of smoking in their own right (Parrott & Craig, 1995). When decoupled from the traditional mode of delivery that is the smoking of a cigarette, nicotine does not always provide positive mood effects (Dar & Frenk, 2004). Amongst the methods utilised to disassociate the sensory from the nicotine effects from smoking is comparing the effects of nicotine administered intravenously and the smoking of cigarettes from which nicotine content was removed. For instance, it has been demonstrated that the smoking of denicotinised cigarettes is capable of alleviating smoking withdrawal symptoms and reducing craving (Barrett, 2010; Caggiula et al., 2002b; Perkins, Karelitz, Conklin, Sayette, & Giedgowd, 2010), as well as providing levels of satisfaction greater than those elicited by intravenous nicotine (Rose, Behm, Westman,

& Johnson, 2000; Rose, Salley, Behm, Bates, & Westman, 2010). In contrast, attenuating these sensory cues seems to diminish self-rating satisfaction (Perkins et al., 2001; Rose, Tashkin, Ertle, Zinser, & Lafer, 1985). As noted earlier, the NAcc core/ventral striatum has been implicated in mediating the positive responses to conditioned stimuli and visual cues, such as images of a burning cigarette, have been demonstrated to activate the ventral striatum in smokers but not in non-smokers (David et al., 2007). David and colleagues further suggested that activation of the ventral striatum elicited by smoking related stimuli and other physiological effects (such as craving) may be caused by neuroadaptations (or persistent changes) in the mesoaccumbens DA area (David et al., 2007). These neuroadaptations derive from sensitisation thought to be caused by long term and repeated exposure to nicotine (Balfour, 2002) and, because sensitisation is amenable to conditioned stimuli, presenting the cues which have, a priori, been paired with smoking, may themselves become reinforcers even with no or negligible doses of nicotine (Rose et al., 2006). This proposition corroborates with a long-established theory, advanced by Robinson and Berridge, the 'Incentive sensitization theory'. This theory posits that the ability of addictive drugs to activate the mesolimbic DA system induces attentional bias and affects perceptions related to the drug or the drug-related stimuli (Robinson & Berridge, 1993; 2008). This psychological mechanism which is referred to as incentive salience, is defined as a pathological motivation to seek and self-administer a given drug and, and is understood to be manifested implicitly via 'wanting' or explicitly through 'craving' (Robinson & Berridge, 1993; 2008). It is further suggested that chronic administration of the drug creates increasing neuroadaptations thereby sensitising the DA system incrementally, increasing the incentive value of the drug and drug-related cues and rendering drug 'wanting' into 'excessive craving' (Robinson & Berridge, 1993).

Indeed, it has been demonstrated that drug related cues elicit increases in DA release in the mesolimbic pathway in animals, but also presentation of these stimuli can induce craving in human smokers (Di Chiara, 2000). Thus, aside from its directly reinforcing properties, it appears that nicotine is able to amplify the incentive and reward value of stimuli (such as sensory, motor and visual) that have been previously paired with its administration. Although this model has received substantial support and provides a comprehensive explanation of cravings and the persistent motivation to self-administer, it does not provide a full account of the compulsive nature of drug-seeking and -taking that are typical in addicted individuals despite the known adverse consequences.

Although non-nicotine stimuli (for example nicotine-free tobacco smoke) have shown to reduce craving and induce positive subjective effects (Rose, Behm, Westman, & Johnson, 2000), aside the fact that these are contrived to the laboratory settings and standardised protocols that arguably may not be a true reflection of the real world smoking behaviours, these positive effects remain significantly modest compared to nicotine-containing conventional cigarettes, which typically evoke greater levels of euphoria and reduction in withdrawal symptoms (Rose, Behm, Westman, Bates, & Salley, 2003; Westman, Behm, & Rose, 1996). That higher levels of nicotine can be discriminated against lower or no nicotine-content cigarettes underlines the primacy of the pharmacological over the sensory stimuli which act in concert to reinforce smoking behaviours. This is in keeping with findings from a study in which a standardised puffing regime was used to delineate the rewarding effects of nicotine from that of tobacco smoke constituents by measuring self-reported reward within 7 seconds of inhalation (before nicotine reaches the brain and the pharmacological effects are felt) (Naqvi & Bechara, 2005). Puffs from the nicotine-containing cigarettes were found to elicit greater reward compared to puffs from the denicotinised cigarettes (Naqvi &

Bechara, 2005). The notion that the pharmacological rewards may be more driven by nicotine per se compared to the airway sensory effects is also supported in earlier studies which found that blocking peripheral nAChRs (with an antagonist trimethaphan) diminishes smoking satisfaction (Rose, Westman, Behm, Johnson, & Goldberg, 1999), whilst increasing nicotine concentrations in tobacco cigarettes increases satisfaction (Pritchard, Robinson, Guy, Davis, & Stiles, 1996b, 1996a). These manipulations indicate that subjective effects are dose-dependent, but also that the route of nicotine delivery (i.e. inhalation) is of equal importance for reward. Indeed, throughout the current discussion the primacy of nicotine per se becomes evident, but it is also apparent that stimuli other than nicotine are of key importance in the maintenance of smoking. Henceforth, the focus of the discussion will turn to the importance of the delivery of nicotine in reinforcing smoking behaviours. The first part will offer a brief description of the importance for a rapid nicotine absorption before discussing how the route and pace of delivery (that is inhalation) render the act of smoking a cigarette the most effective vehicle for nicotine contributing to most of the addictiveness of smoking.

The mode of nicotine delivery as a key reinforcer: The Bolus theory

An average cigarette contains between 3 and 10 mg, and following the smoking of a cigarette an average of 1 to 1.5 mg of nicotine is absorbed in the course of taking 10 puffs per cigarette (Benowitz & Jacob, 1984). The amount delivered can vary between 0.3 and 3.2 mg per cigarette (Benowitz & Henningfield, 1994) (Benowitz & Jacobs, 1984; Gori & Lynch, 1985; Jones, 1987), and this not determined by the cigarette content but largely by the way the cigarette is smoked (Benowitz & Jacob, 1984b; Benowitz, Kuyt, & Jacob, 1982). This is supported by empirical evidence suggesting that smokers of low yield tobacco cigarettes (determined by machine estimations) do

not systematically consume less nicotine and blood nicotine concentrations do not correlate with machine yield estimations (Benowitz et al., 1983). Furthermore, the inter-variabilities in biomarkers of nicotine absorption, with some smokers capable of achieving 100 ng/mL arterial nicotine levels following the smoking of a single cigarette (Henningfield & Keenan, 1993), whilst others' peak at 20 ng/mL (Rose, Behm, Westman, & Coleman, 1999), suggest wide inter-individual variabilities in puffing topography also.

With each puff and tobacco smoke particles inhaled, nicotine passes through the arterial circulation and quickly reaches the alveoli of the lungs due to the large surface of the lungs and the elevated pulmonary capillary blood circulation. This facilitates a rapid absorption and allows nicotine to reach the brain in approximately 10 to 20 seconds, which is followed by sharp increases in arterial blood nicotine concentrations levels and an ultimate peak at the end of the cigarette (Benowitz, 1990). The average nicotine concentrations vary between 20 and 60 ng/mL, but can reach up to 100 ng/mL (Henningfield & Keenan, 1993).

The fast nicotine absorption combined with the high nicotine concentration levels achieved are of particular importance in producing what is referred to as a 'hit' or 'bolus' the intense psychoactive effect of the drug and desired effect sought by the smoker (Benowitz, 1990). That the remarkably fast transit from the lungs to the brain and high arterial nicotine concentration are central to the reinforcing properties of nicotine, is known as the 'bolus theory' (Russell & Feyerabend, 1978). The act of inhaling provides a unique route for nicotine to be delivered rapidly and efficiently to target organs and is thought to mediate the pharmacological effects of nicotine as reflected in the greater levels of arterial versus venous nicotine concentrations (Henningfield, Stapleton, Benowitz, Grayson, & London, 1993). The greater magnitude

and higher pace of the arterial route enhances the reinforcing effects of nicotine. Inhalation produces more rapid and pronounced physiological and psychological effects than nicotine delivered transdermally or via the buccal mucosa (Cone, 1995; Evans et al., 1996; Fant et al., 1999; Henningfield et al., 2004; Henningfield & Benowitz, 2004). This is reflected in the differences in kinetic profiles of the transdermal and oral nicotine products compared to tobacco cigarettes; the latter are typically characterised by a sharp peak at around the end of a cigarette and is followed by a sudden drop, whilst use of the former is associated with a steadier and more gradual release of nicotine (Benowitz, Hukkanen, & Jacob Iii, 2009). As a consequence, biomarkers of nicotine absorption in nicotine products are typically lower than those related to tobacco cigarettes (Schneider, Olmstead, Franzon, & Lunell, 2001), with the exception of high content nicotine products which can exceed levels reached with tobacco cigarettes albeit at a much slower pace (McNabb, 1984; Schneider et al., 2001).

The ability to fully control the pace and amount of the drug delivered through each puff provides a highly effective mode of delivery which allows a rapid absorption through the lungs and produces a desired nicotine concentration bolus to the brain. With each puff, the smoker can regulate their nicotine intake to a desired, satisfactory and constant level (Russell, 1980) thereby achieving optimal level of cognitive arousal and mood regulation (Benowitz, 2001). This rapid absorption is thought to be key in regulating smoking and puffing topography. The hypothesis that regular smokers seek to regulate their blood nicotine levels is apparent in the fact that supplementary nicotine administration suppresses smoking. For instance, a study by Benowitz and Jacob (1990) found that cigarette consumption could be significantly reduced following intravenous nicotine administration, likewise increasing the dosage of nicotine replacement therapy products (hereafter referred to as NRT) could further decrease

cigarette consumption (Benowitz, Zevin, & Jacob Iii, 1998). Although plasma nicotine concentrations tend to fluctuate considerably, since nicotine accumulates over a period of six to eight hours including during sleep, by regulating their smoking, smokers are able to maintain their nicotine blood levels (Benowitz, Kuyt, & Jacob, 1982). This further supports the notion that smokers need to regulate their nicotine intake. In line with this, a typical smoker does not allow his/her blood nicotine to drop below habitual levels thereby avoiding withdrawal by continuing smoking; and abstinence periods are characterised by increases in craving and withdrawal symptoms quickly followed by a rapid escalation in desire to smoke sometimes in remarkably short succession of a cigarette (Schuh & Stitzer, 1995).

NRT formulated to assist an equilibrium in blood nicotine levels, prevent relapse in the short term only; this disappointing outcome may reflect the absence of a nicotine bolus. Conversely higher doses better promote smoking cessation (Hughes et al., 1999). This provides further credence to the notion that smokers' puffing behaviours are driven by their nicotine needs. Moreover, it is recognised that NRT is not used long term and does not cause dependence. NRT's low abuse liability potential may be largely due to the slow nicotine delivery and the fact that the user has little or no control over the nicotine release at least in the case of transdermal patches. There is substantial evidence supporting the hypothesis that the reinforcing properties of addictive drugs can be greatly enhanced by a rapid and direct delivery to the brain in high concentrations (Benowitz, 1990b; Benowitz, 1999; Henningfield & Keenan, 1993). Notably, smokers typically report greater levels of craving for cigarettes and nicotine dependence compared to snuff users (Dole, 1980). This theory suggests that the sudden high level of nicotine in the brain acts as a positive reinforcer thereby contributing to smoking maintenance.

In summary, the evidence pointing to inhalation as an important reinforcer partly explains why smoking is a unique and preferred mode of delivery. By providing greater control over the pace and flux of nicotine, it allows the smoker to regulate the intake of the drug thereby ensuring individualistic and satisfactory blood nicotine levels which is, arguably, at the heart of smoking reinforcement.

Self-titration and compensatory puffing

The evidence cited thus far suggests that smoking behaviours are largely driven by a need to achieve rapid blood nicotine hit, and, to maintain a constant blood nicotine level that is sufficiently high to be satisfactory and prevent withdrawal but also falls below their individualistic threshold not to produce aversive or toxic effects. One mechanism by which smokers achieve individual and satisfactory blood nicotine levels is by adjusting their puffing topography (e.g. puff number, duration and inter-puff intervals) according to the nicotine content of each tobacco cigarette. Evidence for the idiosyncratic nature of puffing topography in smoking is reflected through the stark contrast between the inter-variability (Benowitz et al., 1982) and intra-consistency in nicotine absorption and puffing topography (Gust, Pickens, & Pechacek, 1983). This bio-behavioural mechanism reiterates that smokers need to maintain constant and satisfactory nicotine blood levels by adjusting their puffing topography; this forms the basis for the theory of self-titration. The terminology self-titration is commonly defined as a smoker's individual need to maintain characteristic nicotine levels in the body, whilst compensation or compensatory puffing behaviours denotes active changes in puffing behaviours in response to alterations of nicotine exposure in an effort to maintain characteristic nicotine levels in the body (McMorrow & Foxx, 1983).

Evidence for the self-titration theory is well documented (Ashton et al., 1979, 1970; Herning et al., 1981; Russell, Jarvis, Iyer, & Feyerabend, 1980; Strasser et al., 2007; West, Russell, Jarvis, & Feyerabend, 1984; Woodward & Tunstall-Pedoe, 1993). By increasing the length and frequency of puffs, increasing the number of cigarettes, smoking more intensively (Kolonen, Tuomisto, Puustinen, & Airaksinen, 1991) or smoking each cigarette to a shorter butt length, manipulating vent holes (Kozlowski, Pillitteri, & Sweeney, 1994; Kozlowski, Pope, & Lux, 1988) and cigarette filters (Zacny & Stitzer, 1996), smokers will attempt to compensate for the lower nicotine delivery from low-yield (*light*) cigarettes (machine-measured yield) (Ashton et al., 1979, 1970; Collins et al., 2010; Hammond et al., 2005; Kolonen, Tuomisto, Puustinen, & Airaksinen, 1992; Strasser et al., 2007; Woodward & Tunstall-Pedoe, 1993). In a cross-sectional survey of 2754 smokers, an increase in exhaled carbon monoxide (CO) was positively correlated with tar yield, whilst being inversely correlated with nicotine yield, which implies that a decrease in nicotine availability ensues in an increase in smoke intake (Woodward & Tunstall-Pedoe, 1993). Additional evidence reveals that switching to low nicotine yield cigarettes can induce an increase in the number of cigarettes smoked, whereas a switch to high nicotine yield cigarettes lead to a decrease, which supports the notion that smokers adjust their puffing patterns to regulate their nicotine uptake (Russell, Wilson, Patel, Cole, & Feyerabend, 1973). Likewise, smokers' puffing patterns increase when nicotine is blocked through nicotine antagonist administration (Stolerman et al., 1973); conversely, smoking decreases when nicotine is supplemented via oral route with nicotine gums (Ebert, McNabb, & Snow, 1984).

In line with the titration theory, the way in which each tobacco cigarette is smoked also suggests a drive to maintain desired blood nicotine levels. There is robust evidence that the first puffs on a single cigarette are typically longer and larger and inter-puff

intervals (intervals between puffs) are shorter compared with the subsequent puffs; as a cigarette is smoked, puff duration and volume decrease and inter-puff intervals increase (Chait & Griffiths, 1982; Kolonen et al., 1992). This is suggestive of an attempt to raise fallen blood nicotine levels since the last cigarette (Collins et al., 2010). Likewise, the smoking of two succeeding tobacco cigarettes typically results in a decrease in puff intensity and frequency as the subsequent cigarette is smoked, suggesting a satiation effect (Kolonen et al., 1992). Conversely, abstinence of a few hours may lead to a more intensive puffing regimen. Thus, the behavioural mechanism of compensation is not strictly defined as an adjustment of nicotine intake by consuming more or fewer cigarettes but rather extends to a seemingly perpetual mechanism exerted on a puff-by-puff basis (Woodward & Tunstall-Pedoe, 1993).

Another prediction of this theory posits a propensity for heavier smokers to smoke soon after waking proposedly in response to depleted blood nicotine levels owed to overnight abstinence (Kozlowski, Director, & Harford, 1981); and, the latency of this first cigarette of the day seems a good predictor of nicotine exposure and dependency (Heatherton, Kozlowski, Frecker, Rickert, & Robinson, 1989). This may account for the many self-reports that the first cigarette of the day is the most satisfactory and also concords with the aforementioned findings that the latency of the first cigarette of the day correlates with nicotine dependency and nicotine exposure (Baker et al., 2010; Fagerström, 2003). Although contrary to the previous assumption that the first cigarette of the day would be puffed on more intensively, some have found that cigarettes smoked during the night and the first cigarette of the day are smoked less intensively (Grainge, Shahab, Hammond, O'Connor, & McNeill, 2009) and this coincides with the fact that craving peaks are typically observed at midday as opposed to mornings in highly deprived smokers (Teneggi et al., 2005). It has been proposed that this

adjustment in puffing topography could be driven by a reduction in nicotine tolerance due to the overnight prolonged period of abstinence (Grainge et al., 2009). Of note, the sample used in Grainge et al.'s study was comprised of smokers with the characteristics of a median time to first cigarette of 30 minutes, a sample of heavier smokers with shorter time (that is ≤ 5 minutes) could have yielded opposite results. Equally, it could be argued that cigarettes smoked during the night are smoked less intensively because of a satiation effect of nicotine and that the less intensive puffing is a reflection of the smoker's need to maintain, rather than to raise, blood nicotine levels. Likewise, although current evidence suggests that light cigarette smokers are less dependent compared to heavy smokers, some studies suggest that due to more intensive puffing they may be subject to greater exposure to nicotine (Krebs et al., 2016), suggesting that the fewer cigarettes smoked may lead to a need to puff more intensively on each cigarette, which again lends support to the self-titration theory.

The most commonly used investigative approach to explore the phenomenon of self-titration in tobacco smokers has been to manipulate the nicotine yield of cigarettes. This has been achieved through various methods, i) altering the size of the experimental cigarettes and observing puffing topography following the smoking of the experimental versus the full-size cigarette (Goldfarb, Jarvik, & Glick, 1970 in McMorrow & Foxx, 1983), ii) using manufactured cigarettes for which the nicotine yield was predetermined by smoking machines. A large body of research has suggested that smoking machine-determined nicotine yield are not valid estimates of relative human exposure to toxicants. Whilst smoking machines produce uniform and consistent sets of toxicant yields, the differing nature of smokers' puffing patterns will result in varying levels of toxicants emission so any claims on reduced human exposure based on smoking machines maybe erroneous (Burns et al., 2008). Invalid claims vis-à-vis the reduced

harms associated with the use of low tar low nicotine yield cigarettes have led to a plethora of studies utilising biomarkers such as human blood, urine and saliva whilst measuring puffing topography. Such an approach represents a powerful metric to investigate real human exposure to nicotine as well as toxicants aerosolised. A more complete discussion on compensatory puffing behaviours including further evidence is offered in Chapter II.

Harm Reduction: Nicotine Replacement Therapy Products (NRT)

The renowned Professor Michael Russell stated in 1976 that "People smoke for the nicotine but die from the tar" (Russell, 1976, p. 1431). This often quoted dicta continues to be the basis, not only for the core theories and models related to smoking behaviours, but has also for the development of NRT. In addition to comprehensive tobacco control strategies (through media education campaigns, excise taxes, prevention and cessation services and so on), it has become apparent that nicotine could play a key role in reducing the harms of smoking. NRT emerged as a means to support smokers in their attempt to quit. Because nicotine can be absorbed through cell membranes albeit not as fast as through inhalation, various forms of NRT (patches, gums, lozenges, sprays, inhalators and so on) have been developed to enhance cessation. To enhance their absorption, NRTs were made of an aqueous nicotine solution buffered to alkaline pH (Hukkanen et al., 2005). Transdermal patches are placed on the skin and nicotine release to the blood is constant but very slow with a T_{max} (Time at which maximum concentration peaks) varying between 3 and 8 hours depending on the dose (Fant, Henningfield, Shiffman, Strahs, & Reitberg, 2000). As a result the user has no control over the nicotine release and is unable to self-titrate (Balfour & Fagerström, 1996). In contrast, nicotine gum allows the user a modest degree of self-titration; mastication

causes rise and troughs in blood nicotine levels and time to peak can be achieved after approximately 30 minutes (Balfour & Fagerström, 1996) but control over the nicotine intake is modest and not comparable to smoking a cigarette (Benowitz, 1988). Compared to the transdermal patch, the nicotine delivery profile resembles cigarette smoking albeit at much lower levels (Keenan, Henninfield & Jarvik, 1995). The nasal spray is the fastest nicotine delivery amongst all NRT; with a T_{max} of 5-10 min, its nicotine delivery peak curve is the closest to resemble that of a tobacco cigarette (Sutherland et al., 1992). Although designed to emulate tobacco cigarettes by drawing through a mouthpiece, nicotine inhalators have poor nicotine delivery and rewarding effects compared with cigarette smoking (Balfour & Fagerström, 1996).

Nicotine inhaled via smoking is quickly absorbed and characterised by a peak blood nicotine level at 5 minutes thereby providing a 'hit' to the smoker. By contrast, evidence suggests that such speed of delivery is not achievable via transdermal route (Hukkanen et al., 2005). Nonetheless, some evidence suggests that a higher dosage of NRT can increase one year smoking cessation rate (Tonnesen et al., 1999). That said, if nicotine was the sole cause of smoking maintenance, one might have expected the development of such safer nicotine products to have had a much greater impact on smoking cessation rates. Instead despite many decades on the market, long term smoking cessation using NRT remain fairly modest (Rose, Lokitz, Garg, Turkington, & Minton, 2006) and NRT has increasingly become the least preferred smoking cessation aid (West, Beard, & Brown, 2018; West & Brown, 2018). Although combining NRT with regular behavioural support can increase its efficacy for smoking cessation (Moore et al., 2009), their success rate has been modest with fewer than 20% of users remaining abstinent for a year (Stead et al., 2012). Only 1 in 10 users remain abstinent after 1 year (Etter & Stapleton, 2006), and more recent data suggest that the expected 1 year

abstinence rates using licensed nicotine products is only 5% (Kotz, Brown, & West, 2014). This could be due to the slow and gradual release of nicotine which completely differs from the pharmacokinetic profile of combustible cigarettes typified by a sharp peak as opposed to a flatter and lower curve (Digard, Proctor, Kulasekaran, Malmqvist, & Richter, 2013). A further drawback is their inability to provide the sensory and behavioural cues associated with the ritual of smoking which are deemed highly significant in reinforcing smoking (Perkins, Karelitz, Giedgowd, Conklin, & Sayette, 2010; Rose, 2006) as previously discussed. Empirical evidence suggests the way in which nicotine is delivered plays a major role in the reinforcement of nicotine use and tobacco addiction (Henningfield et al., 1993; Henningfield & Keenan, 1993). As noted previously, smoke inhalation directly through the lungs optimises nicotine delivery and the positive effects of nicotine (Henningfield & Keenan, 1993). Indeed, since many smokers are unwilling or unable to quit, there is a growing consensus that new and more effective cessation methods are needed. Given the literature reviewed above, effective nicotine delivery and replacement of the behavioural aspects of smoking are likely to be key features of a successful cessation method. This sets the context for the emergence of the new 'disruptive' technology: e-cigarettes.

The Emergence of E-cigarettes

First developed in 2004 by the Ruyan Group (Holdings) Ltd, a Chinese company in 2004 (Bullen, McRobbie, Thornley, Glover & Laugesen, 2010; Bullen, et al., 2013; Dawkins, Turner, Roberts & Soar, 2013), electronic-cigarettes (hereafter referred to as e-cigarettes) are today the most popular (West, Beard, & Brown, 2017) and amongst the most effective at supporting smoking reduction in the UK (McRobbie et al., 2014; Brown, Beard, Kotz, Michie, & West, 2014; Beard, West, Michie, & Brown, 2016;

Farsalinos, Poulos, Voudris, Le Houezec, 2016). Although there remains concerns vis-á-vis their use (including the notion that e-cigarettes may renormalize smoking and preclude or reverse tobacco control efforts). Since their introduction they have dramatically increased in popularity from 700000 in 2012 (ASH, 2016) to 2.9 million users in the United Kingdom in 2017 (ASH, 2017).

E-cigarettes are battery-powered devices which closely emulate the action of smoking by releasing an inhalable aerosol through pressurising a solution commonly comprising propylene glycol, vegetable glycerine, and flavourings (Bullen et al., 2013). Although this solution (hereafter referred to as e-liquid) does not contain tobacco, it may contain nicotine (extracted from tobacco leaves), with levels varying from 0 mg/mL up to 20 mg/mL as sold in Europe since the introduction of a cap by the European Union Tobacco Product Directive regulations (EU-TPD) in May 2016. Prior to the EU-TPD, concentrations could range from 0 to 36 mg/mL (European Union, 2014).

Although there is considerable variation between device types, commonly e-cigarettes comprise i) a lithium-ion battery (which can be pre-filled or rechargeable); ii) a micro-electrical circuit containing a sensor which can be activated upon a single draw or button press; iii) a heating component called atomiser which houses a wick and vaporises the nicotine solution; iv) a mouthpiece which acts as the e-liquid reservoir and delivers the aerosol to the mouth (in the earliest models it incorporates the cartridge); in early models v) an optional LED diode which simulates the lighting of a tobacco cigarette upon activation of the sensor, and vi) a manual switch (Bullen et al., 2010; Etter, Bullen, Flouris, Laugesen & Eissenberg, 2011; Goniewicz, Kuma, Gawron, Knysak & Kosmider, 2012; Polosa et al., 2011) (see Figure 1.1, appendix 1). A draw or button press activates the atomiser which is detected by the airflow sensor, in turn

prompting the wick to become saturated with e-liquid, before releasing the inhalable aerosol (Goniewicz, Kuma, Gawron, Knysak & Kosmider, 2012).

Early models were made to resemble conventional tobacco cigarettes in size and shape and housed relatively smaller batteries (in comparison to later models). These are commonly referred to as cigalikes, first generations or closed systems (see Figure 1.2, appendix 1) (Farsalinos, Romagna, Tsiapras, Kyrzopoulos, & Voudris, 2013a). When first introduced, cigalikes could be single or two-piece units with re-chargeable or pre-filled cartridges (thus can be rechargeable or disposable); however, in the later cigalikes, the fluid-filled reservoir cartridge and the atomizer were merged into a single cartomizer (Dawkins, Turner, Roberts & Soar, 2013).

Later e-cigarette models have evolved considerably and differ significantly from cigalikes, this indeed reflects the highly heterogeneous nature of e-cigarettes; the differences in function and effectiveness between models will be discussed in the 'e-cigarette characteristics' section later on. Second generation e-cigarettes differ from the appearance of conventional cigarettes however operate and share many of the characteristics of the cigalikes in terms of internal components. They tend to be slim in appearance, typically resembling pens hence the term 'pen-like', they contain a fillable reservoir for the e-liquid, and operate on a manual switch (refer to Figure 1.3 and 1.4 in appendix 1). Although considered as tank systems because of their fillable reservoirs, for the sake of clarity, these will be referred to as second generation or pen-likes in this thesis. This will allow for a clear distinction between second and third generations. Third generations on the other hand will be referred to as tank systems.

Third (hereafter referred to as tank systems) generation e-cigarettes depart considerably from the appearance of conventional cigarettes, are bulkier and larger than cigalikes and pen-likes, and house more optimal and larger capacity batteries (Figure

1.5, appendix 1). Referred to as open, tank systems (or mods), they can be modified and mounted on various combinations of tanks and mouthpieces. Indeed, this category also includes devices which contain rebuildable atomisers (Grana, Benowitz, & Glantz, 2014). Tank systems have sophisticated features which allow the user to adjust the voltage and/or wattage output, control the temperature, whilst some tanks contain an adjustable airflow rate and are compatible with atomisers of varied voltage resistance. Third generation tank systems also encompass devices for which the e-liquid can be added directly to the heating coil to allow for dripping, this is commonly referred to as 'drip-tip' or 'direct dripping' (Talih, Balhas, Salman, Karaoghlanian, & Shihadeh, 2016). Some models are equipped with downloadable software or memory chips allowing data collection such as history of puff counter. These additional features are thought to considerably enhance the user's experience (Baweja et al., 2016; Yingst et al., 2015). For example, the coil and wick material and arrangement (parts of the atomiser), and the atomiser resistance combined with the voltage output conducted through the atomiser will affect the velocity, density and quality of the aerosol produced as well as the temperature of the device during use (Brown & Cheng, 2014).

Fourth generation e-cigarettes also known as sub-ohm devices are seen as an enhancer of the third generations. They do not differ dramatically from their predecessors (third generation tank systems), are similar in size and shape but differ in terms of their ability to be compatible with atomisers of very low resistance (< 1 ohm) (Figure 1.6, see appendix 1). This allows a greater wattage output to be conducted through the coil thereby resulting in an increase in power and greater volume and density of the aerosol.

E-cigarettes in the context of Smoking cessation

In the UK, smoking prevalence has been in decline from approximately 19% in 2010 to 15.8% (around 7.6 million adults over the age of 18) in 2017 (Office for National Statistics, 2017). In contrast, e-cigarettes uptake has soared to an estimated 2.9 million adult users (ASH, 2017). Males and adults aged 35 to 49 were more likely to be users (Office for National Statistics, 2017). Their ability to closely simulate the psycho-motor actions and rituals of cigarette smoking, and to provide a comparable method of nicotine delivery have certainly contributed to their surge in popularity (Polosa et al., 2014). There is empirical evidence which suggests that in comparison to NRT users, e-cigarette users have greater odds to achieve and sustain abstinence (Brown et al., 2014). Though it cannot be said that e-cigarettes are the main contributor, the decline in smoking prevalence has coincided with the rise in EC use (Office for National Statistics, 2017). Recent statistical data suggest e-cigarettes as the most popular method of smoking cessation over NRT, prescribed medicines and behavioural support (Brown et al., 2014; West et al., 2017). Similarly, in England, an estimated 2.5% (approximately 22,000 smokers) achieved complete cessation using an e-cigarette where they would have failed using no aids or a licensed nicotine product purchased over the counter (West, Shahab, & Brown, 2016). Although past studies do not attribute the decrease in use of more conventional licensed medicinal products to the rapid rise in e-cigarette use (Beard, Brown, McNeill, Michie, & West, 2015), they have certainly coincided. Such coincidences add to concerns that e-cigarettes may detract from the use of licenced nicotine products and impede tobacco control efforts by renormalizing smoking thereby attracting youths and non-smokers and causing a gateway effect to smoking (Bell & Keane, 2014; Kozlowski & Warner, 2017; Leventhal et al., 2015). Some of the opponents expressing concerns, have cited cross sectional study data (Dutra & Glantz, 2014) to conclude that youths who 'ever used' an e-cigarette (even

one puff) were more likely to later become smokers. Such causal inferences are highly inappropriate, ignore many possible confounders whilst failing to consider plausible alternative explanations. Besides, recent statistical data suggest otherwise. For instance, the prevalence in youth smoking in the UK (Bauld et al., 2017; Bauld, MacKintosh, Ford, & McNeill, 2016) and the US (Harrell, Naqvi, Plunk, Ji, & Martins, 2017; Kozlowski & Warner, 2017) suggests that the modest increase in e-cigarettes use has coincided with a decrease in smoking prevalence amongst youths, which is indicative of a gateway from smoking. In fact, many studies alluding to a gateway effect, use flawed methodologies including questionnaire items measuring 'ever use' or 'past 30 day use' which fail to capture actual frequency and intensity of use (Polosa, Russell, Nitzkin, & Farsalinos, 2017). In fact longitudinal data suggest that 'ever use' rarely translates into regular use but rather relates to experimentation (Bauld et al., 2017; Greenhill, Dawkins, Notley, Finn, & Turner, 2016). This is in agreement with the fact that the prevalence of e-cigarette use amongst youths (Jamal et al., 2017) as well as amongst non-smoker adults in the EU (Farsalinos, Poulas, Voudris, & Le Houezec, 2016) remains fairly low and appear to be decreasing. Furthermore, longitudinal data from the US found that prohibition of e-cigarettes sale to adolescents in some States tended to be positively associated with increased smoking rates amongst these groups (Friedman, 2015; Pesko, Hughes, & Faisal, 2016). In addition, the number of ex-smokers using e-cigarettes rose to 52% (approximately 1.5 million) compared to 45% of dual users (smokers using e-cigarettes) (ASH, 2017), this adds further evidence that e-cigarettes are providing a route away from smoking. Of particular interest, in 2014, of a total of 27 460 of EU current and former e-cigarette users, only 10.8% reported being former smokers against 31.1% classified as dual users (Farsalinos, Poulas, Voudris, & Le Houezec, 2016). Altogether this suggests a clear trend of an increase in departure

from smoking to dual use which translates into complete cessation. This is supported by the Eurobarometer data, which found an estimated 35.1% and 32.2% of current e-cigarette users reporting smoking cessation and reduction (respectively) with the help of an e-cigarette, based on 48.5 million EU e-cigarette users (Farsalinos, Poulas, et al., 2016). Of particular importance to this thesis, most of this sample (76.8%) used nicotine containing e-cigarettes as opposed to nicotine-free-ones; the widespread use of nicotine in this sample could be seen as a continued nicotine dependence and a willingness to substitute a well-known harmful mode of nicotine delivery for a safer one. Encouragingly, studies suggest that e-cigarette use may be less addictive than smoking. This is supported by studies comparing nicotine dependence as an e-cigarette vaper against dependence as a smoker; former smokers who made a complete substitution were found to be less dependent on their devices compared to their tobacco smoking (Foulds et al., 2015). This is in agreement with findings suggesting that the latency of the first cigarette of the day, a good indicator of dependence (Heatherton et al., 1989), is longer for e-cigarettes than for tobacco cigarettes (Dawkins, Turner, Roberts, & Soar, 2013; Farsalinos et al., 2013a,b; Goniewicz et al., 2013).

Evidence on the effectiveness of e-cigarettes has not always been so clear and, as they have gained in popularity, opinions have become more polarised. Although early studies suggesting that e-cigarettes could promote cessation were deemed too few thus inconclusive (McRobbie, Bullen, Hartmann-Boyce, & Hajek, 2014), as e-cigarettes have gained in popularity worldwide (Brown et al., 2014; de Lacy, Fletcher, Hewitt, Murphy, & Moore, 2017; Delnevo et al., 2015; Giovenco & Delnevo, 2018; Hummel et al., 2015; King, Patel, Nguyen, & Dube, 2015) including in unfavourable regulatory climates wherein sales of nicotine-containing e-cigarettes are prohibited unless classified as medicinal (Li, Newcombe, & Walton, 2015), the evidence has mounted and

strengthened considerably. In fact findings of early clinical studies (Bullen et al., 2010; Vansickel, Cobb, Weaver, & Eissenberg, 2010) suggested they were inefficient at delivering nicotine, whilst early randomised controlled trials (RCT) and cohort studies found they had very modest effects on abstinence rates (Bullen et al., 2013) casting doubt on their efficiency to become viable as nicotine replacements products. The 2014 Cochrane review, based on evidence from two RCTs with just under 1000 smokers, suggested that 6 months abstinence was more likely in smokers using nicotine e-cigarettes compared to those using placebo e-cigarettes whilst one study found no difference between transdermal nicotine products and e-cigarettes (McRobbie, Bullen, Hartmann-Boyce, & Hajek, 2014). In contrast, evidence from nine cohort studies of just under 2000 smokers did suggest that e-cigarettes could reduce smoking significantly; the limited number of studies and lack of certainty in the results rendered the evidence inconclusive (McRobbie, Bullen, Hartmann-Boyce, & Hajek, 2014). However, one of the key issues with these early studies involved the use of early generation models with poor nicotine delivery highlighting the need to consider device efficiency and reliability more carefully (Lopez & Eissenberg, 2015). Since these early trials and clinical studies, the technology has advanced considerably, and better developed and more efficient devices have enhanced nicotine delivery (Farsalinos et al., 2016). Substantial numbers of experimental studies have found that e-cigarettes can, not only deliver nicotine efficiently (D'Ruiz, Graff, Yan, Sherwin Yan, & Yan, 2015; Dawkins & Corcoran, 2014; Farsalinos et al., 2014; Farsalinos, Spyrou, Tsimopoulou, et al., 2015; Hajek, Przulj, Phillips, Anderson, & McRobbie, 2017; Lee, Gawron, & Goniewicz, 2015; Lopez et al., 2016; Wagener et al., 2016) but sometimes at levels that exceed tobacco cigarettes (Ramôa et al., 2015). Subsequently, it became apparent that measuring the impact of e-cigarettes on smoking cessation using RCTs may have

inherent limitations. E-cigarette use is a highly complex phenomenon. Given the rapid and constant evolution of the product (Lopez & Eissenberg, 2015), the considerable choice in products, the large variation within and between devices combined with the changing trends in use patterns and the possible peer-influence encountered via e-cigarette forums and social media, RCTs may be subject to non-compliance. Thus, as many confounders may arise, it becomes apparent that RCTs are not always appropriate and may not reflect 'real life' scenarios. In fact, in a small (N = 40) 'real life' observational study, in which smokers were followed up for two years, 27.5% of the sample reduced their smoking by more than 50% (reducing their cigarettes smoked per day from 24 to 4), whilst 40% were abstinent at 2 years (Polosa et al., 2014). Further controversies adding to the misconceptions about e-cigarettes' efficiency as smoking cessation aids, stem from a meta-analysis from the US which included cross-sectional and prospective cohort studies (Kalkhoran & Glantz, 2016). The odds of quitting were 28% lower using e-cigarette compared to those who did not. However, this meta-analysis failed to capture crucial variables related to participants' past quit attempts, such as models used, frequency and duration of use (that is casual versus regular use) conflating all different types of e-cigarette users and devices. Firstly, many of the studies included, suffered a sample selection bias wherein ex-smokers who quit using an e-cigarette would have been excluded, whereas smokers who may have made many attempts to quit and used an e-cigarette prior to taking part in the study could be included. In addition, the study did not take into account reasons for e-cigarettes use. For example, one study included reported low cessation rates amongst those who used e-cigarettes for a purpose other than cessation (Vickerman et al., 2017). Secondly, frequency and duration of use were not captured; in fact research suggests that greater frequency of use is associated with a higher likelihood to quit (Hitchman, Brose,

Brown, Robson, & McNeill, 2015). Finally, in the analysis, all devices were treated as one type, a number of studies have found that considerable variations between e-cigarette types in terms of their efficiency (Caponnetto et al., 2017; Farsalinos, Spyrou, et al., 2014; Hajek et al., 2017; Hitchman et al., 2015; Kozlowski, Lynn Homish, Homish, Homish, & Homish, 2017); and that most successful attempts will involve transitioning from one type of device to a more efficient one (Yingst et al., 2015). Clearly, all the aforementioned are crucial components when determining the effectiveness of e-cigarettes, and have all been neglected in this meta-analysis. Given the highly heterogeneous nature of e-cigarettes and e-liquid disparity combined with the different materials and varied methodologies similarly to RCTs, meta-analysis are highly susceptible to misinterpretations. Thus, population level studies may be better suited to assess the true impact of e-cigarettes on the population. In contrast, the 2014 PATH study using a methodology that allows distinguishing between casual and regular use and the inclusion of former smokers who used e-cigarettes, found clear differences between experimentation and former smokers who used their devices on a daily basis (Rodu & Plurphanswat, 2017a); this highlights the need for careful consideration to clearly discriminate users such as regular users versus from experimenters.

A substantial number of studies suggested e-cigarettes to have the potential to promote cessation (Beard, West, Michie, & Brown, 2016; Bell & Keane, 2012; Koch, 2012; Meier, Tackett & Wagener, 2013; Weinberg & Segelnick, 2011), including a large English cross-sectional study (N = 5863), which found that those using an e-cigarette were more than twice as likely to remain abstinent at one year compared to those who used NRT or no aid (Brown et al., 2014). This was echoed in a recent US study which, using the National Health Interview Survey (NHIS) (and a sample of current and former smokers, N = 15,532), found that those using an e-cigarette daily

were more likely to have quit compared to those who did not use an e-cigarette (Giovenco & Delnevo, 2018). In the UK, the emerging evidence has accompanied a growing consensus that e-cigarettes can help reduce smoking and promote cessation. For instance, in 2015, based on a review of 158 studies, Public Health England (PHE) have endorsed them as less harmful than smoking and state that their use should be encouraged for those who are unable or unwilling to quit (McNeill et al., 2015). The Royal College of Physicians echoed PHE's endorsement, other public organisations (such as NICE and NCSCT the National Centre for Smoking Cessation and Training) followed to endorse e-cigarettes and encourage smokers who are unable to quit using conventional cessation aids to try the products. The fact that PHE incorporated e-cigarettes in national cessation campaigns such as 'Stoptober', suggest a change in attitudes with regard to advertisement regulations (NHS, n.d.). Evidence on the effectiveness of e-cigarettes to be helpful in a quit attempt is also captured in qualitative data which suggest that e-cigarettes can help prevent complete relapse to smoking (Notley, Dawkins, Holland, Jakes, & Ward, 2017). The potential of e-cigarettes to help reduce and promote cessation (Etter & Bullen, 2014) more so than NRT (Beard, Bruguera, McNeill, Brown, & West, 2015; Brown et al., 2014) is also reflected in self-reports from long term users stating that they are more effective than NRTs in promoting to smoking cessation and reducing withdrawal symptoms (Nelson et al., 2015).

Based on the International Tobacco Control (ITC) Netherlands Survey and data collected between 2008 and 2014 (N = 1820; N = 1550) the reported chief reason for trying an e-cigarette was to reduce the number of cigarettes smoked per day (hereafter referred to as CPD) (Hummel et al., 2015). In the UK, the ASH report highlighted some distinctions, but clear similarities, between current (dual users) and former

smokers. Reducing and stop smoking were the main reasons reported by dual users and former smokers respectively; whilst for both groups 'saving money' was the second reason, followed by "to help me stop smoking entirely" and "to help keep me off tobacco" given by former and current smokers respectively (ASH, 2017). Other reasons for using an e-cigarette include seeking health improvements (Brown et al., 2014), past failed attempts and concerns regarding the consequences of passive smoking (ASH, 2017).

Aside from reasons for using e-cigarettes, it is relevant to briefly note the key characteristics sought after in e-cigarettes. Past studies have noted that for smokers who have tried e-cigarettes, features such as the design would contribute to increase their appeal (Dawkins, Kimber, Puwanesarasa, & Soar, 2015); whilst for experienced e-cigarette users i) the ergonomics, ii) the compatibility with varied components, iii) the ability to adjust the voltage and wattage, iv) durability and reliability are key desirable characteristics (Baweja et al., 2016; McQueen, Tower, & Sumner, 2011). Of key importance is the 'more satisfying hit', battery capabilities and the affordability to greater e-liquid choice seem highly important features (Baweja et al., 2016; Yingst et al., 2015). The latter will be later discussed in the context of nicotine delivery and differences in device types. These factors are important as they may further the understanding of dissatisfaction and product discontinuation or the need to transition to other models (Yingst et al., 2015); this can be informative to smokers who are yet to transition away from smoking.

E-cigarettes Safety

As there is no combustion and tar, and far fewer toxicants compared with tobacco cigarettes (Goniewicz et al., 2014; McAuley, Hopke, Zhao, & Babaian, 2012; Schripp, Markewitz, Uhde, & Salthammer, 2013; Stephens, 2017), e-cigarettes are

deemed a safer alternative to tobacco smoking (McNeill et al., 2015). This is echoed through discourses amongst vapers who report significant health improvements after switching to e-cigarettes (Dawkins, Turner, Roberts, & Soar, 2013; McQueen, Tower, & Sumner, 2011).

Data from toxicological studies suggest that e-cigarette use is far less harmful than smoking combustible cigarettes (Farsalinos & Polosa, 2014) and subtituting smoking for e-cigarettes use significantly reduces exposure to selected toxicants and carcinogens (Goniewicz et al., 2017) as well as leads to considerable health benefits (Caponnetto, Polosa, Morjaria, Caruso, Strano, & Russo, 2015). Encouragingly, a recent study shows that biomarkers of exposure to selected toxicants and carcinogens in exclusive e-cigarette users are equivalent to those found in exclusive NRT users and significantly lower than those found in smokers (Shahab et al., 2017). Furthermore, several studies (Caponnetto et al., 2015; Farsalinos et al., 2016; Polosa et al., 2016) have found that in individuals with asthma, chronic obstructive pulmonary disease (COPD) or high blood pressure, patients who switched to e-cigarettes, showed a significant improvement in symptoms and physical activity levels. In 2015, PHE estimated that e-cigarette use would unlikely exceed 5% of the risks associated with smoking. This was based on an assessment of 185 studies (McNeill et al., 2015) and the estimate was supported in the Royal College of Physicians subsequent report (Royal College of Physicians & Group, 2016). It is worth noting that the widely cited estimate that e-cigarettes are 95% safer than smoking has received criticisms (The Lancet, 2015; McKee & Capewell, 2015) and these have been addressed in a comprehensive response by the authors (McNeill, Brose, Calder, Hitchman, et al., 2015; McNeill & Hajek, 2014).

The mechanism by which e-cigarettes operate consists of heating a solution at a temperature not exceeding 290°C, which involves little chemical alterations under commonly used operating temperature (Pankow et al., 2017). Conversely, side- and mainstream smoke from tobacco cigarettes involve combustion which can reach up to 900°C and creates physical and chemical alterations generating more than 7000 known and potentially harmful chemicals (United States Department of Health and Human Services, 2014). In fact, studies suggested that many of the chemicals present in tobacco smoke appear to be absent, at trace levels or much lower levels in e-cigarette aerosols in comparison (Flora et al., 2016; Goniewicz et al., 2014; Pankow et al., 2017; Tayyarah & Long, 2014). However, specific circumstances such as long puff duration in combination with high voltage applied to the coil can lead to over-heating the atomizer (Geiss, Bianchi, & Barrero-Moreno, 2016) which can in turn produce carbonyls (Farsalinos, Voudris, & Poulas, 2015; Kosmider et al., 2014; Pankow et al., 2017) listed as harmful or potentially harmful constituents (HPHC) in tobacco products and tobacco smoke (FDA, 2012).

Though e-cigarette aerosols have been found to yield greater number of particles compared to aerosols produced by tobacco cigarettes, studies suggest minimal risks from first and second hand compared to tobacco cigarettes smoke given that the particle concentrations deposited onto surfaces are significantly lower (Scungio, Stabile, & Buonanno, 2018). Likewise, based on previously published chemical analyses, a recent study suggested the potential for carcinogenic cell production from e-cigarette aerosols exposure was found to be only one percent of that from tobacco cigarettes smoke (Stephens, 2017).

Conflicting studies can also be found in the literature with regards to their safety. In one study, sputum samples from e-cigarette users, smokers and non-smokers

were analysed to measure total and individual mucins concentrations and neutrophil extracellular trap formation rates. Findings of greater levels of the target analytes in the e-cigarette group in relation to the smoking group allowed the authors to conclude that the use of e-cigarettes may increase the risk of immune system alteration in the lung system (Reidel et al., 2017). However, it is unclear if and how smoking history of the e-cigarette users was accounted, treated or adjusted for in the analysis. Indeed a sample which included heavy smokers who switched to e-cigarettes would have influenced the results. Moreover, some ex-smokers in the e-cigarette user group were reported to smoke occasionally which alludes to a possible conflation between smokers and e-cigarette users and raises further concerns in relation to the interpretations of these findings. A better sampling method would include e-cigarette users with no history of smoking or the introduction of a control group (for example smokers who quit with the aid of NRT or medication). Further limitations are that sputum cultures may not be the best specimen to determine causative organisms in lung disease as they have not always yielded clear conclusions (García-Vázquez et al., 2004). More importantly, previous studies suggest that increases in mucins concentrations and sputum neutrophils in COPD suffering quitters with inflamed airway may be part of a repair mechanism of tissue damage and this was found to persist a year following cessation (Willemse et al., 2005). Therefore, the authors' interpretation that e-cigarettes use may lead to alterations of the immune system may be premature.

Other critics refer to concerns vis-á-vis the e-liquid safety following publications that some flavours may be associated with high levels of toxicants due to oxidation or thermal degradation (Behar et al., 2016; Leigh, Lawton, Hershberger, & Goniewicz, 2016); whilst others found greater levels of aldehyde in e-cigarette aerosols compared to that of combustible cigarettes (Jensen, Luo, Pankow, Strongin, & Peyton, 2015).

However, it appeared that occurrences of thermal degradation often relate to extreme temperature and misuse of the voltage (Farsalinos, Kistler, et al., 2017; Gillman, Kistler, Stewart, & Paolantonio, 2016). Replicating the methodologies used in these studies, it was later demonstrated that in many instances, the investigators failed to employ realistic conditions replicating how these devices are used in everyday lives, namely a failure to change the coil after each replicate and excessive power applied to the coil, which resulted in greater toxicants and carbonyls production (Stephens, 2017); referred to as the '*dry puff*' phenomenon (Farsalinos, Voudris, & Poulas, 2015). Besides, empirical research has shown that *dry puffs* are discernible and aversive thus avoided by e-cigarette users (Farsalinos, Voudris, Spyrou, & Poulas, 2017) which highlights the need for continued research and more importantly for the inclusion of realistic usage conditions.

Thus altogether, although it may be a little premature to determine the long term health effects associated with e-cigarettes, there is substantial evidence to suggest that e-cigarettes are much safer than smoking, and it is this existing evidence that is informing the current regulatory framework applied in the UK.

E-Cigarette Regulations

The evidence thus far that e-cigarettes are a viable substitution to smoking is vastly encouraging, thus they are likely to play a favourable role in tobacco harm reduction. To this effect, regulations should ensure a minimum quality product standard. The European commission introduced a range of regulations through article 20 from the EU-TPD, with the intentions of ensuring safety and a minimum standard of quality product (European Union, 2014). These included limiting the tank and cartridge sizes to 2 mL and a restriction on nicotine concentrations to 20 mg/mL unless subject to

an application to be classified and sold as medicinal devices which can be an onerous procedure. Although intended to ensure safety, these restrictions may deter potential users (i.e. current smokers) rendering e-cigarettes less appealing. Although, many e-cigarettes users gradually reduce their nicotine concentration over time (Polosa, Caponnetto, Cibella & Le-Houezec, 2015), highly dependent smokers may need to increase their nicotine concentration in order to achieve complete smoking abstinence at least in the early stages of a cessation attempt (Farsalinos, Romagna, Tsiapras, Kyrzopoulos & Voudris, 2013). Although data suggest that only a small proportion of e-cigarette users use nicotine e-liquid higher than the EU-TPD cap (ASH, 2017), given the large population of e-cigarette users overall (2.9 million), the number affected remains large (approximately 170 000 users) and must not be ignored. However, the recent decision to exit the EU might provide some flexibility for the UK to respond to emerging evidence with regards to evaluating the legislative provisions introduced within the TPD. Besides, other countries have gradually softened their stance and started to recognise the potential benefit of e-cigarettes in reducing the burden of smoking. In the process of legalising e-cigarettes, Canada has published an amendment to its *Tobacco Act* to allow for a clear differentiation between the act of smoking from vaping (Senate Government Bill, 2017), whilst New Zealand, recognising the potential of e-cigarettes to contribute towards the *Smoke free 2025* target, is adopting regulations more in line with the UK (New Zealand Ministry of Health, 2017). In contrast, e-cigarettes are still banned in many other countries including in Brazil, Argentina, Colombia and Indonesia.

Ensuring policies in place remain appropriate and proportionate are highly important, since studies show that the regulatory environment may influence the impact of e-cigarettes on smoking cessation (Yong et al., 2017). A comparison was made on

the real-world effectiveness of e-cigarettes in two countries (Canada and Australia) wherein nicotine e-cigarettes sales are prohibited, with two countries (USA and UK) with far less restrictive policies (Yong et al., 2017). It was found that smokers in less restrictive policy climate were more likely to quit using e-cigarettes, compared with making an attempt unaided. In contrast, those using e-cigarettes to quit in more restrictive policy climate were less likely to succeed in their quit attempt (Yong et al., 2017).

Nicotine concentrations, puffing topography, and user's experience

As previously mentioned, the slow and gradual release of nicotine from NRT (Digard et al., 2013) may not be so appealing to smokers and may explain NRT's low abuse liability (Henningfield & Keenan, 1993; Nelson et al., 2015) and the desire for smokers to have greater control over their nicotine delivery at a 'finger-tip' level. Indeed, the troughs and peaks observed following the smoking of a cigarette tend to be strongly associated with greater satisfaction levels (Berridge et al., 2010) and reversal of withdrawal symptoms (Nelson et al., 2015) compared to NRT, which suggests the need for immediate reward namely a 'hit' and lends support to the bolus and titration theory.

Nicotine Delivery and Puffing Topography

Similar to smoking, nicotine delivery in e-cigarettes use is likely to be a key determinant to dictate puffing topography, blood nicotine levels and satisfaction. Early studies to investigate the effectiveness of e-cigarettes found that the devices delivered no to little levels of nicotine compared to tobacco cigarettes (Bullen et al., 2010; Eissenberg, 2010; Vansickel et al., 2010). Differences between tobacco cigarettes' and e-cigarettes' nicotine delivery could be largely attributed to the differences in mechanism suction between cigarette smoking and e-cigarette use with e-cigarettes

requiring a stronger vacuum than combustible cigarettes (Trtchounian, Williams, & Talbot, 2010). This relates to the delay between the activation of the atomiser and the time of the aerosol generation, and to the lower volume of aerosols produced compared with tobacco cigarettes (Farsalinos, Romagna, Tsiapras, Kyrzopoulos, & Voudris, 2013). Indeed some found that despite a higher frequency in e-cigarette puffs compared to tobacco smokers, e-cigarettes failed to match the nicotine delivery of tobacco cigarettes (Hajek et al., 2017). It is worth noting though, that many other variables could have contributed to these differences namely differences between individuals' nicotine absorption, the type of the devices, and so on.

Whilst early studies suggested that e-cigarettes had poor nicotine delivery in naïve users (Bullen et al., 2010; Eissenberg, 2010), subsequent studies in experienced users both, with cigalikes (Dawkins & Corcoran, 2014) and tank systems (Vansickel & Eissenberg, 2013) have yielded significantly higher blood nicotine levels, providing craving relief and satisfaction compared to naïve users using cigalikes or tanks (Farsalinos et al., 2014; Farsalinos et al., 2015). This might be explained by different puffing topographies between experienced users who reportedly take longer puffs compared to naïve users (Farsalinos, Spyrou, Stefopoulos, et al., 2015). Likewise, naïve users tend to increase their puffing over time, possibly to optimise blood nicotine delivery (Lee et al., 2015). Aside from puffing patterns, higher nicotine concentrations in e-cigarette liquids may be preferable to maximise effective blood nicotine delivery. Indeed, studies found that high nicotine concentrations are associated with more effective nicotine delivery (Farsalinos et al., 2013a; Hajek et al., 2017; Lopez et al., 2016), greater satisfaction and greater reduction in withdrawal and desire to smoke (D'Ruiz et al., 2015) and, many studies suggest that the addictiveness of nicotine delivery systems is dependent upon the speed at which nicotine is delivered (Le

Houezec, 2003). That said, e-cigarette users tend to decrease their nicotine concentrations over time (Lechner et al, 2015), however the drivers of this phenomenon are currently unclear. This shift in behaviours may be driven by the negative perception surrounding the long term use of nicotine, the regulatory environment (that is the cap on nicotine concentrations imposed by the EU-TPD) or the increasing popularity of 'sub-ohming'.

To reverse the negative effects of withdrawal symptoms (e.g. lack of concentration, depressed mood and irritability) experienced during abstinence, smokers need to maintain a constant plasma nicotine level by means of continued nicotine intake (Collins et al., 2010). This is supported by the large body of evidence which shows that smokers regulate levels of nicotine in their blood system by adjusting their puffing behaviours when given a lower or higher nicotine concentrations compared to their habitual ones, as discussed earlier (Ashton et al., 1979, 1970; Herning et al., 1981; Strasser et al., 2007; Woodward & Tunstall-Pedoe, 1993). The hypothesis that e-cigarette users may similarly titrate to regulate their blood nicotine levels, although not explicitly, is alluded to in empirical studies. Large individual differences were found in plasma nicotine levels in e-cigarettes users using the same device, concentrations and prescribed standardised puffing protocols (Dawkins & Corcoran, 2014). This suggests that participants may have adjusted the way they puffed by taking more, longer and/or more intensive puffs compared to others in order to extract their desired amount of nicotine. If e-cigarette users are able to self-titrate in the same manner as smokers do with tobacco cigarettes, enabling 'finger-tip' control over their nicotine intake, this might explain the increased appeal of e-cigarettes over traditional NRT.

Nicotine concentrations

Aside from puffing topography and device efficiency, another key driver in nicotine delivery is the concentration of nicotine in the e-liquid. Likewise, pharmacokinetic studies suggest that greater craving and withdrawal relief are associated with high-powered tank systems, more intensive puffing patterns and higher nicotine concentration e-liquids (Etter, 2015). That higher nicotine concentrations are associated with greater craving relief is of some importance, given that the second reason for discontinuation of e-cigarettes was their inefficiency to deal with craving relief (ASH, 2017). Indeed, like nicotine yield in tobacco cigarettes (Hammond, Fong, Cummings, & Hyland, 2005; Russell, Epstein, & Dickson, 1983), e-liquid nicotine concentration is an important factor that is likely to influence puffing topography. In a later study, researchers compared puffing topography between 0, 8, 18 and 36 mg/mL and the interactions with plasma nicotine levels following two vaping bouts of ten puffs each in experienced users (Ramôa et al., 2015). Participants increased their puff duration in the 0 mg/mL condition compared with the 36 mg/mL nicotine concentration (however not in the 8 or 18 vs. 36 mg/mL condition), suggesting an attempt to compensate for the deprivation of nicotine (Ramôa et al., 2015). However, although the study supports the hypothesis of compensatory puffing, utilising a standardised ten-puff protocol may have influenced users' natural puffing behaviours and/or reduced their ability to compensate fully.

There is also evidence that higher nicotine concentrations products (including NRT) can increase smoking cessation (Tunnesen et al., 1999); and that in the early stages of a cessation attempt, smokers need to increase their nicotine concentration in order to achieve and sustain complete smoking abstinence (Farsalinos, Romagna, Tsiapras, Kyrzopoulos & Voudris, 2013). This highlights the importance of high nicotine concentrations over low concentrations.

Longitudinal studies suggest whilst long term e-cigarettes users gradually reduce their nicotine concentration over time (Farsalinos et al., 2013a; Farsalinos, Romagna, Tsiapras, Kyrzopoulos, & Voudris, 2014), this tends to be associated with an increase in nicotine intake (Etter, 2016). Indeed, in a sample of 98 experienced users, although nicotine concentrations decreased from a median cotinine level of 11 to 6 mg/mL after a period of 8 months, salivary cotinine levels increased from 252 ng/mL to 307 ng/mL at follow-up (Etter, 2016). This increase in nicotine consumption may be explained by a need to compensate for the drop in nicotine concentrations and self-titrate in order to maintain a satisfactory and constant level of blood nicotine level. In fact, similarly to smoking (Gritz, Rose, & Jarvik, 1983), the number of puffs do not determine consumption, rather volume consumed is a better predictor; this is acknowledged by e-cigarette users (Baweja et al., 2016), suggesting that other components of puffing topography such as duration may dictate consumption. This adds further evidence for the inter-variability in puffing topography and that self-titration may occur in e-cigarette use.

Differences in device types

Another key element of nicotine delivery is the proficiency of the device used. In recent years, e-cigarettes have evolved and improved considerably. Recent studies using tank systems as opposed to cigalikes have demonstrated rapid nicotine delivery approximating tobacco cigarettes (D'Ruiz, Graff, Yan, Sherwin Yan, & Yan, 2015; Farsalinos et al., 2014; Farsalinos, Spyrou, Tsimopoulou, et al., 2015; Hajek, Przulj, Phillips, Anderson, & McRobbie, 2017; Lee, Gawron, & Goniewicz, 2015; Lopez et al., 2016; Wagener et al., 2016). Nonetheless, there are still marked variations in devices' efficacy to deliver nicotine and their ability to induce a satisfactory hit (Baweja et al.,

2016), and to alleviate craving and withdrawal symptoms (Farsalinos et al., 2014; Hajek et al., 2017; Yingst et al., 2015). Furthermore, studies suggest that successful quit attempts are seldom associated with cigalikes (Brose, Hitchman, Brown, West, & McNeill, 2015; Brown et al., 2014) and the use of tanks are more likely to lead to a quit attempt (Hitchman et al., 2015). The popularity of tank systems over cigalikes with experienced users (Dawkins, Turner, Roberts, & Soar, 2013; Hitchman, Brose, Brown, Robson, & McNeill, 2015; Polosa, Caponnetto, Cibella, & Le-Houezec, 2015; Polosa, Caponnetto, Maglia, Morjaria, & Russo, 2014) may be partly due to their added features, sophistication and more importantly their superiority in delivering nicotine (Farsalinos et al., 2014; Farsalinos, Yannovits, Sarri, Voudris, & Poulas, 2016). This accords with empirical findings suggesting that successful quit attempts often involve and may require transitioning from cigalikes to tanks systems (Yingst et al., 2015).

Although e-cigarettes address both the pharmacological and the psycho-behavioural aspects of smoking, for many smokers they are not as satisfying as smoking. Forty-five percent are dual users whilst 65% of smokers who have tried have discontinued use. Of these 25% reported that they do not feel like smoking and 20% reported they did not help reducing craving for tobacco cigarettes (ASH, 2017). Altogether, the need to monitor, report and ensure efficiency across all device types is warranted.

Furthermore, as previously mentioned, there are key characteristics that are favourable to e-cigarette users (Baweja et al., 2016; Dawkins et al., 2015; McQueen et al., 2011; Yingst et al., 2015) and which may help increase the appeal of these devices to existing smokers. The following characteristics, the compatibility with varied components, the ability to adjust the voltage and wattage, (Baweja et al., 2016; McQueen et al., 2011), ‘more satisfying hit’, battery capabilities and the affordability to

greater e-liquid choice (Baweja et al., 2016; Yingst et al., 2015) are associated with tank systems and are absent from the cigalikes.

User experience: Effects on puffing topography and nicotine delivery

Similarly to tobacco cigarettes smokers (Hammond et al., 2005), there are inter-individual variabilities in e-cigarette users' puffing topography whilst puff number and duration tend to remain constant within individuals across brands or days (Behar, Hua, & Talbot, 2015). Consistency in puffing behaviours within each e-cigarette user may be indicative of a need to maintain steady and constant blood nicotine levels as per the self-titration theory (Russell, 1980; Russell & Feyerabend, 1978).

A key factor in the efficacy of delivering nicotine and in providing satisfaction is the way in which the device is used. Experienced users typically achieve higher levels of plasma nicotine, craving relief and satisfaction compared to naive users; they do so by taking longer and larger puffs thus drawing greater volume of nicotine liquid compared to e-cigarette naïve smokers (Farsalinos, Spyrou, Stefopoulos, et al., 2015b). The efficacy of obtaining satisfactory blood nicotine levels also depends upon the way the e-cigarette is used and studies have shown that both are likely to improve with practise (Hajek et al., 2015). Naïve users tend to increase their puffing patterns over time (Hajek et al., 2015; Polosa et al., 2014), which can lead to marked improvements in blood nicotine levels (Hajek et al., 2015; Lee et al., 2015). This long term adjustment in puffing patterns support the notion that e-cigarette use is a learning curve (McQueen et al., 2011) and practise is required in order to obtain satisfaction.

Puffing Topography Measurements and Methods

For the purpose of the current thesis, the focus here will remain on measurements and methods used to collect human data in e-cigarettes use as opposed to smoking machines which mimic human puffing patterns for the purpose of compounds (smoke/aerosol) analyses. Puffing topography data provide direct evidence of a change in puffing patterns thus is central to compensatory puffing behaviours research (Scherer, 1999). Though puffing topography measures may comprise several variables (i.e. puff duration, numbers, inter-puff intervals, puff volume/velocity, inhalation/exhalation time, pressure drop, flow rate and peak flow rate (see Table 1.1), studies documenting e-cigarette users' behaviours have put more emphasis on puff duration (Hua et al., 2013) and puff number (Farsalinos et al., 2013b, 2015, 2017; St Helen et al., 2016; Strasser et al., 2016) in addition to volume consumed (Norton et al., 2014). However, there is evidence to suggest puff number as a poor indicator of puffing topography and volume consumed and puff duration as stronger assessors for compensatory puffing (Baweja et al., 2016).

Historically, a variety of different methods have been used to capture puffing topography data in smokers; these include observational methods and more precise computerised systems, for instance instruments measuring flow and pressure rate and puff velocity in milliseconds (e.g. Puustinen, Olkkonen, Kolonen, & Tuomisto, 1987). In the nascent era of e-cigarettes research, observational methods such as Youtube videos (Hua et al., 2013) and frame-by-frame analysis (Farsalinos et al., 2013b) were used to report puffing behaviours of e-cigarette users. These methods (Hua et al., 2013; Farsalinos et al., 2013) were in good agreement in that they both suggested differences in puffing characteristics between e-cigarettes users and smokers (Farsalinos, Romagna, Tsiapras, Kyrzopoulos, Voudris, 2013b; Hua et al., 2013). Although frame-by-frame time analysis of video recordings is a useful and reliable method, it present some

limitations. It does not allow the measurements of puff velocity and volume and is susceptible to visual interpretation bias. This is reflected in earlier reports which found longer puff duration compared to mouth-piece devices (Blank et al., 2009). Moreover, the frame-by-frame time analysis renders this method relatively onerous.

Attempts have been made to employ the handheld machine CReSS (Clinical Research Support System) to capture puff count, duration, IPI, puff volume, rate and peak flow rate in e-cigarette users (Behar et al., 2016). However, since the machine was originally developed for tobacco cigarettes and due to in-built features such as a cut-off point for the number of puffs and other technical issues such as leakages, use of the device has led to underestimation of puff counts and duration in some instances (see Behar et al., 2016 for example). Eventually with technological advancement, some studies have been able to overcome these issues by the addition of a manufacturer adaptor for e-cigarettes (Norton et al., 2014). Portable mouth-piece instruments have also been used to capture real world (and daily) puffing topography outside of the lab (e.g. Robinson et al., 2015); and these have been found to offer a more pragmatic and less intrusive method compared to desktop computerised systems (Spindle et al, 2015). However, findings that such modes of data collection may be intrusive thereby confound puffing behaviour data (Ross & Juliano, 2016; Spindle et al., 2015), these have highlighted a need for technologies that offer greater ecological validity. For instance, it has been found that the use of mouth-piece devices negatively affects experiences in many ways including making e-cigarette use difficult (Spindle et al., 2015) and diminishing pleasure from smoking (Blank et al., 2009). Thus, the need for reliable, pragmatic and cost-effective methods with good ecological validity is warranted. Equipped with an in-built downloadable software and memory chip that automatically records and stores the start and end of single puff upon each button press,

the eVic e-cigarette has recently entered the market (at the time of Study 1 planning); these features make the e-Vic a well suited choice of instrumentation to measure puffing topography. Table 1.1 offers an overview of publications reporting used methods and measures in chronological order of methods used; note that care has been taken to differentiate publications that preceded Study 1 planning and data collection and publications that followed.

Table 1.1
Publications reporting puffing topography measures and methods in e-cigarettes research

Author (s) / Publication date	Variables measured	Method used	Study settings
Observational methods			
Hua et al., 2013*	Puff duration	*Youtube* videos	Real world / Ad lib
Farsalinos et al., 2013b*	Puff number and duration	Video recordings	Lab / Ad lib
St Helen et al., 2016	Puff number and duration, IPI	Video recordings	Lab / Ad lib
Strasser et al., 2016	Puff number and duration, IPI	Video recordings	Lab / Standardised protocol
Mouth-piece connecting instruments			
Norton et al., 2014*	Puff number and duration, IPI, Puff volume	CReSS Micro	Lab / Ad lib
Spindle et al., 2015	Puff number and duration, IPI, Puff volume, Flow rate	Non-commercial computerised instrument	Lab / Standardised protocol
Behar, Hua & Talbot, 2015	Puff number and duration, IPI, Total volume, Volume/puff, Flow rate, Peak flow rate	CReSS pocket device with EC adaptor	Lab / 2 separate days of 2 ad lib sessions consisting of 10-min bouts
Lee et al., 2015	Puff number and duration, IPI, Puff volume, Flow rate	CReSS micro	Lab / Ad lib
Robinson et al., 2015	Puff number and duration, Number of puff/day, IPI, Puff volume, Flow rate	Wireless portable monitor	Real world / Ad lib
Ramôa et al., 2015	Puff number and duration, IPI, Puff volume, Flow rate	Non-commercial computerised instrument	Lab / Standardised protocol

Spindle et al., 2016	Puff number and duration, IPI, Puff volume, Flow rate	Non-commercial computerised instrument	Lab / Standardised protocol
Robinson et al., 2016	Puff duration, Number of puff/day, Puff volume, Flow rate	Wireless portable monitor	Real world / Ad lib
Cunningham et al., 2016	Puff number and duration, IPI, Puff volume, Flow rate	Non-commercial SA7 puff flow-induced device	Lab / Ad lib
Lopez et al., 2016	Puff number and duration, IPI, Puff volume, Flow rate	Non-commercial computerised instrument	Lab / Standardised protocol
Hiler et al., 2017	Puff number and duration, IPI, Puff volume, Flow rate	Non-commercial computerised instrument	Lab / Standardised protocol
Lee et al., 2017	Puff number and duration, IPI, Puff volume, Flow rate	Wireless portable monitor	Real world / Ad lib
Integrated e-cigarette devices			
Farsalinos et al., 2015	Puff number and duration	EVic e-cigarette	Lab / Standardised protocol
Dautzenberg & Bricard, 2015	Puff duration, IPI, Number of puff/day	Smokio monitor (Smokio®; http://us.smokio.com)	Real world / Ad lib
Farsalinos et al., 2017	Puff number and duration	EVic e-cigarette	Lab / Standardised protocol

*Note * indicates publications available at the time of study planning and data collection of Study 1.*
Real world signifies real world environment; IPI signifies inter-puff intervals; Lab signifies Laboratory; Ad lib signifies ad libitum

Summary of Rationale and Primary Aims

Altogether, evidence suggests users' experience, device characteristics and nicotine concentrations as likely factors influencing nicotine delivery and the way a device is puffed on (e.g. puff duration and frequency) (Talih et al., 2014). As previously discussed, nicotine delivery in e-cigarettes use is likely to be a key determinant to dictate puffing topography, satisfactory blood nicotine levels and craving reduction for tobacco cigarettes. Effective nicotine delivery therefore is likely to promote sustained e-cigarette use and tobacco cigarettes substitution, and eventually lead to a successful smoking cessation. In this chapter it has also come to light that similarly to smoking, e-cigarette use may involve self-titration; this is reflected in the stark differences in puffing topography between e-cigarette use and the smoking of a tobacco cigarette (Farsalinos et al., 2013b). Others have found some evidence (albeit not direct due to the use of prescribed protocol puffing) of compensatory puffing under the use of 0 compared to 36 mg/mL nicotine concentrations (Ramôa et al., 2015). There is further evidence to suggest that blood nicotine under the use of low nicotine concentrations (including 18 mg/mL) do not equate levels delivered via tobacco cigarettes (Farsalinos, Spyrou, Tsimopoulou, et al., 2014) and this concords with the fact that e-cigarette users are more likely to achieve complete cessation when initiating use with higher nicotine concentration prior to a gradual reduction over time (Farsalinos et al., 2013a).

Aside higher nicotine concentrations, tank devices that allow power adjustment, have been found to be associated with more effective nicotine delivery compared to cigalikes (Farsalinos, Spyrou, Tsimopoulou, et al., 2014). Thus it follows that the use of tank devices combined with higher nicotine concentrations will be more likely to

promote levels of satisfaction close to that achieved under tobacco smoking and alleviate cigarette craving and withdrawal symptoms more effectively compared to cigalikes.

In the same light, given that tank devices and higher nicotine concentrations have been found to be associated with greater craving reduction (Etter, 2015), which in turn is associated with more intensive use, these factors will be more likely to be associated with greater odds of smoking cessation in the long term. Whilst there is ample studies that suggest motivation and cigarette dependence as predictors of smoking cessation, to date, very few studies have explored the potential factors that can help predict quit with the aid of e-cigarettes. Given the difficulty of quitting and remaining abstinent, the struggle for many to achieve a complete transition away from smoking remains. Thus, further understanding of the factors that can predict successful cessation outcomes will be informative and can add to the support provided to smokers and dual users to facilitate complete cessation.

The current thesis aims to shed light on the inter-relationships between puffing topography of e-cigarette users, nicotine concentrations and e-cigarette characteristics. Further aims are to further the understanding of the complementary roles of subjective effects namely satisfaction, the alleviation of craving and reduction of withdrawal symptoms in e-cigarette use. The findings of this thesis will be informative to smoking cessation services, smokers, e-cigarette users and policy makers, specifically vis-à-vis regulatory decisions on nicotine concentrations in e-liquid including assessing whether the decision to restrict nicotine concentrations to 20 mg/mL (Article 20 of the EU-TPD) has merit.

Study 1 will ascertain whether e-cigarette users are able to self-titrate by exploring compensatory puffing behaviour when regular users are given a lower nicotine concentration compared to the one they are accustomed to.

Study 2 will explore how puffing behaviours and nicotine delivery in naïve e-cigarette users (that is, smokers who have no or very little experience in e-cigarette use) change over time and vary according to device type and nicotine concentration. It will also explore effects of device type/nicotine concentration on satisfaction, craving alleviation, product acceptability.

In Study 3, factors such as device types, nicotine concentrations, craving reduction and satisfaction at initial use, baseline motivation to quit and cigarette dependence will be explored in a logistic regression model to assess whether they have any predictive value for smoking cessation with e-cigarettes use.

CHAPTER II

"SELF-TITRATION BY E-CIGARETTE USERS: PUFFING TOPOGRAPHY, PLASMA NICOTINE AND SUBJECTIVE EFFECTS"

Abstract

Background: Self-titration is well documented in the tobacco literature. The extent to which e-cigarette users self-titrate is unknown. This study explored the effects of high and low nicotine concentrations on puffing topography, nicotine delivery and subjective effects in experienced vapers.

Methods: **Pilot study:** In order to inform the procedural aspects of the main experiment, between October and December 2014, a pilot study (N = 5) was conducted using a single-blinded within-participants design to compare resulting puffing topography in a low (6 mg/mL) versus a high (18 mg/mL) nicotine concentration. Puff duration, number and IPI were recorded via video-recordings and an eVic™ e-cigarette with an in-built puff timer/counter. Craving, withdrawal symptoms were measured at the start and end of the 45 min ad lib vaping session, and other subjective (positive and adverse) effects at the end of the session. **Study 1**: In a double-blind within-participants design experiment, between June and July 2015**,** 12 experienced e-cigarette users completed 1 hour of *ad libitum* e-cigarette use under low (6 mg/mL) and high (24 mg/mL) nicotine concentrations. An eVic™ e-cigarette mounted with an Aspire tank containing 2 mL of e-cigarette liquid recorded, puff number and duration. Blood samples and subjective craving/withdrawal symptoms were collected at baseline, 10, 30 and 60 minutes from the first puff. Adverse (e.g. nausea, headache) and positive effects (e.g. hit and satisfaction) were reported at 60 minutes.

Results: **Pilot study:** Means in puff number, duration and volume were greater

in the low condition as per the eVic and also as per the video recording data; conditions did not differ in craving and withdrawal symptoms alleviation or subjective effects. Puffing topography data recorded from the eVic correlated highly with the video recordings data in the low condition for puff number $r = .98$, $p = .005$ and puff duration $r = .90$, $p = .037$, and in the high condition for puff number $r = 1.00$, $p < .0001$, but not for puff duration $r = .67$, $p = .22$. There was a statistically significant correlation between volume consumed and puff duration in the low condition only [$r = .90$, $p = .037$]. **Study 1:** Liquid consumption and puff number were higher and puff duration longer in the low nicotine concentrations condition (all ps < 0.01). Mean (SD) nicotine boost for the high nicotine condition from baseline were 32.35 (34.88) ng/mL, 35.48 (28.31) ng/mL and 43.00 (34.78) ng/mL. Corresponding values for the low nicotine condition were 8.74 (7.52) ng/mL, 16.75 (11.72) ng/mL and 21.96 (16.19) ng/mL at 10, 30 and 60 minutes respectively (ps < 0.05). There were no statistically significant differences between conditions in self-reported craving, withdrawal symptoms, satisfaction, hit or adverse effects.

Conclusion: That both methods correlated highly suggest good reliability of the eVic as an instrument to measure puffing topography and the frame-by-frame time analysis. Both the pilot study and Study 1 found direct evidence of compensatory puffing with lower (6 mg/mL) nicotine concentration liquids. Although self-titration was incomplete with significantly higher plasma nicotine levels in the high condition, compensatory puffing was sufficient to reduce craving and withdrawal discomfort.

Introduction: Self-Titration and Nicotine Delivery - Effects on Puffing Topography

One mechanism by which smokers are able to control their nicotine intake and compensate for a decrease or increase in nicotine availability is by altering their puffing patterns in order to obtain and maintain desired and constant levels of nicotine. There is ample evidence in the literature which provides support to this hypothesis (Scherer, 1999; Scherer & Lee, 2014). Other factors likely to be central to effective nicotine delivery and acceptance of e-cigarettes as a substitution for tobacco smoking are nicotine concentrations and the speed and absorption rate of nicotine.

In Chapter 1, differences between nicotine delivery from tobacco cigarettes and e-cigarettes were described. It was noted that tobacco cigarettes are generally associated with higher and faster blood nicotine delivery although more recent studies using newer generation devices have found better nicotine delivery.

Differences in puffing topography

Whilst early studies reported poor nicotine delivery by e-cigarettes, later studies in long term users (Dawkins & Corcoran, 2014) found that later models can be effective in increasing blood nicotine levels (Farsalinos et al., 2014a; Nides, Leischow, Bhatter, & Simmons, 2014; Vansickel & Eissenberg, 2013), and, experienced users can achieve 50% higher blood nicotine levels compared with naïve users (Farsalinos et al., 2015) in parallel with significant craving alleviation (Etter, 2015; Fearon et al., 2017). This suggests that e-cigarette types differ in their efficacy to deliver nicotine and effective delivery depends largely on the manner in which the device is used (that is puffing patterns). This is in line with later studies which found that regular use and practice can significantly increase blood nicotine levels (Hajek et al., 2015). Using a sample of

naïve users to explore the effects of practice on nicotine absorption, a significant increase in nicotine intake was observed following a four-week period practice, which resulted in a significant increase in blood nicotine levels (Hajek et al., 2015). This increase in nicotine intake may be explained by a need to compensate for the less effective nicotine delivery of e-cigarettes which is characterised by a greater draw resistance in comparison with tobacco cigarettes. Although the nicotine delivery from e-cigarettes appears less effective compared to combustible cigarettes, increasingly it becomes evident that this could be reversed with practice; by adjusting puffing patterns (for example, increasing the duration and intensity of each puff), e-cigarette users can maintain constant and satisfactory blood nicotine levels, and withdrawal and craving relief (Dawkins & Corcoran, 2014; Dawkins, Turner, Hasna, & Soar, 2012; Etter & Bullen, 2011).

Puffing topography (such as puff duration, volume and frequency) therefore, plays a large role in the effectiveness of nicotine delivery. Experienced users typically take longer puffs and exhale larger volume of aerosols (Hua, Yip, & Talbot, 2011) compared with smokers (inexperienced e-cigarette users) using tobacco cigarettes or e-cigarettes (Farsalinos et al., 2013; Talih et al., 2014). Smokers however, tend to adjust their puffing patterns within a week of adopting e-cigarettes, chiefly by increasing their puff duration and adjusting each puff to a slower pace which in turn, result in a decrease in puff flow rate (Lee et al., 2015). Others found that experienced e-cigarette users' inhalation time is shorter compared to that of smokers when smoking combustible cigarettes (Farsalinos et al., 2013). In a study comparing experienced users' puffing topography versus smokers' smoking and vaping topography, experienced users' puffing duration were two-fold higher (M = 4.2, SD = 0.7 s) compared with smokers smoking a tobacco cigarette (M = 2.1, SD = 0.4 s) and smokers using an e-cigarette (M

= 2.3, *SD* = 0.5 s). These results are in agreement with previous findings (Hua, Yip, & Talbot, 2011) and in line with the hypothesis that there is a "learning curve" involved in vaping (McQueen et al., 2011), and suggest that the required adjustment in puff duration is largely dictated by the need to compensate for the poorer nicotine delivery.

Pharmacokinetic profiles of e-cigarettes and nicotine delivery

Likewise, pharmacokinetic (PK) studies suggest that more effective craving and withdrawal relief are associated with high-powered tank systems, more intensive puffing patterns and higher nicotine concentration e-liquids (Etter, 2015). Indeed, like nicotine yield in tobacco cigarettes (Hammond, Fong, Cummings, & Hyland, 2005; Russell, Epstein, & Dickson, 1983), e-liquid nicotine concentration is an important factor that is likely to influence puffing topography. Indeed, studies found that high nicotine concentrations are associated with more effective nicotine delivery (Farsalinos et al., 2013a; Hajek et al., 2017; Lopez et al., 2016), greater satisfaction and greater reduction in withdrawal and desire to smoke (D'Ruiz et al., 2015).

Of particular interest, in a clinical study, documenting the pharmacokinetic effects of an early e-cigarette device, experienced e-cigarette users were invited to a one-hour ad libitum vaping session followed by an hour resting period (Dawkins & Corcoran, 2014). Following a ten-minute ten-puff standardised puffing protocol, large individual variabilities were observed, with a maximum peak of 13.4 ng/mL plasma nicotine levels and a minimum of 2.50 ng/mL despite maintaining the device (a cigalike model) and nicotine concentration (18 mg/mL) constant across all participants and sessions (Dawkins & Corcoran, 2014). This suggests that although participants took the same number of puffs, their puffing patterns differed through adapting each puff to suit their nicotine intake needs (manipulating their puff duration and intensity) which led to the wide individual variability in plasma nicotine levels. The ten-minute standardised

protocol was followed by an ad lib vaping session in which individual variabilities further increased with one participant reaching 25.6 ng/mL and another participant only achieving 4.35 ng/mL plasma nicotine levels at the end of the 60 minute ad lib puffing period (Dawkins & Corcoran, 2014), suggesting that similar to smokers, vapers have desirable blood nicotine levels and titrate their nicotine intake to adapt to their personal nicotine needs. In a later study, researchers compared puffing topography between 0, 8, 18 and 36 mg/mL and the interactions with plasma nicotine levels following two vaping bouts of ten puffs each in experienced users (Ramôa et al., 2015). Participants increased their puff duration in the 0 mg/mL condition compared with the 36 mg/mL nicotine concentration (however not in the 8 or 18 vs. 36 mg/mL condition), suggesting an attempt to compensate for the deprivation of nicotine (Ramôa et al., 2015). However, although the study supports the hypothesis of compensatory puffing, utilising a standardised ten-puff protocol may have influenced users' natural puffing behaviours and/or reduced their ability to compensate fully. Compensatory puffing behaviours have also been documented in studies in which the nicotine levels were moderately reduced from 18 to 16 mg/mL (Behar et al., 2015). In a within-participants design study, participants were provided a 18 (V2 Cig) and a 16 mg/mL (Blu Cig) cartomisers and asked to vape ad libitum during two ten-minute vaping sessions separated by 10-15 minutes interval on two separate days. Nicotine intake and puff volume were significantly higher in the 16 compared to the 18 mg/mL nicotine concentrations, which is indicative of compensatory puffing (Behar et al., 2015). In contrast, others report opposing findings. For instance, D'Ruiz' group found no evidence of compensation between the use of 16 and 24 mg/mL (D'Ruiz et al., 2015) and Hajek et al. (2017) found that a 24 mg/mL nicotine content e-cigarette generated the same PK profile as a 16 mg/mL concentration. However, similar to Ramôa and colleagues', D'Ruiz and

colleagues utilised a 30-minute standardised puffing protocol prior to the ad lib session which may have caused a saturation effect and influenced puffing behaviours.

Self-titration model (in the smoking population)

As discussed in Chapter I, the concepts of self-titration and compensation are well established in the tobacco literature. Although puffing patterns remain fairly constant within a smoker (Gust et al., 1983), generally smokers tend to regulate their nicotine intake by altering their puffing patterns to adapt to the nicotine availability and obtain desired and satisfactory nicotine levels.

Compensation does not necessarily occur through consuming more cigarettes but by smoking more vigorously (that is increasing puff duration and/or inhalation) (Woodward & Tunstall-Pedoe, 1993). This is of high relevance when comparing tobacco smoking topography and puffing topography of e-cigarettes since the former could be defined as a finite activity (the smoking of a single cigarette lasting about five minutes) unlike the latter. Thus, although smoking differs from e-cigarette use, it is reasonable to argue that, like a smoker compensating to raise blood nicotine levels through the first puffs on one cigarette, an e-cigarette user will display a similar pattern when availability of nicotine is reduced (for example, after a period of abstinence).

However, the literature also suggests that compensation through alterations in puffing patterns, may not be driven by nicotine alone, but by the reduction in tar yield (Sutton, Russell, Iyer, Feyerabend, & Saloojee, 1982). That said, studies show that in nicotine deprived states, smokers tend to exert more intensive puffing patterns (Sutton et al., 1982). Likewise acceleration of nicotine renal elimination, induces smokers to augment their smoking patterns (Benowitz & Jacob, 1985). In a study investigating the effects of accelerated nicotine clearance via ammonium chloride administration (or urinary acidification loading) in a 24-hour period, smokers' nicotine intake resulted in

an 18 % increment compared to the placebo group (sodium bicarbonate) during the urinary acidification loading, which lends support to the compensation theory.

Nicotine titration and metabolism

Smoking topography and nicotine intake are also influenced by the rate at which smokers are able to metabolise nicotine. In most smokers, seventy to eighty percent of nicotine absorbed is metabolised by C-oxidation into cotinine which is further metabolised by hydroxylation into trans-3-hydroxy-cotinine (Tricker, 2003). The ratio of 3-hydroxy-cotinine to cotinine (therafter OH-cot/cotinine) determined in saliva or blood samples is commonly utilised as biological markers of nicotine metabolism and for phenotyping. The lower ratio in body fluids is indicative of a slower nicotine metabolism. The major enzyme involved in the clearance and partial conversion of nicotine to cotinine and OH-cot/cotinine has been identified as cytochrome P450-dependent monooxygenases (CYP) or CYP2A6 (Benowitz, Swan, Jacob, Lessov-Schlaggar, & Tyndale, 2006; Nakajima et al., 1996). Smokers with reduced CYP2A6 activity typically smoke fewer cigarettes, have lower plasma nicotine and carbon monoxide levels (Zhu et al., 2013) and, since cotinine levels accumulate faster, they achieve similar plasma cotinine levels compared to those with higher CYP2A6 activity (Strasser et al., 2011) despite smoking fewer cigarettes per day (Schoedel, Hoffmann, Rao, Sellers, & Tyndale, 2004). Consistent with the titration theory, many studies have found that smokers with reduced CYP2A6 activity reduce the intensity of their puffing regimes presumably in order to maintain desired blood nicotine levels (Strasser, Malaiyandi, Hoffmann, Tyndale, & Lerman, 2007). Altogether, the evidence suggests that CYP2A6 to a great extent influences smoking topography. Due to the acute nature of the current study, it will not be possible to infer any effects of nicotine metabolism, nonetheless knowledge of participants' nicotine metabolism profiles could be useful for

interpreting the current study's findings. Thus, baseline cotinine and OH-cot/cotinine levels will be measured to confirm that any changes in puffing topography will not be solely explained by nicotine metabolism but largely due to the availibility of nicotine.

Taken together, the aforementioned studies are suggestive, although not conclusive, of compensatory puffing behaviour in e-cigarette users. Although, this is well researched in tobacco smoking, no such direct empirical evidence from ad libitum protocols in e-cigarette use has come to light yet.

By increasing their puffing duration and intensity, users might increase their exposure to possible carbonyl compounds present in the emitted aerosol. Although these levels are much lower than in tobacco cigarettes (Farsalinos & Polosa, 2014) and equivalent to levels found in NRT (Shahab et al., 2017), longer puff duration combined with higher voltage can lead to increasing temperature which can overheat the atomiser coil (Geiss et al., 2016), and result in increased toxicant emission (Kosmider et al., 2014b). Whilst e-cigarette users show a tendency to gradually reduce the nicotine concentration in their liquids over time (Polosa, Caponnetto, Cibella, & Le-Houezec, 2015), in line with the titration model, studies suggest that the use of higher nicotine liquid is required in order to achieve satisfaction, obtain craving and withdrawal relief (Etter, 2015), and sustain abstinence specifically at the early stages of a cessation attempt (Farsalinos, Romagna, Tsiapras, Kyrzopoulos, & Voudris, 2014). The Tobacco Product Directive (EU-TPD) in Europe implemented in May 2016 imposes restrictions on high nicotine concentration (> 20 mg/mL). This may compel long term e-cigarette users to reduce their nicotine intake or new users (smokers) to adopt a lower nicotine concentration, when switching from smoking, than one which may be optimal for craving reduction and sustained cessation. If e-cigarettes are to be successful in promoting tobacco cigarette cessation, it is crucial that smokers are given the nicotine

concentration that they require. Therefore, investigating the effects of a switch to lower nicotine concentrations on vapers' puffing patterns and documenting the pharmacokinetic effects is warranted and will be informative to users, policy-makers and smoking cessation service advisors.

Thus the primary aims are to explore whether and to what to extent experienced e-cigarette users self-titrate, that is, alter their puffing patterns to adapt to changes in availability of nicotine concentrations in order to achieve a desired blood nicotine level. In this chapter, two empirical studies will be presented. In order to inform the procedural aspects of the main experiment, a pilot study was conducted with the specific aims of i) checking the eVic e-cigarette puffing topography reliability against the frame-by-frame time analysis of video recordings ii) calculating the sample size that would be required for the main study iii) testing the functionality of the eVic, features such as voltage and air hole settings against preferences of experienced e-cigarette users, iv) testing the parameters for the main study such as the blinding process for the nicotine concentrations, and, v) informing the mitigation plan for the main study (e.g. spillages and timing issues of questionnaires administration and weighing of tanks at the commencement and end of each session).

Aims and Hypotheses

It is hypothesised that participants will attempt to compensate by consuming more liquid and take longer and more frequent puffs in the lower nicotine concentration condition. To this effect, puffing topography (puff number and duration), and self-reported (positive and adverse) effects will be measured in the pilot study on two separate occasions under two conditions: a 'high nicotine concentration liquid' (18 in the pilot study and 24 mg/mL in Study 1) and a 'low nicotine concentration liquid (6

mg/mL)'. Plasma nicotine concentrations will be measured in addition in Study 1. Additional aims are to explore the effects of condition (high vs. low nicotine concentration e-liquid) on subjective effects (craving, withdrawal symptoms, positive and adverse effects).

Hypothesis 1 (H_1):

Puffing topography will differ across conditions with a higher number of puffs and longer puff duration in the 6 mg/mL condition.

Hypothesis 2 (H_2):

Plasma nicotine levels will remain similar across both conditions, (i.e. 24 and 6 mg/mL) evidencing compensatory behaviours and complete self-titration.

Hypothesis 3 (H_3):

Craving and withdrawal symptoms should show equivalent levels of reduction across both conditions, evidencing self-titration.

Pilot Study - Do E-Cigarette Users Compensate?

Methods

Design and ethical considerations

Ethical approval was granted (reference number: UREC_1415_02; approval date: 8th October 2014; see appendix 3) from the University of East London's ethics committee (UREC_1415_40) and was conducted in accordance with the ethical standards outlined in the 1964 Declaration of Helsinki. A single blind within-participants design with two conditions: 'high strength' (18mg/ml) and 'low strength' (6mg/ml) nicotine e-liquid was used. The order of presentation was counterbalanced.

Participants

Participants were all daily e-cigarette users recruited via word-of-mouth or known to the researcher. The sample comprised five former smokers (2 females and 3 males), all above 18 years of age, fluent in English and currently using a second or third generation e-cigarette and 18 mg/ml nicotine concentration minimum[a]. Exclusion criteria included current smokers, non-daily e-cigarette users, under the age of 18, pregnant or lactating females, of those with neurobiological or heart conditions.

Measurements and Materials

- Carbon Monoxide (CO) levels were measured using the Bedfont piCO Smokerlyzer®
- The third generation e-cigarette device has many useful features which allows research data collection with the aid of a downloadable software which records the duration, frequency and times in seconds per puff. The device comprises the following 2 components:

[a] Note, 18mg/ml was first selected on the basis that, at the time of the study, it was the most commonly used strength amongst e-cigarette users (Dawkins, Turner, Roberts, Soar, 2013; Etter & Bullen, 2011).

1. The main component the ‘eVic Supreme’ from ‘Joyetech’ comprises a battery tube which houses a lithium-ion battery, has a manual button and a OLED display screen (see figure 2.1, see appendix 2)
2. A ‘Nautilus’ Pyrex glass tank has a capacity to hold 5ml of fluid, is equipped with a measuring gauge and a four-port adjustable airflow control system (figure 2.2, appendix 2). The tank housed a BVC atomiser (Bottom Vertical Coil) of 1.8 ohm resistance which is built to withstand high wattages, therefore is less likely to lead to overheat (figure 2.3, appendix 2).

- Nicotine e-liquid: 6 bottles of 10 ml nicotine e-liquid of 6 and 18 mg/mL nicotine concentration were purchased from an e-cigarette website, ‘Totally Wicked’. The following flavours, lemon sherbet, Columbian coffee, ‘Tutti Frutti’ were chosen based on participants’ preferred choices.
- Precision scales were used to weigh the tank at baseline prior to and at the end of each session to measure the amount of liquid consumed.
- A questionnaire (see appendix 6) was used to collect e-cigarette daily patterns of use from each participant and other information such as type of device used, e-liquid concentrations and daily consumption. Examples of items included “Which strength of nicotine fluid/cartridge are you currently using?” The latter part of the questionnaire contained one item from the Fagerström Test for Cigarette Dependence (thereafter referred to as FTCD) (Fagerström, 2012) which was modified and renamed eFTND to apply dependence related to e-cigarettes use and asked “*How soon after you wake up do you use your electronic cigarette?*”. The available options were ‘*within 5 minutes*’ (corresponds to the highest possible score of 4), whilst the option ‘*after 60*

minutes' scores the lowest as per the FTND (Heatherton et al., 1991). The higher overall scores signify a higher dependence.

- The Mood and Physical Symptoms Scale (thereafter referred to as MPSS) (West & Hajek, 2004) was altered to measure 'urge to vape', as opposed to 'desire to smoke', measured on a scale from 1 to 7 (appendix 7). This questionnaire also measure the presence and severity of the five DSM-IV nicotine withdrawal symptoms. These were 'depressed mood', 'irritability', 'anxiety', 'restlessness', 'hunger' and 'inability to concentrate', each measured using a five-point rating scale (scores range from 1 to 5 "*Not at all*" to "*Extremely*"). Thus, total score can range from 5 to 25.
- Subjective effects of nicotine were assessed using a two-part questionnaire measuring positive and adverse effects.
    1. 'Positive' effects were measured using an 11-item visual analogue scale (VAS) derived from Blank, Sams, Weaver, & Eissenberg' s paper (2008) (appendix 8). Participants were required to rate each statement by drawing a cross or a small line adjacent to the 20 cm-line with the far left representing 'not at all' and the opposite right end 'extremely'. Examples include 'I feel a definite hit from the e-cigarette', 'The e-cigarette is satisfying. Scoring is obtained by measuring from the far left corner to the drawn cross or line in millimetres then the score is translated in percentages. A higher score is indicative of more positive effect.
    2. Adverse effects were measured with a 21-item visual analogue scale (appendix 8). This has been used in previous work (Vansickel & Eissenberg, 2013). Examples of items include 'confused', 'dizzy',

'nausea', 'headache', 'salivation', 'sweaty'. As per the positive effects, participants were required to place a cross or adjacent line through a 20 cm-line. Percentage score is obtained as per the positive effects questionnaire.

Procedure:

Based on participants' preferences and following consultation with other experienced e-cigarette users, the e-cigarette was set to the following parameters across both sessions. The voltage was set at 3.9 Volt for an atomiser with a resistance of 1.8 ohms which resulted in 8.5 Watts. The device was adjusted to the biggest airflow (following recommendation from vaping colleagues). To obtain a measure of the liquid consumed, the tank was removed from the main unit and weighed using precision scales before and after filling up the tank, at the commencement and end of each session. For hygiene purposes, each participant provided their own mouth-piece to be attached to the device.

Participants were asked to abstain from all tobacco products and nicotine intake including use of e-cigarettes for a period of at least 10 to 12 hours prior to each session. Upon arrival, participants were provided with an information sheet then required to provide written informed consent. Exhaled CO levels were collected from participants via a breathalyser to confirm non-smoker status (cut off: 10 ppm).

Participants were given the e-cigarette with instructions to use the device ad libitum. There was no practise period as all participants were current e-cigarette users with prior experience of a similar device. Participants were reminded of their right to withdraw at any time and have a break before the session commenced. The entire

session from the first puff to the last puff was videoed using a 'Toshiba Camileo X400' recorder. At the end of the session, 'urge to vape' was measured and subjective effects of nicotine questionnaires were administered. Following completion of the task, the e-cigarette was attached to a secure and password protected UEL PC and all data on puff duration and frequency were downloaded and backed up onto university drives.

The full session was repeated the following working day for each participant using the other nicotine concentration.

Data and Statistical Analysis

Outcome measurements

The primary outcomes were 'puff number', 'puff duration', 'IPI' in milliseconds and estimated 'volume consumed' in millilitres. Secondary outcomes were changes in 'urge to vape', 'withdrawal' and each of the withdrawal symptom dimensions (e.g. depressed, irritable, anxious), 'satisfaction', 'hit' and other subjective positive and adverse effects. Volume consumed was obtained by first calculating the mass of the liquid (in grams) added to the tank, which was calculated by subtracting the weight of the filled tank before use by the weight of the empty tank [$mass_1$ = X (g) – Y (g)]. Thereafter, liquid density was calculated by dividing the mass of the liquid (in grams) by the quantity of liquid (in mL) contained in the tank [d = $mass_1$ (g) / Z (mL)]. Then, the weight of liquid consumed (in grams) was calculated by subtracting the weight of the filled tank prior to and at the end of the vaping session [$mass_2$ = X (g) – N (g)]. Lastly, the value of the volume consumed in mL was obtained by dividing the mass (in grams) by the density of the liquid consumed [Vol (mL) = $mass_2$ / d].

Due to the small sample size, Wilcoxon Signed Rank tests were used to compare scores between conditions. Alpha level was adjusted to $p < .10$ to address the issue of

small sample size (eg. N < 20) and insufficient power, in accordance with Stevens (1996).

Given 'urge to vape' was measured before and after using the device, variables were computed to obtain values of the changes between urge to vape at baseline and urge to vape at 'Time 2' by calculating the difference between scores at baseline minus the scores at 'Time 2'. Higher scores on 'urge to vape' are indicative of greater reductions. Positive effects (Hit, Satisfied, Pleasant, etc) and subjective adverse effects (Nauseous, Dizzy, etc) under both conditions were compared to investigate the effects under 'low' and 'high concentrations'.

A frame by frame analysis of 29.97 fps using the 'Adobe Premiere Pro CS5' software was used to analyse timing measurements. Puff duration was demarcated by the time at which the e-cigarette was clearly in the mouth with both lips closed (start of the puff) until the frame at which the e-cigarette was removed from the mouth. The end of the puff was defined by the time frame preceding the first time frame of the start of the inter-puff interval (interval between each puff; hereafter referred to as IPI). This coincided with the first time frame when the e-cigarette mouth tip could be clearly seen and the e-cigarette had left the lips. There are instances wherein e-cigarette users press the activation button before (Behar et al., 2015) or whilst bringing the device to the mouth, whilst others may do the opposite (Farsalinos et al., 2013b). In such instances, the time frame was captured only when the device was clearly seen in the mouth with both lips closed. Extra care was taken to ensure clear visibility of the start and end of puff (start / end of inhalation and exhalation). In cases where some aerosol escaped and was visible during a puff (and whilst the e-cigarette was still in the mouth and the user was clearly inhaling), the puff was considered as one puff. To increase accuracy of

measurements from the video-recordings' data, an inter-observer reliability were calculated by taking the average of scores obtained by two different researchers.

Spearman's Rank correlation tests were used to explore relationships between puffing topography variables as well as the relationship between the video and the eVic data (see Table 2.3).

Results

Participants' characteristics

Participants' demographics and baseline characteristics are displayed in Table 2.1. All, except one participant, were current users of nicotine concentrations equal to or exceeding 18 mg/mL and accustomed to using a 3rd generation device, although the participant who reported currently using a cigalike was familiar with 3rd generation devices. Based on the first item of the FTND questionnaire a mean (SD) of 3 (1.23) is indicative of relatively moderate e-cigarette dependence (possible scores range: 1 - 4).

Table 2.1

Participants' Demographics and Baseline Characteristics

	N	%	Mean	SD	Min	Max
Gender	5	-	-	-	-	-
Male	3	60	-	-	-	-
Female	2	40	-	-	-	-
Ethnicity	5	-	-	-	-	-
White	4	80	-	-	-	-
South Asian	1	20	-	-	-	-
Occupational status	5	-	-	-	-	-
Employed	2	40	-	-	-	-
Studying/Part time employed	1	20	-	-	-	-
Self-employed	2	40	-	-	-	-
***eFTND*[1] - First puffs of the day**	5	-	3.00	1.23	1.00	4.00
Within 5 mins	2	40	-	-	-	-
6-30 mins	2	40	-	-	-	-
31-60 mins	0	0	-	-	-	-
After 60 mins	1	20	-	-	-	-
Baseline CO (ppm) high strength	5	-	3.40	2.07	1.00	6.00
Baseline CO (ppm) low strength	5	-	3.00	1.23	2.00	5.00
Daily Liquid Vol consumed (mL)	5	-	2.3	0.76	1.00	3.00
Nicotine concentration most used	5	-	-	-	-	-
12mg/mL	1	20	-	-	-	-
18mg/mL	3	60	-	-	-	-
24 mg/mL	1	20	-	-	-	-
Current model most used	5	-	-	-	-	-
1st gen / Rechargeable cigalike	1	20	-	-	-	-
2nd gen / Penlike/Clearomiser	2	40	-	-	-	-
3rd gen / Tank/Mod systems	2	40	-	-	-	-

Note. [1] ***eFTND*** *= First item of the Modified version of Fagerström Test of Nicotine Dependence for e-cigarettes use "How soon after you wake up do you use your electronic cigarette?"*

Puffing topography

Table 2.2 presents the puffing topography data for: volume consumed, puff number and duration, and IPI (Inter-puff intervals) based on the video recording analysis as well as puff number and duration obtained from the eVic. Minimum and maximum values as well as median scores are presented. Means in puff number and puff duration were very slightly greater in the low condition as per the eVic and also as per the video recording data.

Table 2.2.
Puffing topography data based on the Video analysis and the eVic

	Mean (SD)		Min		Max		Median	
	High	**Low**	**High**	**Low**	**High**	**Low**	**High**	**Low**
Vol. cons.	0.33 (0.20)	0.48 (0.24)	0.14	0.27	0.61	0.85	0.25	0.39
			Video Data					
P. Number	35.60 (15.52)	42.80 (11.69)	19	30	55	55	28	47
P. Duration	3.71 (1.22)	4.52 (1.72)	1.96	2.27	5.10	6.63	3.54	4.61
IPI	146.31 (150.11)	69.74 (29.48)	46.07	43.89	408.50	104.58	112.20	55.31
			eVic Data					
P. Number	38 (17.88)	43.20 (11.49)	20	31	63	55	30	47
P. Duration	4.10 (0.68)	4.67 (1.04)	2.90	3.24	4.50	5.90	4.36	4.81

Note. Legend: Vol. cons.: volume consumed; P. Number: puff number; P. Duration: puff duration; IPI: inter-puff interval.
Volume consumed is expressed in millilitre; Puff duration and IPI are expressed in seconds

Six instances of extremely short button presses (i.e. < 1s) were observed (in 3 separate sessions involving 3 individuals) in the data collected from the eVic. These were verified against backup video data and deleted in cases where the device was not clearly in the mouth. In all instances wherein a short puff (< 1 s) preceded or succeeded a puff of more than 1 second, the former was merged with the latter. Based on the video data, six instances of extremely long puffs exceeding 10 seconds were observed. Participant 5, had the highest record of puffs exceeding 10 seconds, 5 were in the low condition and 1 in the high condition.

Based on the data analysed from the video recordings, there were significant differences between the high (18 mg/mL) and low (6 mg/mL) conditions in mean volume of liquid consumed (mL) [z = -2.02, p = .04] with a large effect size where r = .64 (Figure 2.1), puff number [z = -1.83, p = .07, r = .58] (see Figure 2.2), puff duration [z = -1.75, p = .08, r = .55] (Figure 2.3) and IPI [z = -2.02, p = .04, r = .64] (Figure 2.4). Although very similar to the video recording data and in the same direction, puffing topography data collected from the eVic did not reach statistical significance, mean puff number [z = -1.21, p = .23] and puff duration [z = -1.48, p = .14]).

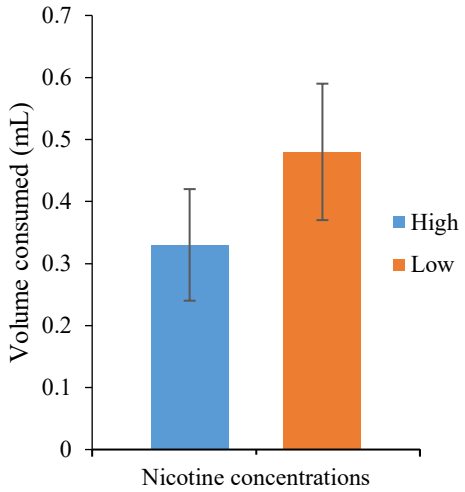


Figure 2.1 Mean (SE) in Volume consumed in high and low nicotine concentrations after the ad lib vaping session

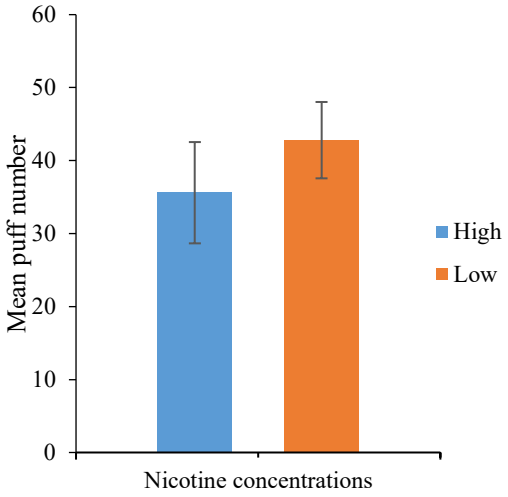


Figure 2.2 Mean (SE) puff number in high and low nicotine concentrations after the ad lib vaping session (Video data[b])

[b] Graphs presented here are based on the data collected from the video recordings only, since values from the eVic were very similar

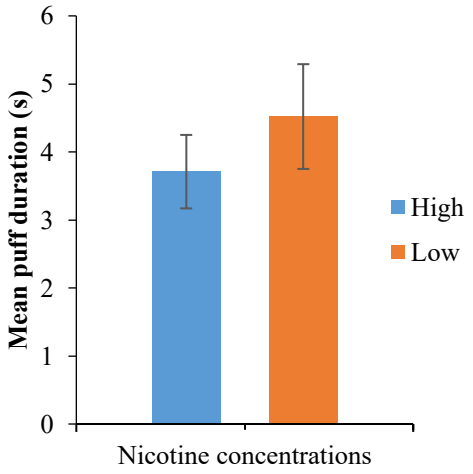


Figure 2.3 Mean (SE) puff duration in high and low nicotine concentrations after the ad lib vaping session (Video data[2])

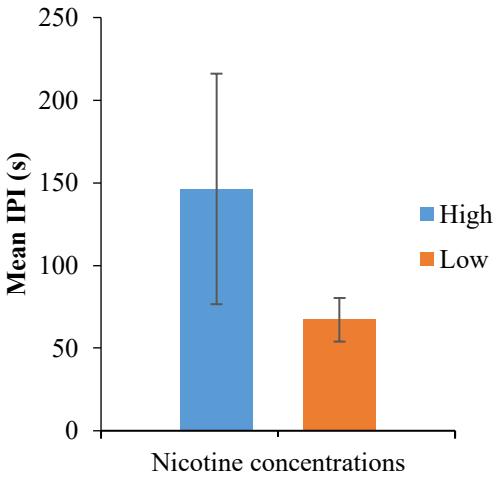


Figure 2.4 Mean (SE) IPIs in high and low nicotine concentrations after the ad lib vaping session (Video data[2])

Table 2.3 Correlations between mean scores in puff number, duration, volume consumed and between the video data and the eVic data

		High Video		High Evic			Low Video		Low eVic	
High		**Puff no**	**Puff d**	**Puff no**	**Puff d**	**Vol.**	**Puff no**	**Puff d**	**Puff no**	**Puff d**
	Vol.	.70	.80	.70	.56	1.00	.70	1.00**	.67	.90*
Video	**Puff no**	-	.50	1.00**	.10	.70	1.00**	.70	.98**	.50
	Puff d	-	-	.50	.67	.80	.50	.80	.56	.50
eVic	**Puff no**	-	-	-	.10	.70	1.00**	.70	.98**	.50
	Puff d	-	-	-	-	.56	.10	.56	.24	.46
Low		**Puff no**	**Puff d**	**Puff no**	**Puff d**	**Vol.**	**Puff no**	**Puff d**	**Puff no**	**Puff d**
	Vol.	-	-	-	-	-	.70	1.00**	.67	.90*
Video	**Puff no**	-	-	-	-	-	-	.70	.98**	.50
	Puff d	-	-	-	-	-	-	-	.67	.90*
eVic	**Puff no**	-	-	-	-	-	-	-	-	.41

Note. *p ≤ 0.05 (2-tailed), **p ≤ 0.01 (2-tailed)

Legend: High Video / eVic: Video / eVic data in the high condition; Low Video / eVic: Video / eVic data in the low condition; Vol. corresponds to Volume consumed; Puff no corresponds to puff number; Puff d corresponds to puff duration

There was a statistically significant correlation between volume consumed and puff duration in the low condition only [r = .90, p = .037]. Puffing topography data recorded from the eVic correlated highly with the video recordings data in the low condition for puff number r = .98, p = .005 and puff duration r = .90, p = .037, and in the high condition for puff number r = 1.00, p < .0001, but not for puff duration r = .67, p =.22.

Subjective effects

Reduction in urge to vape and withdrawal

Means (SD) in changes in 'urge to vape' (M = 2.8, SD = 1.30) and (M = 2.40, SD = 1.52) and changes in withdrawal symptoms (M = 2.8, SD = 1.79 and M = 5.4, SD = 3.78) in the high and low conditions respectively suggest that both nicotine concentrations equally reduced withdrawal symptoms (Note that higher scores are indicative of greater reductions). There were no statistically significant differences in changes in 'urge to vape' (z = -1.00, p = .32) (see figure 2.8). Differences in changes in withdrawal symptoms between conditions just fell short of statistical significance (z = -1.83, p = .068) (see figure 2.9). There were no significant differences between conditions in changes in depressed mood, irritability, anxiety, drowsiness, restlessness, hunger and inability to concentrate (all ps > .05).

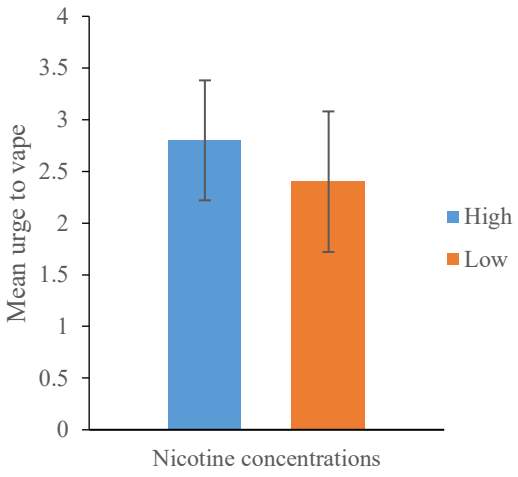


Figure 2.5 Means (SE) in changes in 'urge to vape' in the high and low concentrations[c]

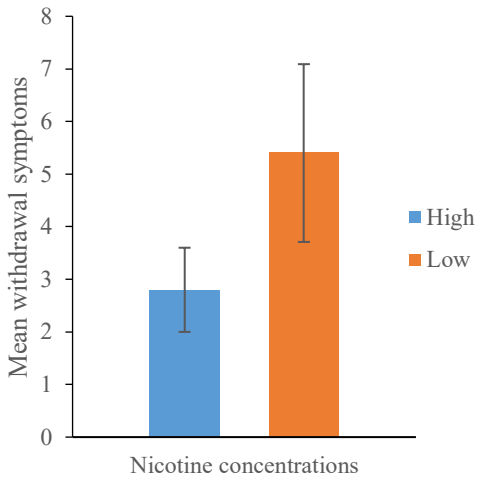


Figure 2.6 Means (SE) in changes in withdrawal symptoms in the high and low concentrations

Satisfaction, Hit and Other positive effects

There were no statistically significant differences between conditions (all p values > 0.10) on self-reported positive effects. Data on satisfaction, hit and other positive effects are presented in Table 2.4.

[c] The higher scores in 'urge to vape' and in 'withdrawal symptoms' indicate greater reductions

Table 2.4

Mean (SD) and median (%) in subjective positive effects in the high and low conditions

	High		**Low**	
	Mean (SD)	**Median**	**Mean (SD)**	**Median**
'Hit'	65.5 (13.31)	73.5	51.25 (16.64)	54.5
'Satisfaction'	70.85 (16.96)	72.25	54.35 (17.91)	54.5
'Pleasant'	71.65 (12.87)	71.5	64.05 (16.59)	74.5
'Tastes good'	68.65 (14.01)	67	70.4 (19.25)	66.75
'Reduced my craving'	59.31 (23.52)	69	58.35 (19.34)	66.75
'helped my concentration'	61.6 (16.27)	67	55 (22.21)	65
'helped me feel calmer'	55.2 (21.88)	61.5	57.35 (18.10)	64.5
'feel more awake'	47 (18.05)	42.5	51.41 (17.80)	41
'reduced my hunger'	35.2 (9.39)	32.75	35.15 (30.54)	25
'tastes like my usual brand/model'	34.3 (22.22)	33.5	48.3 (18.61)	45
'feels like using my usual brand/model'	38.3 (23.96)	23.5	42.57 (24.02)	31.1

Note. Values are mean scores in percentage for each dimension of the VAS questionnaire on positive subjective effects related to e-cigarette use.

Adverse effects

Wilcoxon signed rank tests revealed no statistical differences between conditions in adverse effects (all p values > .10) (all means and standard deviations are presented in Table 2.5).

Table 2.5

Mean (SD) and median (%) in subjective adverse effects in the high and low conditions

	High		Low	
	Mean (SD)	**Median**	**Mean (SD)**	**Median**
Confused	9.25 (8.22)	7.5	11.5 (10.60)	10
Dizzy	22.35 (24.53)	10	18.8 (18.37)	18.75
Headache	15.55 (15.61)	10	16.4 (19.02)	9.25
Pounding heart	15.4 (15.45)	10	11.3 (9.17)	9.5
Light headed	29.3 (31.22)	15	26.2 (25.86)	21.5
Nausea Feeling sick	20.6 (25.20)	9.5	12.3 (13.73)	9
Nervous	12.7 (14.02)	5	7.1 (6.19)	6
Salivation	6.8 (5.03)	6	11.4 (15.22)	6
Sweaty	6.3 (4.98)	7	7.2 (5.89)	6
Weak	10.35 (13.02)	6	6.504 (5.11)	6
Mouth irritation	5.07 (4.20)	5.5	6.2 (3.90)	6
Throat irritation	8.05 (6.36)	7.5	6.3 (3.90)	6.5
Aching jaws	13.95 (21.41)	6	8.65 (9.31)	6
Vomiting	15.25 (17.49)	9.5	9.24 (9.03)	6.5
Flatulence bloating	9.65 (7.86)	9.5	7.6 (4.49)	9
Stomach ache	7.75 (5.26)	9.5	7.6 (4.35)	9
Heartburn	8.75 (6.76)	9.5	8.35 (5.42)	9
Diarrhoea	5.1 (4.51)	4.25	6.35 (4.01)	7.75
Hiccups	5.8 (4.62)	6	7.21 (4.13)	9
Cold hands feet	7.1 (4.59)	9.5	12.81 (14.35)	9
Palpitations	13.1 (12.68)	9.5	6.71 (3.93)	7.55

Note. Values are mean scores in percentage for each dimension of the VAS questionnaire on negative subjective effects related to e-cigarette use.

Discussion

Summary of findings

The current pilot study was primarily designed to inform procedural aspects of Study 1. To this effect, video recordings were used to measure puffing topography in conjunction with the eVic, to check for reliable eVic recordings, so any risk of data distortion due to pre-puffing instances could be minimised. The eVic data were found to correlate highly with data collected via the video recordings in the low condition for puff number [r = .975, p = .005] and puff duration [r = .900, p = .037], and in the high

condition for puff number, $r = 1.00$, $p < .0001$, but not for puff duration, $r = .67$, $p = .22$. Video recordings have been used in previous studies and found to be reliable (Farsalinos et al., 2013b) against other automated puffing recorder machines for example the CReSS pocket device which consists of a mouthpiece equipped with a computerised system (Blank, Disharoon, & Eissenberg, 2009; Spindle, Breland, Karaoghlanian, Shihadeh, & Eissenberg, 2015). In these previous studies, participants reported dislike of such automated machines, adding the device made use of the e-cigarette difficult; such fact is likely to influence participants' puffing patterns. Thus arguably, the eVic is a less invasive method and may have greater ecological validity compared to puffing machines such as the CReSS device. In the current study, puff number and duration correlated highly in the low condition for puff number and puff duration as well as puff number in the high condition. This strengthens the validity of the eVic as a method for puffing data collection. The non-statistical significant relationship in the high condition for puff duration may be explained by a lack of statistical power given the shorter puff duration.

Other aims of this pilot study, were to investigate whether and to what to extent experienced e-cigarette users alter their puffing patterns to adapt to changes in nicotine availability so they can achieve desired blood nicotine levels. As per hypothesis H_1, participants made an attempt to self-titrate by increasing their liquid consumption, puff duration and frequency in the lower nicotine concentration condition. As per hypothesis H_3, compensatory puffing behaviours were effective in relieving 'urge to vape' and withdrawal discomfort, there was no statistical difference between conditions. Similarly, there were no differences in subjective positive effects such as 'satisfaction' and 'hit'. Adverse effects did not differ between conditions. These findings seem to support the titration theory and suggest that in the same manner as smokers, vapers have

a tendency to adjust their puffing patterns and obtain an optimal and satisfactory level of blood nicotine, sufficient to alleviate craving and withdrawal symptoms.

Puffing topography

Participants consumed a greater quantity of liquid, increased their puff numbers and duration, and took shorter inter-puff intervals in the low nicotine condition compared with the high nicotine concentration. These findings concur with previous studies, average puff duration in the low condition observed here are similar to those previously reported in experienced users (Hua et al., 2011) with newer generation devices (Farsalinos et al., 2014), and longer than those reported for cigalike (first-generation) devices (Behar et al., 2015) and combustible cigarettes (Hua et al., 2013). The proposition that longer puff durations and shorter IPI (Williams et al., 2011) are conducive to more efficient nicotine delivery (Hajek et al., 2015) seem to be supported by the current data. The significant increase in puff duration suggest that participant did unconsciously or consciously felt the need to exert a more intensive puffing pattern in the low condition to obtain and maintain a constant and satisfactory nicotine blood level, likewise in the high condition, they adjusted their puff numbers and duration to regulate their nicotine intake.

Effects on craving, withdrawal and subjective effects

Means in changes in 'urge to vape' and withdrawal symptoms in the high and low conditions respectively suggest that both nicotine concentrations reduced withdrawal symptoms and 'urge to vape' equally. Indeed, there were no differences between conditions in '*depressed mood*', '*irritability*', '*anxiety*', '*drowsiness*', '*restlessness*', '*hunger*' and '*inability to concentrate*'. This suggests that low nicotine concentration levels may be sufficient for the relief of craving and withdrawal discomfort at least subjectively.

There were no statistically significant differences in subjective positive effects. This suggests that, although participants scored fairly high on the nicotine dependence scale (as per '*first puff of the day*' from the *eFTND*), the low nicotine concentration and efforts made to compensate were sufficient to achieve satisfaction and feel a hit from the low nicotine concentration, at least in the short term. Likewise, there were no statistically significant differences in adverse effects.

Concluding remarks

This pilot study was set to inform the procedural aspects of Study 1 and, as such suggests that the e-cigarette eVic offers a reliable method of data collection. These findings also suggest, that in line with the titration model, experienced e-cigarette users, like smokers, have a tendency to self-titrate by controlling their puffing patterns to maximise nicotine delivery and regulate their nicotine intake to a desired and constant level; this is supported by the large effect sizes for the puffing topography variables. Here, compensatory puffing appeared to be effective in alleviating subjective 'urge to vape' and withdrawal discomfort and achieving satisfaction, at least in the short term and under acute conditions. However, in this pilot work, biomarkers were not measured thus there is no clear indication that greater quantity of liquid consumed and indeed the increase in puffing patterns translated into greater nicotine absorption. This forms the focus of the subsequent study.

Study 1 - Nicotine Titration by E-Cigarette Users: Puffing Topography, Plasma Nicotine and Subjective Effects

The pilot study has achieved two aims i) confirmed that the use of the eVic provides a reliable method for data collection on puffing topography, ii) informed other procedural aspects (including sample size) for Study 1, iii) suggested that experienced e-cigarette users adjust their puffing patterns in response to changes in nicotine availability. To this effect, several methodological changes were implemented to strengthen the design of Study 1. In Study 1, it is anticipated that the findings of the pilot study will be replicated using a series of biomarkers in addition to measuring puffing topography namely baseline salivary cotinine to ensure participants are habituated to high nicotine concentrations, plasma nicotine levels to observe differences between conditions and substantiate findings of compensatory behaviours and, 3-hydroxy cotinine and 3-hydroxy-cotinine to cotinine ratio were measured to eliminate these possible confounders and ensure any indications of compensatory puffing behaviours observed were not the result of previous nicotine use or phenotypic status. In addition, unlike the pilot study which was single blinded, Study 1 used a double blind design to minimise expectancy effects and researcher bias. A team of 3 researchers will be in place to facilitate the double blind procedure and ensure the smooth running of Study 1 (e.g. nicotine liquid and the eVic and questionnaires will be handled by one researcher whilst blood samples by another, a third researcher held responsibility for timings). Statistical power of the study was increased with the inclusion of a larger sample (N = 12 from N = 5 in the pilot study). Final changes included an increase in nicotine concentrations. At the time of the pilot study planning, 18 mg/mL was the most commonly used concentration amongst e-cigarette users (Dawkins, Turner, Roberts, Soar, 2013; Etter & Bullen, 2011). However due to the imminent EU-TPD

legislations to limit nicotine concentrations to 20 mg/mL, the decision was made to increase the nicotine concentration to 24mg/ml in Study 1, as it was a worthwhile opportunity to document puffing behaviours in the EU with nicotine concentrations that would soon be in excess of the legal limit.

The aims of Study 1 are to observe how experienced e-cigarette users respond to a high versus a low nicotine concentration by measuring i) puffing topography, ii) urge to vape and withdrawal symptoms, ii) subjective effects (positive and adverse effects) and iii) plasma nicotine levels.

Methods

Design

A double-blind within-participants, counterbalanced design with 2 conditions: 'low' (6 mg/mL) and 'high' (24 mg/mL[d]) nicotine concentration liquid, was employed.

Ethical considerations

The study received full ethical approval from the University of East London's ethics committee (UREC_1415_40; approval date: 29th January 2015, see appendix 9) and was conducted in accordance with the ethical standards outlined in the 1964 Declaration of Helsinki. All participants provided written informed consent (appendices 10 and 11) to take part in the study.

Full risk and COSHH assessments were conducted in addition to the ethics consideration. The week leading to the first study session, laboratory walk-through were performed in consultation with the technical laboratory support staff. Prior to the

[d] Note, 18mg/ml was first selected on the basis that, at the time of the study, it was the most commonly used strength amongst e-cigarette users (Dawkins, Turner, Roberts, Soar, 2013; Etter & Bullen, 2011). However due to the EU-TPD legislations that nicotine concentration will be capped at 20 mg/mL, the decision was made to increase the nicotine concentration to 24mg/ml which were subsequently used in study 1.

commencement of each session, the research team and qualified doctor or phlebotomist nurse discussed all aspects and each step of the study's procedure so all were fully informed and aware of each person's remit. All medical equipment was checked by the phlebotomist prior to participants' arrival.

All data were coded, treated confidentially and retained in accordance with the Data Protection Act, stored on password protected hard drives. To facilitate any withdrawal, each participant was given an identification number which they had to memorise should they wish to withdraw. Hard copies of questionnaires were stored in a locked filing cabinet in a lockable room then destroyed in accordance with the university's data protection policies. Signed consent forms were stored separately from questionnaires and video data were accessible to only the research team. Video recordings were analysed then entered into a computerised dataset before being disposed of securely.

Participants

Twelve regular e-cigarette users (11 male and 1 female, all ex-smokers; see sample size calculation below), attended 2 separate sessions between 2 and 7 days apart at the University of East London. Participants responded to advertisements on social media sites, e-cigarette forums and emails. Eligibility criteria were: aged 18+; experienced e-cigarette user (daily use for > 3 months); currently using a second or third generation e-cigarette; familiar with 24 mg/mL nicotine concentration liquid (i.e. used 24mg/mL at least once in the last 6 months); baseline salivary cotinine levels > 100 ng/mL; CO levels ≤ 10 ppm; willing to provide saliva and blood samples and abstain from using nicotine for 12 hours prior to study commencement.

Randomisation and Masking

A double-blind design was utilised. To facilitate the double blind process, nicotine liquid was decanted into plain bottles and re-labelled X and Y, so brand and nicotine concentration were unknown to participants and participant-facing researchers. Ten most popular brands of nicotine liquid were selected using various e-cigarette review internet sites, during January and February 2015. One brand (Halo Smokers' Angels) was selected at random by one member of the research team with nicotine concentrations of 6 and 24 mg/mL (both 60/40 propylene glycol and glycerine). Order of presentation was counterbalanced and participants were randomly allocated to receive either nicotine concentration on their first session.

Statistical analysis

An a priori estimation of power for the required sample size and to detect an effect on puffing topography was calculated using the statistical software GPower 3.1 with the pilot data. To detect a difference between conditions in mean number of puffs, puff duration and volume consumed with > 80% power at $p < 0.05$ with 95% CI, a sample of between N = 9 and 17 was required. The initial target was set to recruit fifteen participants but due to participant drop out and failure to meet required baseline criteria, twelve were tested.

Data were analysed using 'IBM SPSS Statistics 23'. Normality of the distribution of scores was checked using the following approaches, Skewness and Kurtosis values, Kolmogorov-Smirnov and Shapiro-Wilk tests and, histograms and normal quantile-quantile (Q-Q) plots. Given the sample size ($N < 20$) combined with the lack of normality in various variables, transformations were not appropriate (Field, 2013) and non-parametric statistics were conducted to compare means between the high

and low nicotine conditions. Two-tailed Wilcoxon signed-rank tests were performed to explore differences in puffing topography (puff number, duration and volume consumed), nicotine boost, changes in self-report craving and withdrawal symptoms and subjective (positive and adverse) effects. Spearman's correlations coefficients were used to explore the relationship between a) plasma nicotine levels (nicotine boost) and puffing topography data at each time point, b) between cotinine to 3-hydroxy-cotinine ratio and plasma nicotine levels and c) cotinine to 3-hydroxy-cotinine ratio and puffing topography. The accepted alpha level was $p < .05$.

Outcomes

The primary outcomes were puffing topography: i) mean number of puffs; ii) mean puff duration (as recorded by the eVic) and, iii) volume of liquid consumed (in millilitres) and nicotine boost. Nicotine boost, a measure of nicotine exposure, was calculated by subtracting plasma nicotine levels at each time points from baseline levels (Patterson et al., 2003). Secondary outcomes were subjective effects which include: changes in craving (urge to vape) and withdrawal symptoms and, self-reported hit, satisfaction, other positive and adverse effects. Changes in craving, withdrawal and each dimension of the MPSS were computed by subtracting baseline levels from levels at each time point. Volume consumed was calculated in the same way as for the pilot study.

Measures

In addition to the materials, apparatus and measures in the pilot study, the following measures were employed (For a full list of materials and apparatus used for the blood collection, handling and storage as well as for the collection of the puffing topography data refer to Appendix 12).

Questionnaires:

- Baseline questionnaire to collect demographic information including age, gender, ethnicity, occupational status and highest qualification (appendix 13).
- The Fagerström Test for Cigarette Dependence (hereafter referred to as FTCD) measuring Cigarette dependence (Fagerström, 2012) was modified and renamed eFTND to measure dependence on e-cigarette, vaping history and e-cigarette use. Examples of items include measurements of self-report behavioural markers such as "*How soon after you wake up, you use your e-cigarette?*" The available option '*within 5 minutes*' corresponds to the highest score, whilst the option '*after 60 minutes'* scores the lowest as per the FTCD 3 (appendix 14). High scores signify high dependence. Other items were more subjective and aimed at measuring dimensions of loss of control, for instance: "Do you find it difficult to refrain from using your electronic cigarette in places where it is forbidden?"
- A single-item rating of addiction from 0-100% (scored from 0 = low addiction to 5 = very high addiction taken from the Cigarette Dependence Scale (CDS) (Etter, Le Houezec & Perneger, 2003) was also used (see appendix 14).

Procedure

Figure 2.7 illustrates the design of the study. Prior to testing, participants were sent an information sheet and screened via email and telephone. Pre-test saliva samples were collected by post and assessed for cotinine at Advanced Bioanalytical Service (ABS) Laboratories Ltd in order to verify that participants were habitual users of high nicotine concentrations and minimise the risks of aversive effects. Consistent with the pilot study, the e-cigarette was set at 3.9 Volt for an atomiser with a resistance of 1.8

ohms resulting in 8.5 Watts. The device was adjusted to the biggest airflow. To obtain a measure of the liquid consumed, the tank was removed from the main unit and weighed using precision scales before and after filling up the tank, at the commencement and end of each session. Liquid was pipetted in the tank prior to the start of each session.

Participants were asked to abstain from all tobacco products and nicotine intake including use of e-cigarettes for a period of at least 10 to 12 hours prior to each session and were tested individually. Upon arrival, each participant was welcomed by the research team, introduced to the phlebotomist and invited to take a seat on a medical plinth. Participants were offered a glass of water and provided written informed consent. Smoking status was verified via carbon monoxide breath test (cut off was < 10 ppm) and nicotine abstinence via baseline blood sample.

Baseline and demographic characteristics including vaping history, e-cigarette dependence, current craving ('urge to vape') and withdrawal symptoms were collected before a phlebotomist inserted a venous cannula into the participant's forearm and collected a baseline blood sample. Thereafter, the participant was presented with the eVic™ e-cigarette and asked to vape *ad libitum* for 60 minutes. During this time the participant read quietly, worked, engaged with social or other media via his/her own device. Further blood samples were taken, and craving and withdrawal symptoms recorded, at 10, 30 and 60 minutes after the first puff. Only the phlebotomist dealt with the blood samples collection assisted by a researcher, whilst another member of the research team handled data collection, running of the video recordings and the eVic™. At the commencement of each session and prior to replenishment, the tank was removed so the e-cigarette could be thoroughly cleaned and a new atomiser mounted. A third member of the research team was responsible for time monitoring and overseeing

the smooth running of the study's procedures. Puffing topography (puff number and puff duration) was recorded by the eVic™ and downloaded to 'My Vapors Joyetech 1.4'. The venous catheter was removed following the last blood sample collection at 60 minutes, before completion of a Visual Analogue Scale assessing positive (including hit and satisfaction) and adverse effects associated with nicotine and e-cigarette use (Dawkins & Corcoran, 2014). Participants were then offered a snack and refreshment, thanked and reimbursed financially for their time (£50 compensation) at the end of the second session.

Blood collection and nicotine analysis

Blood samples were collected using 4 mL BD K2EDTA vacutainer tubes and put on ice upon collection in an polystyrene igloo with the lid tightly closed at all times. All pre-labelled vacutainer tubes were kept in a closed container away from the vaping area. Only the phlebotomist and a designated researcher handled blood samples. Prior to handling vacutainer tubes, hands were disinfected (with Virucidal alcohol hand gel) and gloves were worn and changed every time to avoid contamination. A different researcher was responsible for handling the e-cigarette and loading with liquid to avoid blood sample contamination. After each testing session all blood samples were transported to an on-site forensic laboratory for plasma extraction, using PPE (gloves, safety spectacles) within 5 hours maximum of being collected.

Surface was disinfected using clinell and Blue roll. The MSE Falcon 6/300R centrifuge system was set and pre-run as follows, 2000 RCF, Temperature 4º Celsius, for 10 minutes. All blood samples were weighed and arranged in pairs before being placed in centrifugation machine diagonally to render samples acellular. The latter was set to run for 10 minutes at 4º Celsius, (2000 RCF). Thereafter, plasma was extracted

from the cell pellet using sterile Pasteur pipette heads and pipetted into sterile pre-labelled microvials. All samples were kept at -20°C pending transportation to ABS Laboratories Ltd. for analysis using a validated LC-MS/MS method with a lower limit of quantification (LOQ) of 0.5 ng/mL.

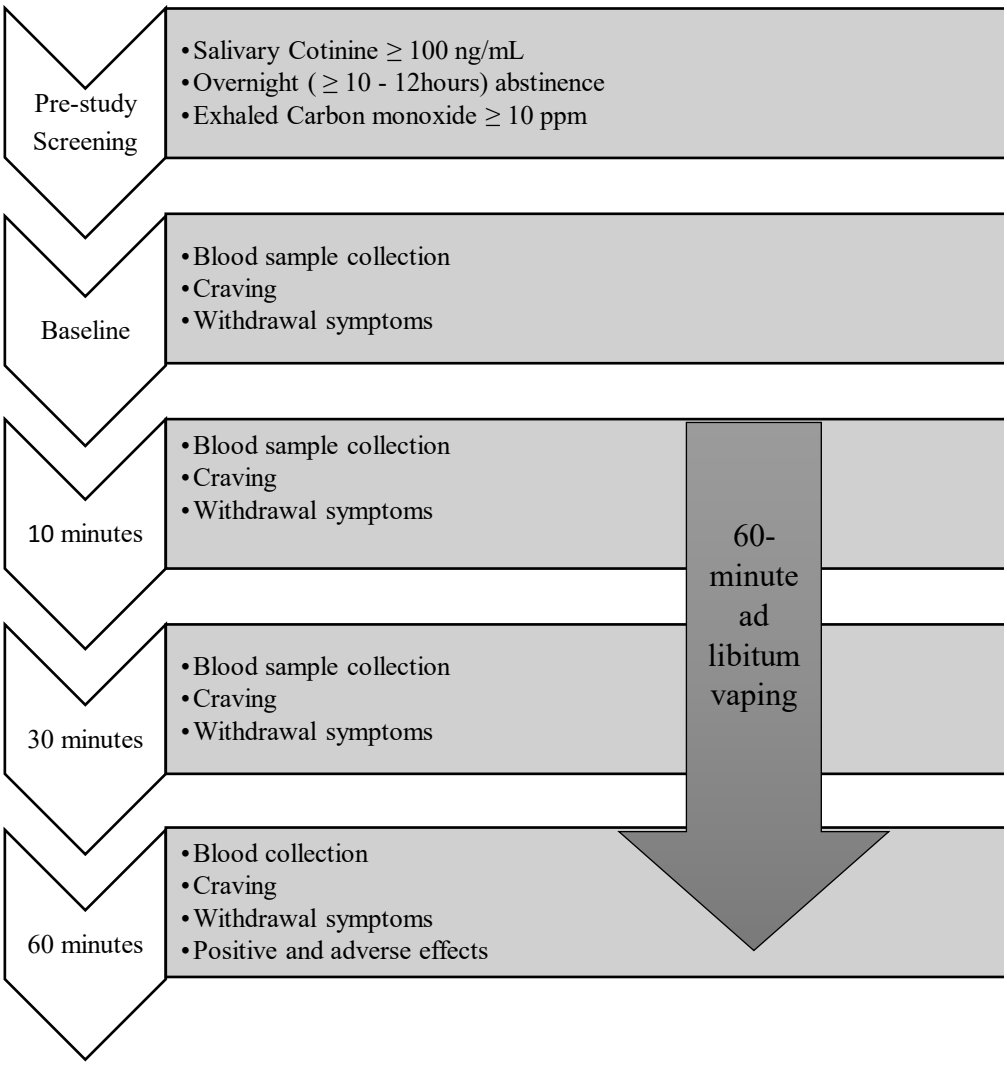


Figure 2.7 Experimental procedure (repeated at both sessions)

Results

Participant Characteristics

Table 2.6 provides demographics baseline characteristics of the sample.

Table 2.6

Participants' demographics and Baseline characteristics (Study 1)

	N	%	Mean	SD	Min	Max
Age (years)	12		42.33	12.60	21	56
Gender						
Male	11	91.67	-	-	-	-
Female	1	8..33	-	-	-	-
Ethnicity			-	-	-	-
White	12	100	-	-	-	-
Qualification			-	-	-	-
GSCEs level	5	41.7	-	-	-	-
A levels	2	16.7	-	-	-	-
Undergraduate level (5 to 6)	5	41.7	-	-	-	-
Occupational status			-	-	-	-
Employed	7	58.3	-	-	-	-
Non-employed	2	16.7	-	-	-	-
Self-employed	3	25.0	-	-	-	-
eFTND[1]	12		3.83	1.12	2	6.00
E-cig Addiction[2]	12		3.25	1.13	1	5.00
Baseline cotinine (ng/mL)	12		451.97	243.93	134.2	890.6
3-hydroxy-cotinine (ng/mL)	12		159.33	124.00	41.90	458.10
Cotinine to 3-hydroxy-cotinine ratio	12		0.37	0.22	0.09	.86
Baseline CO (ppm) high strength	12		2.00	1.21	1.00	4.00
Baseline CO (ppm) low strength	12		2.08	1.31	1.00	5.00
Daily Liquid Vol consumed (mL)	12		4.00	1.73	2.00	7.00
6 mg/mL	4	33.3	-	-	-	-
10 mg/mL	1	8.3	-	-	-	-
11mg/mL	1	8.3	-	-	-	-
18mg/mL	1	8.3	-	-	-	-
24 mg/mL	3	25	-	-	-	-
30 mg/mL	2	16.7	-	-	-	-
Current model most used			-	-	-	-
Rechargeable non-cigalike (2nd gen)	1	9.1	-	-	-	-
Modular systems (incl.sub-ohms[4])	11	90.9	-	-	-	-

Note. [1] eFTND = Modified version of Fagerström Test for Cigarette Dependence for e-cigarettes use with item regarding consumption removed (score range: 0-7)

[2] Self-rated addiction: 0-20% = 1; 21-40% = 2; 41-60% = 3; 61-80% = 4; 81-100% = 5

[3]Note: Most participants indicated using multiple strengths.

[4] i.e. atomiser resistance < 1ohm used with higher wattage

Puffing Topography

Some instances of extremely short puffs/button presses (i.e. < 1s) (9/568 puffs in the high condition and 15/887 puffs in the low condition; 1.58% and 1.69% respectively) were observed. These were verified against backup video data and deleted in cases where the device was not clearly in the mouth (N = 4). In all other instances wherein a short puff (< 1 sec) preceded or succeeded a puff of more than 1 second, the former was merged with the longer puff. Individual puffing topography data are presented in Tables 2.7 and depicted graphically in Figures 2.8 to 2.12. All participants (with the exception of P.4) increased their number of puffs and puff duration (with the exception of P1) from the high to the low condition. Likewise, volume consumed (mL) increased from the high to low condition for all participants.

Table 2.7

Puffing topography (individual and overall scores in mean number of puffs and duration during the 60 mins ad lib vaping period)

	Puff number (*SD*)		**Puff Duration (s) (*SD*)**		**Volume consumed (mL) (*SD*)**	
	High	**Low**	**High**	**Low**	**High**	**Low**
P1	51	85	4.60	4.51	.61	1.19
P2	17	32	4.00	7.31	.84	1.74
P3	61	89	4.02	6.77	.70	1.46
P4	80	61	5.76	6.84	.56	1.34
P5	65	97	3.94	4.53	.82	.97
P6	63	131	3.94	5.17	.88	2.38
P7	50	94	2.15	3.20	.45	.73
P8	36	109	2.36	3.35	.43	.85
P9	12	14	3.50	5.09	.41	.45
P10	16	31	1.92	3.62	.34	.50
P11	57	66	2.01	4.00	.58	.99
P12	60	78	3.80	6.16	.82	1.77
Overall means	47.33 (22.08)	73.92 (34.66)	3.76 (1.01)	5.04 (1.43)	0.62 (0.18)	1.20 (0.57)

Note. Values are mean scores on puff number and duration (in seconds) and volume consumed (in millilitre). Standard deviations are presented for the overall sample

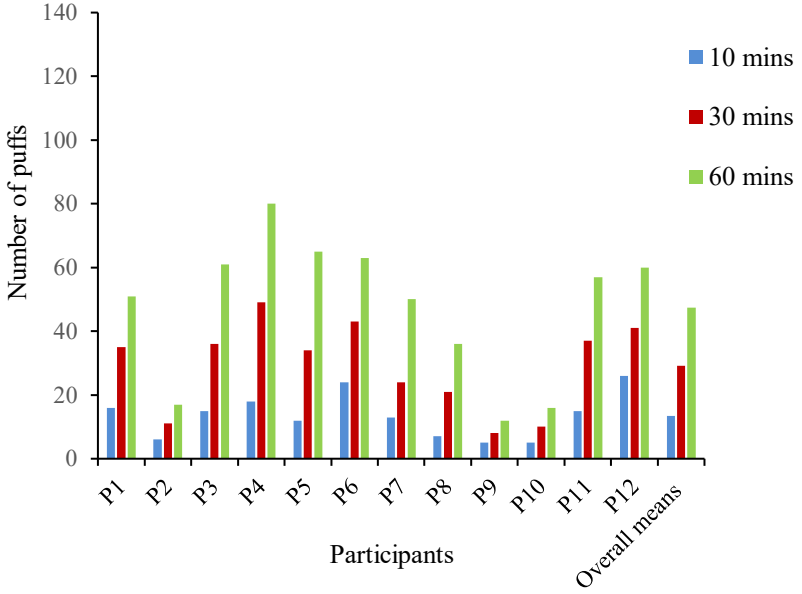


Figure 2.8 Puff number per participant and overall means at each time point in the high condition[e]

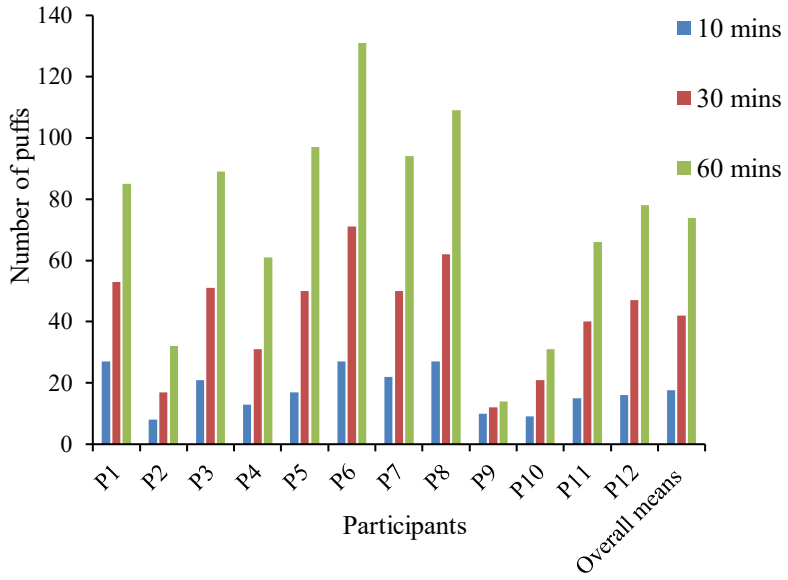
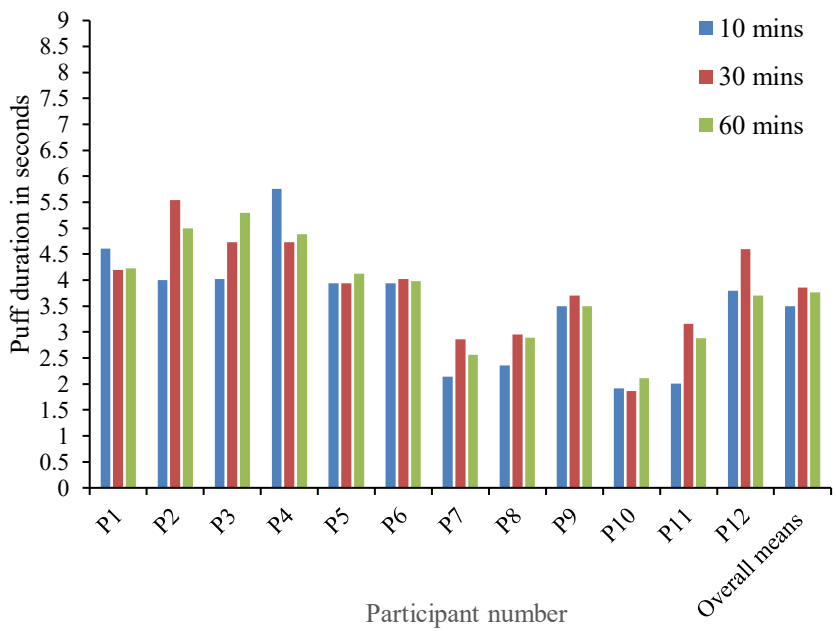
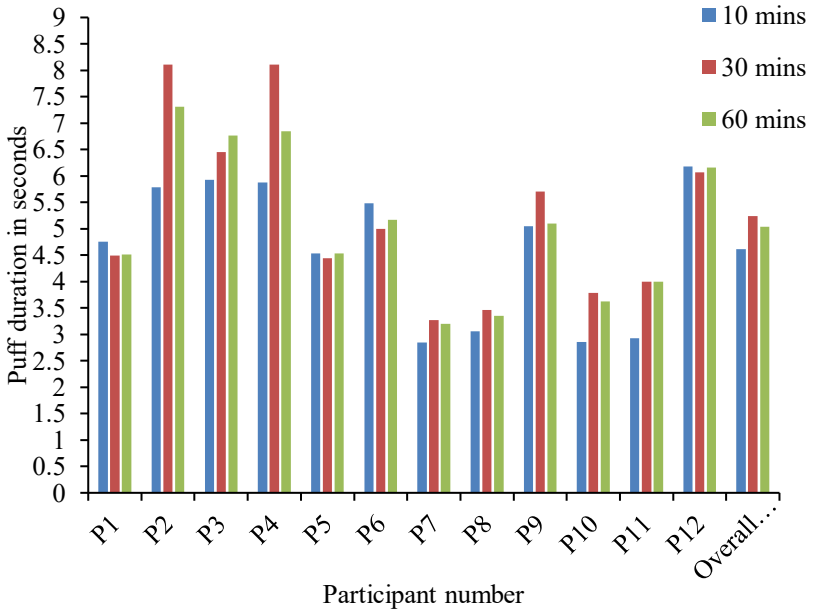


Figure 2.9 Puff number per participant and overall means at each time point in the low condition

[e] Figures 2.8 and 2.9 Means here are cumulative scores and represent total number of puffs taken from baseline to the end of the 60 min ad lib session



Figures 2.10 Average puff duration per participant and overall means at each time point in the high condition[f]



Figures 2.11 Average puff duration per participant and overall means at each time point in the low condition

[f] Figures 2.10 and 2.11 Means here are cumulative scores from baseline to the end of the 60 min ad lib session

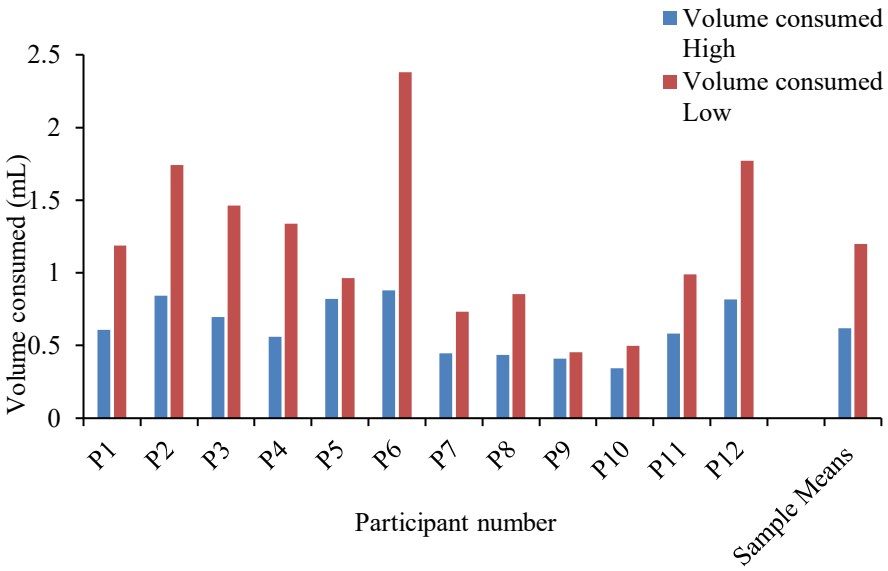


Figure 2.12 Volume consumption (in mL) per participant in high and low conditions ($p < 0.05$)

A Wilcoxon Signed Rank Test revealed statistically significant differences between the high and low conditions in mean puff number [$z = -2.59$, $p < .05$] (see figure 2.8 and 2.9) with a large effect size ($r = .53$), in mean puff duration [$z = -3.06$, $p = .002$] (figure 2.10 and 2.11) with a large effect size ($r = .62$), and mean volume of liquid (mL) consumed [$z = -3.06$, $p = .002$] (Figure 2.12) and a large effect size ($r = .62$). The median scores in volume consumed $Md = .59$ mL increased almost two-fold from the high to the low condition $Md = 1.08$ mL. Median scores in puff number also increased from $Md = 54$ to $Md = 81.50$, and in puff duration from $Md = 3.84$ to $Md = 4.81$ from the high and to the low condition respectively, indicative of compensatory puffing.

Spearman's rank correlation tests indicated a statistical strong relationship between volume consumed and overall means in puff duration, in the high, $r = .63$, $n = 12$, $p < .05$ and in the low condition respectively, $r = .69$, $n = 12$, $p < .05$. There was no

statistically significant correlation between volume consumed and overall means in puff numbers in the high or in the low condition (all $p > .05$).

In order to explore how participants adjusted their puffing topography in response to the given nicotine concentrations, means in non-cumulative[g] puff numbers and duration in high versus low condition were compared using Wilcoxon Signed Rank tests. Detailed puffing topography data at each time points with mean (SD) puff numbers and duration are presented in Table 2.8. There was a statistically significant difference between conditions in mean puff numbers at 30 [$z = -2.35$, $p < .05$] and 60 minutes [$z = -2.83$, $p < .05$], and in puff duration at 10 [$z = -3.06$, $p < .05$], at 30 [$z = -3.06$, $p < .05$] and at 60 minutes [$z = -3.06$, $p < .05$]. The greater mean values in puff duration in the low compared to the high condition suggest that participants increased their puff duration in response to the drop in nicotine concentration (see Table 2.8 for means (SD). The difference between conditions in mean puff number at 10 minutes was not statistically significant ($p > .05$). From the high to the low condition respectively, median scores in puff number at 10 minutes increased from *Md* = 14 to *Md* = 16.50, at 30 minutes from *Md* = 17 to *Md* = 27, at 60 minutes from *Md* = 19.5 to *Md* = 31.5, and in puff duration at 10 from *Md* = 3.87 to *Md* = 4.90, at 30 from *Md* = 3.98 to *Md* = 4.75 and at 60 minutes from *Md* = 3.62 to *Md* = 4.66 suggesting compensatory puffing at all time points.

[g] Non-cumulative puff number scores correspond to the total of puffs taken at each vaping sequence (from baseline to 10, from 10 to 30 and from 30 to 60 minutes), and non-cumulative scores in puff duration correspond to averages in puff duration per vaping sequence.

Table 2.8

Individual scores in mean puff numbers and duration at each time point

	Puff number (SD)						**Puff duration (SD)**					
	High			**Low**			**High**			**Low**		
	10 mins	**30 mins**	**60 mins**	**10 mins**	**30 mins**	**60 mins**	**10 mins**	**30 mins**	**60 mins**	**10 mins**	**30 mins**	**60 mins**
P1	16	35	51	27	53	85	4.61	4.20	4.23	4.75	4.49	4.51
P2	6	11	17	8	17	32	4.00	5.54	5.00	5.79	8.11	7.31
P3	15	36	61	21	51	89	4.02	4.73	5.29	5.93	6.45	6.77
P4	18	49	80	13	31	61	5.76	4.74	4.88	5.88	8.11	6.85
P5	12	34	65	17	50	97	3.94	3.94	4.12	4.53	4.44	4.53
P6	24	43	63	27	71	131	3.94	4.02	3.98	5.48	5.00	5.17
P7	13	24	50	22	50	94	2.15	2.86	2.57	2.85	3.28	3.20
P8	7	21	36	27	62	109	2.36	2.95	2.89	3.06	3.46	3.35
P9	5	8	12	10	12	14	3.50	3.70	3.50	5.05	5.70	5.09
P10	5	10	16	9	21	31	1.92	1.86	2.11	2.86	3.78	3.62
P11	15	37	57	15	40	66	2.01	3.16	2.88	2.93	4.00	4.00
P12	26	41	60	16	47	78	3.80	4.60	3.70	6.18	6.07	6.16
Overall means	13.50 (7.03)	29.08 (13.91)	47.33 (22.08)	17.67 (7.08)	42.08 (18.31)	73.92 (34.66)	3.50 (1.17)	3.86 (1.02)	3.76 (1.01)	4.61 (1.33)	5.24 (1.67)	5.04 (1.43)

Nicotine boost: Changes in Plasma Nicotine levels

All plasma nicotine level (nicotine boost) results and correlational analyses included eleven participants due to exclusion of one participant due to an inability to withdraw blood ($N = 11$).

A Wilcoxon Signed Rank Test revealed a statistically significant increase in nicotine boost at all time points in the high (10 mins: $Z = -2.85$, $p = .04$; 30 mins: $Z = -2.70$, $p = .007$; 60 mins: $Z = -2.58$, $p = .01$ with large effect sizes, $r = .61$, $r = .59$, $r = .55$ at 10, 30 and 60 minutes respectively) compared with the low condition. The median scores in the high condition increased from 10 ($Md = 18.12$) to 30 ($Md = 36.11$) and 60 minutes ($Md = 37.23$). Mean (SD) nicotine boost for the high nicotine condition from baseline were 32.35 (34.88) ng/mL, 35.48 (28.31) ng/mL and 43.00 (34.78) ng/mL. Corresponding values for the low nicotine condition were 8.74 (7.52) ng/mL, 16.75 (11.72) ng/mL and 21.96 (16.19) ng/mL at 10, 30 and 60 minutes respectively (see Figure 2.13). This suggests that compensatory puffing was not complete with significantly higher plasma nicotine boost in the high condition.

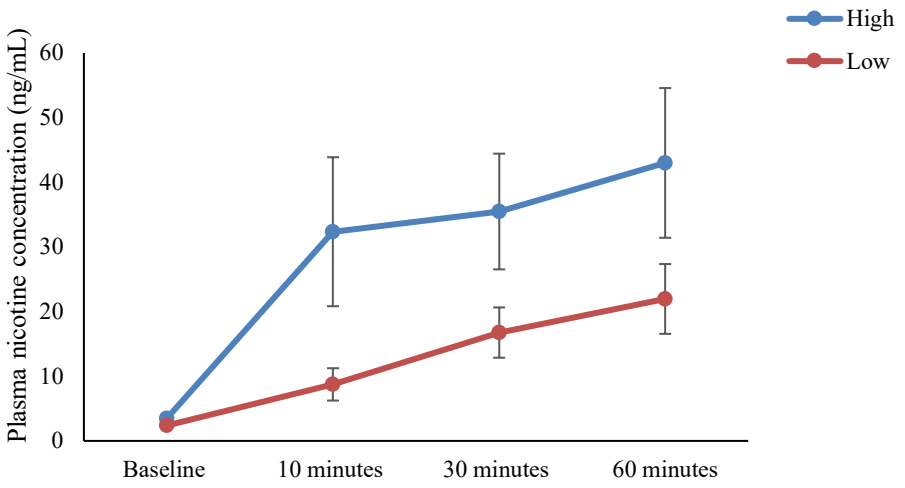


Figure 2.13 Plasma nicotine concentration at baseline, 10, 30 and 60 mins from baseline, for the high and low nicotine conditions (N = 11; Error bars are ± 1 SE)

Correlation between plasma nicotine boost and cumulative scores in puffing topography

Spearman's Rank correlation tests indicate that plasma nicotine boost correlated with mean scores in puff number at all time points, and with mean puff duration only at 10 minutes in the high condition. Correlations were significant only at 30 minutes in the low condition (see Table 2.9).

Table 2.9

Correlations between the cumulative mean scores in puff number, duration, liquid consumption and plasma nicotine boost, at each vaping sequence in the high and low nicotine conditions.

High Nicotine Liquid	**Plasma Nicotine Boost**			
	10 mins	**30 mins**	**60 mins**	**Total scores**
Puff number	.91**	.86**	.85**	.85**
Puff Duration	.62*	.59	.56	.63*
Low Nicotine Liquid	**10 mins**	**30 mins**	**60 mins**	**Total scores**
Puff number	.49	.61*	.56	.56
Puff duration	.39	.33	.35	.32

Note. *p ≤ 0.05 (2-tailed); **p ≤ 0.01 (2-tailed). Mean scores on Puff number and duration are averaged from baseline at each time point.

Individual puffing topographies in relation to plasma nicotine levels

In order to explore how participants adjusted their puffing behaviours in response to the given nicotine concentrations and resulting rise in their plasma nicotine levels, non-cumulative puff numbers and duration are plotted at each time point and presented per individual graphs (Figures 2.14 to 2.37 in appendix 15). The graphs suggest fairly similar fluctuations in puffing patterns per vaping sequence across both conditions. In the low condition it seems that means in puff number and duration accompanied an increase in plasma nicotine levels in most cases. Whereas in the high condition, increases in plasma nicotine levels were followed by downward trends in puffing patterns but not for all participants. Two participants (P6 and P10) seemed to achieve complete self-titration raising their plasma nicotine in the low condition to similar levels achieved in the high condition (see Figures 2.24 - 2.25 and 2.32 - 2.33, appendix 15).

Correlations between plasma nicotine and puffing topography (in non-cumulative scores)

Puff number correlated with plasma nicotine boost at each vaping sequence in the high (all ps < .01, see Table 2.5) and only at 30 minutes in the low condition. Puff duration correlated with plasma nicotine boost at 10 minutes in the high condition and not at all in the low condition. Volume consumed also correlated positively with nicotine boost at 60 minutes in the low condition only (see Table 2.10).

Table 2.10

Correlations between the non-cumulative mean scores in puff number, duration, liquid consumption and plasma nicotine boost, at each vaping sequence in the high and low nicotine conditions.

High Nicotine Liquid	**Plasma Nicotine Boost**			
	10 mins	**30 mins**	**60 mins**	**Total scores**
Puff number	.91**	.78**	.61*	.85**
Puff Duration	.62*	.59	.56	.63*
Volume (mL) consumed	-	-	-	.47
Low Nicotine Liquid	**10 mins**	**30 mins**	**60 mins**	**Total scores**
Puff number	.49	.68*	.57	.56
Puff duration	.39	.36	.35	.32
Volume (mL) consumed	-	-	-	.75**

Note. *p ≤ 0.05 (2-tailed); **p ≤ 0.01 (2-tailed). Volume (mL) consumed was only measured at 60 mins.

Correlational analysis between 3-hydroxy-cotinine to cotinine ratio and plasma nicotine

There was a statistically significant positive correlation between 3-hydroxy-cotinine and non-cumulative scores in puff numbers at 30 [$r = .62$, $p < .05$] and at 60 minutes [$r = .66$, $p < .05$]. Baseline cotinine, 3-hydroxy cotinine and 3-hydroxy-cotinine to cotinine ratio did not correlate with plasma nicotine boost under high or low conditions at any time point (all *ps* > .05*)* or with puffing topography variables *(*all *ps* > *.05)* which suggest that previous nicotine use or phenotypic status to metabolise nicotine did not affect puffing behaviours.

Changes in 'Urge to vape' and withdrawal symptoms

For 'changes in urge to vape, median scores indicated that participants generally reported greater reduction in the high versus low conditions at 10 minutes *Md* = 2 versus *Md* = 1, 30 minutes *Md* = 2.5 versus *Md* = 2 and 60 minutes *Md* = 3 versus *Md* = 2.5. However, these differences were not statistically significant [at 10 z = -.51, 30 z = -.67 or 60 minutes z = -.79, all p < .05]. There was no statistically significant differences between conditions in 'changes in overall withdrawal symptoms' at any time points [10 z = -.36, 30 z = -.42 and 60 minutes z = -.56, all p < .05]. There was no statistically significant differences between conditions in changes in depressed mood, irritability, anxiety, drowsiness, restlessness, hunger and inability to concentrate (all ps > .05). These results suggest that although plasma nicotine levels failed to equate those achieved in the high condition, compensatory puffing was sufficient to alleviate craving and withdrawal symptoms.

Subjective Effects: Self-report positive effects

Mean (*SD*) and median scores are presented in table 2.11. There were no statistically significant differences between conditions for hit $z = 1.726$, $p = .084$ and satisfaction $z = 1.883$, $p = .060$ nor for any other positive effects (all $ps > .05$).

Table 2.11

Mean (SD) and Median scores in the high and low conditions in changes in positive effects associated with e-cigarette use.

	Low condition		**High condition**	
	Mean (*SD*)	**Median**	**Mean (*SD*)**	**Median**
'Hit'	45.21 (22)	45	61.96 (30.03)	68.5
'Satisfaction'	47.31 (16.21)	46.25	62.02 (17.11)	64.38
'Pleasant'	46.46 (23.77)	49.25	48.06 (15.26)	49
'Tastes good'	41.33 (23.38)	38.25	27.07 (22.41)	27.75
'Reduced my craving'	59.63 (19.76)	60.75	71.33 (22.83)	74
'helped my concentration'	48.29 (15.81)	46.75	57.93 (21.47)	59.50
'helped me feel calmer'	48.40 (18.35)	51	51.35 (20.97)	50.25
'feel more awake'	37.83 (20.16)	34.75	45.46 (25.23)	45
'reduced my hunger'	25.77 (15.19)	23.25	29.02 (23.80)	27.75
'tastes like my usual brand/model'	15.08 (18.36)	9	13.79 (17.69)	9.75
'feels like using my usual brand/model'	25.01 (27.16)	19.75	38.08 (25.14)	42.25

Note. Values are mean scores in percentage for each dimension of the VAS questionnaire on positive subjective effects related to e-cigarette use.

Self-report adverse effects

Overall mean adverse effect self-ratings did not differ statistically across conditions: $z = -1.33$, $p = 0.18$. Analysis of individual symptoms revealed no significant differences across conditions (all $ps > 0.05$; see Table 2.12). There was one incident in which a participant felt unwell and vomited 30 minutes into his first testing session following use of the e-cigarette in the high condition. To prevent such incidents and ensure that participants were daily users and accustomed to the high nicotine concentration specified in the inclusion criteria, all were pre-screened with a cut-off

point for cotinine ≤ 100mg/mL and advised to drink plenty and to eat breakfast. This participant admitted failing to follow these recommendations. His previously experienced anxiety over difficulty locating the study venue on a very hot day, may have also contributed to his sickness. After a period of 20 minutes symptoms eased away and the participant chose to continue with the study.

Overall adverse effects did not correlate with plasma nicotine boost at any time points (all *ps* > .05).

Table 2.12

Mean (SD) and Median scores in the high and low conditions in adverse effects associated with e-cigarette use (in order from the most to the least severe symptoms based on means).

	Low condition		**High condition**	
	Mean (*SD*)	**Median**	**Mean (*SD*)**	**Median**
'Sweaty'	11.71 (14.17)	5.00	18.46 (15.73)	12.50
Throat irritation	13.58 (19.28)	10.50	18.25 (26.76)	11.75
'Light headed'	11.92 (12.23)	8.25	16.17 (16.16)	12.75
'Mouth irritation'	14.79 (19.04)	12.75	15.35 (27.10)	9.00
Nausea, feeling sick	8.71 (9.38)	5.50	14.88 (17.46)	11.00
'Dizzy'	7.56 (6.48)	6.00	14.25 (14.39)	10.75
'Weak'	10.21 (11.84)	5.00	13.98 (14.37)	11.88
'Salivation'	10.19 (15.21)	4.63	12.50 (13.91)	8.50
'Confused'	7.04 (5.61)	5.75	11.96 (14.07)	9.00
'Pounding heart'	10.44 (11.47)	8.75	6.79 (7.14)	6.00
'Nervous'	9.08 (8.28)	7.75	9.79 (13)	6.00
'Cold hands and feet'	6.21 (5.63)	5.00	9.04 (13.96)	4.50
'Palpitations'	6.29 (5.73)	5.00	9.00 (13.29)	5.00
'Stomach ache'	6.17 (5.50)	5.00	8.15 (7.80)	6.00
'Headache'	7.04 (5.63)	6.00	6.96 (6.73)	6.00
'Aching jaws''	6.17 (5.50)	5.00	6.13 (6.63)	4.75
'Hiccups'	6.94 (6.85)	5.00	5.96 (6.52)	4.25
'Diarrhoea'	6.31 (5.62)	5.00	6.04 (6.64)	4.25
'Heartburn'	6. 29 (5.52)	5.00	6.25 (6.70)	5.00
'Flatulence, Bloating'	5.96 (5.21)	5.00	6.19 (6.73)	5.00
'Vomiting'	6.00 (5.30)	5.00	5.96 (6.65)	4.25

Note. Values are mean scores in percentage for each dimension of the VAS questionnaire on negative subjective effects related to e-cigarette use.

Discussion

Summary of findings

The aims of the current study were to investigate whether and to what to extent experienced e-cigarette users adjust their puffing patterns to adapt to changes in nicotine availability so they can achieve desired blood nicotine levels. As per hypothesis H_1, participants made an attempt to self-titrate by increasing their liquid consumption, puff duration and frequency in the lower nicotine concentration condition. However, as for hypothesis H_2, which posited that there will be no differences between conditions, plasma nicotine levels were significantly higher in the high nicotine concentrations suggesting that titration was only partial and that compensatory puffing was not sufficient to raise plasma nicotine levels to match levels achieved in the high condition. On the other hand, as per hypothesis H_3, the use of both nicotine concentrations were equally effective in alleviating 'urge to vape' and withdrawal discomfort. Similarly, there were no differences in subjective positive effects such as 'satisfaction' and 'hit' or in adverse effects.

Puffing topography and plasma nicotine levels

Participants consumed almost double the amount of liquid in the low compared with the high nicotine concentration, increased their puff numbers and duration. Mean puff durations found here are in good agreement with previous documentations of experienced e-cigarette users' puff duration (Farsalinos et al., 2013b; Hua et al., 2013). Others found much shorter puffs whilst comparing plasma nicotine levels with 0, 8, 18 and 36 mg/mL nicotine concentrations. Following the first 10-puff (30s IPI) standardised protocol, mean scores in puff duration (across 0, 8, 18 and 36 mg/mL conditions) differed statistically only between 0 and 36 mg/mL; the significant increase in puff duration from 36 mg/mL to the placebo nicotine condition is suggestive of

compensatory puffing (Lopez et al., 2016). However, the same group later reported similar differences in mean puff duration between 0 and 36 mg/mL (Ramôa et al., 2015). The argument that longer puff duration and shorter IPI (Williams et al., 2011) are conducive to more efficient nicotine delivery (Hajek et al., 2015) seem to be supported by the current data; mean puff durations were significantly longer in the low condition and here significantly higher plasma nicotine levels were achieved with 6mg/mL nicotine e-liquid compared to previous studies. In a previous study, following a 60 min ad lib puffing session, a mean puff duration of (M = 2.3, SD = 0.2 s) yielded an average plasma nicotine level of 13.8 ng/mL (SD = 1.6) in naïve e-cigarette users (Farsalinos et al., 2015), which are moderate compared to the mean level seen here 21.96 ng/mL (SD = 16.19) at 60 minutes. The higher number of puffs taken in the 6 mg/mL condition in an attempt to compensate is echoed in previous findings. Following a 30-minute controlled, rapidly succeeded by a 60-minute ad lib, puffing session, higher puff numbers were associated with lowest nicotine e-liquid concentrations and with cartridge models (Hajek et al., 2017) which typically have a poor nicotine delivery compared with tank models (Farsalinos et al., 2014).

These puffing topography results concur with previous findings; the average puff duration in the high condition observed here was slightly longer than those previously reported in experienced users (Hua et al., 2011) using newer generation devices (Farsalinos et al., 2014) and longer than those reported for cigalike (first-generation) devices (Behar et al., 2015) and combustible cigarettes (Hua et al., 2013). Previous studies have found longer puff duration in first-generation cigalike models to be associated with greater discomfort compared with second-generation eGo models (Dawkins, Kimber, Puwanesarasa, & Soar, 2015) suggesting that the increased sophistication of the newer generation devices may promote longer puffing patterns.

Nevertheless, there is emerging data which suggests that puffing patterns may be influenced by factors other than nicotine concentration such as the ingredients present in the liquids. For instance, studies have found a higher ratio of propylene glycol (compared to vegetable glycerine) to be associated with shorter puff number and duration, but result in higher plasma nicotine levels suggesting that a combination of propylene glycol (PG) and vegetable glycerine (VG) is a better vehicle for nicotine compared to vegetable glycerine alone (Yan & D'Ruiz, 2015). Here this was controlled by using a 60/40 (PG/VG) ratio across both conditions. In the same light, the high ratio of PG may have contributed to the unusually high plasma nicotine observed here.

These findings suggest that, in line with the self-titration model, experienced e-cigarette users will compensate by adjusting their puffing patterns to maximise nicotine delivery from a lower nicotine concentration liquid. Furthermore, statistically significant differences in mean values of non-cumulative puffing number and duration suggest that participants had to adjust their puffing patterns across time points during the course of using the e-cigarette according to the nicotine availability; this provide support for the 'finger-tip' control that is often ascribed to smoking.

Nicotine boost

Nicotine boost was significantly higher in the high compared with the low nicotine concentration (see Figure 2.7). Although puffing topography data are an indication of attempts to compensate for the low nicotine concentration, from the latter results it is clear that self-titration was not complete with significantly higher levels in the high nicotine content concentration. This is in line with previous reports from tobacco cigarette studies. Self-titration tend to be complete when smoking higher yield cigarettes, but only partial when smoking lower yield cigarettes (Scherer, 1999). A likely explanation for the partial titration could be that the high nicotine concentration

was sufficiently high to exceed desired levels. This is supported by the non-cumulative puffing data which shows that as plasma nicotine boost increased, participants decreased their puffing intensity.

Plasma nicotine levels achieved here were unusually high compared to those in previous reports and equate to levels observed in tobacco smoking (Russell et al., 1980; Russell, Wilson, Patel, Feyerabend, & Cole, 1975). These levels exceed those reported in previous studies in which similar (Farsalinos et al., 2014; Farsalinos et al., 2015; Yan & D'Ruiz, 2015) and higher nicotine concentrations (Hajek et al., 2017; Lopez et al., 2016; Ramôa et al., 2015) were used. For comparison, in a recent study the smoking of a tobacco cigarette in a five-minute puffing period (10 puffs, 30 seconds apart) resulted in Cmax of 13.4 ng/mL (Fearon et al., 2017), which seems negligeable compared to levels achieved by some individuals from the current study (means ranged from 5 to 110.12 ng/mL); although much greater levels (that is, ≥ 30 ng/mL following the smoking of 32 mg/mL nicotine yield tobacco cigarettes) are reported in earlier tobacco research studies (Russell et al., 1975). In a study investigating PK profiles of a cigarette versus different e-cigarette brands, none of the e-cigarette matched the PK profile of the tobacco cigarette (C_{max} = 17.9 ng/mL), that is including a 48 mg/mL nicotine concentration e-cigarette which yielded a mean C_{max} of 13.6 (*SD* = 9.7) ng/mL following 19 puffs during a five-minute prescribed vaping protocol (Hajek et al., 2017). In one study, higher puff numbers tended to be associated with the lowest PK profiles and the lower nicotine concentrations (Hajek et al., 2017), which concurs with the compensatory puffing hypothesis. Such high plasma nicotine levels may be due to the one hour ad libitum vaping design combined with the required 10 to 12 hour nicotine abstinence and a self-selective recruitment method of high nicotine concentration vapers with baseline salivary cotinine levels equal or exceeding 100 ng/mL. Due to the rapid

advancement in technology, recent findings suggest that the shape of pharmacokinetic curve of the nicotine delivery of e-cigarettes is increasingly approaching that of combustible cigarettes (St Helen, Havel, Dempsey, Jacob 3rd, & Benowitz, 2015); findings of the present study certainly seem to support this trend.

Nicotine boost and puff numbers were highly correlated at all time-points in the high but not in the low nicotine concentration condition. The failure to reach statistical significance may be due to the small sample size or due to a saturation effect in the low nicotine concentration condition. In the high condition, non-cumulative puffing data suggests that, as plasma nicotine boost was increasing, participants tended to decrease their puffing. The number of puffs taken from baseline to 30 minutes was significantly lower than the number of puff taken during the remaining 30 minutes, and in some cases, the trajectory of the nicotine boost goes downwards after the 30 minutes, which is suggestive of an attempt to regulate (downwards) to a desired and satisfactory plasma nicotine levels. Already the first 10 minutes were marked by a significant difference in puff duration, with a higher overall mean (SD) in the low (M = 4.61, SD = 1.33 s.) compared to the high condition (M = 3.50, SD = 1.17 s.) which lends support to the self-titration theory.

The notion of self-titration (or self-regulation) is well established and extensively evidenced in smokers, typically in up-regulation attempts, titration is seldom complete (Scherer, 1999). In the present study with vapers, despite the several fold reduction in the low nicotine concentration (24 to 6 mg/mL), corresponding nicotine boost was only two-fold lower at 30 and 60 minutes. One possible explanation for the failure to fully self-titrate could be that the large difference in nicotine liquid content may require too great an effort to compensate fully combined with a time constraint of one hour which may have caused a satiation effect. That is, any given time

period imposes a limit on the quantity of nicotine liquid that an individual is able to consume comfortably. It is unknown whether a more prolonged ad libitum session would have led to similar plasma nicotine levels.

Individual differences

Puffing topography and plasma nicotine levels varied widely amongst participants as has previously been demonstrated in smokers (Hammond et al., 2005; Russell, 1980), and e-cigarette users (Dawkins & Corcoran, 2014; Farsalinos et al., 2015; Yan & D'Ruiz, 2015). However, like in the smoking population (Gust et al., 1983), puffing patterns were consistent across conditions within participants (Behar et al., 2015). As indicated by the standard deviations, individual variabilities were more pronounced in the low nicotine concentration with the lowest individual mean in puff number of 14 and the highest 131 puffs. Mean volume consumed ranged from .45 to 2.38 mL and mean puff duration ranged from 3.20 to 7.31 s, in some cases exceeding 10 s. Such extreme long puffs are not uncommon in e-cigarette use, Hua and colleagues observed puff duration of 8 s in experienced e-cigarette users (Hua et al., 2013). As was the case for most of the sample, participant 6 increased his puffing frequency by almost double from 63 to 131 puffs and his puff duration from 3.94 to 5.17 s and his liquid consumption from 0.88 to 2.38 mL. Likewise participant 4 increased his liquid consumption from 0.56 to 1.34 and his puff duration from 5.76 to 6.84 s, inversely his mean puff number reduced from 80 to 61 puffs. Though, the latter must be interpreted with caution as participant 4 did feel unwell half way through the session and his puffing behaviour in the repeated session may have been impacted by a possible recall of the event in his previous session. The lack of statistical significance in correlational analysis between puffing topography and plasma cotinine levels suggests that inhalation depth may have differed widely between participants and influenced nicotine

absorption. The individual variations are likely to be due to the small size of the sample and also be a reflection of the variations in nicotine dependence within the sample with baseline cotinine levels varying from 134.2 to 890.6 ng/mL.

Participants' puffing patterns seemed to fluctuate in the same fashion across vaping sequence and conditions. In the low condition, means in puff number and duration accompanied an increase in nicotine boost in most cases. Whereas in the high condition, increases in nicotine boost were followed by downward trends in puffing patterns but not for all participants. Two participants (P6 and P10) seemed to achieve complete self-titration raising their plasma nicotine in the low condition to similar levels achieved in the high condition, which suggests that it is possible for some e-cigarette users to self-titrate by adjusting their puffing patterns in order to obtain desired and satisfactory plasma nicotine levels.

Relationships between nicotine metabolism, nicotine intake and puffing topography

Mean in trans-3-hydroxy-cotinine to cotinine ratio (M = .37, SD = .22) fell within the common range amongst smokers (.2 to .75) (Dempsey et al., 2004), however, the sample varied widely with 3-hydroxy cotinine to cotinine ratio ranging from .09 to .86. As per previous reports in tobacco smokers (Levi, Dempsey, Benowitz, & Sheiner, 2007), puffing patterns were not influenced by 3-hydroxy-cotinine to cotinine ratios. Puff number and duration were not associated with baseline cotinine levels, 3-hydroxy cotinine or 3-hydroxy-cotinine to cotinine ratio. Neither did baseline cotinine, 3-hydroxy cotinine or 3-hydroxy-cotinine to cotinine ratio correlate with plasma nicotine boost under high or low conditions at any time point. This suggests that previous nicotine use or phenotypic status to metabolise nicotine did not affect puffing behaviours. This is in line with previous studies in tobacco smokers, the wide individual variabilities in the relationships between nicotine intake, saliva cotinine and

could be largely explained by i) the individual differences in percentages in nicotine to cotinine conversion (usually between 55 - 92 %) and also ii) individual differences in the rate of cotinine metabolism (Benowitz & Jacob, 1994). Besides, whilst nicotine metabolism has been shown to influence nicotine intake, the one hour ad libitum vaping session would not allow sufficient time for the metabolism of nicotine or cotinine conversion to exert any effect on puffing behaviours.

Effects on craving, withdrawal and subjective effects

Although compensatory plasma nicotine levels in the low nicotine concentration failed to equate to those in the high nicotine concentration, in line with tobacco smoking (Sutton et al., 1982), such attempts to self-titrate appeared to be effective in alleviating subjective urge to vape and withdrawal discomfort. Median scores in reduction in '*urge to vape*' were greater in the high conditions compared with the low condition, but, these differences did not reach statistical significance. Similarly, there was no statistically significant differences between conditions in any of the individual withdrawal symptoms. These results suggest that although plasma nicotine levels failed to equate those achieved in the high condition, compensatory puffing was sufficient to alleviate craving and withdrawal symptoms. Findings of the current study concur with others who found significant differences in plasma nicotine levels from tobacco cigarettes smoking versus e-cigarettes use, but no differences between groups in reduction in '*desire to smoke*' (Fearon et al., 2017); this suggests that low nicotine concentration levels may be sufficient for the relief of craving and withdrawal discomfort, nonetheless, with the caveat of consuming a much greater amount of liquid and exerting a more intensive puffing regime. In contrast, previous studies found craving alleviation to be associated with more intensive puffing patterns (that is greater puff numbers and volume consumed) and the use of higher nicotine concentrations and are in turn linked

to achieving higher satisfaction (Etter, 2015). Likewise, early studies also report greater relief of '*urge to smoke*' in e-cigarettes which contain nicotine compared with placebo e-cigarettes (Dawkins et al., 2012). Others found that higher plasma nicotine levels were associated with greater reduction in craving relief and higher '*satisfaction'* and '*throat hit*' (Farsalinos et al., 2014). However, the small sample size could not be dismissed as an alternative explanation for the lack of differences between conditions, since the study was powered to detect an effect on puffing topography variables but not on subjective effects.

There were no significant differences in subjective effects, although both, means in hit and satisfaction were numerically higher in the high compared with the low condition and in the high. These results reiterate that although self-titration was incomplete with significantly lower plasma levels in the low condition, compensatory puffing was sufficient to achieve subjective satisfaction at least in the short term. In fact, differences could have emerged from a longer ad libitum session or a larger sample. Likewise, there were no statistically significant differences in adverse effects and the highest means tended to be in the high nicotine condition however.

Limitations

This study is one of the first (Farsalinos et al., 2015) to use an e-cigarette with a downloadable software to record puffing topography data, which, unlike the CReSS machine (Ross & Juliano, 2016; Spindle et al., 2015) is non-evasive and may have greater ecological validity. However, the device used here has its limitations, it does not measure puff velocity and volume and has a safety cut off point which prevent any presses of more than 10 seconds. That said, previous studies suggest that puff volume (Kosmider, Madej, Garwon, Sobczak, 2016) and velocity alone do not influence liquid evaporation (Talih et al., 2014) and, here, liquid consumption was measured using other

means. Moreover, the current study was conducted in a controlled laboratory environment which may not be a true reflection of real puffing behaviour (Robinson et al., 2015). For instance, previous studies suggest that smokers and e-cigarette users have a tendency to display more intensive puffing patterns in laboratory settings compared to when in their natural environment (June et al., 2012; Robinson et al., 2015). However, to postulate that the intensive puffing patterns and unusually high plasma nicotine levels could be an over-estimation of real puffing behaviour, may not hold true since the current study is concerned with changes in puffing patterns within individuals and the same controlled laboratory setting across both conditions was utilised. Similarly, in an attempt to hold variables constant and maintain experimental control, the e-cigarette device was set with fixed parameters, however this may not reflect real-life use of third generation e-cigarettes, specifically given the surge in the use of sub-ohm tanks (mounted with atomisers with less than one ohm resistance) which are typically used with very low nicotine concentrations. In fact, participants stated that in natural conditions they would alter device parameters (increase the voltage/wattage and reduce atomiser resistance) when using a lower nicotine concentration e-liquid. Thus, these findings cannot be generalised to other e-cigarette device types given the wide variability in puffing patterns across device types (Behar et al., 2015). Furthermore, to maximise the true effect of the ad libitum session, participants were not allowed to get accustomed to the eVic™, however, this may have been offset by the fact that all were experienced and regular e-cigarettes users accustomed to third generation devices. A further limitation may lie in the use of 6 and 24 mg/mL nicotine concentrations only which are at opposing extremes of the nicotine concentration spectrum. It is not clear whether the use of 18 and 24 mg/mL for example would have allowed participants to self-titrate fully and achieve matching plasma nicotine levels.

Finally, participants were mostly all Caucasian 91.67 % males; this does not reflect the wider population, given that studies suggest gender differences in nicotine dependence (Dawkins et al., 2012), in e-cigarette use (Jorenby, Smith, Fiore, & Baker, 2017) and ethnic variations in nicotine disposition kinetics (Benowitz, Hukkanen, & Jacob III, 2009).

Concluding remarks

Findings here suggest that, similar to smokers, experienced e-cigarette users have a tendency to self-titrate by altering their puffing patterns when switching to a lower nicotine concentration liquid. Similarly to previous tobacco cigarette studies which suggest that upwards regulation is more commonly achieved compared to downwards regulation, titration was not complete; despite significantly lower puff number and duration, plasma nicotine levels were significantly higher in the high nicotine concentration condition. However, it is unclear whether a more prolonged ad libitum session would have led to equal plasma nicotine levels; future work may focus on more naturalistic settings to obtain more realistic puffing topography data and biomarkers.

There were no differences in subjective craving, withdrawal, satisfaction and other positive effects, which infers that participants were able to obtain satisfactory plasma nicotine levels at least subjectively. This suggests that matching plasma nicotine levels and indeed very high nicotine content liquids may not be an absolute necessity for alleviation of craving or withdrawal at least for experienced vapers in acute conditions in the laboratory. However, the findings suggest that when given lower nicotine concentrations, vapers would (consciously or subconsciously) exert compensatory puffing behaviours which carry greater financial expense (consuming a greater quantity of e-liquid) and potential health risks. Whilst studies suggest that e-cigarette emissions

carry only 5 % of the health risks of tobacco cigarettes (Mcneill et al., 2015), more intensive puffing pattern as documented here, may lead to increased toxicant exposure and adverse long term health effects (Kosmider, Kimber, Kurek, Corcoran & Dawkins, 2017).

Findings of the current study also suggests that under certain conditions, extremely high plasma nicotine levels can be achieved; this is indicative of a remarkable improvement in e-cigarette nicotine delivery. Thanks to the rapid advancement in technology, recent findings suggest that the shape of pharmacokinetic curve of the nicotine delivery of e-cigarettes can match and indeed exceed that of combustible cigarettes. This is encouraging given the importance of nicotine delivery in the maintenance of tobacco use and the ability of alternative products such as e-cigarettes to rival tobacco cigarettes.

Summary of Chapter II

Whilst previous studies have used standardised puffing protocols either exclusively or preceding an ad lib session, here it is argued that the use of prescribed puffing regimes do not reflect real-life device use and will affect nicotine delivery and absorption. Thus, although they are suggestive of compensatory puffing behaviours, they do not provide clear evidence of titration. This study is the first to directly explore self-titration by allowing experienced e-cigarette users, customarily using high nicotine concentration e-liquid, to use an e-cigarette device ad libitum in the lab. In fact, the findings provide direct empirical evidence of a clear attempt to self-titrate; when given a lower nicotine concentration, users increased their puff frequency and duration and consumed more e-liquid. As with tobacco smoking, compensation was partially effective; users reported equivalent reduction in craving and withdrawal symptoms

between conditions although blood nicotine levels remained significantly higher in the high nicotine condition. Indeed, the current study shows that experienced users using third generation e-cigarettes and a 24 mg/mL nicotine concentration e-liquid can achieve high blood nicotine levels very quickly, matching and even exceeding those reported in tobacco smokers. Such patterns of nicotine delivery, approximating those achieved in cigarette smoking may bolster cessation rates, but may raise concerns over nicotine dependence for some.

When considering the phenomenon of self-titration or nicotine regulation there is a need for longitudinal studies. Furthering the understanding of how smokers adapt their smoking and puffing behaviours in response to nicotine availability certainly merit attention. Study 3 (in the following chapter) is not investigating the compensation theory directly, rather is exploring the self-titration theory through the lens of inter-intra variability of puffing topography in response to varying nicotine concentration levels in e-cigarettes and, to what extent if any smokers alter their puffing topography over time to adapt to nicotine delivery.

CHAPTER III

"CIGALIKES VERSUS TANK SYSTEMS: EFFECTS OF PUFFING TOPOGRAPHY, DEVICE CHARACTERISTICS AND NICOTINE CONCENTRATION DURING THE EARLY STAGE OF A QUIT ATTEMPT"

Abstract

Background: Although the previous study demonstrated evidence of self-titration through compensatory puffing, this was in experienced users and only over a short duration in the lab. There is also a need to understand how smokers adapt their smoking and puffing behaviours in response to nicotine availability and different e-cigarette models over time as this may have implications for e-cigarette satisfaction, acceptance and ultimately cessation. This study aimed to measure changes in puffing topography over 2 weeks in naïve e-cigarettes smokers and subsequent cigarette reduction, comparing cigalikes and tanks and low versus high nicotine concentrations.

Methods: E-cigarette naïve smokers, (N = 70; 62.9% female) were randomly allocated to 1 of 3 conditions, cigalike (18mg/mL), tank-high (18mg/mL) or tank-low (6mg/mL). Participants were given the e-cigarette to take home and recorded cigarettes smoked per day (CPD) and subjective symptoms in a diary during the 2-week period. In three additional lab sessions, they used the e-cigarette ad lib for 20 min, at baseline, one week later (Time 1) and 2 weeks later (Time 2). Puff duration, puff number and inter-puff intervals (IPI) were recorded along with exhaled carbon monoxide (CO), cigarette dependence, craving and withdrawal (pre- and post- e-cigarette use), and subjective effects at each session.

Results: Puff duration increased significantly between Baseline and Time 2, whilst puff numbers and IPI decreased. Cigalikes were associated with longer puff duration, shorter IPI and greater number of puffs; the use of tank-high led to longer IPI and shorter puff duration. The tank-high and cigalikes were more efficient in reducing craving compared to the tank-low at baseline. Participants rated the tanks (both high & low) as more satisfying at baseline and Time 1 compared with the cigalike. CPD, CO and nicotine dependence reduced significantly, but did not differ between conditions

Conclusion: Smokers increased their puff duration over time, presumably as they learned puff-technique in order to obtain satisfactory blood nicotine levels in line with the self-titration theory. Whilst higher nicotine concentrations were more effective in reducing craving, tanks were associated with higher satisfaction. Although nicotine concentration did not have a direct effect on puffing topography, the longer puff duration and puff number associated with the cigalike suggest nicotine delivery is a primary determinant of puffing topography. E-cigarettes helped reduce tobacco smoking in the initial weeks of a quit attempt regardless of the device and, the effects on long term smoking reduction and abstinence are reported in Chapter IV.

Introduction

Differences in device types

A key element of nicotine delivery is the proficiency of the device used, and as devices have evolved, so has nicotine delivery improved (D'Ruiz, Graff, Yan, Sherwin Yan, & Yan, 2015; Farsalinos et al., 2014; Farsalinos, Spyrou, Tsimopoulou, et al., 2015; Hajek, Przulj, Phillips, Anderson, & McRobbie, 2017; Lee, Gawron, & Goniewicz, 2015; Lopez et al., 2016; Wagener et al., 2016).

Long term users can also achieve 50% higher blood nicotine levels compared with naïve users (Farsalinos et al., 2015) coupled with significant craving alleviation (Etter, 2015; Fearon et al., 2017). Thus, there are marked differences in device efficacy to deliver nicotine and their ability to induce a satisfactory hit (Baweja et al., 2016) and alleviate craving and withdrawal symptoms (Farsalinos et al., 2014; Hajek et al., 2017; Yingst et al., 2015). This indicates that the type of e-cigarette plays a significant role in nicotine delivery and subjective effects, and, variations in efficiency are also contingent upon the manner in which the device is used (that is puffing patterns).

In England, e-cigarettes have enhanced cessation rates by an estimated 2.5% (22,000 of smokers) (West, Shahab, & Brown, 2016), however the likelihood of cessation does not seem to be associated with cigalikes but rather, with tank systems (Hitchman et al., 2015). In a survey of 1643 smokers, those using a tank device on a daily basis were more likely to quit than those using cigalikes or using any device types on a non-daily basis. Furthermore, although most smokers initiate use with a cigalike (at least prior to 2016), most successfully reduce their smoking to eventually quit only after transitioning to a more advanced later generation model to achieve '*a more satisfying hit*' (Yingst et al., 2015). Thus, it soon became evident that these initial reports of poor nicotine delivery were an attribute of cigalike models.

In the UK, in 2017, the number of regular e-cigarette users, that is former smokers who completely switched to solely using e-cigarettes, exceeded the proportion of smokers using e-cigarettes concurrently (hereafter referred to as dual users) (52% versus 45% respectively) (ASH, 2017). Since a reduction in smoking compared to complete cessation does not necessarily reduce mortality risks (Inoue-Choi et al., 2017), this is an encouraging trend and, equally further reiterates that e-cigarettes use can promote cessation. In contrast, in the US, where cigalikes has occupied a large proportion of the market share (Department of Health et al., 2016; Shiffman, Sembower, & Kim, 2018), the number of dual users is still greater than the proportion of former smokers who quit using an e-cigarette (54% versus 34% respectively) (Rodu, 2017). Though there may be several factors at play, the greater prevalence of cigalikes in the US compared to the UK may partly explain these opposite trends. It is worth noting however that due to the fast evolving nature of this market, the figures reported herein are likely to fluctuate thus become rapidly outdated.

In 2017, the Eurobarometer found that of the 15% of European smokers who declared trying an e-cigarette, 9% did so only once or twice, whilst 4% who used them regularly in the past had discontinued their use (Eurobarometer, 2017). That smokers try an e-cigarette once or twice or even regularly before discontinuing use suggests dissatisfaction of the product. This is alluded to in the recent ASH Fact sheet in which dissatisfaction, more explicitly that 'the device did not feel like smoking' or that 'it did not help dealing with craving for smoking', was reported as an important driver for discontinuation of use (ASH, 2017). Although, these data do not provide information that enables one to attribute device type to discontinuation, this highlights the importance for all e-cigarette devices to be efficient in delivering nicotine, reduce craving for tobacco cigarettes and successfully replace smoking. The implications for

these continuing smokers are that first negative encounters with e-cigarettes may deter future cessation attempts using an e-cigarette. Consequently, that some smokers who succeed in their cessation attempts with the aid of e-cigarettes whilst others fail, certainly warrants further investigation to assist this population.

Importantly, the visual appearance of the device may have a role to play in enhancing their appeal and acceptability to smokers. In a previous study, it was found that when offered a choice of cigalike or tank model, smokers were equally likely to choose either (50% opted for cigalike; 50% tank-like) but following use of the device, tank models were associated with greater satisfaction compared to the cigalikes (Dawkins, Kimber, Puwanesarasa, & Soar, 2015). Qualitative data from the same study included reports from smokers that it is important that the device '*feels and looks like smoking*'; this highlights the high importance of the behavioural aspects of smoking addiction. On the other hand, there are reports that the complexity associated with tank systems may be a deterrent and impedes acceptability of e-cigarettes (Mckeganey & Dickson, 2017), hampering smoking cessation. Qualitative studies suggest that the bulky and oversize appearance combined with the complex technology of the tank-models may be a barrier for some users (Wadsworth, Neale, Mcneill, & Hitchman, 2016). This therefore provides a strong rationale for smaller cigarette-type devices such as cigalikes to remain on the market as well a large variety of devices to reflect the heterogeneity of preferences from different users. Given the appeal for their close resemblance to conventional cigarettes for some smokers, investigating whether cigalike models can be well received is therefore important.

User's Experience: the Effect of Practise

The efficacy of obtaining satisfactory blood nicotine levels also depends upon the way the e-cigarette is used and studies have shown that both are likely to improve

with practise (Hajek et al., 2015). Although the nicotine delivery from e-cigarettes appears less effective compared to combustible cigarettes, at least for some models, it is becoming increasingly evident that this could be reversed with practice; by adjusting puffing patterns (for example, increasing the duration and intensity of each puff), e-cigarette users can maintain constant and satisfactory blood nicotine levels, withdrawal and craving alleviation (Dawkins & Corcoran, 2014; Dawkins, Turner, Hasna, & Soar, 2012; Etter & Bullen, 2011). This is in line with later studies which found that regular use and practice can significantly increase blood nicotine levels (Hajek et al., 2015). This increase in nicotine intake may be explained by a need to compensate for the less effective nicotine delivery of e-cigarettes which is characterised by a greater draw resistance in comparison with combustible cigarettes.

Puffing topography (such as puff duration, volume and frequency) therefore, plays a large role in the effectiveness of nicotine delivery. Experienced users typically take longer puffs and exhale larger volume of aerosols (Hua, Yip, & Talbot, 2011) compared with smokers (inexperienced e-cigarette users) using tobacco cigarettes or e-cigarettes (Farsalinos et al., 2013; Talih et al., 2014). Smokers however, tend to adjust their puffing patterns within a week of adopting e-cigarettes, chiefly by increasing their puff duration and adjusting each puff to a slower pace which in turn, results in a decrease in puff flow rate (Lee et al., 2015). Others found that experienced e-cigarette users' inhalation time is shorter compared to that of smokers when smoking combustible cigarettes (Farsalinos et al., 2013). In a study comparing experienced users' puffing topography versus smokers' smoking and vaping topography, experienced users' puffing duration were two-fold higher (M = 4.2, SD = 0.7 s) compared with smokers smoking a tobacco cigarette (M = 2.1, SD = 0.4 s) and smokers using an e-cigarette (M = 2.3, SD = 0.5 s). These results are in good agreement with

online video data (Hua, Yip, & Talbot, 2011) and accords with the hypothesis that there is a '*learning curve'* involved in vaping (McQueen et al., 2011), which suggests that the required adjustment in puff duration is largely dictated by the need to compensate for the lesser effective nicotine delivery or more demanding suction mechanism.

The importance of nicotine concentrations in the e-liquid: a key factor in nicotine delivery

Another factor influencing how e-cigarette use is likely to be effective and accepted as a substitute to smoking is the nicotine concentration in the e-liquid. Although e-cigarette users tend to gradually reduce the nicotine concentration in their e-liquids over time (Farsalinos et al., 2014), higher concentrations of nicotine may be important in the initial stages of transitioning from smoking to vaping. In a study of 111 experienced e-cigarette users, 42% using nicotine concentration liquids exceeding 15 mg/mL, and 16.2% reported that they had to increase the initial nicotine levels used in order to achieve complete smoking abstinence (Farsalinos, Romagna, Tsiapras, Kyrzopoulos, & Voudris, 2013). There is also evidence that higher nicotine concentrations in alternative nicotine delivery products (ANDS) and NRT can increase smoking cessation (Tunnesen et al., 1999).

Likewise, pharmacokinetic studies suggest that greater craving and withdrawal relief are associated with high-powered tank systems, more intensive puffing patterns and higher nicotine concentration e-liquids (Etter, 2015). In a cross-sectional survey of 374 regular users, greater craving relief were associated with the use of more advanced models and higher voltages, as well as higher nicotine concentrations and greater puffing frequency (Etter, 2015). That higher nicotine concentrations are associated with greater craving relief is of some importance given that the second cause of discontinuation of the product was reported as the inability of the product to relieve craving (ASH, 2017).

Indeed, like nicotine yield in tobacco cigarettes (Hammond, Fong, Cummings, & Hyland, 2005; Russell, Epstein, & Dickson, 1983), e-liquid nicotine concentration is an important factor that is likely to influence puffing topography. Clinical studies, and Study 1 of this thesis, suggest that experienced e-cigarette users may adjust their puffing in response to nicotine concentrations in the e-liquid. However, whether naïve e-cigarette users can do so, is unclear.

Longitudinal studies suggest whilst long term e-cigarettes users gradually reduce their nicotine concentration over time (Farsalinos et al., 2013a; Farsalinos, Romagna, et al., 2014; Polosa et al., 2015), this tends to be associated with an increase in nicotine intake (Etter, 2016). Indeed, in a sample of 98 experienced users, although nicotine concentrations e-liquids decreased from 11 to 6 mg/mL after a period of 8 months, salivary cotinine levels increased from 252 ng/mL to 307 ng/mL at follow-up; of particular interest the author also report an increase in the volume of liquid consumed (Etter, 2016). This increase in nicotine intake may be explained by a need to compensate for the drop in nicotine concentrations from the e-liquid and to self-titrate via compensatory puffing in order to maintain a satisfactory and constant level of blood nicotine level. Similarly to smoking (Gritz et al., 1983), the number of puffs do not determine consumption, rather volume consumed is a better predictor in comparison and this is acknowledged by e-cigarette users (Baweja et al., 2016), suggesting that other components of puffing topography such as puff duration may be a better predictor of consumption.

Altogether, much evidence suggests users' experience, device characteristics and nicotine concentrations are likely factors influencing nicotine delivery and the way a device is used or puffed on (Talih et al., 2014). Given that nicotine is the primary reinforcer of smoking behaviour (Harvey et al., 2004), the implications are that e-

cigarette devices with poor nicotine delivery will preclude product acceptability, influence puffing behaviour and possibly put users at risk of relapsing to tobacco smoking. If e-cigarettes are to help in alleviating the huge burden of smoking prevalence, it is important to further explore their potential as smoking cessation aids. To date there is little evidence which helps understand the dynamic interplay of the factors and mechanisms which influence nicotine delivery positively or negatively and result in success at quitting smoking. Although previous studies (Dawkins & Corcoran, 2014; Dawkins, Kimber, Doig, Feyerabend, & Corcoran, 2016; Farsalinos et al., 2015) have documented the influence of puffing patterns and device characteristics on blood nicotine delivery (Farsalinos et al., 2014) and also suggest that puffing behaviours evolve over time (Hajek et al., 2017), how all these factors influence smoking cessation success has not been explored.

Although e-cigarettes address both the pharmacological and the psycho-behavioural aspects of smoking, for many smokers they are not as satisfying as smoking. Forty-five percent are dual users whilst 65% of smokers who have tried have discontinued use. Of these, 25% reported that they do not feel like smoking and 20% reported they did not help reduce craving for tobacco cigarettes (ASH, 2017). Altogether, the need to monitor, report and ensure efficiency across all device types is warranted.

Furthermore, as previously mentioned, there are key characteristics that are favourable to e-cigarette users (Baweja et al., 2016; Dawkins et al., 2015; McQueen et al., 2011; Yingst et al., 2015) and may help increase the appeal of these devices to existing smokers. The following characteristics, customisability, the ability to adjust the voltage and wattage, (Baweja et al., 2016; McQueen et al., 2011), more satisfying hit, battery capabilities and the affordability to greater e-liquid choice (Baweja et al.,

2016; Yingst et al., 2015) are associated with tank systems and are absent in cigalike models. These factors are important as they may further the understanding of dissatisfaction and product discontinuation or the need to transition to other models (Yingst et al., 2015); this can be informative to smokers who are yet to transition away from smoking.

There is increasing concerns that e-cigarettes may re-normalise smoking, preclude smoking cessation and reverse efforts made by tobacco control. Indeed, due to their close similarity to combustible cigarettes, these worries could be more justified in the case of cigalikes. Concerns that e-cigarettes may re-normalise smoking and act as a gateway to smoking have remained despite the evidence suggesting otherwise. Opponents fear that they may preclude smoking cessation and reverse tobacco control efforts. Specifically given the close similarity of the cigalike model to combustible cigarettes, these concerns could be more justified.

Although the evidence outlined above suggests that cigalikes may not be as effective in promoting cessation, they are clearly serving as an introductory product and facilitating the transitional phase to more advanced devices to subsequently increase the likelihood to quit. Thus, this next study will explore how puffing behaviours and nicotine delivery in naïve e-cigarette users (that is, smokers who have no or very little experience in e-cigarette use) change over time (as a user becomes more experienced in vaping) and vary according to device type and nicotine concentration. It will also explore satisfaction, craving and withdrawal symptoms alleviation, and other subjective effects. Study 3 (Chapter IV) will continue to follow up this cohort of smokers over a 6-month period to explore factors associated with smoking cessation.

Taking a more naturalistic approach (relative to Study 1; Chapter II), the aims of this current study are threefold, to explore: i) how naïve users' puffing behaviours a)

differ between devices (cigalike vs. tank), and b) change over time, c) differ according to nicotine concentration (high vs. low); ii) to explore smoking reduction over a two-week period, and the effects of device type and nicotine concentrations on smoking reduction and, iii) to explore a) satisfaction, craving and withdrawal symptoms alleviation and product acceptability.

A sample of 70 current smokers, willing to initiate a quit attempt were recruited. Participants were randomly allocated (using 'IBM SPSS Statistics 22' software) to either condition: 1) a cigalike, 2) a tank with low nicotine concentration (6 mg/mL; Tank 6), 3) a tank with high nicotine concentration (18 mg/mL; Tank 18). Participants were invited to attend 3 sessions: at baseline, week-1 and week-2. They were given the e-cigarette to keep along with refill cartridges/bottles at baseline and week-1 and told to substitute as many cigarettes as possible with the e-cigarette. Each participant was given a diary to record their daily e-cigarette use, cigarettes smoked per day (CPD) and subjective effects associated with the e-cigarette. Socio-demographic data, craving and information on cigarette dependence were collected. At the 2-week session participants were invited to keep the device and encouraged to continue use, substituting for as many cigarettes as they can in order to be followed up for Study 3.

Aims and Hypotheses

Aside higher nicotine concentrations, tank devices that allow power adjustment, have been found to be associated with more effective nicotine delivery compared to cigalikes (Farsalinos, Spyrou, Tsimopoulou, et al., 2014). Thus it follows that the use of tank devices combined with higher nicotine concentrations will be more likely to promote levels of satisfaction close to that achieved under tobacco smoking and alleviate cigarette craving and withdrawal symptoms more effectively compared to cigalikes.

Hypothesis 1 (H_1):

Puff duration will increase overtime from baseline to Time 2 across all conditions evidencing an attempt to self-titrate.

Hypothesis 2 (H_2):

Puffing topography will differ across conditions with a higher number of puffs, longer puff duration and shorter IPI in Tank 6 compared to Tank 18 and Cigalike in order to compensate for the lower nicotine concentration. Tank 18 will be associated with few and shorter puffs and longer IPI.

Hypothesis 3 (H_3):

CPD and Cigarette dependence will reduce overtime.

Hypothesis 4 (H_4):

The Tank 18 will be associated with greater reduction in craving and withdrawal symptoms and greater levels of satisfaction and other positive effects.

Methods

Design

The study received full ethical approval by the University ethics committee (UREC 1516-04_5th October 2015, see appendix 16). A mixed-participants design with the between-subject factor (cigalikes, tank 18 and tank 6) and the within-subject factors of time: baseline, one-week and two-week period was used (Figure 3.1). Participants were randomly allocated to one of the following conditions: 1) a cigarette-like model (referred to as cigalike thereafter) with a high nicotine concentration liquid housed in a

cartomiser, (18 mg/mL), each participant received a week supply of 15 cartridges; 2) a tank model with a high nicotine concentration of 18 mg/mL, and a supply of 20 mL of liquid per week; 3) a tank model with a low nicotine concentration (6 mg/ml), and a supply of 30 mL of liquid per week (see Figure 3.1).

Participants

Seventy (62.86% female) smokers (smoking 5 cigarettes or more per day for at least in the preceding year), not currently using an e-cigarette (i.e. have not used an e-cigarette more than 5 times in the past) and willing to make a quit attempt, were recruited between December 2015 to December 2016. Other inclusion criteria included aged 18 or over, fluent in English. Exclusion criteria were pregnant or lactating females, any known neurobiological or heart conditions. Participants were included regardless of their gender, ethnicity and SES (collected via questionnaires). Recruitment was conducted via advertisements on social media (Twitter and Facebook), leaflets distribution and posters on and outside the University of East London premises.

Questionnaires / Measures:

Puffing topography preliminary analysis

As the devices used in this study did not have inbuilt puff counters, puffing topography (number of puffs, puff duration and IPI) was measured by video-recording at each session then using a frame by frame analysis of 29.97 fps (frame per second) to analyse timing measurements (using the 'Adobe Premiere Pro CS5' software). Puff duration was demarcated as the time at which the e-cigarette was clearly placed in the mouth with both lips closed until the first frame capturing when the e-cigarette was removed from the mouth. IPI was demarcated as the time frame when the e-cigarette was removed from the mouth until the time at which the e-cigarette was repositioned in

the mouth with both lips closed. This method has high inter-rater-reliability as observed in the pilot study for Study 1.

Measures:

- CO measured before each ad libitum lab session to calculate smoking reduction from Baseline to Time 1 and Time 2.
- Puffing topography (puff number, duration and IPI) at Baseline, Time 1 and Time 2 (1 and 2 weeks after first use; measured via video-recordings during the 20-minute ad libitum puffing lab sessions).
- CPD (Self-reported cigarette per day) at Baseline, Time 1 and 2
- Volume consumed measured in mL
- Baseline socio-demographic questionnaire (appendix 13).
- Smoking history questionnaire (appendix 19) to collect information such as number of years of smoking, number of cigarette smoked per day, ever use of an e-cigarette.
- A single item from the Motivation To Stop Scale (MTSS) (Kotz, Brown, & West, 2013) to assess motivation to stop smoking, measured on a 7-point Likert scale. It contains items such as "I don't want to stop smoking" (coded as 1 the lowest possible score) and "I REALLY want to stop smoking and intend to in the next month" (coded as 7 the highest possible score) (appendix 19). The highest score reflects 'a strong desire and short term intention' to initiate a quit attempt whilst the lowest reflects the absence of any desire or intention to do so.
- A single item to measure self-confidence to be successful in a quit attempt (appendix 19).
- The Fagerström Test for Cigarette Dependence (Fagerström, 2012) (appendix 20).

- Craving reduction (urge to smoke) and Withdrawal symptoms relief at Baseline, Time 1 and 2. These were measured via the Mood and Physical Symptoms Scale (MPSS) questionnaire (West & Hajek, 2004) (See Chapter II, p. for further details, appendix 7).
- Subjective effects at Baseline, Time 1 and 2, positive such as satisfaction, hit and adverse effects rated on the VAS for positive and negative effects associated with nicotine use as described in Chapter II. Possible maximum scores are 100 (see appendix 8).
- A diary to record daily e-cigarette use, number of cigarettes smoked per day, craving and withdrawal symptoms related to tobacco cigarettes, other subjective effects associated with the use of nicotine (e.g. satisfaction, hit, pleasant, based on the VAS questionnaire (appendix 21). These dimensions were collected and measured at Week 1 and 2.
- Follow-up Interview questions for Study 3 (appendix 22)

Equipment:

A carbon Monoxide (CO) Bedfont piCO Smokerlyzer® was used to measure carbon monoxide. 'Salivette' kits were procured through ABS Labs to measure salivary cotinine. A Samsung video recorder was used to record each session. The Totally wicked mini curve tank system e-cigarette by Totally Wicked was mounted with a 2 mL capacity tank which housed a standard atomiser of 1.8 ohm resistance; the kit contained a charger and a manual instructions. Initially, the TECC 'Go e-cigarette' was used (N = 11), however due to repeated issues of leakages, this model was replaced by the BLU cigalike model device (N = 13). Participants in the two respective cigalike conditions were recorded and allocations were taken into account during data collection and entry. Repeated measures ANOVA revealed a statistically significant difference between the 'TECC' and 'Blu' cigalikes in CO reduction over time ($p = .027$). Whilst means

stagnated for the TECC cigalike model (M = 8.88, SD = 3.40 at baseline to M = 7.13, SD = 4.08 at 2-week follow up), those allocated the BLU cigalike device saw a decrease in CO from 21.25 at baseline (SD = 14.66) to 13.75 (SD = 12.06) at 2-week follow up. There were no further statistically significant difference for all the remaining measures. Thus primary analyses were conducted and presented with both cigalike models collapsed. Both cigalike kits contained a user manual, battery (main body) and charger. Tobacco flavour 'Go e-cig' Refill cartridges 18 mg/mL nicotine concentration and refill bottles tobacco flavour 'Totally Wicked Red Label' 18 and 6 mg/mL nicotine concentration were provided to the participants in the cigalike, tank-high and tank-low condition respectively. All e-cigarette products were purchased online between July 2015 and October 2016.

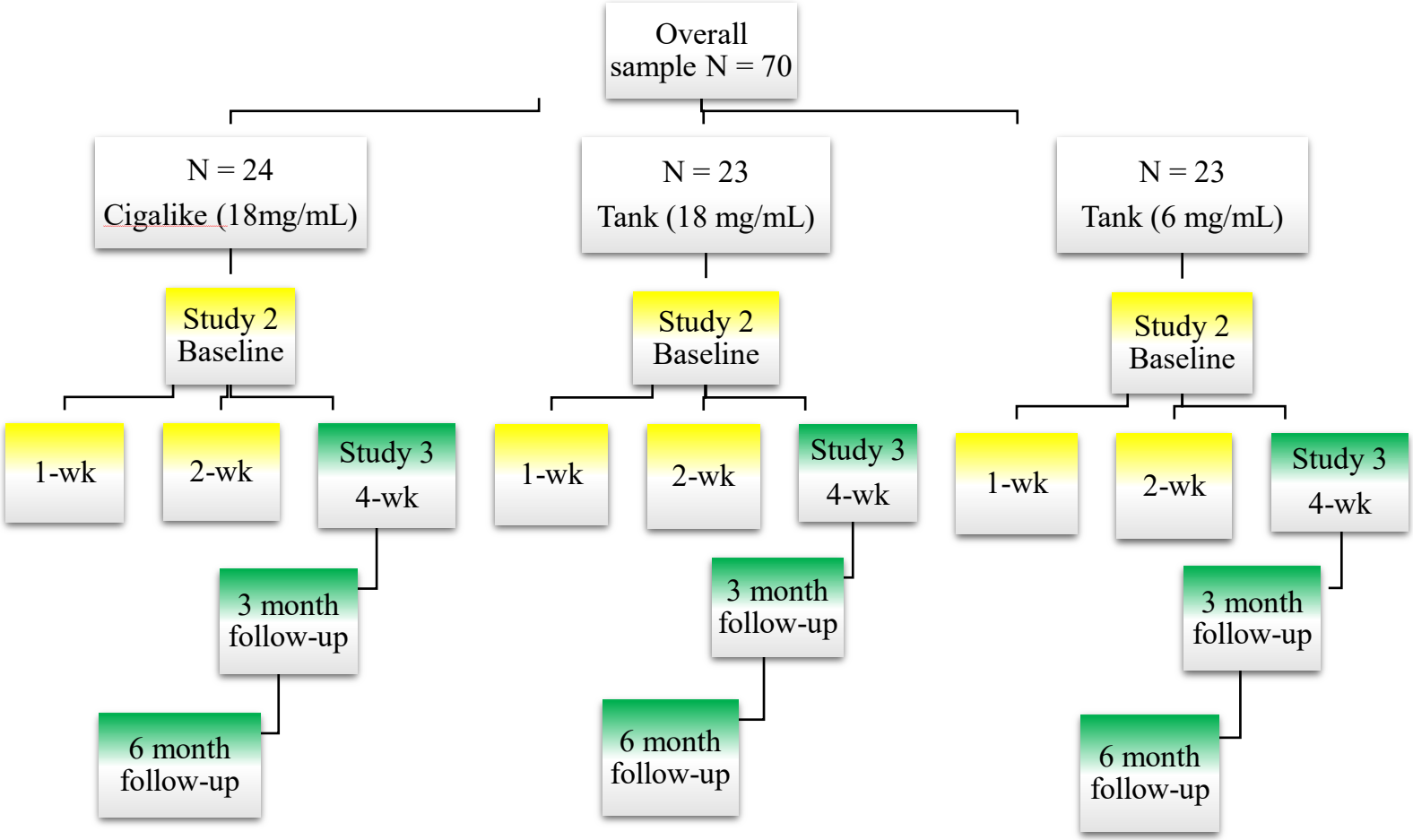


Figure 3.1. Outline of Study 2 (yellow gradient boxes) and Study 3 (green gradient boxes)

Procedure

Potential participants who responded to the advert via email or phone calls, were screened via phone interviews to ensure all inclusion criteria were met and an information sheet was provided via email. Participants were invited to attend baseline and 1 and 2-week individual testing sessions. Sessions followed a one hour abstinence period, so participants would not feel satiated before the start of the vaping session. Upon arrival, the researcher went through the information sheet (appendix 17) and ensured all aspects of the procedure were understood. Participants were asked to provide written informed consent (appendix 18), informed of their right to withdraw and invited to ask further questions. Salivary cotinine and exhaled CO tests were administered before collecting baseline, socio-demographic data and information about their nicotine/cigarette dependence. Participants were asked to place the test swab in their mouth for 5 minutes, then the fully saturated cotton swab was placed in a capped centrifugation tube and in an individually sealed plastic bag for storage in a -20ºC freezer. The MPSS was completed at the start and end of each session. Participants were allocated a condition, given the e-cigarette with instructions on how to use it and to vape *ad libitum* for 20 minutes. The entire vaping session was recorded. At the end of the first session, participants completed the subjective effects questionnaires. Lastly, participants were instructed to keep a record of their e-cigarette use and number of cigarettes smoked in the diary at the end of each day, given a supply of e-liquid bottles (20 mL) or cartridges (10 units) and asked to return all used and unused refills. The session was repeated one then two weeks later. At week-2 (Time 2), supply of e-liquid/cartridges ceased. Participants were asked to keep the device and encouraged to continue use; there was no other reward or motivational incentives. Participants were asked for their consent to be contacted via phone calls for further follow-up phone interviews at one, three and six months in order to provide information regarding their

smoking status and e-cigarette use (see Chapter IV). See Figure 3.2 for an outline of the procedure of each of the sessions.

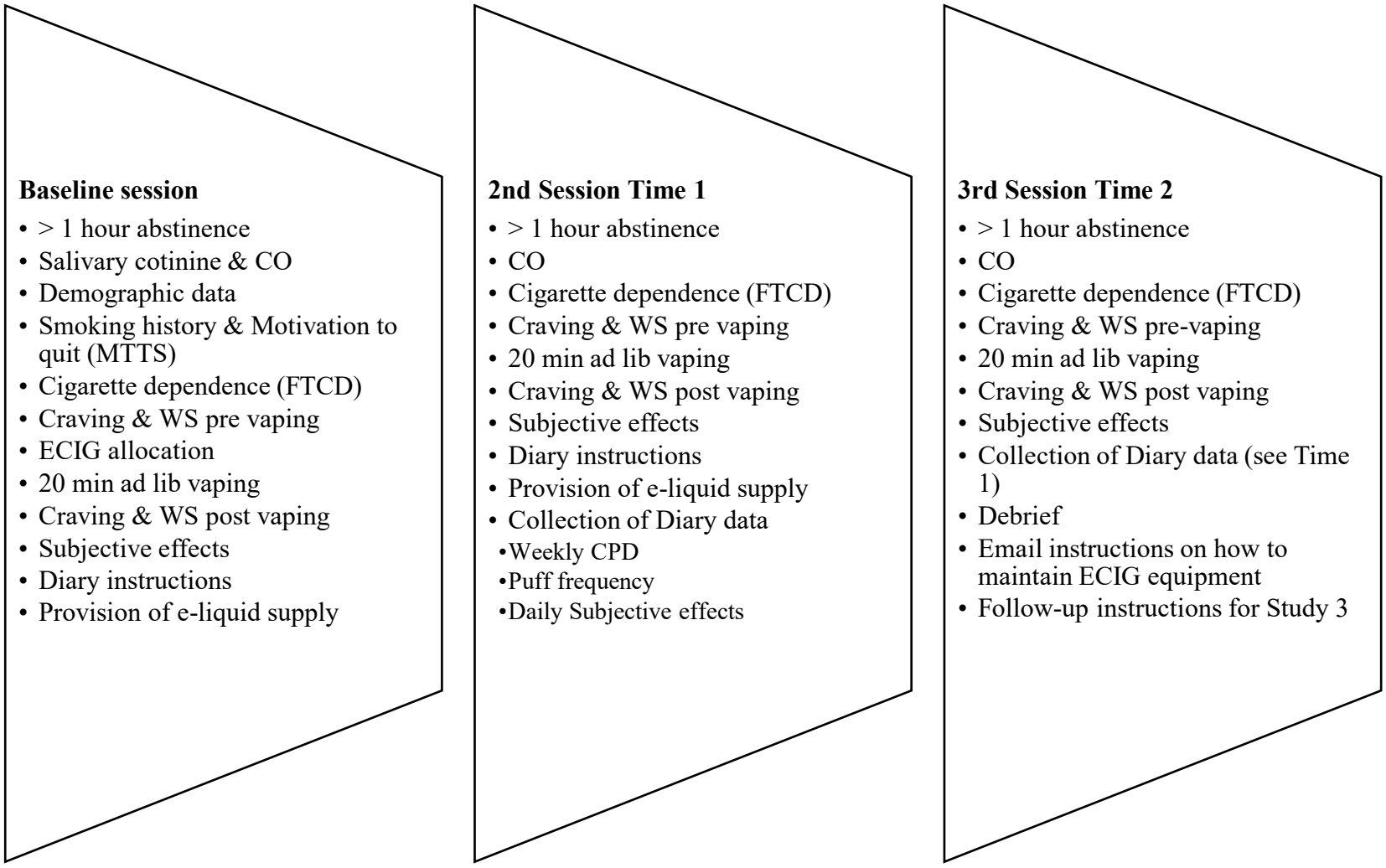


Figure 3.2 Diagram of Experimental procedure

Salivary cotinine analysis

Salivary cotinine is an accurate method that is highly correlated with blood cotinine levels (and can be detected in low range) for measuring active exposure to nicotine (Abrams, Follick, Biener, Carey, & Hitti, 1987; Benowitz, 1996; Etter, Due, & Perneger, 2000). More importantly, compared to blood sampling, it is less invasive and presents less difficulty in terms of collecting body fluids from a large sample of individuals.

All analyses were conducted by ABS labs and disposed of in accordance with the MHRA, GLP (Good Laboratory Practise) and GCP (Good Clinical Practise) accreditation protocols. Samples were frozen (-20ºC) and stored in UH006/7 at on site forensic laboratory. All samples were sent in appropriate containers to the Advanced Bioanalytical Service (ABS) Laboratories Ltd., Welwyn Garden City, UK, for the determination and quantitation of cotinine in saliva. All samples were disposed of in accordance with the MHRA, GLP (Good Laboratory Practise) and GCP (Good Clinical Practise) accreditation protocols. A certificate of destruction was issued and provided by ABS laboratories Ltd.

Statistical analyses

Changes in scores

Variables were computed to calculate reduction from pre- to post-use for craving, withdrawal symptoms, and cigarette dependence (Fagerström Test for Cigarette Dependence) overall mean scores at each time point. In instances where variables were not normally distributed, and assumptions were violated, non-parametric tests were used and medians, as opposed to means were reported. The number of outliers to remove was too great in relation to the sample size distribution for each of the group. Thus, where appropriate, variables were transformed using logarithm transformation to avoid affecting the size of the sample and minimise Type 1 error.

Unless otherwise specified, each variable was analysed using a mixed analysis of variance, with the between subjects factor as condition (Cigalike, Tank 18 and Tank 6) and the within-subjects factor of time (Baseline, Time 1 and Time 2). Given e-cigarette type is the variable of key interest, Pairwise comparisons for the main effect of condition is reported. Where a significant interaction effect was found, post hoc t-tests were conducted.

Results

Participants Characteristics

Table 3.1 displays the baseline characteristics for the whole sample (N = 70). Eleven of the initial 70 participants failed to attend the follow-up sessions and were therefore excluded from further analyses. Reported reasons for drop out included health, conflicting commitments, one participant wished not to vape during a second session due to sore throat, 3 participants wished to discontinue use of the device due to adverse effects (including sore throat and elevated depressed mood), and one did so following negative stories read in an online newspaper. Others wished to withdraw whilst consenting for their data not to be discarded, others gave no reasons. A flow diagram of participation is presented below (see Figure 3.3).

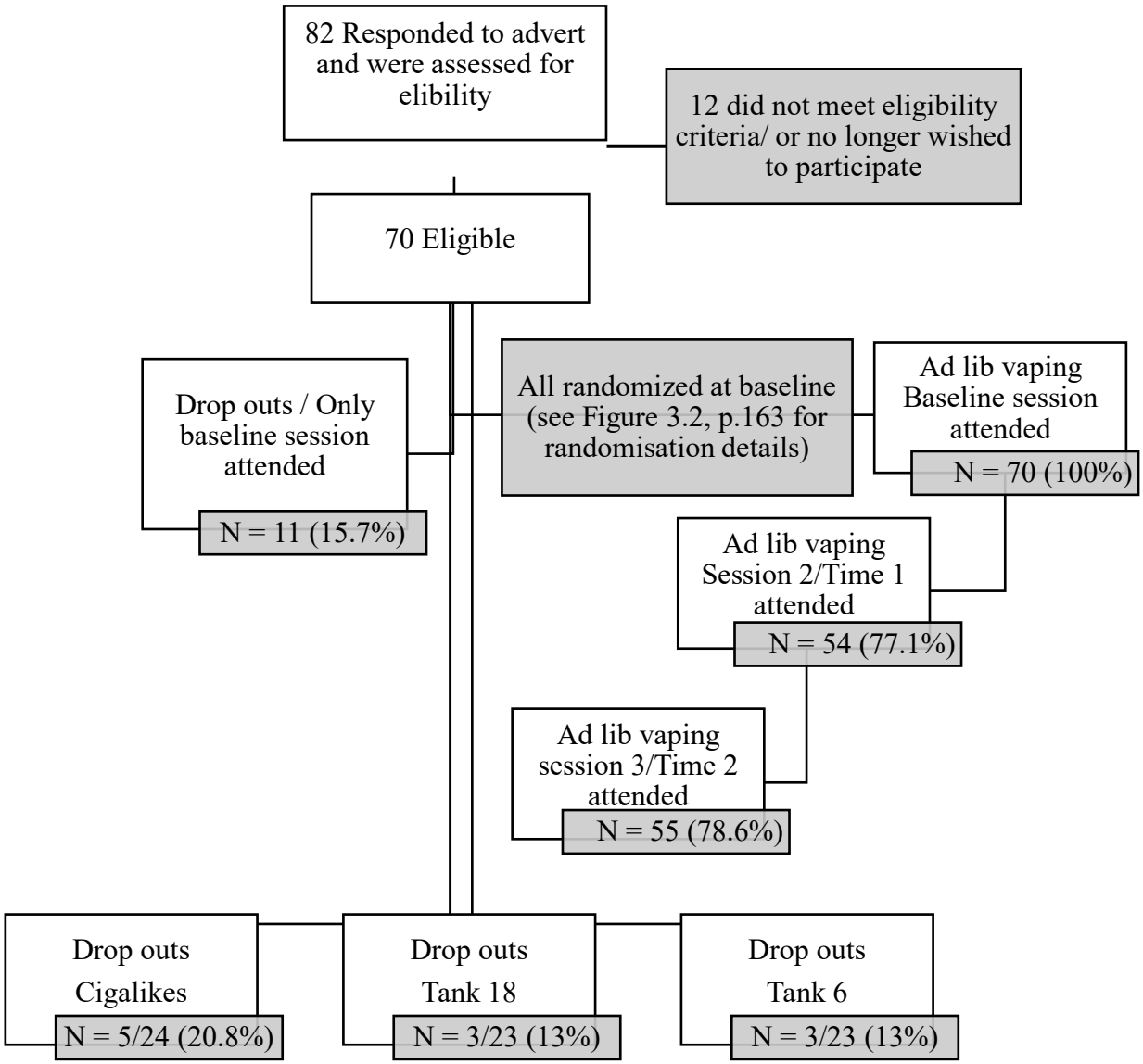


Figure 3.3 Flow chart of study completion. Flow chart representing the total of participants attending Session 2 and 3 differ as some participants were unable to attend the 2nd session but attended the baseline and 3rd session, whilst others attended the baseline and 2nd session only.

Table 3.1 Participants characteristics

	N	%	Mean	SD	Min	Max
Age (years)	70	-	29.47	9.19	18	60
Gender	70	-	-	-	-	-
Male	26	37.1	-	-	-	-
Female	44	62.9	-	-	-	-
Ethnicity	70	-	-	-	-	-
White/Caucasian	42	60	-	-	-	-
Black Afro-Caribbean	8	11.4	-	-	-	-
Mixed Race	4	5.7	-	-	-	-
Asian (Indian/Pakistani/Bangladeshi)	4	5.7	-	-	-	-
Nth/Sth East Asian (Chinese)	1	1.4	-	-	-	-
Arab-Nth African	1	1.4	-	-	-	-
Greek/Turkish/Cypriot/Kurd	2	2.9	-	-	-	-
Other	8	11.4	-	-	-	-
Qualification	70	-	-	-	-	-
None	1	1.4	-	-	-	-
GSCEs or equivalent	5	7.1	-	-	-	-
Vocational qualification (BTECs)	8	11.4	-	-	-	-
A-levels/O-levels or equivalent	24	34.3	-	-	-	-
Undergraduate level (5 to 6)	22	31.4	-	-	-	-
Postgraduate study (≥ level 7)	10	14.3	-	-	-	-
Occupational status	70	-	-	-	-	-
Employed	19	27.1	-	-	-	-
Non-employed	1	1.4	-	-	-	-
Self-employed	2	2.9	-	-	-	-
Studying	48	68.9	-	-	-	-
Tried an ecig before	70	-	-	-	-	-
Never used it	32	45.7	-	-	-	-
Fewer than 5 times	37	52.9	-	-	-	-
More than 10 times	1	1.4	-	-	-	-
Type of ecig tried before	36	-	-	-	-	-
Cigalikes	17	24.28	-	-	-	-
Later generations	13	18.57	-	-	-	-
Both	6	8.57	-	-	-	-
Past quit attempts	70	-	-	-	-	-
Yes	60	85.7	-	-	-	-
No	10	14.3	-	-	-	-
Numbers of quit attempts	70	-	2.41	2.07	0	10
Baseline cotinine (ng/mL)	70	-	140.33	84.84	4.70	438.20
Baseline CO (ppm)	70	-	14.89	9.60	2	42
Cigarettes smoked per day (CPD)	70	-	12.83	6.50	4	35
Smoking history (years)	70	-	12.31	9.16	2	44
[1] **FTCD**	70	-	4.23	2.37	0	9
[2] **MTSS**	70	-	4.76	1.53	2	7
[3] **Confidence in successful quit**	70	-	2.07	.97	0	4

[1] FTCD = Fagerström Test for Cigarette Dependence (score range: 0-10)
[2] MTSS = Motivation to Stop Scale (Kotz, Brown & West, 2013) (score range:1 – 7)
[3] Self-rated confidence in quitting (score range: 0 – 4)

Puffing topography

Ad libitum laboratory sessions

A mixed analysis of variance (ANOVA) was conducted to assess the variability between conditions and changes in puffing patterns across Baseline, Time 1 and 2. Data on the puff number and IPI variables were not normally distributed, and the assumptions of the analysis of variance were violated. Thus, variables were transformed using logarithm to avoid affecting the size of the sample and minimise Type 1 error as the number of outliers to remove was too great in relation to the sample size distribution for each of the group. All means (SD) for the puffing topography variables are displayed per conditions and session in Table 3.2.

Puff number

ANOVA revealed a significant main effect of condition $F(2,47) = 8.48$, $p = .001$, $\eta^2 = .27$; suggesting that the device type influenced participants' puff numbers. Post Hoc tests (Least Significant Difference LSD) revealed that overall across all 3 sessions, participants took a greater number of puffs in the Cigalike ($M = 39.51$, $SD = 20.93$, 95% CI [31.90, 47.12]) compared to participants in the Tank 18 ($M = 19.87$, $SD = 11.80$, 95% CI [14.22, 25.56]) ($p < .0001$) and those in the Tank 6 condition ($M = 22.97$, $SD = 11.31$, 95% CI [17.17, 28.76]) ($p = .003$). There was no statistically significant difference between the Tank 18 and Tank 6 conditions ($p = .28$).

There was a main effect of time, Wilks Lambda $F(2,46) = 5.13$, p = .010, $\eta^2 = .18$. Across all conditions, puff number was greater at Baseline ($M = 31.39$, $SE = 2.59$, 95% CI [26.18, 36.59]) compared to Time 1 ($M = 26.07$, $SE = 2.08$, 95% CI [21.89, 30.26]) ($p = .004$) and Time 2 ($M = 24.89$, $SE = 1.62$, 95% CI [21.63, 28.15]) ($p = .017$). This suggests that participants puffing frequency changed over time at least in the acute

laboratory session. However, puff number did not differ between Time 1 and 2 (p = .604). There was no significant interaction effect between time and condition on puff number, Wilks lambda $F(4,92) = 0.69$, $p = .59$, $\eta^2 = .03$.

Puff duration

Mauchly's test indicated that the assumption of sphericity had been violated for the main effect of time, χ^2 (2) = 6.87, p = .032. Therefore, Greenhouse-Geisser corrected values are reported and revealed a main effect of time $F(1.76, 82.55) = 5.91$, p = .006, $\eta^2 = .11$. Post Hoc tests (LSD) revealed that across all conditions, participants' puff duration increased from Baseline (M = 2.52, SD = 1.09, 95% CI [2.23, 2.88]) to Time 1 (M = 2.82, SD = 1.46, 95% CI [2.48, 3.34]) and to Time 2 (M = 2.88, SD = 1.39, 95% CI [2.64, 3.41]). These increases were statistically significant between Baseline and Time 1, and Baseline and Time 2 (p = .026 and p = .004 respectively) but not between Time 1 and 2 (p = .310).

There was no main effect of condition $F(2, 47) = 2.14$, $p = .129$, $\eta^2 = .083$. Although, mean puff duration in Cigalike were greater (Estimated Marginal Means; M = 3.36, SE = .35, 95% CI [2.66, 4.07]) compared with Tank 18 (M = 2.47, SE = .26, 95% CI [1.95, 3.00]) (p = .048). Cigalike did not differ from Tank 6 (p = .117) and the latter did not differ from Tank 18 (p = .626). There was no significant interaction effect between time and condition on puff duration, Wilks lambda $F(4,92) = 1.82$, $p = .133$, η^2 = .073.

IPI (Inter-puff intervals)

Using logarithm transformation for the IPI variable, ANOVA revealed a significant main effect of time, Wilks Lambda $F(2,46) = 6.172$, $p = .004$, $\eta^2 = .212$. IPI increased significantly from Baseline (M = .62, SD = .47, 95% CI [,]) to Time 1 (M = 1.03, SD = 16.22, 95% CI [,]) ($p < .001$) then significantly decreased at Time 2 (M =

.72, SD = .50, 95% CI [,] (p = .027). (Means (SD) with all 3 conditions collapsed). There was no statistically difference between Baseline and Time 2 on IPI (p = .166).

There was a main effect of condition, $F(2,47) = 10.68$, p < .0001, $\eta^2 = .31$. Post Hoc tests (LSD) revealed that across all 3 sessions, participants took shorter inter-puff intervals in the Cigalike (Estimated marginal means, M = .36, SE = .15, 95% CI [0.06, 0.66]) compared to those in the Tank 18 (M = 1.03, SE = .11, 95% CI [.81, 1.26]) (p < .0001) and those in the Tank 6 condition (M = .79, SE = .11, 95% CI [.57, 1.02]) (p < .001). There was no statistically significant difference between the Tank 18 and Tank 6 conditions (p = .208).

There was no significant interaction effect between time and condition on IPI, Wilks lambda $F(4,92) = 0.66$, p = .67, $\eta^2 = .025$.

Table 3.2
Means (SD) for Puff Number, Puff Duration and IPI per condition at baseline, Time 1 and 2

Session	Condition	Puff Number Mean	SD	Puff Duration Mean	SD	IPI Mean	SD	N
Baseline	Cigalike	46.09	27.53	2.77	1.07	.31	.18	11
	Tank 18	23.80	16.79	2.40	1.03	.80	.57	20
	Tank 6	24.26	9.72	2.49	1.20	.62	.37	19
	Total	28.88	19.60	2.51	1.09	.62	.47	50
Time 1	Cigalike	39.09	20.32	3.46	1.55	.40	.36	11
	Tank 18	17.65	11.98	2.57	1.46	1.38	1.15	20
	Tank 6	21.47	12.03	2.71	1.38	1.04	.84	19
	Total	23.82	16.22	2.82	1.46	1.03	.97	50
Time 2	Cigalike	33.36	14.94	3.86	1.53	.36	.30	11
	Tank 18	18.15	6.64	2.45	1.25	.92	.55	20
	Tank 6	23.16	12.18	2.77	1.22	.72	.45	19
	Total	23.40	12.27	2.88	1.39	.72	.50	50

Self-reported daily puff number at Week 1 and Week 2

Changes in weekly mean puff numbers were calculated by first computing the variables for the total number of self-reported puffs per day into weekly averages at

Week 1 (Time 1/Session 2) and Week 2 (Time 2/Session 3). Exploratory analysis including Q-Q plots and Kolmogorov-Smirnov revealed clear deviations from a normal distribution with outliers at Time 1 and unusual cases at Time 2. Removal of outliers and transformations of data did not rectify the distribution, therefore non-parametric tests were conducted to explore changes in self-reported daily puffing patterns and differences between conditions. Friedman's ANOVA revealed no statistically significant changes in scores over time ($p > .05$), daily puff number averaged at Week 1 ($M = 41.07$, $SD = 38.04$; $Md = 33.50$) and Week 2 ($M = 43.29$, $SD = 43.04$, $Md = 29.64$). Kruskal-Wallis tests indicated no statistically significant differences between conditions ($p > .05$).

Effects on Smoking reduction

Changes in cigarettes smoked per day (CPD) per condition

Table 3.3 displays Means (SE) in CPD for all time points are displayed in Figure 3.4. Mauchly's test indicated that the assumption of sphericity had been violated, therefore degrees of freedom were corrected using Greenhouse-Geisser estimates of sphericity ($\varepsilon = .67$ for the main effect of time). There was a main effect of time, $F(1.35, 52.49) = 66.39$, $p < .0001$, $\eta^2 = .63$, a large effect size suggesting that self-report CPD reduced over time. Pairwise comparison (LSD) tests revealed that CPD reduced from Baseline $M = 12.53$, $SE = 1.04$, 95% CI [10.44, 14.63] to Time 1 $M = 6.61$, $SE = .88$, 95% CI [4.83, 8.39] ($p < .0001$) and Time 2 $M = 5.82$, $SE = .81$, 95% CI [4.18, 7.46] ($p = .033$). Differences were also statistically significant between Baseline and Time 2 $p < .0001$. However, there was no main effect of condition $F(2,39) = .754$, $p = .477$, $\eta^2 = .037$. There was a significant interaction effect between time and conditions, Greenhouse-Geisser $F(2.69, 52.49) = 2.88$, $p = .05$, $\eta^2 = .129$. Means (SE) of CPD reduction were greater in the Tank 6 compared $M = 9.68$, $SE = 1.26$, 95% CI [7.12,

12.23] to the Tank 18 $M = 8.00$, $SE = 1.80$, 95% CI [5.37, 10.64] and the Cigalike $M = 7.28$, $SE = 1.74$, 95% CI [3.77, 10.79] ($p = .033$) (all $p < .05$). At Time 1, there were 2 self-reports of complete cessation (CO < 10 ppm verified) in the Tank 18, 1 in the Tank 6 and none in the Cigalike condition, these remained consistent at Time 2.

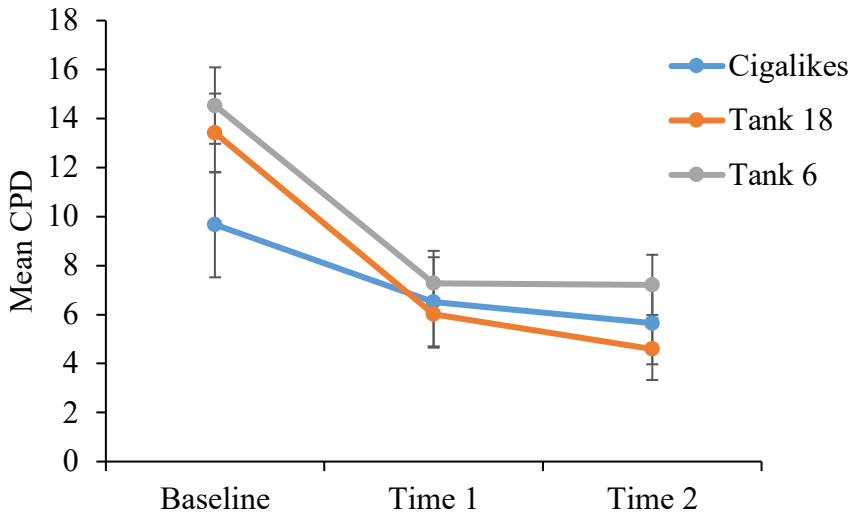


Figure 3.4. CPD per condition at Baseline, Time 1 and 2.

Changes in Carbon monoxide levels (CO) per conditions

Means (SD) per conditions and across all sessions are displayed in Table 3.3. A mixed ANOVA revealed a main effect of time. Participants' CO reduced steadily across all 3 sessions; from Baseline $M = 14.86$, $SE = 1.37$, 95% CI [12.11, 17.63] to Time 1 $M = 11.45$, $SE = 1.32$, 95% CI [8.79, 14.11] ($p < .001$) and Time 2 $M = 10.44$, $SE = 1.17$, 95% CI [8.10, 12.79] ($p < .0001$). The reduction between Time 1 and 2 was not statistically significant, $p = .226$. Wilks' Lambda indicates a main effect of time $F(2, 49) = 14.12$, $p < .0001$, $\eta^2 = .366$ suggesting that CO significantly reduced over time. However, there was no main effect of condition $F(2, 50) = .245$, $p = .783$, $\eta^2 = .010$. There was no significant interaction effect Wilks' Lambda, $F(4, 98) = .513$, $p = .726$, $\eta^2 = .021$.

Table 3.3.
Mean (SD) in CPD (cigarette per day) and CO (Carbon Monoxide) per conditions at Baseline, Time 1 and 2

		CPD			CO		
Session	Condition	Mean	SD	N	Mean	SD	N
Baseline	Cigalike	9.67	4.03	9	13	10.15	12
	Tank 18	13.41	5.25	16	15.90	9.58	21
	Tank 6	14.53	8.21	17	15.70	9.50	20
	Total	13.06	6.56	42	15.17	9.57	53
Time 1	Cigalike	6.52	4.00	9	10.83	11.28	12
	Tank 18	6.02	5.68	16	11.52	10.12	21
	Tank 6	7.29	5.88	17	12.00	6.91	20
	Total	6.64	5.36	42	11.55	9.16	53
Time 2	Cigalike	5.65	3.72	9	9.33	7.81	12
	Tank 18	4.59	5.30	16	10.10	9.13	21
	Tank 6	7.21	5.39	17	11.90	7.47	20
	Total	5.88	5.07	42	10.60	8.15	53

Craving reduction

All means (SD) are displayed in Table 3.4 and Means (SE) per condition are illustrated in Figure 3.5. Within each session, change scores were computed by subtracting craving scores post e-cigarette use from pre e-cigarette use (i.e. 1 hr abstinence scores). A series of one-way ANOVAs were conducted to assess craving reduction at each time point.

Within-session craving reduction at each time point

A One way ANOVA revealed a statistically significant difference in craving reduction from pre to post e-cigarette use at baseline [$F(2, 63) = 3.05$, $p = .05$] with a small effect size Cohen's d = .01. Post Hoc comparisons Tukey HSD tests suggest a statistically significant difference between Tank 18, $M = 2.17$, $SD = 1.77$ and Tank 6, $M = .95$, $SD = 1.63$, $p = .043$. Cigalikes did not differ from either conditions, $M = 1.60$, $SD = 1.43$. There were no statistically significant differences in craving reduction at Time 1 [$F(2, 51) = 0.571$, $p = .568$, $\eta^2 = .022$], nor were there any statistically significant differences in craving reduction at Time 2 [$F(2, 53) = 1.422$, $p = .250$, $\eta^2 = .051$].

Craving reduction across all sessions

Mauchly's test indicated that the assumption of sphericity had been violated, therefore degrees of freedom were corrected using Greenhouse-Geisser estimates of sphericity (ε = .89 for the main effect of time). A mixed ANOVA revealed no main effect of time, $F(1.77, 85.02) = 0.204$, $p = .790$, $\eta^2 = .004$ suggesting that craving reduction did not differ across all 3 sessions. There was no main effect of condition $F(2, 48) = 2.259$, $p = .115$, $\eta^2 = .086$, although Pairwise comparisons (LSD) indicated that Means (SD) in Tank 18 was significantly greater, $M = 2.17$, $SE = .28$, 95% CI [1.61, 2.72] compared to Tank 6, $M = 1.33$, $SE = .28$, 95% CI [.76, 1.90] ($p = .041$). This difference was less pronounced and not statistically significant ($p > .05$) in comparison with the Cigalike condition $M = 1.37$, $SE = .45$, 95% CI [.92, 2.36]. There was no significant interaction between Time and Condition, Greenhouse-Geisser, $F(3.54, 85.02) = .165$, $p = .942$, $\eta^2 = .007$.

Table 3.4.
Mean (SD) Craving reduction in the lab (pre vs. post e-cigarette use) by condition at Baseline, Time 1 and 2

	Conditions	Means	SD	N
Baseline	Cigalike	1.42	1.68	12
	Tank 18	2.20	1.88	20
	Tank 6	1.21	1.78	19
	Total	1.65	1.82	51
Time 1	Cigalike	1.83	1.70	12
	Tank 18	2.05	1.90	20
	Tank 6	1.37	1.34	19
	Total	11.55	9.16	51
Time 2	Cigalike	1.67	1.37	12
	Tank 18	2.25	1.86	20
	Tank 6	1.42	1.39	19
	Total	1.80	1.60	51

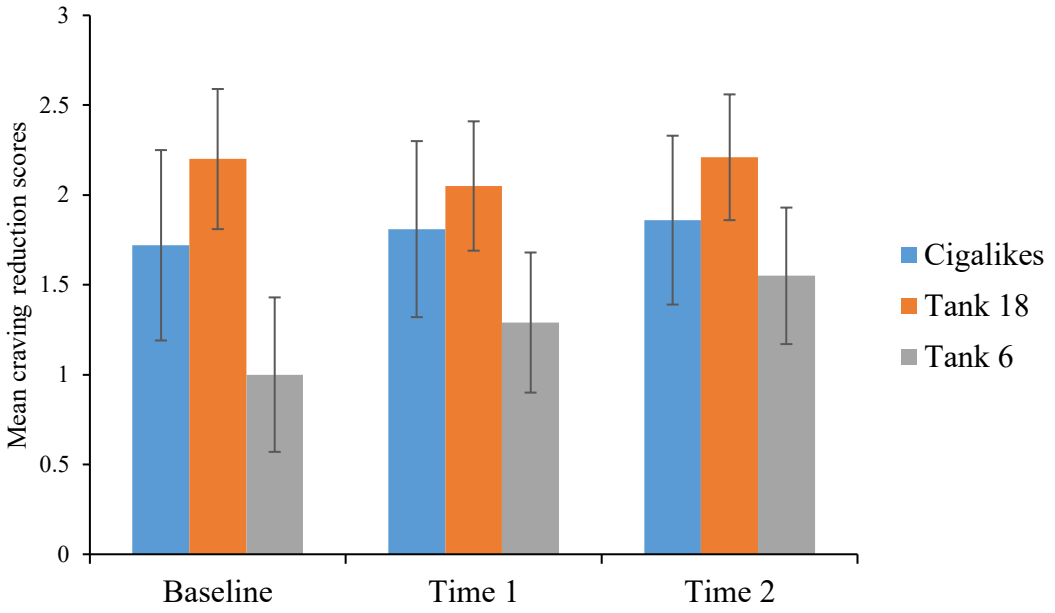


Figure 3.5. Mean (SE) in Craving reduction per condition at Baseline, Time 1 and 2 (i.e. pre e-cig use score minus post e-cig use score).

Withdrawal symptoms

As for craving, change scores were computed by subtracting withdrawal scores post e-cigarette use from pre e-cigarette use (i.e. following 1 hr abstinence).

Within-session withdrawal symptoms at each time point

A One way between-groups analysis of variance revealed no statistically significant differences in overall withdrawal symptoms reduction at baseline [$F(2, 67) = 1.786$, $p = .176$, $\eta^2 = .051$], Time 1 [$F(2, 51) = 1.414$, $p = .253$, $\eta^2 = .053$] or Time 2 [$F(2, 53) = 0.956$, $p = .391$, $\eta^2 = .035$].

Withdrawal symptoms across all sessions

Exploratory analysis including Q-Q plots and Kolmogorov-Smirnov indicated a non-normal distribution. The assumptions of analysis of variance were violated even following the removal of outliers, therefore non-parametric tests were conducted. Friedman test revealed a statistically significant difference across time χ^2 (2, n = 51) = 19.348, $p < .0001$. Medians increased from *Md* = -1.00 at Baseline to *Md* = 1.00 at

Time 1 and *Md* = 0 at Time 2, suggesting that regardless of the device type, withdrawal states following use of the e-cigarette improved with time. Kruskal-Wallis revealed no statistically significant differences between conditions at Baseline χ^2 (2, N = 70) = 4.92, p = .085, Time 1 χ^2 (2, N = 54) = 2.36, p = .307 and Time 2 χ^2 (2, N = 56) =.89, p = .640 (see Table 3.5).

Table 3.5
Means (SE) Withdrawal symptoms across time with all conditions collapsed

Time	Means	SD	Md	N
Baseline	-.60	2.31	-1.0	70
Time 1	.89	2.38	1	54
Time 2	1.04	2.69	0.0	56

Changes in Cigarette dependence at all time points per condition

Mauchly's test indicated that the assumption of sphericity had been violated, therefore degrees of freedom were corrected using Greenhouse-Geisser estimates of sphericity (ε = .85 for the main effect of time). A mixed between-within analysis of variance revealed a main effect of Time, F(1.71, 83.71) = 13.72, p = .0001, η^2 = .219, suggesting that cigarette dependence decreased over time. Means (SD) decreased from Baseline M = 4.11, SE = .35, 95% CI [3.41, 4.80] to Time 1 M = 3.26, SE = .35, 95% CI [2.56, 3.96] (p < .0001) and Time 1 to Time 2 M = 2.83, SE = .34, 95% CI [2.16, 3.51] (p < .0001). The decrease between Time 1 and 2 was not statistically significant (p = .089).

There was no main effect of condition F(2, 49) = .66, p = .52, , η^2 = .026 or interaction between time and condition (p > .05).

Effects on 'time to first cigarette'

Levene's test revealed that the variance across groups were unequal at Time 2, therefore non-parametric Friedman test was conducted and revealed a statistically significant effect of Time χ^2 (2, n = 52) = 21.432, p < .0001. Means (SD) decreased

from Baseline M = 1.65, SD = .95, Md = 2.00 to Time 1 M = 1.33, SD = 1.06, Md = 2.00 and Time 2 M = 1.17, SD = .99, Md = 1.00, suggesting that the latency of the first cigarette of the day delayed to a later time in the day.

Subjective effects

Positive effects following e-cigarette use in the lab

Means (SD) for all positive effects are tabulated individually (Table 3.6, appendix 23). Mixed ANOVAs were conducted for the overall mean scores on Hit and Satisfaction as per commonly reported in the literature (Cox et al., 2016; Dawkins et al., 2016, 2015; Rose, Behm, et al., 1999; Yingst et al., 2015).

For the overall scores on all positive effects collectively, there was a main effect of Time $F(2, 94) = 10.159$, $p < .0001$, $\eta^2 = .178$. Following e-cigarette use across all 3 sessions, overall scores (all conditions collapsed) on positive effects reduced from M = 47.27, SE = 2.28, 95% CI [42.69, 51.85] at Baseline to M = 42.01, SE = 2.12, 95% CI [37.75, 46.27] at Time 1 then to M = 38.20, SD = 2.55, 95% CI [33.08, 43.32] at Time 2. Post Hoc tests using Pairwise comparisons Least Significant Difference revealed a statistically significant difference between Baseline and Time 1 (p = .015), Baseline and Time 2 ($p < .0001$) and Time 1 and 2 (p = .043). This suggests that overall scores on all positive effects altogether worsened across the 2 weeks post initiation of e-cigarette use. There was no main effect of Condition $F(2, 47) = .981$, p = .383, $\eta^2 = .040$ but there was a significant interaction between Time and Condition $F(4, 94) = 2.623$, p = .040, $\eta^2 = .100$ which demonstrated that the decrease in positive effects was largely driven by the cigalike condition (illustrated in Figure 3.6). There was no statistically significant main effect of Time or Conditions for each of the positive effects singly (all means are reported in Table 3.6; appendix 23).

Hit

There was a main effect of time $F(2, 94) = 4.134$, $p = .019$, $\eta^2 = .081$. Following e-cigarette use across all 3 sessions, overall Hit scores (all conditions collapsed) reduced from $M = 58.11$, $SE = 3.65$, 95% CI [50.76, 65.45] at Baseline to $M = 50.53$, $SE = 3.01$, 95% CI [44.47, 56.58] at Time 1 then to $M = 49.18$, $SE = 3.50$, 95% CI [42.15, 56.21] at Time 2. Post Hoc tests revealed a statistically significant difference between Baseline and Time 1 ($p = .048$) and Baseline and Time 2 ($p = .013$). There was no statistically significant difference between Time 1 and Time 2 ($p = .630$).

There was no main effect of condition $F(2, 47) = 1.938$, $p = .155$, $\eta^2 = .076$. There was no significant interaction between time and condition for the overall mean scores of positive effects $F(2, 47) = 2.749$, $p = .074$, $\eta^2 = .105$. This suggests that overall scores in Hit declined over time regardless of the device type used.

Satisfaction

Mauchly's test indicated that the assumption of sphericity had been violated, therefore degrees of freedom were corrected using Greenhouse-Geisser estimates of sphericity ($\varepsilon = .82$ for the main effect of time). There was a main effect of time $F(1.64, 77.25) = 6.24$, $p = .005$, $\eta^2 = .117$. Following e-cigarette use across all 3 sessions, overall scores on Satisfaction (all conditions collapsed) reduced from $M = 61.73$, $SD = 17.38$, 95% CI [55.42, 65.49] at Baseline to $M = 56.29$, $SD = 18.58$, 95% CI [49.23, 59.60] at Time 1 then to $M = 51.90$, $SD = 23.82$, 95% CI [42.64, 55.22] at Time 2. Post Hoc tests using multiple comparisons Least Significant Difference revealed a statistically significant difference between Baseline and Time 2 ($p = .004$) and Time 1 and Time 2 ($p = .033$). There was no statistically significant difference between Baseline and Time 1 ($p = .074$). This suggests that satisfaction worsened overall across the 2 weeks post initiation of e-cigarette use.

There was a main effect of condition $F(2,47) = 7.42$, $p = .002$, $\eta^2 = .240$. Following e-cigarette use across all 3 sessions, overall scores on Satisfaction were greater in the Tank 18, $M = 61.75$, $SE = 3.06$, 95% CI [55.60, 67.90] in comparison to Cigalikes $M = 42.77$, $SE = 4.12$, 95% CI [34.47, 51.06] and Tank 6, $M = 59.29$, $SE = 3.14$, 95% CI [52.98, 65.60]. Post Hoc tests using multiple comparisons Least Significant Difference revealed that the differences were statistically significant between Tank 18 and Cigalikes ($p < .001$) and Cigalikes and Tank 6 ($p = .003$). The difference between Tank 18 and Tank 6 was not statistically significant ($p = .577$). Together, the results suggest that satisfaction was greater following the use of the tank models compared to Cigalikes. There was no significant interaction between time and condition for the overall mean scores of positive effects $F(2, 47) = 1.60$, $p = .193$, $\eta^2 = .064$.

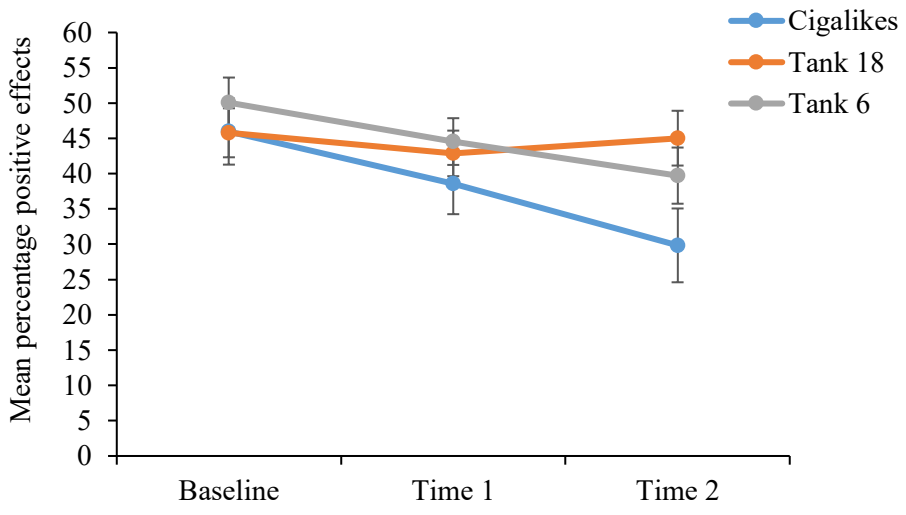


Figure 3.6. Means (SE) for overall scores on Positive effects per condition and Time

Self-report adverse effects

All means (SD) are reported for overall adverse effects in the appendices (Table 3.7, appendix 23). Mauchly's test indicated that the assumption of sphericity had been violated, therefore degrees of freedom were corrected using Greenhouse-Geisser estimates of sphericity (ε = .754 for the main effect of time). A mixed ANOVA revealed no main effect of Time for the overall adverse effects, $F(1.51, 70.92) = .875$, $p = .394$, $\eta^2 = .018$. There was no main effect of condition $F(2, 47) = .864$, $p = .428$, $\eta^2 = .035$ and no significant interaction between Time and Condition $F(3.02, 70.92) = .636$, $p = .595$, $\eta^2 = .026$.

For each of the individual adverse effects, Mixed ANOVAs were also conducted and revealed no main effect of Time or Condition and no significant interaction (all ps >.05).

Changes in subjective effects from Week 1 to Week 2 based on the diary data

Weekly averages of daily subjective effects were computed for week 1 (from baseline to day 7) and week 2 (day 7 to day 14) by averaging daily scores in a weekly total for Week 1 then this was repeated for Week 2.

Daily ratings of satisfaction

Mixed between-within ANOVA was conducted to measure changes in subjective effects from Week 1 to Week 2 for satisfaction. There was no main effect of Time $F(1, 34) = .249$, $p = .621$, $\eta^2 = .007$. There was a near significant effect of Condition $F(2, 34) = 3.242$, $p = .051$, $\eta^2 = .160$. Following e-cigarette use after a week, self-rated scores on satisfaction differed but failed to reach statistical significance between conditions. Means were higher in Tank 18 $M = 51.65$, $SE = 5.44$, 95% CI [40.60, 62.70] compared to the Cigalikes $M = 33.15$, $SE = 6.01$, 95% CI [20.93, 45.37] ($p = .029$). Equally, participants in the Tank 6 condition rated the device as more

satisfying $M = 50.05$, $SE = 4.38$, 95% CI [41.16, 58.94] compared to the Cigalikes (p = .029). The difference between Tank 18 and Tank 6 was not statistically significant (p = .829) (see Figure 3.7). There was no significant Time X Conditions interaction $F(2, 34) = 2.373$, $p = .108$, $\eta^2 = .122$.

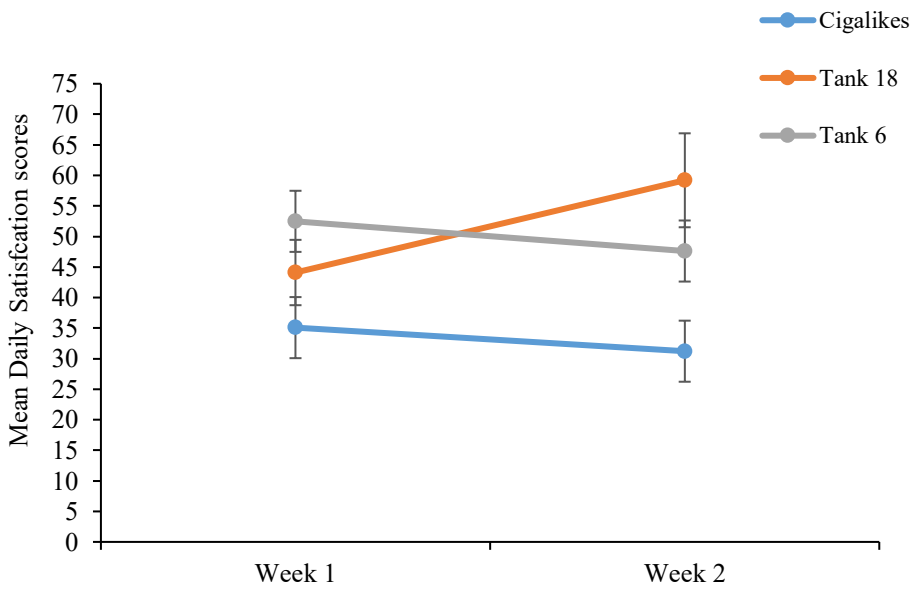


Figure 3.7. Means (SE) in Daily ratings of Satisfaction per condition at Week 1 and 2

Daily ratings of hit

A Mixed ANOVA on 'hit' ratings, indicated no main effect of Time $F(1, 33)$ =.058, $p = .812$, $\eta^2 = .002$, no main effect of Condition $F(2, 33) = 1.574$, $p = .222$, $\eta^2 = .087$, nor interaction $F(2, 33) = 1.333$, $p = .277$, $\eta^2 = .075$.

Daily ratings of 'Reduced craving'

A Mixed ANOVA revealed no main effect of Time $F(1, 33) = .075$, $p = .786$, $\eta^2 = .002$. There was no significant Time X Condition interaction $F(2, 33) = .246$, $p = .783$, $\eta^2 = .015$. There was a main effect of Condition $F(2, 33) = 3.369$, $p = .047$, $\eta^2 = .170$. Post Hoc Tests Multiple Comparisons revealed that following e-cigarette use after a week, self-rated scores on 'reduced craving' differed between conditions. Scores in

Tank 18 were greater $M = 49.19$, $SE = 6.04$, 95% CI [36.91, 61.47] compared to the Cigalikes $M = 27.30$, $SE = 6.36$, 95% CI [14.36, 40.25] ($p = .018$). Equally, participants in the Tank 6 condition rated the Tank device higher $M = 43.36$, $SE = 4.63$, 95% CI [33.94, 52.78] compared to the Cigalikes ($p = .049$). The difference between Tank 18 and Tank 6 was not statistically significant ($p = .449$). Means (SE) are represented in Figure 3.8.

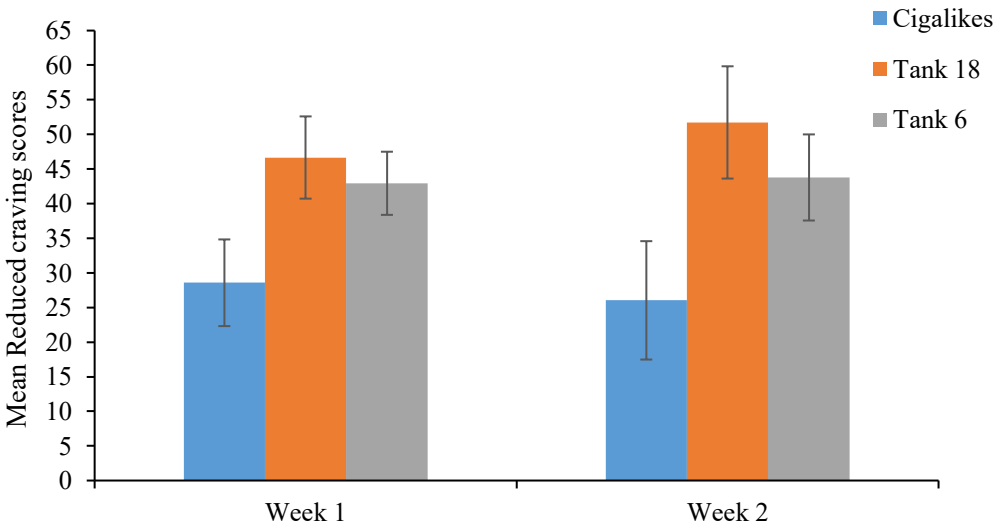


Figure 3.8. Means (SE) daily ratings of 'Reduced craving' for cigarettes per condition at Week 1 and 2

Daily ratings of pleasant

Mixed ANOVA revealed no main effect of Time $F(1, 34) = .037$, $p = .849$, $\eta^2 = .001$, no main effect of Condition $F(2, 34) = .833$, $p = .444$, $\eta^2 = .047$, nor interaction $F(2, 34) = .809$, $p = .454$, $\eta^2 = .045$.

Discussion

Summary of findings

The aims of Study 2 were threefold to explore: i) how e-cigarette naïve smokers' puffing behaviours differ a) between device types (cigalike vs. tank), b) according to nicotine concentration (18 mg/mL vs. 6 mg/mL) and c) evolve over time; ii) the effects of device types and nicotine concentrations on a) smoking reduction and cigarette dependence, b) craving and withdrawal symptoms alleviation, and d) satisfaction and other subjective effects.

Effects on Puffing topography

As hypothesised, participants increased their puff duration one week after e-cigarette initiation (Time 1); there was no further increase at Time 2. Conversely, they decreased the number of puffs after a week of initial use. As for puff duration, there was no further change at Time 2. A greater number of puffs were observed in the Cigalike condition compared to both Tank 18 and Tank 6 which did not differ from each other. Similarly puff duration was greater in the Cigalike condition compared to both the Tank 18 and Tank 6. Thus, it could be argued that there was no effect of nicotine concentrations per se but an effect of device type since statistically significant differences emerged between the Cigalike and the Tank 18 and the Cigalike and the Tank 6 but not between Tank 6 and Tank 18. That shorter IPI were observed in the cigalikes compared to both the Tank 18 and the Tank 6 reiterates the effect of device type and suggests that cigalikes are associated with a harder mechanism suction compared to the tank systems.

Effects on smoking reduction

Cigarettes smoked per day (CPD) decreased over time regardless of the device type. This was echoed in the CO results suggesting that regardless of the device type or

nicotine concentration, initiation of e-cigarette use contributed to a reduction in smoking.

Effect on Cigarette dependence

Cigarette dependence decreased over time from Baseline to Time 1. There was no further decrease at Time 2 and no differences between conditions. Similarly to smoking reduction, regardless of the device type or nicotine concentration, the e-cigarette contributed to a reduction in cigarette dependence. Likewise, participants reported a longer time to their first cigarette of the day at Week 1 compared to baseline, this did not differ between conditions and there was no difference at Week 2.

Effects on craving reduction and withdrawal symptoms

Craving reduction (i.e. pre minus post e-cig use) scores remained the same at Baseline, Time 1 and 2. Participants in the Tank 18 reported greater levels of reduction compared to those in the Tank 6. Interestingly, there was no difference between the Tank 18 and the Cigalike. Given the Cigalike helped reduce craving equally, to the same magnitude than the Tank 18 and that the Tank 6 performed the poorest, it could be argued that there was an effect of nicotine concentration rather than an effect of device type. Thus, for craving, higher nicotine concentrations seemed to be more effective although this assertion cannot be corroborated given that actual nicotine delivery was not measured. Besides, daily self-report data are slightly divergent and seem to also suggest a device type effect. Participants in the Tank 6 reported greater craving reduction compared to those in the Cigalikes who reported the lowest craving reduction. Consistent with the lab-based data, participants using the Tank 18 reported greater craving reduction, which reiterates the superiority of the tank systems. In relation to withdrawal symptoms, although the magnitude of the reduction improved over time, this did not depend on device type.

Effect on subjective effects

Overall subjective effect scores decreased from Baseline to Time 1 and Baseline to Time 2 with no significant changes between Time 1 and 2, which indicates a short term effect following a week of e-cigarette use. There was a significant interaction between time and conditions, whilst overall scores on positive effects remained fairly constant for the Tank 18, showing a marginal decrease at Time 1 then an increase at Time 2, scores for the Tank 6 and Cigalikes decreased considerably from Baseline to Time 2, this was more pronounced in the Cigalike condition.

Contrary to expectation, ratings of hit and satisfaction decreased over time but this seemed to be largely due to the cigalike condition and, to a lesser extent the Tanks. In relation to Hit, scores were greater at Baseline compared to Time 1 but no further significant reduction at Time 2. There were no differences between conditions. Similarly, for Satisfaction, following the ad lib vaping session in the lab, participants rated the e-cigarette higher at Baseline than they did at Time 2, there was no significant changes between Baseline and Time 1. Tank 18 and Tank 6 were associated with greater scores on Satisfaction compared to Cigalikes, suggesting that tank systems are preferred in terms of satisfaction. Consistent with the acute condition in the ad libitum lab session, data from diaries indicated that Tank 18 and Tank 6 were associated with greater scores on Satisfaction compared to the Cigalikes.

For the overall adverse effects, there was no changes over time or differences between conditions. Amongst all adverse effects, throat irritation was the most exacerbated symptom in each condition. This is consistent with other reports that throat and mouth irritation are the most commonly reported adverse effects associated with e-cigarette use (Dawkins & Corcoran, 2014; Dawkins et al., 2013).

E-cigarette type, Nicotine Concentrations and Practise Effects on Puffing Topography
Puff duration

Mean puff duration at initial use (M = 2.52 s, SD = 1.09) are in good agreement with previous findings (Behar et al., 2015; Farsalinos, Spyrou, et al., 2014; Lee et al., 2015; Talih et al., 2014).

Herein, participants in the Cigalike condition took longer puffs compared to both those using the Tank 6 and Tank 18, which is suggestive of a device type effect as opposed to a nicotine concentration effect. Against expectations and in contrast to the findings in study 1, there was no significant difference between Tank 18 and Tank 6 suggesting some degree of homogeneity in the puffing topography of participants in the two Tank conditions compared with the cigalike. Nevertheless, that mean puff numbers and puff duration were higher in the Tank 6 in comparison with the Tank 18, but failed to reach statistical significance, suggest that the findings do not depart significantly from that of previous studies. In previous research lower nicotine concentrations (Farsalinos, Romagna, Tsiapras, Kyrzopoulos, & Voudris, 2013a) and shorter puff duration were found to be associated with poorer nicotine delivery (Farsalinos, Spyrou, Tsimopoulou, et al., 2015; Hajek, Przulj, Phillips, Anderson, McRobbie, 2017; Hiler, 2016b). Furthermore evidence from the nicotine titration literature also suggests the importance of dosage in compensatory smoking and higher nicotine concentrations as exerting a stronger influence on puffing topography (McMorrow & Foxx, 1983).

Akin to the large standard deviations found between conditions the current findings echo the inter-variations in e-cigarette puffing topography reminiscent of those seen in previous studies (Behar et al., 2015; Hajek et al., 2015) including in Study 1, but also in smoking behaviours (Hammond et al., 2005). Importantly, these large individual variations may provide some explanation for the non-statistically significant differences between the Tank 6 and Tank 18.

The findings that cigalikes were associated with greater puff duration but lower satisfaction provide new evidence for the literature and align with previous findings that cigalikes tend to be associated with a less efficient nicotine delivery compared to tank systems (Farsalinos, Spyrou, Tsimopoulou, Stefopoulos, Romagna, & Voudris, 2014; Rüther et al., 2017). Consistent with the current study, in Rüther et al. (2017)'s study, a tank model successfully reduced craving to the same magnitude, as did the smoking of the combustible cigarette which reiterates tank models' efficiency compared to cigalikes. The fact that participants in the cigalike condition displayed greater puff duration may possibly be due to their stronger suction mechanism and differing airflow-activated system compared to tanks (Williams, Ghai, & Talbot, 2015) which unavoidably will constrain the nicotine e-liquid delivery and affect sensory factors.

Consistent with previous studies, participants increased their puff duration after a week following initial use from 2.52 s to 2.82 s then plateaued at Time 2 to an average of 2.88 s. This increase in puff duration is suggestive of an adjustment in puffing topography by naïve users over time which corroborates the self-titration theory at least in the short term, in so far as the shorter puffs exerted at initial use may not have yielded satisfactory blood levels. This increase in puff duration is a key finding specifically as puff duration along with IPI have been reported as the most influential drivers of aerosol yield (Kośmider, Madej, Gawron, & Sobczak, 2016). Indeed, a week seems to be sufficient to observe an evolution in puffing topography and for e-cigarette naïve smokers to develop an awareness for the need to adjust their puffing from smoking to e-cigarette use. This is consistent with previous findings in which a sample of 20 smokers who were introduced to a 18 mg/mL cigalike increased their puff duration from 2.2 s (0.1) to 3.1 s (0.3) after a week of initial use (Lee et al., 2015).

Although puff duration increased at Time 1, this increase remained unchanged at Time 2 to an average of 2.88 s. This mean puff duration is comparable to means reported in experiments which employed directed puffing protocols using naïve users and 18 mg/mL nicotine concentrations (M = 2.85, SD = 1.49) though negligible compared to the commonly reported 4s puff duration seen in experienced users (Spindle et al., 2015) outside of the laboratory (Hua et al., 2011). It would be reasonable to posit that should the current study extend to a longer period, more practise could have resulted in longer puff duration to match the puffing seen in real world behaviours by experienced users. Indeed, it has been reported that 4 weeks of practise using a 24 mg/mL cigalike resulted in significant blood nicotine increase which is a good indication that this period is amply sufficient for a successful adjustment in puffing topography (Hajek, et al., 2015). The increase in puff duration over one week period found here is echoed in previous studies (Lee et al., 2015).

The increase in puff duration herein is in line with the early suggestion that there is a learning process required for an effective use of e-cigarettes (McQueen, Tower & Summer, 2011). Similarly, others have found that the efficiency of obtaining satisfactory blood nicotine levels depends upon the way the e-cigarette is used and is likely to improve with practise (Hajek et al., 2015). Studies suggest that e-cigarette naïve smokers are less able to obtain systemic raised blood nicotine levels, unlike experienced users (Farsalinos, Spyrou, Stefopoulos, et al., 2015; Fearon et al., 2017). This is largely due to their typical shorter puff duration compared to experienced users at initial use (Farsalinos, Romagna, Tsiapras, Kyrzopoulos & Voudris, 2013). That shorter puff duration tend to be associated with poorer nicotine delivery profile is evidenced in previous studies (Talih et al., 2015) in which lower plasma nicotine concentrations were found in e-cigarette naïve smokers who displayed lower puff

duration of 2.3 s compared to experienced users who displayed a mean puff duration of 3.5 s (Farsalinos, Spyrou, Tsimopoulou, et al., 2015).

IPI

Other changes in puffing behaviour over time were the increase in IPI from Baseline to Time 1 and plateaued at Time 2. In line with the longer puff duration in the Cigalike, IPI were greater in the Tank 18 compared to the latter; and although this was not statistically significant, mean scores were greater in Tank 18 compared to the Tank 6. This suggests that the participants in the higher nicotine concentrations condition in the tank exerted a longer pause between puffs, which coincides with the literature suggesting that nicotine concentrations relate directly to nicotine delivery (Lopez et al., 2016) and to a great extent dictate puffing topography. Likewise, the puffing topography derived from the cigalikes reiterates the proposition of a more erratic or intense puffing profile associated in the cigalikes compared to the tank systems and supports the aforementioned proposition of the interwoven relationship between nicotine delivery and puffing topography.

Puff number

Of particular interest, in the current study, was a significant decrease in the number of puffs over the two-week period which, combined with a significant increase in puff duration, strongly suggest a dramatic change in puffing behaviour characterised by a shift to slower, longer more paced puffs from a more erratic puffing style at initial use. Like in the case of puff duration, there was no difference between Time 1 and 2 suggesting a plateau effect after a week following initial use. There was no difference between conditions, the importance of this finding may however be less relevant since some reports suggest that puff numbers have no or little effect on nicotine delivery (Kośmider et al., 2016; Spindle, 2015).

In the current study, participants took an average of 39.51 puffs in the cigalike condition across the 3 sessions together. This is in good agreement with a previous study in which 20 experienced e-cigarette users took an average of more than 20 puffs in a 10-minute ad lib session (Behar et al., 2015) which, compares well with the mean of 39.51 in the 20-minute session in the current study. Indeed, the likening of this observed adjustment in puffing topography to an attempt to compensate for the less efficient nicotine delivery compared to tobacco cigarettes is further evidenced in a previous study in which 50 puffs from a 24 mg/mL containing cigalike were required to raise blood nicotine to levels analogous of those obtained after the smoking of one combustible cigarette (which consisted of approximately 10 puffs) (Yan & D'Ruiz, 2015). That cigalikes are associated with poorer nicotine delivery is also documented in pharmacokinetic studies in which modest increases in plasma nicotine were obtained in naïve users' and experienced users in relation to combustibles (Fearon et al., 2017). It can be posited that the greater number of puffs exerted in e-cigarettes compared to when smoking a single cigarette may be caused by a stronger suction mechanism and/or the need to generate sufficient heat to the heating element of the atomiser. Further differences can also be seen in puffing topography between e-cigarette naïve smokers and the smoking of a cigarette (M = 2.3, SD = 0.5 s Vs M = 2.1, SD = 0.4 s) versus e-cigarette users' puffing topography (M = 4.2, SD = 0.7 s) (Farsalinos, Romagna, Tsiapras, Kyrzopoulos & Voudris, 2013).

Effects on Smoking Reduction and Cigarette Dependence

Effects on smoking behaviour

Self-reported CPD significantly reduced over time from Baseline to week 2. Similarly, there was a significant reduction in measured CO one week after initial use and a further decrease 2 weeks later, albeit to a lesser extent,

The current findings add to the literature which suggests that e-cigarettes can be an effective aid to support reducing smoking for those who do not intend to quit or are unable to do so. . The question addressed herein of whether e-cigarettes promote smoking reduction is pertinent and as such has been extensively researched in the past (Glasser et al., 2016).

In the current study, both e-cigarette types, the 6 and 18 mg/mL nicotine concentrations led to a reduction in smoking. Although it is worth noting that there was differential drop out across conditions with 20.8% drop out rate in the cigalike compared to 13% respectively in both the Tank 6 and 18. The greater drop-out rate in the cigalike compared to the tanks conditions may suggest that those in the Cigalikes who successfully maintained use of the device and reduced their CPD may have had greater motivation to do so compared to those who dropped out.

The reduction in smoking found overall, is consistent with previous findings. In a 2 part 8-month RCT, 48 smokers unwilling to quit who received a 2nd generation e-cigarette achieved a reduction in CO and CPD from the baseline to 4 weeks whilst an increase was observed in the control group. Salivary cotinine levels in e-cigarette users showed an initial decrease but then increased by 32 weeks. Similarly, in an 8-month pilot study, 34 smokers unwilling to quit received medical assistance training on correct use of a cigalike e-cigarette. At each follow-up session (1, 4 and 8-month) cotinine levels remained unchanged whilst CO and CPD reduced significantly. Although these two studies have no account of puffing topography, one of the mechanisms by which cotinine levels remained stable despite the drop in CO, may have been through an adjustment in puffing. This provides further evidence for the titration theory and accords with empirical evidence that more intensive use typically achieve greater

smoking reduction (Biener & Hargraves, 2015; Biener, Hargraves, & Hargraves, 2015; Brose et al., 2015; Hitchman et al., 2015; Manzoli et al., 2015).

Effects on Cigarette dependence

By virtue of its reinforcing properties from which dosage, mode (i.e. inhalation) and speed of delivery are key determinants of its dependence liability (Benowitz, 1990b; Benowitz, 2010), it is anticipated that nicotine delivered via e-cigarettes will have the potential to reduce cigarette dependence by acting as substitutes to combustible cigarettes.

In a similar fashion to smoking, in the current study, cigarette dependence reduced (as measured by the FTCD) from baseline to after a week after e-cigarette use initiation; a further, although less pronounced decrease was observed from week 1 to week 2. Surprisingly, the decrease did not differ between conditions. Similarly, regardless of the device type or nicotine concentration, at the second session, participants' habitual first cigarette of the day was reported to be smoked at a later time compared to the time reported in the baseline session.

The reduction in cigarette dependence corroborates with the reduction in smoking and suggest that e-cigarettes were well accepted and offered an effective coping mechanism at least at the initiation stage; whether participants were able to maintain this reduction in cigarette dependence will be explored in the following chapter. These findings align well with previous findings. In a 26-day mixed study, dual users and smokers were instructed to reduce their smoking by 75% before instructions to abstain completely from smoking for 3 days with 2 ad libitum vaping weeks in between each condition (Jorenby, Smith, Fiore, & Baker, 2017). In the smoking restriction period, dual users were more likely to sustain abstinence compared to smokers and did so by increasing their e-cigarette use 4-fold; this suggests that e-cigarettes were used as a coping mechanism and avoid nicotine deprivation.

Interestingly, cigarette dependence was rated lower by dual users in comparison to smokers. This concurs with the view that e-cigarettes may help reduce cigarette dependence.

The lack of difference between device types in the current study is in sharp contrast with the existing literature suggesting the difference in nicotine delivery between device types. Indeed, given the relationship between optimal nicotine delivery and dependence potential, one would expect a greater reduction in cigarette dependence in the Tank 18 group over the Tank 6 and Cigalikes groups. Many studies have associated cigalikes with modest nicotine delivery (Bullen et al., 2010; Farsalinos, Spyrou, et al., 2014; Rüther et al., 2017; Vansickel et al., 2010) and significantly lower dependence potential in comparison to tank systems, even when used with nicotine concentrations deemed sufficient to raise blood nicotine levels and induce satisfaction (Foulds et al., 2015). In one study, mean nicotine plasma concentration of 20 participants using 3 different cigalike models and a tank model on 4 separate occasions were compared against those of tobacco smokers (Rüther et al., 2017). Mean plasma nicotine concentrations within the first five minutes increased significantly following use of the tank compared to the cigalikes which led to modest increases; suggesting the ability of tanks to induce dependence certainly over cigalikes.

Although e-cigarette users' vaping patterns are as frequent as their cigarette consumption (Foulds et al., 2015), e-cigarette users are less dependent on their devices in comparison to their tobacco cigarettes as smokers (Etter & Eissenberg, 2015; Farsalinos, Romagna, Tsiapras, Kyrzopoulos, & Voudris, 2013b; Foulds et al., 2015; Liu, Wasserman, Kong, & Foulds, 2017). Given that e-cigarettes are associated with considerable reduced harm compared to smoking, it is reasonable to posit that the

benefits far outweigh existing concerns that e-cigarettes serve to maintain dependence and encourage continued use of an addictive substance (Rüther et al., 2017).

Notwithstanding the importance of nicotine, the current findings suggest that other non-nicotine factors may also be important in cigarette dependence (e.g. the look, tactile components, taste, feeling of smoke in the throat), secondary factors which have become closely associated with the primary reinforcer, nicotine. That use of a cigalike was associated with a comparable reduction in CPD compared with the tank devices suggests that some of these non-nicotine aspects of dependence may have been transferred to the e-cigarette (this is also supported by the lab-based data on craving discussed thereafter). These factors however, may become less important over a more protracted period of abstinence wherein nicotine delivery becomes more important. This will be explored further in the next chapter.

Effects on Craving reduction, withdrawal symptoms alleviation

The inability of the e-cigarette to deal with craving was given as the one of the chief reasons for discontinued use, therefore greater emphasis must be put on educating smokers how to use their devices to obtain craving relief for tobacco. That nicotine intake has a direct and linear effect on craving reduction has been suggested in previous clinical studies which found IPI to be inversely related to serum nicotine and cotinine levels as well as craving scores (Williams et al., 2011); this is suggestive of the influence of puffing topography and nicotine delivery over craving reduction.

Following the ad lib vaping baseline session, craving reduction scores were greater in the Tank 18 compared to the Tank 6, differences between the Cigalikes did not differ from Tank 18 or Tank 6. Across all 3 sessions and consistent with the baseline session, participants in the Tank 18 tended to report greater craving reduction than those in the Tank 6 but Cigalikes did not differ from Tank 18 or Tank 6, which is suggestive of a

nicotine concentration rather than a device type effect and that higher nicotine concentrations were more effective at reducing craving.

Many studies have suggested that e-cigarettes can be effective at alleviating craving and withdrawal symptoms related to smoking (Bullen et al., 2010; Dawkins & Corcoran, 2014; Dawkins et al., 2015; Dawkins, Turner, & Crowe, 2013; Dawkins et al., 2012; Etter, 2015; Foulds et al., 2015; Lechner, Meier, et al., 2015; Spindle et al., 2015; Vansickel, Weaver, & Eissenberg, 2012; Vansickel et al., 2010). The nicotine concentration effect in craving scores is in line with previous studies which suggest that greater craving reduction is associated with higher nicotine concentrations (Etter, 2015). In an early study, a first generation cigalike model filled with 16 mg/mL was compared to a combustible cigarette, an inhalator and a placebo cigalike, the 16 mg/mL cigalike was found to be the second most effective at reducing craving after the combustible cigarette (Bullen et al., 2010) suggesting nicotine as more rewarding compared to placebo. That said, because a delayed effect on craving reduction was observed (only 10 mins following the last puff), the authors conjectured that changes in craving were not contingent upon nicotine absorption, rather sensory and tactile cues or an anticipation of the nicotine delivery may be have contributed to the decrease (Bullen et al., 2010). Conversely, the remarkable findings that Cigalikes did not differ from Tank 18 are inconsistent with previous studies which found that newer devices (K E Farsalinos, Spyrou, et al., 2014) and higher nicotine concentrations are more effective at reducing craving than cigalikes, and are capable of inducing greater satisfaction and nicotine dependence (Foulds et al., 2015). However, others found that cigalikes equally reduced craving in e-cigarette naïve smokers to the same magnitude as did a second penlike generation over the short term and concluded that the visual similarities to a cigarette may have been partly responsible (Dawkins, Kimber, Puwanesarasa, &

Soar, 2015). The latter results may have differed should the cigalike been compared to a third, as opposed to a second generation device, since these models use less powerful and smaller batteries. However, it is unclear if, and to what extent, frequency of use had an impact on craving reduction. That the lower nicotine concentration condition was associated with lower scores on craving agrees well with previous studies, which found that nicotine-containing e-cigarettes elicit greater levels of craving reduction compared to placebo e-cigarettes (Dawkins, Turner, Hasna, & Soar, 2012), although placebo e-cigarettes have shown to reduce craving in smokers who were told they contained nicotine (Copp, Collins, Dar, & Barrett, 2015); thus since the experiment was not blind, whether knowledge of the nicotine concentrations administered influenced responses is unclear.

Furthermore, although unexpected, that different device types did not give rise to varying craving reduction scores ought not to be so surprising. In fact, pharmacokinetic studies suggest that nicotine delivery does not always dictate craving scores (D'Ruiz et al., 2015). In 2 sessions, 24 participants were randomised to 5 e-cigarettes with varying e-liquid formulation (16 and 24 mg/mL and differing PG/VG ratio) and a combustible cigarette; plasma nicotine levels and craving (urge to smoke) were measured during a 30-min standardised protocol (50 puffs, 30 s IPI) followed by a 1 h ad lib use of the e-cigarettes. Strikingly, following completion of both sessions, the differences in nicotine exposure (23 to 53% greater from the tobacco cigarette compared to the e-cigarettes) and means nicotine plasma concentrations were not reflective of the homogeneity observed in craving reduction scores (10% greater in the tobacco cigarette in relation to the e-cigarettes following the ad lib period) (D'Ruiz et al., 2015). Given the differences in nicotine exposure, one would expect the differences in craving to be of magnitude greater than the values observed. Thus, although nicotine concentrations

influenced plasma nicotine concentrations, craving did not seem to be impacted to the same extent. Thus, it is reasonable to conclude that although an important determinant of smoking behaviours, craving does not always correlate with nicotine delivery and sensorimotor factors may also be important at least in the early stages of a quit or smoking reduction attempt.

Following use of the e-cigarette in the current study, withdrawal symptoms ameliorated across the 2 weeks post initiation of use, there was an increase in alleviation at week 1 and further increase at week 2. Unlike craving, withdrawal symptoms scores did not differ across conditions. This is divergent from the suggestion that e-cigarette types vary in their ability to attenuate withdrawal symptoms. For example, a cross-over study of 22 smokers who were instructed to use a 16 mg/mL nicotine containing cigalike and second generation e-cigarette (with the same nicotine concentrations) on separate days, found that the latter was more effective at reducing withdrawal symptoms compared to the former (Lechner, Meier, et al., 2015). Based on the diary data, following use of the e-cigarette, withdrawal symptoms ameliorated across the 2 weeks post initiation of use, there was an increase in alleviation at week 1 and further increase at week 2. Unlike craving, withdrawal symptoms scores did not differ across conditions.

The steady increase in withdrawal relief reflects the changes in puffing topography, and may be synonymous of an increment in learning to use the e-cigarette more effectively. These findings add to the current literature which suggests that e-cigarettes are capable of alleviating withdrawal symptoms not only in acute conditions in clinical lab-based studies (Dawkins & Corcoran, 2014; Farsalinos, Spyrou, Tsimopoulou, et al., 2015; Nides et al., 2014) but also in real word conditions following

a period in which users have had the opportunity to accustom themselves with the device.

Additionally, the importance of withdrawal symptoms for these findings is also implied in previous studies which have found that withdrawal symptoms can be a strong predictor of a quit attempt (West, Hajek, & Belcher, 1989). Furthermore, although the presence of nicotine dictates greater craving reduction, there is substantial empirical evidence to suggest that similarly to smoking, conditional cues such as 'throat hit' (Dautzenberg, Scheck, Garelik, Kayal, & Dominique, 2016), handling, olfactory and visual cues may hold some key role in alleviating craving and withdrawal symptoms (Van Heel, Van Gucht, Vanbrabant, & Baeyens, 2017).

Effects on Subjective effects

As suggested in Chapter 1, the dosage and rapidity of nicotine are highly important for smokers' satisfaction and craving reduction. Sensory effects are also an important aspect of smoking behaviours (Carpenter et al., 2007; Rose et al., 2000, 2010) thus are likely to influence e-cigarette product acceptability.

Surprisingly, scores on Hit reduced from baseline to week 1 and was followed by a plateau at week 2 and there was no significant difference between conditions. Similarly, participants rated the e-cigarette as more satisfying following the baseline ad lib vaping session than they did a week and 2 weeks later after initiation of use. Tank 18 and Tank 6 were rated as more satisfying compared to cigalikes, suggesting that tank systems are preferred in terms of satisfaction. Scores on the overall scale of all positive effects together decreased from Baseline to week 1, and Baseline to week 2. The lack of significant changes between the first and the second week indicates a short-term effect at Time 1. Whilst overall scores on positive effects remained fairly constant for the Tank 18, showing a marginal decrease at week 1 then an increase at week 2, scores

for the Tank 6 and Cigalikes decreased considerably from Baseline to Time 2, this was more pronounced in the Cigalike condition. There were no changes over time in the overall adverse effect scores and no significant differences between conditions. Amongst all adverse effects, throat irritation was the most exacerbated symptom in each condition.

Several studies have documented the ability of e-cigarettes to elicit positive subjective effects (Bullen et al., 2010; Spindle et al., 2015; Vansickel et al., 2012; Vansickel et al., 2010; Vansickel & Eissenberg, 2013), however not to the same extent than tobacco cigarettes (Martínez-Sánchez et al., 2014; Norton, June, & O'Connor, 2014); whilst others report differences in their ability to induce satisfaction and other positive effects between device types (Dawkins et al., 2015; Farsalinos, Spyrou, et al., 2014). For example, Farsalinos' group employed a VAS questionnaire to compared subjective effects elicited following the use of a cigalike versus a tank system e-cigarette and found the latter to be more satisfying than the former (Farsalinos, Spyrou, et al., 2014) which corroborates the current findings. Of particular interest to this thesis, means in nicotine plasma levels mirrored findings of satisfaction. That greater satisfaction and higher nicotine plasma levels were both linked to the tank device, evoke a strong correlation between nicotine delivery and satisfaction whilst supporting the titration theory. Likewise, use of the tank induced greater 'throat hit' compared to the cigalikes. In the same light, previous findings identified an individualistic and optimal throat hit (which differs from the rewarding nicotine bolus referred to previously) that is contingent upon e-liquid nicotine concentrations as well as the power applied to the coil, which in turn, all influence the desire to switch to e-cigarettes in smokers (Dautzenberg et al., 2016). This confirms that nicotine concentration is an important determinant and reinforces previous recommendations that higher nicotine

concentrations may be more advisable (Dawkins et al., 2016) at least during initiation of use (Farsalinos, Romagna, & Voudris, 2015).

Limitations

The current study must be considered in the light of the following limitations. Unlike pharmacokinetic studies which by design, use standardised puffing protocol, the current study employed an ad libitum puffing paradigm in order to capture adjustments in puffing during initiation of use. Indeed, such a paradigm is likely to reflect real world puffing behaviours whilst avoiding any satiation effect which could have influenced puffing topography. That said, the instructed one-hour abstinence may have been counterintuitive in this regard, specifically for the low dependent (light) smokers for whom their normal routines may have been to smoke very few cigarettes in the day.

Primary aims of the study were to observe evolution of puffing topography over a period of two weeks and differences in device types based on the rationale that cigalikes would be associated with poorer delivery. To this effect, 24 participants were randomly allocated to the aforementioned condition compared to 23 in each of the 2-separate conditions. This group was associated with a large drop-out rate in subsequent sessions (20.8% vs 13 and 13% in the Tank 18 and 6 respectively) which affected the sample size and resulted in most of the analyses to be run with unequal group sizes. This differential drop-out may therefore explain some of the non-significant findings in relation to CPD, craving and withdrawal symptoms – that is, cigalikes were only helpful for those participants who were sufficiently motivated to continue to use or for whom positive subjective effects were experienced.

Equally, other primary aims were to gain further understanding of the impact of a 6 and 18 mg/mL nicotine concentrations on puffing topography of naïve e-cigarette smokers. However, the accuracy of nicotine exposure could not be established given

that nicotine concentrations contained in the e-liquid were not chemically measured. That said, contrary to early manufactured products (Cameron et al., 2014; Trehy et al., 2011), in recent years, studies report little variability in the accuracy of the labelling of European and Western nicotine containing e-cigarettes and liquids (Etter et al., 2013; Farsalinos, Spyrou, et al., 2014; Goniewicz, Hajek, & McRobbie, 2014) suggesting some improvement in manufacturing practices. One measure which could have counteracted the latter limitation would have been through collecting plasma nicotine concentrations, a rather onerous and invasive procedure.

Furthermore, three sessions were scheduled one week apart during which participants reduced their smoking and mean puff duration increased in the first week. This is reflective of an effective and rapid adaptation in puffing regimen. However, the time at which this adjustment occurred is unclear. It could have been within the first 10 minutes into the baseline session or hours following the end of the session. Future studies should consider incorporating a 24 hr follow-up session.

It is possible that aside from taking longer puffs, the changes in puffing topography occurred through deeper inhalation also. A further limitation may be that no apparatus was used to measure puff velocity and puff volume. Nonetheless, studies suggest that such apparatus, although useful may influence the user's experience thereby hindering the capture of naturalistic behaviours (Ross & Juliano, 2016). In addition, that puff volume (Kósmider, Madej, Garwon, Sobczak, 2016) or velocity do not influence liquid evaporation (Talih et al., 2014) and that puff duration is the most common currency used in this area of research strengthens the validity of the current findings.

Moreover, the current study was conducted in a controlled laboratory environment which may not be a true reflection of real puffing behaviour (Robinson, Hensel, Morabito, & Roundtree, 2015) since previous studies suggest that smokers and e-cigarette users have a tendency to display more intensive puffing patterns in laboratory settings compared to when in their natural environment (June et al., 2012; Robinson et al., 2015). Nevertheless, given that the means puffing duration and number of puffs are in line with those reported in the literature, the behaviours displayed in the lab were likely to be reflective of real world puffing behaviours.

There are instances wherein e-cigarette users press the activation button before or whilst bringing the device to the mouth in order to warm the atomiser and allow a stronger throat hit, which is known as the pre-puff phenomenon (Behar et al., 2015), in contrast, others may do the opposite (Farsalinos et al., 2013b). In such instances, the time frame was captured only when the device was clearly seen in the mouth with both lips closed. Extra care was taken to ensure clear visibility of the start and end of puff and increase accuracy. In terms of the evolution of puffing, the within-participants design would have helped mitigate this limitation since previous studies suggest intra-consistency in puffing behaviours (Behar et al., 2015), any occurrences of such behaviours would have been likely to have been replicated in subsequent sessions.

Furthermore, given the wide variations in device models and brands' ability to deliver nicotine, the heterogeneity in e-liquid formulation, the current findings cannot be generalised to other e-cigarette device types and e-liquids.

Finally, the current study relies heavily on self-reported data specifically for the measure of CPD, which may have rendered these results biased. However, the strong similarity of the biochemical measure of CO levels in relation to CPD means has provided more credibility to these findings.

Summary of Chapter III

The current study was not designed to allow for any direct conclusions on compensatory puffing, since the smokers do not switch between nicotine concentrations; rather it provides evidence on whether e-liquid with different nicotine concentrations and different e-cigarette types are puffed on differently and require different puffing patterns in order to optimise nicotine delivery and increase product acceptability. Indeed, substantial research has demonstrated the importance of and interweaved relationship between puffing topography and nicotine delivery in their ability to elicit reward and maintain smoking behaviours; herein an attempt has been made to shed further light on how these factors interplay in e-cigarette naïve smokers.

Consistent with previous studies, participants increased their puff duration after a week following initial use. This increase in puff duration is suggestive of an adjustment in puffing topography by naïve users over time, which corroborates the self-titration theory at least in the short term, in so far as initial puffs taken at baseline were evidently too short to yield satisfactory blood levels. Indeed, a week seems to be sufficient to observe an evolution in puffing topography and for e-cigarette naïve smokers to develop an awareness for the need to adjust their puffing from smoking to e-cigarette use. Interestingly and consistent with the idea that the vacuum mechanism of cigalikes may be harder and require a stronger draw than tanks, the cigalike was associated with longer puff duration. This reiterates the proposition of a more erratic or intense puffing profile associated in the cigalikes compared to the tank systems. That they require stronger suction may have contributed to their inability to elicit satisfaction to the same magnitude than did the tanks. The significant decrease in the number of puffs over the two-week period which, combined with a significant increase in puff

duration strongly suggest a dramatic change in puffing behaviour characterised by a shift to slower, longer more paced puffs from a more erratic puffing style at initial use.

Of key interest to this thesis, in the lab, cigalikes and 18 mg/mL containing tanks were equally associated with craving reduction, whilst the 6 mg/mL nicotine containing tank performed the poorest. In addition, the 18 mg/mL containing tank elicited greater levels of satisfaction whilst cigalikes were associated with a greater drop-out rates, which may indicate that cigalikes are less satisfying. Given the predictive value of craving and satisfaction on smoking cessation attempts, and as discussed throughout, the influence of nicotine concentrations on puffing topography, in line with the existing literature suggests that higher nicotine concentrations may be more effective in the relief of craving at least during initiation of e-cigarette use.

Taken together, these findings confirm previous observations that puffing patterns differ across device types and change over a one week period as the user learns to use the device. Across all subjective variables, the Tank 18 performed the strongest and, although the cigalike was associated with some promising results, these need to be considered in the context of the high drop-out rate in this condition. To conclude, these findings suggest higher nicotine concentrations and tanks appear the most suited tool to support the early stage of a smoking cessation attempt.

CHAPTER IV

"PREDICTORS OF SMOKING CESSATION USING E-CIGARETTES: DEVICE TYPES, NICOTINE DELIVERY, CRAVING AND CIGARETTE DEPENDENCE"

Abstract

Background: Although factors associated with smoking cessation success have been widely studied, very little is known about the factors that may promote cessation where e-cigarettes are used as aids. This study aimed to identify the possible predictors of smoking cessation in smokers attempting to quit using an e-cigarette.

Methods: 70 e-cigarette naïve smokers (N = 70; 62.9% female) were followed up at 1, 3 and 6 months after a 2-week lab experiment (consisting of 3 separate sessions) in which they received either i) a cigalike e-cigarette model containing 18 mg/mL nicotine concentrations, ii) a tank e-cigarette device (containing 18 mg/mL), iii) a tank (containing 6 mg/mL). Logistic regression analyses were conducted to assess whether device type (initial allocation and device type used at follow up), nicotine concentrations, craving reduction following e-cigarette use in the lab, mean puff duration, cigarette dependence and motivation to quit could predict cessation.

Results: Cigarette dependence, Craving reduction with e-cigarette use and Device type at follow up were significant predictors of cessation at 1, 3 and 6 months respectively. Nicotine concentrations, mean puff duration and motivation to quit at baseline were not significant predictors.

Conclusion: In a sample of 70 e-cigarette-naïve smokers, less dependent smokers were more likely to quit at 1 month. Those who reported greater craving reduction following

e-cigarette use in the lab were more likely to have quit at 3 months; the predictive utility of measures of craving reduction at first use can be fostered to inform smoking cessation programmes. The odds of quitting at 6 months were higher for those using a tank device at the time of follow-up compared to those using a cigalike, which is in line with previous studies suggesting that tank systems are associated with successful cessation.

Introduction

In the previous chapter, the findings of Study 2 (Chapter III) suggest that higher nicotine concentrations and tanks seem more effective to support the early stage of a smoking cessation attempt. Following their final ad libitum vaping session at week 2 (Study 2; Chapter III), participants were followed at 1, 3 and 6 months via phone calls and/or emails to assess their current smoking status and e-cigarette use.

Herein, Study 3 uses the cohort of participants in Study 2 to identify the possible predictors of successful cessation over a longer time period. Based on the literature (Berry et al., 2018; Borland, Yong, O'Connor, Hyland, & Thompson, 2010; Hitchman et al., 2015; Rohsenow et al., 2018; Simonavicius et al., 2017; Vangeli et al., 2011) the following variables were selected as predictors: device types at baseline and follow-ups, nicotine concentrations used at baseline and follow-ups, cigarette dependence, motivation to quit, craving and satisfaction, and previous experience of e-cigarette before the start of the study (i.e. whether they have used an e-cigarette before).

E-cigarettes and smoking cessation

As described in Chapter I, there is some evidence that e-cigarettes are useful in promoting smoking cessation and reduction (Beard, West, Michie, & Brown, 2016; Bell & Keane, 2012; Glasser et al., 2016; Meier, Tackett, & Wagener, 2013; Weinberg & Segelnick, 2011), and help prevent relapse (Biener et al., 2015; Jamie Brown et al., 2014; Giovenco & Delnevo, 2018; Hajek, Etter, Benowitz, Eissenberg, & McRobbie, 2014). However for many, e-cigarettes have not been sufficiently satisfying as substitutes to tobacco cigarettes (ASH, 2017). There may be a number of factors that predict why e-cigarettes help some to quit and not others; the mechanisms by which e-

cigarettes promote smoking reduction and cessation may be multiple. These include: helping to alleviate craving for tobacco smoking and other adverse abstinence related symptoms (Adriaens et al., 2014; Dawkins, Kimber, et al., 2015; Etter, 2015; Hajek, Przulj, Phillips-Waller, Anderson, & McRobbie, 2018; Rüther et al., 2017), delivering nicotine efficiently and in ways that approximate that of combustible cigarettes (Farsalinos, Spyrou, Tsimopoulou, et al., 2015; Hajek et al., 2015, 2017; Lechner, Meier, et al., 2015; Wagener et al., 2016), providing novel features such as device customisability (Vandrevala et al., 2017) or sensory such as olfactory factors (e.g. availability of wide range of flavours) (Farsalinos, Romagna, Tsiapras, Kyrzopoulos, Spyrou, et al., 2013; Ward et al., 2018), and allowing lapses whilst minimising risks of complete relapse (Notley et al., 2017). Given the difficulty of quitting and remaining abstinent the struggle for many to achieve a complete transition away from smoking remains. Thus, further understanding of the factors that can predict successful cessation outcomes will be informative and can add to the support provided to smokers and dual users to facilitate complete cessation.

Predictors of cessation

Efficient Nicotine delivery: Device types, Nicotine concentrations and Puffing duration

Amongst the factors that are likely to determine product acceptance, nicotine delivery occupies a central role. Although tank models tend to be favoured by experienced e-cigarette users, cigalikes have not completely lost their appeal and, though opinions are divergent (Dawkins et al., 2015; McKeganey & Dickson, 2017), many rate their cigarette-like appearance as very important (Dawkins, Kimber, et al., 2015; Dawkins, Munafò, et al., 2015; McQueen et al., 2011). This may partly explain why many choose a cigalike as their entry product during initiation of use (Wadsworth et al., 2016). However, as discussed in previous chapters, cigalikes are associated with poorer nicotine delivery (Farsalinos, Spyrou, et al., 2014; Hajek et al., 2018, 2017;

Rüther et al., 2017) and reduced satisfaction (Dawkins, Kimber, et al., 2015; Rüther et al., 2017) compared with tank models and the latter may be more effective for smoking cessation (Hitchman et al., 2015; Rodu & Plurphanswat, 2017b). However it has been suggested that for some, tank devices could constitute a barrier to e-cigarettes use. The complexity of the technology of the tank systems (Wadsworth et al., 2016) combined with the intricacies of nicotine liquid composition further diminishes acceptability of the product (Mckeganey & Dickson, 2017). Thus, this highlights the importance of device type as a key variable to predict cessation.

Aside from device type, frequency and duration of use are critical in predicting complete transition to e-cigarette use. In a longitudinal online survey of 1643 UK smokers followed up during a period of one year, those using a tank device on a daily basis were more likely to quit in comparison to those using cigalikes or any device type on a non-daily basis (Hitchman et al., 2015). Consistent with this, in a US based study, of 695 (N = 1374 at baseline) users, daily use was 6 times at greater odds to be associated with cessation compared to non-daily use (Biener & Hargraves, 2015). In a separate longitudinal study with 2028 US residents, long term e-cigarette use was associated with a greater cessation rate (Zhuang, Cummins, Y Sun, & Zhu, 2016). The infrequent use of e-cigarettes as demonstrated by many smokers who discontinue e-cigarette use may be driven by a high level of dissatisfaction which may dissuade from future use and promote smoking. In the latest ASH survey, dissatisfaction was one of the main motivations for discontinuing use of e-cigarettes (ASH, 2017). Reasons for discontinuation of use has also been associated with their reduced ability to alleviate craving for tobacco cigarettes (ASH, 2017). Other studies suggest that greater craving and withdrawal relief are associated with high-powered tank systems, more intensive puffing patterns and higher nicotine concentration e-liquids (Etter, 2015), which

reiterates the importance of effective nicotine delivery and suggests nicotine concentrations as a key variable. There is also evidence that higher nicotine concentration NRT products can increase smoking cessation (Tunnesen et al., 1999); and that in the early stages of a cessation attempt, smokers need to increase their nicotine concentration in order to achieve and sustain complete smoking abstinence (Farsalinos, Romagna, Tsiapras, Kyrzopoulos & Voudris, 2013).

Another factor that may influence the likelihood of smoking reduction and cessation is puffing topography such as puff duration. There are several indications that users acquire an optimum puffing technique over time to obtain satisfactory nicotine delivery, craving relief and satisfaction (Dawkins & Corcoran, 2014; Dawkins et al., 2016; Farsalinos, Spyrou, et al., 2014; Farsalinos, Spyrou, Stefopoulos, et al., 2015; Hajek et al., 2015). Further indication pointing to the importance of puffing duration comes from empirical evidence suggesting that naïve users' puffing duration increase over time in order to optimise nicotine delivery and increase satisfaction (Hajek et al., 2017). As reported in Chapter III and as documented previously (Hajek et al., 2015), naïve e-cigarette smokers rapidly learned to increase their puffing duration after a week of initiation of e-cigarette use, arguably to optimise nicotine delivery and obtain satisfactory blood levels.

Craving and Satisfaction

The interrelated association between craving, effective nicotine delivery and satisfaction in e-cigarette use is clear. Previous research suggests that higher nicotine concentrations and better nicotine delivery devices relate to greater craving relief (Etter, 2015). This concords with other research suggesting that nicotine containing e-cigarettes compared to placebo are more effective at promoting cessation (Hartmann-Boyce et al., 2016). That discontinuation of use of the device is commonly attributed to

dissatisfaction and the inability of the e-cigarette to alleviate craving for tobacco cigarettes (ASH, 2017; Simonavicius, McNeill, Arnott, & Brose, 2017) suggest craving as an important variable for predicting cessation.

Cigarette dependence

Historically, theoretical models of smoking have put a great emphasis on cigarette and nicotine dependence. The widespread use of cigarette dependence as a key measure to understand smoking behaviours (Baker et al., 2010; Billieux et al., 2010; Borland et al., 2010; Fagerström, 2012; Foulds et al., 2015; Lechner, Meier, et al., 2015; Lee et al., 2015; Muhammad-Kah, Hayden, Liang, Frost-Pineda, & Sarkar, 2011; Parzynski, Jaszyna-Gasior, Franken, & Moolchan, 2008; Pechacek et al., 2017; Schnoll, Goren, Annunziata, & Suaya, 2013; Ucar et al., 2014; Van Overmeire et al., 2016; Zhang et al., 2012) is testament of its strong predictability value of future quit attempts and likely success. Cigarette dependence therein encompasses two interrelated concepts, motivational factors and a degree of loss of control over tobacco use (West, 2006). Two key items i) Time to the first tobacco cigarette (hereafter TFTC) after waking and ii) the number of cigarettes per day (hereafter CPD) have high theoretical relevance due to the short half-life of nicotine which evokes a need to raise depleted blood nicotine up to habitual 'awaken' levels (Kozlowski et al., 1981). This is corroborated by studies which have found CPD and TFTC to be strong correlates of biomarkers of exposure such as urine nicotine, serum cotinine and blood carboxyhaemoglobin (Muhammad-Kah et al., 2011). Similarly, studies have found that the shortest latency to the first cigarette of the day to be the strongest predictor of lapses and relapse compared to other measures of cigarette dependence (Baker et al., 2010). The Fagerström Test for Cigarette Dependence (FTCD) (also referred to as Fagerström Test of Nicotine Dependence or FTND) (Fagerström, 2012), employed in this thesis,

comprises a total of 6 items including TFTC and CPD and has been widely used due to its predictive validity. Although some studies have found mixed results (Etter, 2005; Ferguson et al., 2003; Kozlowski, Porter, Orleans, Pope, & Heatherton, 1994; Piper, McCarthy, & Baker, 2006), a substantial number of studies, and others pointing to its consistency in predicting cessation outcomes (Vangeli, Stapleton, Smit, Borland, & West, 2011), makes the FTCD a valid measure in predicting quit outcomes.

The potential of e-cigarettes to deliver nicotine as efficiently as tobacco cigarettes under certain conditions (Dawkins et al., 2016; Ramôa et al., 2015; Wagener et al., 2016) may be attributed to e-cigarettes 'puff-by-puff' mode and route of delivery (inhalation route) which closely mimic the delivery afforded by tobacco cigarettes and allow a fast delivery to the brain (Russell & Feyerabend, 1978). Since the ability of a drug to induce addiction is largely determined by the rapidity at which the drug reaches the brain (Le Houezec, 2003), understanding the predictive validity of cigarette dependence on cessation outcomes would be of great value. As echoed in previous studies (Buu, Hu, Piper, & Lin, 2018; Rohsenow, Tidey, Martin, Colby, & Eissenberg, 2018a) and reported in Chapter III, e-cigarette use can reduce cigarette dependence. Online longitudinal surveys (using retrospective data) have found that e-cigarettes tend to be less addictive than cigarettes (Etter & Eissenberg, 2015; Farsalinos, Romagna, Tsiapras, Kyrzopoulos, & Voudris, 2013a; Foulds et al., 2015). For example, in the PATH Study (Population Assessment of Tobacco and Health), in which 2727 exclusive smokers were followed from *Wave 1* (2013–2014) to *Wave 2* (2014–2015), the data suggested that e-cigarette use was associated with a reduction in cigarette dependence (Buu et al., 2018). Another group of researchers, using the PATH data reported a reduction in CPD at *Wave 2* following initiation of e-cigarettes use at *Wave 1* (Berry et al., 2018). Taken conjointly, these findings suggest that e-cigarettes have the potential

to lessen dependence and dependence levels of smokers (including dual users for whom a complete switch to e-cigarettes remains challenging) are greater than that of exclusive e-cigarette users (Dawkins, Turner, Roberts, et al., 2013; Etter & Eissenberg, 2015; Foulds et al., 2015; Goniewicz et al., 2013; Liu et al., 2017). Based on the aforementioned evidence and that greater dependence is associated with reduced odds of quitting (Heatherton et al., 1989; Kozlowski et al., 1981), cigarette dependence a likely candidate for the prediction of smoking cessation outcomes following e-cigarette use initiation.

Motivation to stop smoking

Another likely factor to affect e-cigarette use and a cessation attempt is motivation. Both public preconceptions and the existing literature suggest that motivation could be a key factor to determine the likelihood of successful smoking cessation outcomes. Similarly smokers crucially perceive motivation as essential and also sufficient to successfully quit smoking (Balmford & Borland, 2008). However, despite a strong motivation to quit, many smokers are unable to do so resulting in high relapse rates (Etter & Stapleton, 2006). One of the core theoretical assumptions that is relevant to smoking behaviours posit that a strong intent (motivation) to engage in a given behaviour and the perceived control upon that behaviour are likely to determine future outcomes of this given behaviour (West, 2007).

A robust measure of motivation, Kotz et al's (2013) Motivation to Stop Scale (MTSS), has been widely used in the tobacco and e-cigarette literature. Motivation, herein as measured in the MTSS questionnaire, refers to intentions and desire to perform the behaviour (i.e. initiating a quit attempt). The literature which supports motivation to quit as a central role in predicting smoking cessation is somewhat conflicting. Whilst some studies found an association between motivation and cessation

(Hagimoto, Nakamura, Morita, Masui, & Oshima, 2010; R West, McEwen, Bolling, & Owen, 2001; Zhou et al., 2009), others found no such association (Hellman, Cummings, Haughey, Zielezny, & O'Shea, 1991), and many found that its predictive ability is constrained to predicting future cessation attempts as opposed to predicting successful outcomes or sustained abstinence (Borland, Yong, Balmford, et al., 2010; Vangeli et al., 2011). A recent systematic review of 8 studies from both the UK, US, Canada, Australia (plus the ITC-four countries data), France, Spain, Japan, China, Malaysia and Thailand, and samples varying from n = 267 to 16 469 of smokers trying to quit, examined the best predictors of smoking cessation and found that of all the variables (including demographics), cigarette dependence was the only variable to systematically predict success in quitting whilst *desire to quit* tended to predict future attempts (Vangeli et al., 2011).

As for the impact of e-cigarettes on motivation to quit, the evidence suggesting a positive effect of e-cigarette use on motivation to quit is predominant but not unanimous (Kalkhoran & Glantz, 2016; Pokhrel, Herzog, Muranaka, Regmi, & Fagan, 2015). In one study, a week of e-cigarette use was sufficient to increase '*readiness*' and confidence to quit (Wagener et al., 2014). Consistent with this, in a pilot study, 17 smokers reported an increase in 'contemplating' the idea to quit from baseline towards week 6 and 10 weeks ; this was in line with the observed reduction in CPD, CO and cigarette dependence (Rohsenow et al., 2018a). Although some could argue that financial incentives including the provision of free e-cigarettes may have influenced the results, the fact that smoking and a reduction in cigarette dependence, as well as motivation to quit, were all maintained even after provision of the free e-cigarettes had ceased, suggest a real effect of e-cigarette use initiation (Rohsenow et al., 2018a).

Aims and hypotheses

The current study uses the cohort of participants in Study 2 to identify possible predictors of successful cessation. Based on the most recent and appropriate literature (Berry et al., 2018; Borland, Yong, O'Connor, Hyland, & Thompson, 2010; Hitchman et al., 2015; Rohsenow et al., 2018; Simonavicius et al., 2017; Vangeli et al., 2011) the following variables were selected: device types used at baseline and follow-ups, nicotine concentrations used at baseline and follow-ups, cigarette dependence, motivation to quit, craving and satisfaction, and previous experience of e-cigarette use before the start of the study . Following their final ad libitum vaping session (Study 2; Chapter III), participants were followed at 1, 3 and 6 months via phone calls and/or emails to assess their current smoking status (biochemical verification via CO readings where ≥ 7 days abstinence was self-reported) and e-cigarette use. Participants' device types were recorded at each follow-ups, thus in addition to baseline allocation of the device type, device type at each follow-ups will be entered in the regression model.

It is hypothesised that a greater likelihood of quitting would be associated with participants using a Tank, those using higher nicotine concentrations, those with lower cigarette dependence, higher motivation to quit and those who experienced greater craving reduction during e-cigarette use at initiation.

Methods

Design and ethical approval

The study received full ethical approval from the University of East London's ethics committee (UREC_1516_04; approved on the 15th September 2015, see appendix 16). All participants provided written informed consent to be followed up to 6 months after the start of the study.

The cohort of smokers (from Study 2, see Chapter 3) were contacted via telephone and email between January 2016 and June 2017. At the end of Study 2, participants were instructed that they can change devices and e-liquid nicotine concentrations as they wished. As a result, device type and nicotine concentrations as per self-report at each follow-up point were entered as predictor variables, in addition to the baseline conditions (see predictor variables).

Outcome measures: Smoking cessation at 1, 3 and 6 months (binary coded, 'quit' or 'did not quit').

Predictor variables: i) The primary predictor device types combined with nicotine concentrations as per the baseline allocation Cigalikes (18 mg/mL), Tank 18 and Tank 6; ii) Device types reported at follow-ups (1, 3 and 6 months) coded as Cigalikes and Tanks. Those reporting using Sub-ohming systems (using atomisers with > 1 ohm resistance) were classified as using tanks (as only 5 did so); iii) Nicotine concentration at follow-up, although participants reported a wide variation in nicotine concentrations, to avoid violating the assumption of case numbers per cell, nicotine concentrations were initially grouped into 3 categories a) ≤ 6 mg/mL, b) ≥ 8 but ≤ 16 mg/mL and c) ≥ 18 mg/mL, however, this was still problematic resulting in more than 20% of the expected counts to be less than 5. To address this issue and avoid the loss of statistical power, nicotine concentration categories were revised and grouped into High (≥ 8 mg/mL) versus Low (≤ 6 mg/mL)[h] categories; iv) Baseline cigarette dependence (FTCD), (Fagerström, 2012); vii) Daily consumption of e-liquid (in mL) (at each

[h] Note that at the time of data collection, nicotine concentrations equal to or exceeding 18 mg/mL were considered as high and were commonly used. Advanced technology with devices compatible to atomisers which allow greater wattage to flow to the coil and generate more power, has led to a shift towards an increase in use of lower nicotine concentrations (< 6 mg/mL) with recent reports of 4-8 mg/mL amongst dual and exclusive users (Adriaens et al., 2017), hence the categorization of nicotine concentrations in the present analysis.

respective time-point) and Mean puff duration (in seconds; at the last experimental session); viii) Baseline measures of motivation to quit (MTSS); ix) Baseline satisfaction and craving reduction (measured in the first experimental session); x) past e-cigarette use (binary coded: Yes or No).

Participants

From the cohort of Study 2 (Chapter III), participants were followed up at 3 time-points; 1-, 3- and 6-months. As per West al. (2015), an intention to treat analysis was used wherein lost to follow-up participants were treated as maintaining smoking. To that effect, the initial sample of 70 smokers at Day 1 was included in all subsequent analyses.

Measures

Baseline measures

Baseline data (including demographics, smoking history, motivation to stop smoking, confidence to quit, past e-cigarette use and cigarette dependence were collected as described in Study 2, Chapter III). See Table 3.1 in Results section in Chapter III/Study 2 for baseline participants' characteristics.

Follow up measures at 1, 3 and 6 months

Follow-up CPD by asking number of cigarettes smoked per day in the last 7 days and number of cigarettes smoked per day on average. Follow-up Cigarette dependence was measured using the FTCD (as described in Study 2 Chapter III).

E-cigarette dependence and patterns of use at follow up assessed by employing an adapted version of the FTCD and included items questionnaire such as time of first puffs and so on (as described in Study 1, Chapter II and in appendix 20) (Dawkins & Corcoran; Dawkins et al., 2016). Patterns of use were assessed by asking participants about current device type used and if applicable, date they stopped/resumed use of the

device, nicotine concentrations, flavours and estimate of liquid and cartridges used. Please note that volume consumed is not analysed but only reported due to the inconsistency between units of measurements for tank (mL) and cigalike (cartridges).

The outcome variable, cessation at each follow up point, was measured with self-report CPD (abstinence was denoted by 0 cigarettes in the last 7 days. Participants were then invited to the lab for a CO breath test.

Mixed ANOVA was used to measure changes in cotinine levels at follow-ups from baseline by collecting saliva samples at baseline and upon self-report of quit only.

Procedure

During the final ad lib vaping session in Study 2 (Chapter 3), participants were debriefed (see appendix 16 for debrief letter) and informed about what the 3 follow-ups time points would involve. They also received some guidance on how to maintain their devices (replacing the atomisers, and so on). At 1, 3 and 6 months after their baseline session, each participant was contacted via a 10 minute phone call to collect information on their smoking status, CPD and e-cigarette dependence (outlined above), patterns of e-cigarette use, device type and nicotine concentrations.

Data and statistical analysis

IBM SPSS version 23 was used for all analyses. Frequencies of device types and nicotine concentrations reported at follow ups were examined as well as descriptive statistics (means and SD) for all predictors. Chi square analyses were conducted to examine quit rates per condition and at each time point. A parsimony heuristic approach was adopted for the logistic regression to explore factors predicting cessation. As such the model was built entering predictor variables that are in line with the theoretical basis of this thesis and most prominent in the e-cigarette literature. Binary logistic regression analyses were conducted with the main outcome variables cessation

at 1, 3 then 6 months (Yes or No) and the main predictor variables in the following respective order: i) Device types combined with nicotine concentrations at baseline; ii) Device types at follow-ups (3 categories, see design section); iii) Nicotine concentrations at follow-ups (2 categories, see design section), iv) Baseline cigarette dependence (continuous variable) ; v) Mean puff duration (continuous variable) at 2-week follow up (see Study 2, Chapter III); vi) Baseline measures of motivation to quit (continuous variable); ; vii) Baseline satisfaction and craving reduction following e-cigarette use (continuous variables); viii) past e-cigarette use ('yes' or 'no'); ix) The interaction Device type X Nicotine concentrations. Based on model improvement, predictors were retained and further logistic regression analyses were conducted.

Results

Participation flow chart - Quit and lost to follow-up frequencies

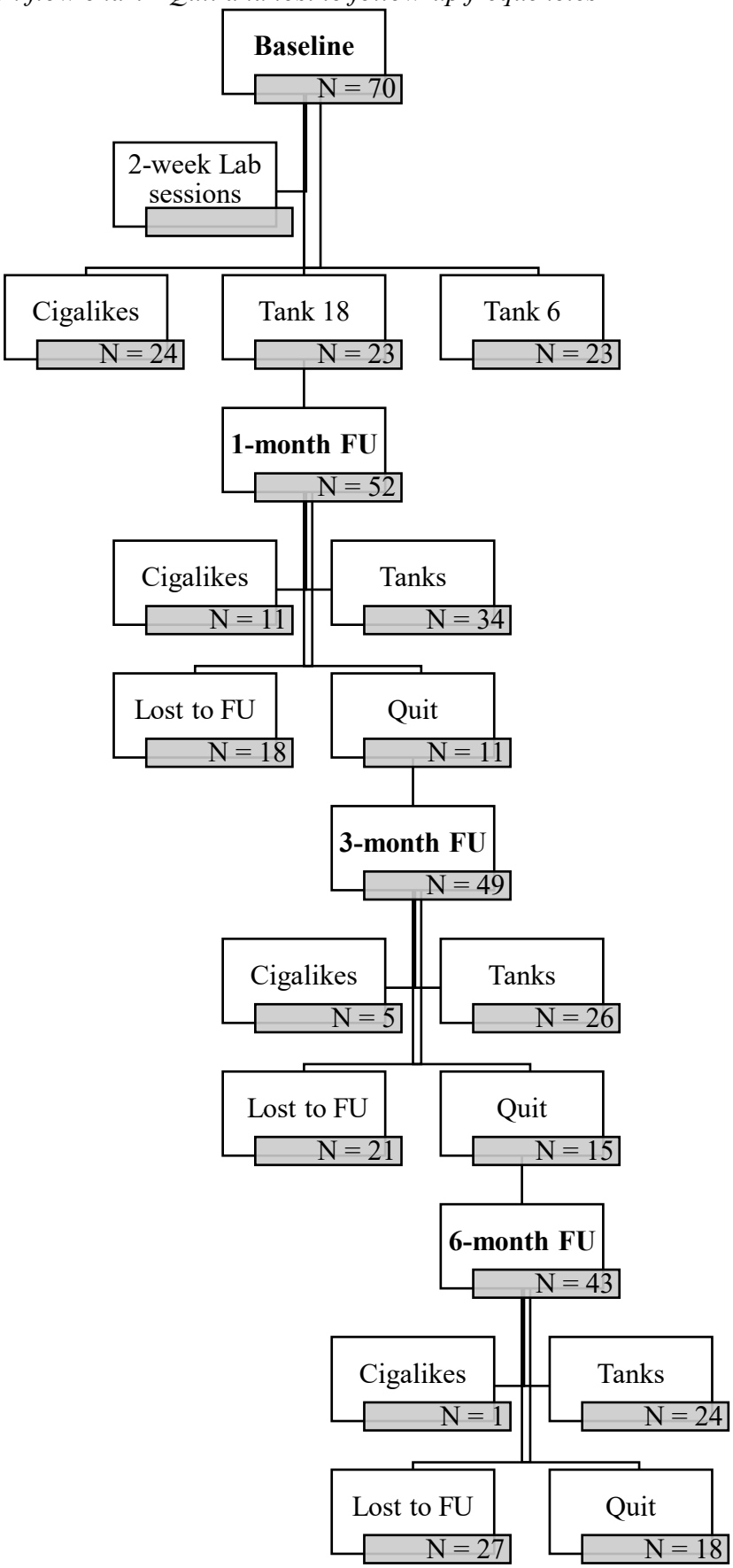


Figure 4.1. Flow diagram of study participation from 1- to 6-month FU (follow-up). Following a 2-week period of 3 lab-based ad lib vaping sessions, in which 70 e-cigarette (EC) naïve smokers were randomly allocated to either i) a cigalike (18mg/mL nicotine concentrations), ii) a tank (18 mg/mL) iii) a tank (6 mg/mL) and encouraged to substitute as many tobacco cigarettes (TC) as they can. Participants were at liberty to upgrade e-cigarette model and change nicotine concentrations, to this effect, tank 18 and 6 were merged into 'Tanks' in subsequent regression models. Participants were followed up at 1, 3 and 6 months to assess smoking status, EC use and levels of dependency. At the end of the 2-week lab-based sessions, smokers were given minimum advice on EC use and product maintenance, and were given permission to upgrade devices and nicotine concentrations (at their own cost and volition). This explains the merging of both tank conditions and the increase in self-report of tanks use and decrease in cigalikes from the 1-month FU upwards. Quit rates encompass self-report dual users, exclusive EC use and quitters (former smokers no longer using an EC).

Predictors: Device type and nicotine concentrations at baseline and at follow-ups

Table 4.2 presents frequencies (number of participants in each condition) for device type and nicotine concentration at baseline and at follow-ups (1, 3 and 6 months). Note that device type at follow up has been reclassified to denote those using a cigalike versus a tank. Table 4.2 highlights the contrast between cigalikes and tanks with a decrease in frequency in cigalike use and an increase in tank use. Changes in nicotine concentrations over time is less clear due to a large amount of missing data at follow-ups.

Table 4.2

Frequencies of Device Type and nicotine concentrations (Nic Concent.) at Baseline, 1, 3 and 6 months follow-up (FU) and Past e-cigarette (EC) use.

Device Type	**Baseline**		**1-month FU**		**3-month FU**		**6-month FU**	
	N	*%*	*N*	*%*	*N*	*%*	*N*	*%*
Cigalikes	24	34.29	22	31.4	21	30	19	27.1
Tanks*	46	65.71	48	68.6	49	70	51	72.9
Total	70	100	70	70	70	70	70	70

Nic concent. (mg/mL)	**Baseline**		**1-month FU**		**3-month FU**		**6-month FU**	
	N	*%*	*N*	*%*	*N*	*%*	*N*	*%*
≤ 6	23	32.86	11	15.7	13	18.6	14	20
≥ 8 (incl. ≥ 18)	47	67.1	33	47.1	19	27.1	12	17.1
Total	70	100	44	62.9	32	45.7	26	37.1
Missing	0	0	26	37.1	38	54.3	44	62.9
Total	70	100	70	100	70	100	70	100

Note **Tanks represent Tank 6 and Tank 18 collapsed*

Changes in Cotinine levels at follow-ups in self-report quit

Of the 70 baseline samples collected, 11 saliva samples were collected at follow-ups for the self-report quit. Thus mixed ANOVA analyses included only the 11 samples that were returned and compared against their respective baseline levels. Mauchly's test indicated that the assumption of sphericity had been violated. Therefore, Greenhouse-Geisser corrected values are reported and revealed no main effect of time $F(1, 8) = .358$, $p = .358$, $\eta^2 = .043$. (Estimated Marginal Means, Time 1: $M = 137.38$, $SD = 97.65$, Time 2: M =167.65, SD = 161.96). There was no main effect of condition $F(2,8) = .255$, $p = .781$, $\eta^2 = .06$ (Estimated Marginal Means, Cigalike: $M = 107.76$, $SD = 172.29$; Tank 18: $M = 172.65$, $SD = 135.50$; Tank 6: $M = 159.25$, $SD = 61.79$). There was no significant interaction effect between time and condition $F(2,8) = .039$, $p = .952$, $\eta^2 = .01$.

Quit frequencies per conditions and time points

As can be seen in Table 4.3, at 1 month, 11 participants (15.71%) reported that they had quit smoking and 59 (84.29%) continued to smoke. Although more participants reported having quit in the tank versus the cigalike condition, there was not a statistically significant association between Device type and Quit at 1-month follow up, $\chi 2$ (1, n = 70) = 1.917, p = .154, phi = .208.

Table 4.3
Crosstabulations of frequencies and percentages for quit rates at 1 month (1-M) and Device type reported at 1 month follow up (FU).

1-M Quit		**Device Type at 1-M FU**		
		Cigalikes	**Tanks**	**Total**
No	**Count**	21	38	59
Yes	**Count**	1	10	11
Total	**Count**	22	48	70

There was no statistically significant association between Device type and Quit at 3-month follow up, χ^2 (1, n = 70) = 1.616, p = .202, *phi* = .190. As displayed in Table 4.4, similar to the one month follow up, there was a greater number of quit reports in those using a tank.

Table 4.4
Crosstabulations of frequencies and percentages for quit rates at 3-month (3-M) and Device type reported at 1 month follow up (FU).

3-M Quit			Device Type at 3-M FU		
			Cigalikes	Tanks	Total
	No	Count	19	36	55
	Yes	Count	2	13	15
Total		Count	21	49	70

There was a statistically significant association between Device type and Quit at 6-month, χ^2 (1, n = 70) = 4.335, p = .016, *phi* = .286. Those who reported using a tank device were more likely to report cessation at 6-month follow up (as can be seen in Table 4.5).

Table 4.5
Crosstabulations of frequencies and percentages for quit rates at 6-month (6-M) and Device type reported at 1 month follow up (FU).

6-M Quit			Device Type at 6-M FU		
			Cigalikes	Tanks	Total
	No	Count	18	34	52
	Yes	Count	1	17	18
Total		Count	19	51	70

There were no significant association between Nicotine concentrations and Quitting at either the 1, [χ^2 (2, n = 44) = 1.011, p = .603, *phi* = .152], 3 [χ^2 (2, n = 32) = 2.245, p = .367, *phi* = .265], or 6 month follow ups [χ^2 (2, n = 26) = 1.489, p = .552, *phi* = .234] (see Tables 4.6 – 4.8for counts). However, note that the assumptions of Chi square were violated due to small expected cell counts.

Table 4.6

Crosstabulations of frequencies and percentages for quit rates at 1 month (1-M) and Nicotine concentrations reported at 1 month follow up (FU).

1-M Quit		Nicotine concentrations (mg/mL) at 1-M FU		
		≤ 6	≥ 8	Total
No	Count	9	27	36
Yes	Count	2	6	8
Total	Count	11	33	44

Table 4.7

Crosstabulations of frequencies and percentages for quit rates at 3-month (3-M) and Nicotine concentrations reported at 1 month follow up (FU).

3-M Quit		Nicotine concentrations (mg/mL) at 3-M FU		
		≤ 6	≥ 8	Total
No	Count	8	13	21
Yes	Count	5	6	11
Total	Count	13	19	32

Table 4.8

Crosstabulations of frequencies and percentages for quit rates at 6-month (6-M) and Nicotine concentrations reported at 6 month follow up (FU).

6-M Quit		Nicotine concentrations (mg/mL) at 6-M FU		
		≤ 6	≥ 8	Total
No	Count	7	8	15
Yes	Count	7	4	11
Total	Count	14	12	26

Table 4.9 displays inter-correlations between variables. To avoid issues of multicollinearity, Cigarette dependence (FTCD scores) was selected instead of CPD since past studies suggest Cigarette dependence as a key predictor of cessation. Likewise, Confidence in quitting was removed as it correlated highly with motivation to quit, another variable of key interest in the literature. Finally, craving reduction and satisfaction correlated, given the critical role of craving in reinforcing of smoking and in

e-cigarette use (ASH, 2017; Etter, 2015; Simonavicius et al., 2017), craving was favoured to be retained in the model instead of satisfaction.

Table 4.9 Correlational analyses between continuous variables targeted as predictors for the initial model

Predictor Variables	CPD	FTCD	MTSS	Conf.	Craving	Satis.
CPD[1]	1	.741**	.091	-.302*	.111	.059
FTCD[2]	-	1	-.120	-.216	.082	-.018
MTSS[3]	-	-	1	.547*	.057	.081
Conf.[4]	-	-	-	1	.224	.061
Craving[5]	-	-	-	-	1	.298*
Satis. [6]	-	-	-	-	-	1

*Note ** Correlation is significant at p = .001 (2-tailed)*
** Correlation is significant at p = .005 (2-tailed).*
[1] CPD corresponds to Cigarette smoked per day
[2] FTCD corresponds to Cigarette dependence
[3] MTSS corresponds to Motivation to quit
[4] Conf. corresponds to Self-reported confidence in quitting at baseline
[5] Craving reduction measured in the last experimental ad lib session
[6] Satis. corresponds to satisfaction

Logistic Regression analyses

Three logistic regressions were run with the primary outcomes Quit at 1 month, Quit at 3, then Quit at 6 months; the results for each of these outcomes will be presented separately. Note that initial analyses were conducted with all the aforementioned predictors (see statistical analysis section), however at each time point, models (at 1, 3 and 6 months) contained issues regarding ratio of cases to variables (too few data in some cases) which gave rise to very large standard errors rendering the outputs uninterpretable (Field, 2013) for the variable nicotine concentrations at follow-up. In order to rectify issues of complete separation (one predictor variable predicting the outcome variable perfectly based on inspection of Chi square results), nicotine concentrations at follow-ups were re-coded as binary (High versus Low; ≤ 6 vs. ≥ 8 mg/mL, see method section for rationale of categorisation) to counteract the issues of few cases in the mid-nicotine concentrations and to align the follow-ups with the initial

allocated condition. In addition, the predictor variable consumption of e-liquid in mL was removed due to a large proportion of missing data at all time points. Furthermore, contrary to expectation, one of the key predictors of interest, the Nicotine concentration at follow-ups X Device type interaction was not significant and did not improve the model. Consequently, models were revised to include baseline condition, baseline measures of motivation to quit and Cigarette dependence, Device types (Tanks vs Cigalikes) and Nicotine concentrations (High vs Low) at follow ups, and Baseline Craving reduction scores collected in the initial ad lib vaping experimental session. Other changes to the analysis for each follow up time point are described below. For all regression analyses, Cigalikes were entered as the reference category.

Predictors of Quit at 1-month follow-up

The initial model was run to include the predictors as described above however, due to issues of complete separation (unusually large standard errors), problematic variables were identified and removed (device type at 1 month follow-up), thus the final model was re-run to include the following: i) Baseline conditions, ii) Nicotine concentrations, iii) Puff duration, iv) Craving reduction, v) Motivation to quit and vi) Cigarette dependence. The overall model was statistically significant (p = .012). Cigarette dependence was found to be a significant predictor; participants who scored the lowest on the FTCD scale were at greater odds of quitting (95% CI = .294 to .992, p = .047). More specifically, for every one point decrease on the FTCD, odds of quitting increased by 0.54. None of the remaining variables predicted quit at 1 month (see Table 4.10).

Table 4.10

Coefficients of the model with Odds Ratio of predictors of cessation at 1 Month

	b		95% CI for Odds Ratio			
		SE	*Lower*	*Odds*	*Upper*	*p*
Included						
Constant	5.172	3.942	-	176.199	-	.190
Tank 18 (vs Cigalikes)	2.398	1.579	.498	11.00	243.074	.129
Tank 6 (vs Cigalikes)	-.970	1.868	.010	.379	14.748	.604
Nicotine concent.	-1.787	1.591	.007	.168	3.791	.262
Puff duration	-.013	.434	.421	.987	2.311	.976
Craving reduction	.888	.614	.729	2.430	8.09	.148
MTSS	-.718	.469	.195	.488	1.223	.126
FTCD	**-.616**	**.311**	**.294**	**.540***	**.992**	**.047**

Note $R^2 =$ 23.430 (Hosmer & Lemeshow) .340 (Cox & Snell) .551 (Nagelkerke). Model $\chi^2(7, n = 43) = 17.89$, $p = .012$.

Bold indicates significant at $p < .05$

Predictors of Quit at 3 months follow-up

The initial model was run to include all predictors as described above previously. Due to issues of complete separation (unusually large standard errors) including the addition of the key predictor variable - baseline device type, problematic variables were identified and removed, thus the final model was re-run to include the following: i) Device used at 3 months follow up, ii) Puff duration, iii) Craving reduction, iv) Motivation to quit, v) Cigarette dependence. The overall model was statistically significant (p = 0.031). Craving reduction with e-cigarette use at baseline was found to be the only significant predictor, participants who reported greater craving relief in the baseline ad lib vaping session were more likely to quit at 3 months (95% CI = 1.118 to 2.913, p = .016). For every one point increase in craving reduction with e-cigarette use at baseline, odds of quitting increased by 1.142. None of the remaining variables predicted quitting at 1 month (see Table 4.11).

Table 4.11

Coefficients of the model with Odds Ratio of predictors of cessation at 3 Months

			95% CI for Odds Ratio			
	b	*SE*	*Lower*	*Odds*	*Upper*	*p*
Included						
Constant	-3.126	1.728	-	.044	-	.070
Device at FU (Tanks vs Cigalikes)	1.729	1.164	.576	5.638	55.212	.137
Puff duration	.009	.255	.613	1.009	1.662	.971
Craving reduction	**.133**	**.233**	**1.118**	**1.142**	**2.913**	**.016**
MTSS	.133	.223	.738	1.142	1.768	.551
FTCD	-.101	.144	.682	.904	1.198	.482

Note. R^2 = 50.086 (Hosmer & Lemeshow) .201 (Cox & Snell) .296 (Nagelkerke). Model $\chi^2(5, n = 55)$ = 12.314, p = .031.

Bold indicates significant at $p < .050$

Predictors of Quit at 6 months follow-up

The initial model was run to include all predictors as previously described. Due to issues of complete separation (unusually large standard errors), problematic variables were identified and removed (Baseline condition, Puff duration), thus the final model was re-run to include the following: i) Baseline condition, ii) Device type at follow up, iii) Craving reduction, iv) Motivation to quit and v) Cigarette dependence. The overall model was statistically significant (p = .035). Type of e-cigarette used at 6 months was statistically significant; participants reporting using a tank device at 6 months were 17.14 times more likely to quit (95% CI = 1.2 to 244.59, p = .036) compared to those using a cigalike. For craving reduction, for every one point increase in reduction with e-cigarette use at baseline, odds of quitting increased by 1.51 although this fell short of statistical significance (p = .053), (see Table 4.12). None of the other variables predicted quitting at 6 months.

Table 4.12

Coefficients of the model with Odds Ratio of predictors of cessation at 6 Months

			95% CI for Odds Ratio			
	b	*SE*	*Lower*	*Odds*	*Upper*	*p*
Included						
Constant	-4.443	1.696	-	.012	-	.009
Tank 18 vs Cigalikes	-.813	.988	.064	.444	3.074	.411
Tank 6 vs Cigalikes	-901	.975	.060	.406	2.744	.355
Device at FU (Tanks vs. Cigalikes)	**2.841**	**1.356**	**1.20**	**17.135**	**244.59**	**.035**
Craving red	.409	.211	.995	1.506	2.279	.053
MTSS	.107	.211	.736	1.113	1.684	.611
FTCD	-.050	.127	.741	.951	1.221	.695

Note. R^2 = 66.261 (Hosmer & Lemeshow) .176 (Cox & Snell) .259 (Nagelkerke). Model χ^2 (6, n = 70) = 13.545, p = .035.

Bold indicates significant at $p < .05$

Discussion

Summary of findings

The aim of the current study was to investigate the variables that best predict smoking cessation at 1, 3 and 6 months in a sample of 70 e-cigarette-naïve smokers. At 1-month follow-up, 11 out of 70 smokers reported quitting, 15 did at 3 months and 18 did at 6-months. The 11 participants who self-reported quit (CO verified) and returned their saliva samples, maintained their cotinine levels after quit. At 1-month follow-up, only cigarette dependence significantly predicted smoking cessation; those with lower scores at baseline were more likely to quit compared to participants with higher baseline dependence scores. Baseline condition (cigalike, Tank 6, Tank 18), nicotine concentrations, craving and motivation to quit did not predict cessation. At the 3-month follow-up, only craving reduction predicted cessation at 3 months. Participants reporting a higher reduction in craving following use of the e-cigarette device at baseline had greater odds of a successful cessation outcome at 3 months. At 6 months, only the type of e-cigarette device at follow-up was a significant predictor of cessation. Thus, regardless of the nicotine concentration, participants using a tank device were at greater odds of quitting smoking compared to those using a cigalike device.

Nicotine delivery a critical factor in the transition to exclusive e-cigarette use: Device types, Nicotine concentrations and puffing behaviours

In a sample of 70 e-cigarette-naïve smokers, the odds of quitting at 6 months were 17.135 times higher for those using a tank device compared to a cigalike at the time of follow up. These findings are in line with previous studies concluding that device characteristics play an important role in promoting sustained use of the device and smoking cessation. In a US based study (N = 923), successful quitters were more likely to be exclusive tank users and report greater craving reduction compared to

current smokers (Chen, Zhuang, & Zhu, 2016). Although, the nature of this study limits any inferences of causation, such associations have been reported elsewhere in larger samples. An online longitudinal survey which followed 1643 smokers over 1 year, found that those using a tank daily were more likely to have quit at 1 year compared to non-daily users of both cigalikes and tanks, whilst non-daily users of cigalikes and tanks were no more or less likely to have quit (Hitchman et al., 2015). This accords with more recent findings which suggest that tank users were more likely to have successfully quit and be heavier users compared to those using cigalikes (Shiffman et al., 2018). Although in agreement with the current findings, both studies point to one aspect that has hitherto received less attention in this field, frequency of use, which is not reported in the current study and may constitute a limitation. Although estimate of volume consumed was collected, it could not be used as a predictor variable as the unit of measurement differed across the groups (mL for tank users and units of cartridges for cigalike users). Other reports of the association between tank users and achievement of complete cessation provide further support for tanks as more efficient devices over cigalikes (Farsalinos, Romagna, Tsiapras, Kyrzopoulos, Voudris, 2014). A large EU cross-sectional survey of 19000 respondents, suggested that cigalike users were less likely to have quit (Farsalinos, Romagna, et al., 2014). Moreover, a relatively small Catalonian sample of smokers (N = 71) reported a remarkably high reduction and cessation rate at 12 months follow-up (25.4 and 40.8% respectively), in smokers purchasing their first tanks; the increase in product transition from standard basic tanks to more sophisticated modifiable tanks (8.5 to 18.4% from baseline to 12 months follow-up) (Polosa et al., 2015) could be attributed to a need for better nicotine delivery products and a more fulfilling vaping experience.

Further cues to the importance of the differences between cigalikes and tanks originate from a number of studies suggesting a transition from cigalikes to more advanced tank models (Tacket et al, 2015) arguably for a more enhanced experience notably '*a more satisfying hit*' (Yingst et al., 2015); but also to address the dissatisfaction with starter kits such as cigalikes (McQueen et al., 2011). Here, the movement over time was also away from cigalikes towards tanks. That the design and greater customisability afforded by tanks are amongst the most valued qualities sought after by experienced users (Baweja et al., 2016) may also help to explain this organic transition to tank systems. In the current study, many reported stopping using the device due to a lack of satisfaction. On the other hand, the ease of use, simplicity of cigalikes (Baweja et al., 2016; Wadsworth et al., 2016) and close resemblance to tobacco cigarettes (Dawkins, Kimber, et al., 2015; Dawkins, Munafò, et al., 2015; Hoek, Thrul, & Ling, 2017) are equally valued qualities that appeal to smokers trying to transition. In fact, some cigalike users have experimented with several tanks only to return to cigalikes in need for a closer enactment of the rituals of smoking (Hoek et al., 2017). For others, it is the complex technology combined with the knowledge required to deal with the intricacies of nicotine liquid composition that are discouraging factors against sustaining e-cigarette use (Mckeganey & Dickson, 2017), which reiterates the heterogeneity of e-cigarette use and users. Whilst much evidence suggests nicotine as central in facilitating the transition to exclusive e-cigarette use, it appears that some smokers/users are able to concede and favour a design that is closer to the rituals of smoking over the importance of effective nicotine delivery. In fact, that nicotine delivery may not be a determinant factor for all is not so surprising given that placebo e-cigarettes have been shown to reduce craving for cigarettes albeit in the short term only (Dawkins et al., 2012). Thus, whilst non-pharmacological cues play an important role,

nicotine delivery remains a central factor in promoting complete substitution. Besides, the sudden surge in popularity of the new e-cigarette device 'JUUL' (developed in the US) suggests a continued demand for improved nicotine delivery products, given that it can be mounted with pods containing nicotine concentrations that largely exceed the 20 mg/mL cap (Willett et al., 2018).

The predicting model at 6 months included device type at follow up as opposed to device type at baseline. The issues with device type at baseline may have emerged due to a lack of statistical power. The differences in categorisation with three conditions at baseline (to include nicotine concentrations, high vs. low) whilst at follow up there were two clear categories (tanks vs. cigalikes). A likely factor to explain why the tanks were more able to promote cessation could have been that they provided greater levels of satisfaction compared to the cigalikes. It is possible that the cigalikes did not provide comparable levels of satisfaction perhaps due to technical issues (e.g. leakages), less pleasant taste or more resistant mechanism suction. This is reflective in the decrease from baseline to 6 months in participants using a cigalike compared to the increase in the number of participants using a tank at 6 months. Other possible explanation could be that tanks were more able to relieve craving for tobacco cigarettes. The greater capability of the tank models to alleviate craving compared to cigalikes may lie in their nicotine delivery efficiency. Several experimental lab-based studies assessing the nicotine delivery of e-cigarette models have found that tanks systematically deliver higher nicotine levels to the user compared to cigalikes (Farsalinos et al., 2014; Hajek et al., 2018; Rüther et al., 2017), even at levels that equate those reached after the smoking of a cigarette (Dawkins et al., 2016; Ramôa et al., 2015; Rüther et al., 2017; Wagener et al., 2016). The reinforcing value of nicotine in smoking (Benowitz, 2010; Henningfield & Keenan, 1993), to alleviate craving,

provide satisfaction (Etter, 2015; Dawkins, Kimber et al., 2015), may be the route by which tanks are better able to facilitate sustained use of the product and provide an effective replacement to smoking (Hitchman et al., 2015; Yingst et al., 2015).

Unexpectedly, nicotine concentrations was not found to significantly predict cessation at 1month and was subsequently removed from the model at 3 and 6 months due its poor predictive ability. This is at odds with the findings reported in Chapter III; where ad libitum use of the 18 mg/mL nicotine concentration containing tank led to greater craving relief compared to use of the 6 mg/mL. Given the predictive ability of craving on relapse to smoking (Zhou et al., 2009), and that higher nicotine concentration is a negative correlate of craving relief for cigarettes (Etter, 2015), one would have expected nicotine concentrations to be a critical in predicting cessation. It is likely that the increase in puff duration over time seen in Study 2 (Chapter III) may have been accompanied by stable nicotine delivery even following a reduction in nicotine concentrations (as found in previous studies see Soar, Kimber, McRobbie, & Dawkins, 2018), and possible moves to more sophisticated devices; together these factors would militate against finding an effect of nicotine concentrations over the longer term. Counterintuitively, the reduction in nicotine concentrations is not accompanied by a reduction in nicotine exposure. Recent studies found that whilst experienced users reduce their nicotine concentrations over time, they increase their consumption of nicotine liquid while maintaining constant cotinine levels (Soar et al., 2018). One likely mechanism that could explain this phenomenon may be that users self-titrate by adjusting their puffing patterns in order to obtain (or maintain) satisfactory nicotine levels in their blood. This accords with the increase in puffing duration seen in Study 2 over time and the findings that those who completely switched from smoking to e-cigarette use maintained their cotinine levels (Study 3, although this

is based on a small subset of the sample). This makes it difficult to consider nicotine concentrations as an isolated variable given its interweaved relationship with device and user characteristics under the umbrella of nicotine delivery.

Puff duration (measured at 2 weeks) was not a significant predictor of cessation at 3-month follow-up and was removed from the model at 1 and 6 months. Given numerous studies have highlighted the importance of puffing duration to optimise nicotine delivery (Dawkins & Corcoran, 2014; Dawkins et al., 2016; Farsalinos, Spyrou, et al., 2014; Farsalinos, Spyrou, Stefopoulos, et al., 2015; Hajek et al., 2015), increase satisfaction (Hajek et al., 2017) and promote sustained use of the e-cigarette in replacement of smoking, the current findings are at odds with the literature. That said, to date, few studies refer to puff duration as a single predictor of cessation. This may be due to the difficulty in detangling puffing duration from other factors (i.e. nicotine concentrations, device characteristics, satisfaction and liking) which when combined exert a great influence over nicotine delivery as discussed in previous chapters.

Craving

The predictive ability of Baseline craving reduction associated with e-cigarette use fell short of significance at 6-months and was not significant at 1 month. Conversely, at 3 months follow-up, craving was found a significant predictor of cessation. Those who experienced greater craving reduction after using the e-cigarette at baseline were more likely to quit at 3 months. Although it's unclear why this effect was found at 3 months but not earlier, it aligns well with the findings of Study 3. That higher nicotine concentrations and the use of tanks elicit greater craving reduction compared to lower nicotine concentrations and cigalikes respectively, provide a good explanation for the greater probability of success in quitting using tanks over cigalikes,

and reiterate the critical role of nicotine delivery in promoting sustained use (of e-cigarettes) and smoking reduction.

That craving reduction associated with e-cigarette use at baseline is a predictor of cessation at 3 months suggests that the initial encounter of e-cigarette use can provide some indication of the ability of the e-cigarette to satisfy craving; thereby facilitate sustained use and promote smoking reduction. Likewise, this also suggests craving reduction as a possible precipitator of relapse. In a Spanish sample of 775 continuing smokers, whilst positive and negative affect were found the predominant factors in predicting relapse, craving was also identified as a significant contributing factor (Piñeiro et al., 2017).

An increasing number of studies suggest that e-cigarettes are capable of alleviating craving (Bullen et al., 2010; Dawkins et al., 2018; Dawkins & Corcoran, 2014; Dawkins et al., 2012; Etter, 2015; Farsalinos, Spyrou, Tsimopoulou, et al., 2015) and more recent studies suggest that tanks are more efficient in doing so compared to cigalikes (Chen et al., 2016; Hajek et al., 2018; Rüther et al., 2017). In a small sample of 20 young New Zealand residents, those using tanks were more likely to report that the e-cigarette satisfied their craving for tobacco cigarettes compared to those using cigalikes (Chen et al., 2016). Others found that e-cigarettes help reduce their craving for cigarettes but lack in authenticity (Robertson et al., 2018). This perceived lack of authenticity is echoed in other studies (Hoek et al., 2017; Mckeganey & Dickson, 2017). It is suggested that the e-cigarette only partly mimics smoking sensory cues but does not compare to the throat hit associated with smoking. Given the central role and hedonic properties of the latter (Dautzenberg et al., 2016) and that for many that e-cigarettes can deliver this throat hit, constitutes as one of the primary motives for initiation of use (Yingst et al., 2015). However, no studies have explored the ability of craving to

predict cessation in a sample of smokers who have been newly introduced to e-cigarette; thus, in this sense the current findings are novel.

Motivation and Cigarette dependence as related predictors

Motivation did not predict cessation at 1, 3 or 6 months follow-up. Whilst previous studies suggest that motivation is predictive of a cessation attempt, this predictive ability seldom translates into predicting success outcomes or sustained abstinence (Borland, Yong, Balmford, et al., 2010; Vangeli et al., 2011). Thus, motivation appears to be insufficient to achieve and maintain cessation. Based on the preconceptions held by smokers that the mere presence of motivation is sufficient to quit, a likely explanation may be that an overreliance on their motivation and/or self-confidence to quit may be an impediment to seeking support and using other effective coping mechanisms (Balmford & Borland, 2008). This evidence provides some explanation for the lack of predictive ability of motivation predicting cessation in this study.

Though motivation to quit measured at baseline has proven a robust measure to predict quit attempts (Borland, Yong, Balmford, et al., 2010b; Hummel, Brown, Willemsen, West, & Kotz, 2016; Kotz, Brown, & West, 2013; Vangeli, Stapleton, Smit, Borland, & West, 2011), it conveys a flawed idea that motivation or intention to quit is static (Hughes et al., 2014). In a sample of 16,657 smokers intending to quit, who responded to questions about their past quit attempt, current motivation to quit and consistency of their motivation in cross-sectional surveys, it was found that the incorporation of measures of the consistency of motivation to quit strengthened the prediction model of cessation above and beyond motivation alone (Perski, Herd, Brown, & West, 2018). Thus, it seems that motivation is fluid and the extent, and the continued presence, of motivation may be better predictors of cessation.

Although cigarette dependence has consistently emerged as a predictor in standard smoking cessation studies, no studies have looked at cigarette dependence as a predictor of quit success using e-cigarettes. Cigarette dependence was a significant predictor at 1 month only. That dependence could predict cessation accords well with the literature, although, that dependence did not successfully predict cessation at 6 months was unexpected. Several studies suggest dependence levels as a predictor of cessation in smokers followed up over a period exceeding 6 months (Hagimoto et al., 2010; Hughes et al., 2014; Rohsenow, Tidey, Martin, Colby, & Eissenberg, 2018b; Vangeli et al., 2011). In a Japanese population sample of smokers (N = 1358) who were followed-up a year later, higher nicotine dependent smokers were less likely to quit smoking (Hagimoto et al., 2010), this is not surprising given that light smokers (based on CPD and first time of TC) have stronger motivation to stop than moderate to heavy smokers (Kotz, Fidler, & West, 2012). That the overall sample in the current study had relatively low to moderate cigarette dependence scores may explain the predictive ability of cigarette dependence in this model; although the quit rate at 1 month was relatively modest (15.71 %).

Cigarette dependence predicted cessation at 1 month but not at 6 months. Like motivation to quit, this is indicative that cigarette dependence is not a static process. Thus not surprisingly, it predicted early success but as the smoker navigates through his/her slow transition towards a vaper's identity, s/he may become less dependent on cigarettes as dependence is transferred over to e-cigs. This is reflected in reports of several studies suggesting reduced and lower cigarette dependence in experienced e-cigarette users in comparison to smokers (Etter & Eissenberg, 2015; Farsalinos, Romagna, Tsiapras, Kyrzopoulos, & Voudris, 2013a; Foulds et al., 2015). At this point, the effectiveness of the device for delivering nicotine and alleviating craving becomes

more important for maintaining cessation. Thus, because e-cigarettes differ from other cessation aids and quitting cold turkey (with very low or no abuse liability), the usefulness of cigarette dependence as a predictor of cessation is unclear.

Limitations

Due to several limitations, the current findings must be interpreted with caution. The cigalike group suffered a large drop-out rate which affected the sample size and gave rise to issues in the statistical analyses. Variables such as nicotine concentrations had to be re-categorized to account for the missing data. The small sample size along with the gender imbalance at baseline (62.9% female) may constitute a further sampling bias since gender differences are well documented in the tobacco (Perkins et al., 1999) as well as in the e-cigarette literature (Jorenby et al., 2017).

In addition, the data relies on self-report and due to limited resources, cessation was not always biologically verified, (as it was not always possible to collect CO). More frequent face-to-face follow-ups would have allowed better monitoring of daily e-cigarette use behaviours including greater understanding of the interactions between device characteristics and users' responses, which would have allowed for a better understanding of how e-cigarettes promote smoking reduction and cessation. Instead, participants were contacted every 2 months over the 6 month period which could have caused recall bias. That said, such approach (telephone contacts) is and continues to be standard practise in many large-scale surveys and has contributed greatly to the literature on predictors of cessation (Bauld et al., 2017; Maddison et al., 2010). Likewise, salivary cotinine was collected at baseline but could not always be collected at self-reported quit to assess nicotine exposure following smoking cessation. A further limitation may come from the lack of representation of lower education smokers,

however it must be noted that the university campus wherein the study took place strives as an inclusive institution made up of a large black and ethnic minority groups from varied socio-economic status, and this is reflected in the current sample. Lastly, given the lengthy process in the running of studies involving human participants (ethical approval, completion of data collection and so on) combined with the rapid evolving nature of this field of research (i.e. technological advancement), the current findings must be limited to the e-cigarette models assessed herein.

Summary of Chapter IV

Despite the above limitations, the current study is informative. In a sample of 70 e-cigarette naïve smokers willing to quit, predictors of smoking cessation were assessed at 1, 3 and 6 months after e-cigarette initiation. From the predictors i) device type and nicotine concentrations combined (as per baseline allocation), ii) nicotine concentrations at follow-up, iii) device type at follow up, iv) baseline craving reduction (measured during e-cigarette initiation), v) baseline motivation to quit and vi) cigarette dependence and vii) puff duration post 2 weeks of initiation, only cigarette dependence was a significant predictor of cessation at 1 month, followed by baseline craving reduction at 3 months and e-cigarette device used at 6 months follow-up. These findings suggest that lower cigarette dependent smokers were more likely to quit at 1 month, whilst those with higher craving reduction at baseline had greater odds of successfully quitting at 3 months. Participants using tanks at follow up were at greater odds of quitting smoking at 6 months compared to those using cigalikes. This is consistent with the literature and aligns well with the findings of Study 3. That higher nicotine concentrations and the use of tanks elicit greater craving reduction compared to lower nicotine concentrations and cigalikes respectively, provide a good explanation for

the greater probability of success in quitting using tanks over cigalikes, and reiterate the critical role of nicotine delivery in promoting sustained use (of e-cigarettes) and smoking reduction.

CHAPTER V

OVERVIEW OF RESEARCH FINDINGS: GENERAL DISCUSSION

Introduction: Summary of key findings

The current thesis aimed to shed light on the inter-relationships between e-cigarette puffing topography, nicotine concentrations and e-cigarette characteristics. Specific objectives were to: i) determine the effects of varying nicotine concentrations (high vs. low) in e-cigarettes on users' puffing topography, ii) explore the effects of device types (tanks vs. cigalikes) on users' puffing topography and smoking related effects and behaviours, iii) document how e-cigarette puffing topography evolves over time and differs in response to device types and nicotine concentrations and, iv) further the understanding of the complementary roles of subjective effects namely satisfaction, the alleviation of craving and withdrawal symptoms. Additional objectives were to explore v) the factors (including puffing topography) that best predict smoking cessation at 1, 3 and 6 months in a sample of 70 e-cigarette-naïve smokers using different device types and varied nicotine concentrations.

In the first main and pilot study (described in Chapter II), findings suggested that like smokers, experienced e-cigarette users will self-titrate, that is, respond to nicotine concentrations by changing their puffing patterns in order to regulate their blood nicotine to satisfactory/habitual levels, sufficient to alleviate craving and withdrawal symptoms. E-cigarette users in this study drew longer puffs and increased their puffing frequency when administered the low, compared to the high, nicotine concentrations. Following a 60 min ad lib use of 6 mg/mL nicotine concentration e-

liquid, titration was only partial, evidenced by marked differential plasma nicotine levels yet no differences in craving and withdrawal symptom alleviations between the low and high nicotine concentrations.

In Study 2, (Chapter III), 70 e-cigarette-naïve smokers increased their puff duration one week following initial use, seemingly to compensate for the less effective nicotine delivery of e-cigarettes relative to their tobacco cigarettes. Those using the cigalikes drew more frequent and longer puffs compared to those using tank devices. Furthermore, cigalikes and tanks both containing 18 mg/mL nicotine concentration did not differ in their ability to alleviate craving, whilst the 6 mg/mL tank performed the poorest. In contrast, regardless of the nicotine concentrations the tank model elicited greater levels of satisfaction compared to the cigalike. Encouragingly, regardless of the device type and nicotine concentrations, e-cigarettes helped reduce smoking and cigarette dependence in the first two weeks following initiation. However, smoking reduction plateaued between the first and second week.

Study 3 (Chapter IV) revealed cigarette dependence as the best predictor for smoking cessation at 1 month, the less dependent smokers were more likely to quit at 1 month. Those who reported greater craving relief at baseline following the 20-minute ad libitum vaping session were at greater odds to have quit at 3 months, whilst those reporting using a tank device at 6-month follow-up were more likely to have succeeded in their quit attempt.

Self-titration and nicotine delivery: Effects of e-liquid nicotine concentrations, device types and practise

The addictiveness of a drug directly relates to the time at which it is administered and the time for the reward to manifest in the brain; this may explain the direct relationship between nicotine dependence and the speed of nicotine delivery (de Wit et al., 1992; Henningfield and Keenan, 1993). Thus, nicotine delivery is a key

determinant in the appeal and effectiveness of e-cigarettes which in turn will influence their potential to be accepted and replace smoking.

A likely mechanism by which users exert control over this delivery is through altering their puffing patterns in order to obtain desired and maintain steady levels of nicotine. Although well documented in the tobacco literature, Study 1 is the first to provide direct evidence of self-titration in experienced e-cigarette users (Dawkins et al., 2016). Like smokers, e-cigarette users will alter their puffing frequency and duration in response to the nicotine exposure in an attempt to match habitual levels. Thus, compensatory puffing behaviours observed in Study 1 suggest that nicotine concentrations play a key role in influencing puffing topography, which in turn affects blood nicotine delivery, satisfaction and craving alleviation. The recent national survey citing the inability of e-cigarettes to reduce craving for tobacco cigarettes as one of the chief reasons for discontinuing use of the device (ASH, 2017), highlights the relevance of the findings of Study 2. In e-cigarette naïve smokers, higher nicotine concentrations (18 mg/mL) were more effective in relieving craving compared to lower ones (6 mg/mL) (see Chapter III).

The efficacy of obtaining satisfactory blood nicotine levels depends upon the way the e-cigarette is used and studies have shown that both are likely to improve with practise (Hajek et al., 2015). Although the nicotine delivery from e-cigarettes appears less effective in e-cigarette-naïve smokers, Study 2 suggests that this could be reversed with a week of practice. By learning to adjust their puffing patterns (increasing the duration of each puff), e-cigarette users can obtain satisfactory blood nicotine levels, withdrawal and craving alleviation (Dawkins & Corcoran, 2014; Dawkins, Turner, Hasna, & Soar, 2012; Etter & Bullen, 2011) and eventually succeed in substituting their tobacco smoking to e-cigarette use. Although nicotine exposure was not measured in

study 2, reflecting on their smoking reduction and craving alleviation, it is reasonable to argue that overall, participants were able to obtain satisfactory blood levels.

Another likely factor to influence nicotine delivery is the proficiency of the device used. As highlighted in chapter I, early studies reported that e-cigarettes could not deliver nicotine efficiently (Bullen et al., 2010; Eissenberg, 2010; Vansickel et al., 2010). In contrast, later studies in long term users (Dawkins & Corcoran, 2014) found that later tank models can be effective in increasing blood nicotine levels and alleviating craving to a greater extent than do cigalikes (Farsalinos et al., 2014a; Nides, Leischow, Bhatter, & Simmons, 2014; Vansickel & Eissenberg, 2013). In Study 2 although cigalikes reduced smoking, cigarette dependence and craving to the same extent than did the tanks, cigalikes were associated with reduced satisfaction at all time points. Given that dissatisfaction with e-cigarettes has been cited as one of the main reasons for discontinuing use (ASH, 2017; Simonavicious, 2017), these findings are important. Consistent with the literature, the findings here help explain the trend for users to transition to tank models. Additionally, Study 3 found that those using tanks at follow-up, were more likely to have succeeding in their quit attempts at 6 months compared to those using cigalikes, which reflects the larger drop-out rate in those using cigalikes, in study 3.

The effects of nicotine concentrations on puffing topography and nicotine delivery

Findings of Study 1 lend support to the proposition that puffing topography is a key determinant of nicotine delivery (Williams et al., 2011) with longer puff duration and shorter IPIs yielding more efficient nicotine delivery (Hajek et al., 2015). There was a near two-fold increase in the e-liquid consumption in the low compared with the high nicotine concentration (see Figure 2.6, Chapter II). This was coupled with a significant increase in puff duration and puff number in the 6 mg/mL compared to the 24 mg/mL nicotine concentrations condition. Nevertheless plasma nicotine levels

reached a much greater level under the 24 mg/mL compared to the 6 mg/mL (See Table 2.2 and Figures 2.2 and 2.3, Chapter II). The near doubling of liquid consumption combined with a more intensive puffing regimen suggest that participants may have felt the need to invest substantial effort in an attempt to raise their blood nicotine to satisfactory levels, although whether this behavioural mechanism is conscious is unclear; this could be a focus for future research. The time constraint (one hour) combined with the four-fold drop in nicotine availability may have caused a saturation effect and impeded their attempt to self-titrate successfully. This could be attributed to the fact that any constrained time period restricts the quantity of nicotine liquid that an individual is able to consume comfortably. A more prolonged ad lib session could have raised plasma nicotine to the same levels as those achieved in the high nicotine concentrations although whether this is achievable and the time course required remain unknown.

The one hour ad libitum use of the low nicotine concentrations (6 mg/mL) in Study 1, led to longer mean puff durations and markedly higher plasma nicotine levels compared to levels observed in naive users in other studies (Farsalinos et al., 2015). However, although slightly longer, the average puff duration in the high condition (24 mg/mL) observed is in good agreement with those previously reported in experienced users (Hua et al., 2011) using newer generation devices (Farsalinos et al., 2014). The differences in puff duration between naïve and experienced users have been previously reported (Farsalinos, Spyrou, Stefopoulos, et al., 2015; Farsalinos, Romagna, Tsiapras, Kyrzopoulos, & Voudris, 2013b; Fearon et al., 2017; Hiler, 2016a; Talih et al., 2014), and are a good reflection of the increased puff duration observed in e-cigarette-naïve smokers a week following e-cigarette initiation (as seen in Study 2, Chapter III). This adjustment in puffing characteristics may be attributed to a lower nicotine flux (that is

the pace to dosage ratio of the aerosol delivered) (Shihadeh & Eissenberg, 2015; Talih et al., 2015) in relation to tobacco cigarettes requiring an increase in puff duration. Thus, altogether, the adjustment seen in the sample of e-cigarette naïve users within this thesis (Study 2, Chapter III) strongly suggests an attempt to compensate for a slower and less efficient nicotine delivery. The significant decrease in the number of puffs over the two-week period seen in Study 2, which combined with a significant increase in puff duration, suggests a dramatic change in puffing behaviour. This appears to be characterised by a shift from a more erratic puffing regimen at initial use to longer and more paced puffs in subsequent sessions which may form part of the learning process required when transitioning from smoking to exclusive e-cigarette use.

In Study 2, mean puff duration at initial use are consistent with previous findings using e-cigarette naïve smokers (Behar et al., 2015; Farsalinos, Spyrou, et al., 2014; Lee et al., 2015; Talih et al., 2014) which suggests some degree of homogeneity in the puffing topography of smokers, but contrasts with the typical topography of experienced users (Farsalinos et al., 2013; 2014; 2015; Talih et al., 2015). Indeed, mean scores in puff duration (both conditions collapsed) found in Study 1 in experienced users were higher than those observed in the e-cigarette-naïve smokers in Study 2. These differences in puffing topography mirror the reduced ability of e-cigarette naïve users to obtain systemic raised blood nicotine levels, satisfaction and craving relief (Farsalinos, Spyrou, Stefopoulos, et al., 2015; Fearon et al., 2017); which affect nicotine delivery (Foulds et al., 2015). That shorter puff duration tend to be associated with poorer nicotine delivery profile is evidenced in previous studies in which lower plasma nicotine concentrations were found in e-cigarette naïve smokers who displayed lower puff duration of 2.3s compared to experienced users who displayed a mean puff duration of 3.5s (Farsalinos, Spyrou, Tsimopoulou, et al., 2015).

In another study comparing e-cigarette naïve smokers' puffing profiles to those of experienced users, Talih and colleagues (2015), found the 2-second duration profile to be associated with much lower nicotine delivery compared to longer puffing duration of 4s commonly exerted by experienced users. Similarly, following a 65 min ad lib vaping session, Farsalinos' group reported significantly greater levels of plasma nicotine levels in experienced users versus e-cigarette naïve smokers; this was mirrored by the difference in puff duration with a mean puff duration of 2.3 s in naïve e-cigarette smokers and 3.5 s in experienced users (Farsalinos, et al., 2015).

Consistent with previous studies, participants increased their puff duration after a week following initial use which subsequently plateaued at the final follow-up session 2 weeks post e-cigarette initiation (Study 2, Chapter III). This increase in puff duration is suggestive of an adjustment in puffing topography by naïve users over time which corroborates the self-titration theory at least in the short term insofar as the shorter puffs exerted at initial use may not have yielded satisfactory blood levels. This increase in puff duration is a key finding specifically as puff duration along with IPI have been reported as the most influential drivers of aerosol yield (Kośmider et al., 2016). Indeed, a week seems to be sufficient to observe an evolution in puffing topography and for e-cigarette naïve smokers to develop an awareness for the need to adjust their puffing from smoking to e-cigarette use. This is consistent with previous findings in which a sample of 20 smokers who were introduced to a 18 mg/mL cigalike increased their puff duration from 2.2 s to 3.1 s after a week of initial use (Lee et al., 2015). The increase in puff duration herein is in line with the early suggestion that there is a learning process required for an effective use of e-cigarettes (McQueen, Tower & Summer, 2011). Similarly, others have found that the efficiency of obtaining

satisfactory blood nicotine levels depends upon the way the e-cigarette is used and is likely to improve with practise (Hajek et al., 2015).

In Study 2, there was a significant decrease in the number of puffs over the two-week period which, combined with a significant increase in puff duration strongly suggest a dramatic change in puffing behaviour characterised by a shift to slower, longer more paced puffs from a more erratic puffing style at initial use. Like in the case of puff duration, there was no difference between time points 1 and 2 suggesting a plateau effect after a week of use. There was no significant difference between the 6 and 18 mg/mL nicotine concentrations tanks or the cigalike, although previous studies suggest that puff numbers have no or little effect on nicotine delivery (Kośmider et al., 2016; Spindle, 2015). The present findings are in line with the findings relating to puff duration thus are informative. Altogether, Studies 1 and 2 provide evidence which lends support to the notion that like smokers, e-cigarette users self-titrate to regulate their nicotine intake to desired levels.

Conversely, puff duration an important factor in nicotine delivery as suggested in Study 1 and 2, was not a significant predictor at 3-month follow-up and was removed from the model at 1 and 6 months, in study 3. Findings of Study 2 suggested adjusting puff duration over time as part of the learning process to help in reducing smoking and for a successful transition to e-cigarettes use. Study 1 found that puffing duration to be a key differential factor that partly contributed to plasma nicotine levels. Thus, given that numerous studies have highlighted the importance of puff duration to optimise nicotine delivery (Dawkins & Corcoran, 2014; Dawkins et al., 2016; Farsalinos, Spyrou, et al., 2014; Farsalinos, Spyrou, Stefopoulos, et al., 2015; Hajek et al., 2015), and increase satisfaction (Hajek et al., 2017), which may help promote sustained use of the e-cigarette in replacement of smoking, the poor predictive ability of puff duration in

study 3 was unexpected. This may be due to the difficulty in distinguishing puff duration from other variables (i.e. nicotine concentrations, device characteristics) which when combined, exert a greater influence on nicotine delivery as discussed in previous chapters.

Plasma nicotine levels

A number of studies have found that smokers are usually successful in regulating their blood nicotine to satisfactory levels in response to higher nicotine content cigarettes (that is they are able to down-regulate and lower their blood nicotine to habitual non-aversive levels) (Russell, 1980; Russell, Sutton, Iyer, Feyerabend, & Vesey, 1982; Sutton et al., 1982). However, when presented with lower nicotine content cigarettes, raising blood nicotine to habitual levels is more challenging and rarely achieved (Ashton et al., 1979; Russell et al., 1975). This was also the case in experienced e-cigarette users in Study 1. Despite the more intensive puffing regimen exerted in the low nicotine concentrations condition, plasma nicotine levels did not reach levels achieved in the high nicotine concentrations; though an alternative and plausible explanation may be that levels achieved in the high nicotine concentrations were unusually high in respect to participants' habitual levels and they were unsuccessful in their attempts to achieve downward titration. However, both conditions, the 6 and 24 mg/mL nicotine concentrations, helped alleviate craving and withdrawal symptoms to the same extent which suggests that the partial titration achieved was sufficient from a subjective perspective. Thus, although it could be tempting to argue that these findings undermine the primacy role played by nicotine delivery in the reinforcement of smoking, the explicit increase in puffing intensity certainly suggests otherwise. Besides, a more plausible explanation would be that the lower plasma nicotine levels are the result of a ceiling effect and that the four-fold drop

in nicotine concentrations was too great to yield equal levels in plasma nicotine. It is unclear whether a more mid-range nicotine concentration (e.g. 12 mg/mL as opposed to 6) would lessen the gap in plasma nicotine levels and result in complete titration; this could be a focus for future research.

Thanks to the rapid advancement in technology, recent findings suggest that the shape of the pharmacokinetic curve of the nicotine delivery of e-cigarettes is increasingly approaching that of combustible cigarettes (St Helen et al., 2015); findings of the present study certainly seem to support this trend. Plasma nicotine levels achieved in the sample of experienced users in Study 1 were remarkably high compared to those in previous reports and exceed levels observed in tobacco smoking (Russell et al., 1980; Russell, Wilson, Patel, Feyerabend, & Cole, 1975) and in previous e-cigarette studies in which similar (Farsalinos et al., 2014; Farsalinos et al., 2015; Yan & D'Ruiz, 2015) and higher nicotine concentrations (Hajek et al., 2017; Lopez et al., 2016; Ramôa et al., 2015) were employed. In a relatively recent study the smoking of a tobacco cigarette in a five-minute puffing period (10 puffs, 30 seconds apart) resulted in Cmax of 13.4 ng/mL (Fearon et al., 2017), which seems negligeable compared to levels achieved by some individuals in study 1 (means ranged from 5 to 110.12 ng/mL); although it is worth noting that earlier titration studies in smokers demonstrated much higher Cmax compared to more recent studies (Russell et al., 1975). Such discrepancies may be attributed to the differences in puffing protocols between studies. In particular, in order to control for baseline nicotine delivery, previous studies imposed a standardised puffing regimen prior to the ad libitum session (Lopez et al., 2016; Ramôa et al., 2015). Although such approach allows for greater control and internal validity on one hand, on the other, it may affect participants' responses and bias the results by imposing unrealistic puffing patterns. Alternatively, in study 1, such high plasma

nicotine levels may be due to the one-hour ad libitum vaping design combined with the requirement to abstain for 10 to 12 hours and a self-selected sample of experienced users with high baseline salivary cotinine levels equal or exceeding 100 ng/mL nicotine concentration. Such prolonged periods of abstinence could have evoked or exacerbated the increased puffing exertion to counter depleted blood nicotine levels and produce rapid elevation (Sutton et al., 1982).

Further evidence of compensation could be found in the per interval puffing topography data in Study 1. In the high condition, the increase in plasma nicotine boost was accompanied by analogous decrease in puffing. The number of puffs taken from baseline to 30 minutes was significantly lower than the number of puffs taken during the remaining 30 minutes, and in some cases, the trajectory of the nicotine boost went downwards after the 30 minutes, suggesting an attempt to down-regulate to habitual comfortable plasma nicotine levels. Already the first 10 minutes were marked by a significant difference in puff duration, with a higher overall mean in the low compared to the high condition. Given the rapid 10 to 20 seconds period in which nicotine typically reaches the brain (Henningfield et al., 1993) and that the greatest amount of nicotine is transported in the first two minutes of e-cigarettes use (Rüther et al., 2017), this first 10 minutes would have been amply sufficient to allow for possible aversive effects to be experienced, triggering a need to reduce the nicotine intake and down-titrate. This puffing behaviour is reminiscent of cigarette smoking. Smokers are able to regulate their smoke and nicotine intake on a puff-by-puff basis by exerting a more intensive puffing regimen on the first puffs of a single cigarette subsequently decreasing the intensity of their puffing as the cigarette is smoked (Collins et al., 2010). Similarly, the results of Study 1 suggest that e-cigarette users will adjust and decrease their puffing in response to nicotine overload or saturation. In fact, although, this did not differ

statistically, mean scores on 'vomiting' were greater in the high nicotine condition compared to the low condition, suggesting that aversive effects may have occurred in the high condition more so than in the low nicotine concentration condition.

Individual differences

Puffing topography and plasma nicotine levels varied widely amongst participants (in Study 1, Chapter II) however, like in the smoking population (Gust et al., 1983) and as seen in previous studies (Behar et al., 2015), puffing patterns were consistent across both conditions (6 and 24 mg/mL nicotine concentrations) within participants. As indicated by the standard deviations (study 1), individual variabilities were more pronounced in the low nicotine concentration condition with the lowest individual mean puff number of 14 and the highest 131. Mean volume consumed ranged from .45 to 2.38 mL and mean puff duration ranged from 3.20 to 7.31 s, in some cases exceeding 10 s. Such extreme long puffs are not uncommon in e-cigarette use, Hua and colleagues observed puff duration of 8 s in experienced e-cigarette users (Hua et al., 2013). As was the case for most of the sample, participant 6 increased his puffing frequency by almost double from 63 to 131 puffs and his puff duration from 3.94 to 5.17 s and his liquid consumption from .88 to 2.38 mL. The lack of statistical significance in correlational analysis between puffing topography and plasma cotinine levels suggests that inhalation depth may have differed widely between participants and influenced nicotine absorption. The individual variations are likely to be due to the small size of the sample and may also be a reflection of the variations in nicotine dependence within the sample with baseline cotinine levels varying from 134.2 to 890.6 ng/mL. Nonetheless, these wide individual differences seen here are consistent with variations observed in tobacco smoking (Hammond et al., 2005) with plasma nicotine levels varying from 5 to 70 ng/mL (Russell, 1980), but also in previous studies in e-cigarette use (Farsalinos et al., 2015; Yan & D'Ruiz, 2015).

Participants' puffing patterns seemed to fluctuate in the same fashion across vaping sequence and conditions. In the low condition, mean puff number and duration accompanied an increase in nicotine boost in most cases whereas in the high condition, increases in nicotine boost were generally followed by downward trends in puffing patterns. . Two participants (P 6 and P 10) seemed to achieve complete self-titration raising their plasma nicotine in the low condition to similar levels achieved in the high condition, which suggests that it is possible for some e-cigarette users to self-titrate by adjusting their puffing patterns in order to obtain desired and satisfactory plasma nicotine levels. Nonetheless this was only achieved by almost a two-fold increase in puff numbers, a significant increase in puff duration and more than two-fold increase in e-liquid consumption, which altogether may be associated with long term adverse health consequences (Dawkins et al., 2018; Kośmider, Kimber, Kurek, Corcoran, & Dawkins, 2017).

Likewise, individual differences were observed in Study 2. Akin to the large standard deviations found between conditions, the findings of Study 2 echo the inter-variations in e-cigarette puffing topography reminiscent of those seen in previous studies (Behar et al., 2015; Hajek et al., 2015) but also in smoking behaviours (Hammond et al., 2005). Importantly, these large individual variations may provide some explanation for the non-statistically significant differences in puffing topography between the Tank 6 and Tank 18 (refer to the section on *E-cigarette type, nicotine concentrations* in the discussion in Chapter III).

Relationships between nicotine metabolism, nicotine intake and puffing topography

In Study 1, baseline cotinine levels and trans-3-hydroxy-cotinine to cotinine ratio (OH-cot/cotinine) were measured to confirm that any changes in puffing topography were not due to nicotine metabolism alone but largely to the availibility of

nicotine. In the sample of experienced e-cigarette users means in OH-cot/cotinine ratio were within the common range seen amongst smokers (Dempsey et al., 2004), with wide inter-individual variation, thus would have little influence on puffing patterns. Puff number and duration were not associated with baseline cotinine levels, 3-hydroxy cotinine or OH-cot/cotinine ratio. Neither did baseline cotinine, OH-cot or OH-cot/cotinine correlate with plasma nicotine boost under high or low conditions at any time point. This suggests that phenotypic status did not affect puffing behaviours. Besides, whilst nicotine metabolism has been shown to influence smoking and nicotine intake (Scherer & Lee, 2014), the one hour ad libitum vaping session probably did not allow sufficient time for the metabolism of nicotine to exert any effect on puffing behaviours.

The effects of device types on puffing topography and nicotine delivery

The average puff duration using the 6 mg/mL by experienced users in Study 1 were longer than those reported for cigalikes in other studies (Behar et al., 2015) and combustible cigarettes (Hua et al., 2013); whilst puff duration associated with the cigalikes in Study 2 exceeded that associated with the tanks. That previous studies found longer puff duration in cigalikes to be associated with greater discomfort compared to slightly later tank models (eGo e-cigarette) (Dawkins, Kimber, Puwanesarasa, & Soar, 2015) reinforces the notion that rather than a nicotine concentrations effect, here the longer puff duration seen in cigalikes may have been driven by device characteristics namely, a more resistant draw mechanism. However, of worthy mention, aside from nicotine concentration and device types, other factors such as the e-liquid ingredients and parameters applied during use have been found to influence puffing patterns and nicotine yield. For instance, liquids containing a combination of propylene glycol (PG) and vegetable glycerine (VG) elicit lower and shallower puffing frequency but higher plasma nicotine levels compared to VG-based e-

liquid due to their chemical properties (Yan & D'Ruiz, 2015). In the same light, flavours have also been found to affect puffing topography (Litt, Duffy, & Oncken, 2016) and nicotine delivery (St.Helen, Shahid, Chu, & Benowitz, 2018). In this experimental work, this was controlled for by ensuring in each study, all products (including atomisers) were purchased in batches, labelled with the same PG/VG ratio, and same flavours within and across conditions and participants, with the exception of the use of two cigalikes brands due to leakage issues (see methods section in Study 2, Chapter III). Nonetheless, variance in device parameters could not be fully controlled as participants were instructed to use the tank within the recommended range of 3.3 to 4 volts; such variances may have affected nicotine delivery.

In Study 2, participants in the Cigalike condition took longer puffs compared to both those using the Tank 6 and Tank 18, which is suggestive of a device type, rather than a nicotine concentration effect. Against expectations and in contrast with the findings in Study 1, there was no significant difference between Tank 18 and Tank 6 at any time point suggesting some degree of homogeneity in the puffing topography of participants in both tank conditions compared with the cigalike, which may be driven by the fact that both are the same model with the same mechanism suction, thus same aerosol density emission; although such comparison between Study 1 and 2 is problematic since participants in Study 1 were experienced users whilst those in Study 2 were naïve users. Given the three-fold drop in concentrations (18 to 6 mg/mL) that Tank 6 did not differ from Tank 18 is surprising; one might have expected significantly longer puff duration in the Tank 6. Nevertheless, although failing to reach statistical significance, mean scores in puff numbers and duration were higher in the Tank 6 in comparison with the Tank 18 across all sessions. This concurs with previous studies in which lower nicotine concentrations (Farsalinos, Romagna, Tsiapras, Kyrzopoulos, &

Voudris, 2013a; Hajek et al., 2017) and shorter puff duration were found to be associated with poorer nicotine delivery (Farsalinos, Spyrou, Tsimopoulou, et al., 2015; Hiler, 2016b).

In Study 2, the inter-puff intervals (IPI) increased from Baseline to Time 1 and plateaued at Time 2. In line with the longer puff duration in the Cigalike, IPI were greater in the Tank 18 in comparison; and although this was not statistically significant, mean scores were greater in Tank 18 compared to the Tank 6. This suggests that the participants in the higher nicotine concentration tank condition exerted a longer pause between puffs, consistent with the literature suggesting that nicotine concentrations relate directly to nicotine delivery (Lopez et al., 2016) and dictate puffing topography. Likewise, the puffing topography derived from the cigalikes reiterates the proposition of a more erratic or intense puffing profile associated with cigalikes compared to the tank systems and are suggestive of an interweaved relationship between nicotine delivery and puffing topography.

In Study 2, participants took an average of 39.51 puffs in the cigalike condition across the 3 sessions together. This is in good agreement with a previous study in which 20 experienced e-cigarette users took an average of more than 20 puffs in a 10-minute ad lib session (Behar et al., 2015) which, compares well with the mean of 39.51 in the 20-minute session. Indeed, the likening of this observed adjustment in puffing topography in an attempt to compensate for the less efficient nicotine delivery compared to tobacco cigarettes is further evidenced in a previous study in which 50 puffs from a 24mg/mL containing cigalike were required to raise blood nicotine to levels analogous of those obtained after the smoking of one combustible cigarette (which consisted of approximately 10 puffs) (Yan & D'Ruiz, 2015). That cigalikes are associated with poorer nicotine delivery is also documented in pharmacokinetic studies in which modest

increases in plasma nicotine were obtained in naïve and experienced users compared to combustibles (Fearon et al., 2017).

An increasing number of studies suggest the superiority of tanks over cigalikes in alleviating craving (Chen et al., 2016; Hajek et al., 2018; Rüther et al., 2017). The results of Study 2 suggesting no differences between tanks and cigalikes in craving alleviation contradict previous findings. An online cross-sectional survey of 374 respondents, found that tanks systems and higher nicotine concentrations were found to be associated with greater craving relief (Etter, 2015) which suggests that the greater capability of the tank models to alleviate craving compared to cigalikes could be attributed to their nicotine delivery efficiency. Several experimental lab-based studies assessing the nicotine delivery of e-cigarette models have found that tanks systematically deliver higher nicotine levels compared to cigalikes (Farsalinos et al., 2014; Hajek et al., 2018; Rüther et al., 2017), even at levels that equate to those reached after the smoking of a cigarette (Dawkins et al., 2016; Ramôa et al., 2015; Rüther et al., 2017; Wagener et al., 2016). The reinforcing value of nicotine in smoking (Benowitz, 2010; Henningfield & Keenan, 1993), to alleviate craving and provide satisfaction (Etter, 2015; Dawkins, Kimber et al., 2015), may be the route by which tanks are better able to facilitate sustained use of the product and provide an effective replacement to smoking (Hitchman et al., 2015; Yingst et al., 2015).

In Study 2, the unexpected lack of differences between Tank 18 and cigalikes in craving reduction scores despite the arguably different nicotine delivery systems may suggest a reduced role for craving in dictating puffing topography. This has been suggested in previous studies. In 2 separate sessions, 24 participants were randomised to 5 e-cigarettes with varying e-liquid formulation (16 and 24 mg/mL and differing PG/VG ratio) and a combustible cigarette; plasma nicotine levels and craving (urge to

smoke) were measured during a 30-min standardised protocol (50 puffs, 30 s IPI) followed by a 1 h ad lib use. Strikingly, following completion of both sessions, the differences in nicotine exposure (23% to 53% greater from the tobacco cigarette compared to the e-cigarettes) and mean scores in nicotine plasma concentrations were not reflective of the homogeneity observed in craving reduction scores (10% greater in the tobacco cigarette in relation to the e-cigarettes following the ad lib period). Given the differences in nicotine exposure, one would expect the differences in craving to be of magnitude greater than the values observed. Thus, as per the authors' comments, although higher nicotine concentrations and the presence of PG influenced means plasma nicotine concentrations, craving did not seem to be impacted. Therefore, it is reasonable to conclude that although an important determinant of smoking behaviours, the alleviation of craving is not always proportional with nicotine delivery. This is in line with the findings of Study 1. Compensatory puffing failed to raise plasma nicotine levels but was sufficient to yield craving alleviation in the 6 mg/mL nicotine concentrations condition; this further suggests that craving would make a poor indicator of efficient or poor nicotine delivery. Alternatively, the aforementioned findings reiterate that non-pharmacological factors such as visual stimuli (e.g. aerosol visibility) play a significant role in the alleviation of craving as suggested in previous studies (Dawkins, Munafò, et al., 2015; Dawkins et al., 2012), and may partly contribute to the appeal for e-cigarettes.

The findings of Study 2 that cigalikes were associated with greater puff duration but lower satisfaction provide novel evidence for the literature and align with previous findings that cigalikes tend to be associated with a less efficient nicotine delivery compared to tank systems (Farsalinos, Spyrou, Tsimopoulou, Stefopoulos, Romagna, & Voudris, 2014). This is consistent with a previous study which compared plasma

nicotine concentrations between device types and a combustible cigarette. In the first five minutes, whilst cigalikes failed to yield comparable increase in nicotine in relation to the tank and the combustible cigarette, the tank model successfully reduced craving to the same magnitude as did the smoking of the combustible cigarette (Rüther et al., 2017). This reiterates tank models' superiority in providing satisfactory nicotine levels to its user compared to cigalikes. The inability of cigalikes to provide satisfaction levels that equal those obtained with tanks may be due to differences in device components' design (Gillman et al., 2016), which may be responsible for the stronger vacuum mechanism of cigalikes compared to tanks. Altogether, these findings highlight the importance of nicotine delivery and align well with the notion that more intensive puffing intensity exerted in cigalikes compared to tanks may be attributed to a more resistant airflow-activated system (Williams, Ghai, & Talbot, 2015) which constrains the nicotine e-liquid delivery and affect sensory factors.

In Study 2 although cigalikes reduced smoking, cigarette dependence and craving to the same extent as tanks, cigalikes were associated with reduced satisfaction at all time points. The latter could have contributed to the larger drop-out rate in those using cigalikes compared to other conditions. This in concordance with the findings of Study 3 indicating that those using tanks at follow-up, were more likely to have succeeded in their quit attempts at 6 months compared to those using cigalikes. Given that dissatisfaction with e-cigarettes has been cited as one of the main reason for discontinuing use (ASH, 2017; Simonavicious, 2017), these findings are important and reiterate the need for better nicotine delivery devices that provide satisfaction. Whilst nicotine remains central in facilitating the transition to exclusive e-cigarette use, it appears that for some, the rituals of smoking are of equal, or even greater importance compared to nicotine delivery. This reiterates the heterogeneity of e-cigarette use, and

users, and the need to maintain a market that offers a wide range of products for those seeking an experience that closely approximates smoking and for those requiring effective nicotine delivery. In line with this proposition, recently, there has been a dramatic surge of a novel vaporiser 'JUUL' (developed in the US); its characteristics, a relatively slim shape, small size and non-rechargeable pod (Willett et al., 2018) do not depart from that of a cigalike. Importantly it can be mounted with pods containing nicotine concentrations that largely exceed the 20 mg/mL cap (Willett et al., 2018). The sudden increase in popularity of this novel device in the US is indicative of a continued need for improved nicotine delivery products which do not diverge greatly from the resemblance of tobacco cigarettes.

Predictors of smoking cessation: the interweaved relationship between device type, nicotine concentration, puffing behaviour and subjective effects

Studies suggest multiple mechanisms by which e-cigarettes help promote smoking reduction and cessation. These may be by i) helping to alleviate craving for tobacco cigarettes and other adverse abstinence related symptoms (Adriaens et al., 2014; Dawkins, Kimber, et al., 2015; Etter, 2015; Hajek, Przulj, Phillips-Waller, Anderson, & McRobbie, 2018; Rüther et al., 2017), ii) delivering nicotine efficiently and in ways that approximate that of combustible cigarettes (Farsalinos, Spyrou, Tsimopoulou, et al., 2015; Hajek et al., 2015, 2017; Lechner, Meier, et al., 2015; Wagener et al., 2016), iii) providing novel features such as device customisability (Vandrevala et al., 2017) or enhanced sensory factors (Farsalinos, Romagna, Tsiapras, Kyrzopoulos, Spyrou, et al., 2013; Ward et al., 2018), and iv) by allowing lapses whilst minimising risks of complete relapse (Notley et al., 2017).

In Study 3, in a sample of 70 naïve e-cigarette smokers, those reporting using a tank device at the time of follow-up were more likely to have quit at 6 months compared to those using a cigalike, regardless of the nicotine concentrations. As

described in Chapter IV, these findings are consistent with previous studies suggesting that tank users are more likely to report craving reduction and complete cessation (Chen et al., 2016; Hitchman et al., 2015; Shiffman et al., 2018). Past studies point to an important factor which has often been neglected in many past correlational studies exploring the relationship between e-cigarettes and subsequent smoking: frequency or heaviness of e-cigarette use (Leventhal et al., 2015). In Study 2, self-reported puffing patterns did not significantly differ between conditions (cigalikes, tank 18 and tank 6), nor were there any significant differences over time. These findings contradict those obtained during the ad libitum lab-based sessions (Study 1), although participants' puffing patterns changed over time and differed between conditions, these did not extend outside of the lab. In Study 3, participants were asked to recall the volume of e-liquid they consume in mL per day for those using tanks and cartridges per week in the cigalike group. However, many had difficulties making such recalls (in addition there were some reports of spillages and refilling with third parties' liquid at times) which lessened the reliability of the variable volume consumed. Such challenges in the reporting of quantity consumed is echoed in the literature (Cooper, Harrell, & Perry, 2016) and suggests that the lack of changes over time or differences in self-report puffing patterns in Study 2 outlined previously may lack accuracy and reliability.

The findings that tanks were associated with complete cessation in relation to cigalikes in Study 3, are consistent with cross-sectional surveys which suggest that cigalike users are less likely to have quit (Farsalinos, Romagna, Tsiapras, Kyrzopoulos, Voudris, 2014). This is echoed in longitudinal studies in which the first purchase of tanks led to just over 40% of cessation rate, whilst transition from standard basic tanks to more sophisticated modifiable tanks increased from 8.5% to 18.4% at 12 months follow-up (Polosa et al., 2015), suggesting a need for better nicotine delivery products.

Unexpectedly, nicotine concentration did not predict cessation. This is at odds with the findings reported in Study 2 where craving relief was greater with a higher nicotine concentration e-liquid, suggesting an important role for nicotine concentrations. Given the predictive ability of craving on relapse to smoking (Zhou et al., 2009), and that higher nicotine concentration is a positive correlate of craving relief for cigarettes (Etter, 2015), one would have expected nicotine concentration to predict cessation. On the other hand, although studies suggest that higher nicotine concentrations better facilitate the complete switch to e-cigarettes, the gradual decrease in nicotine concentrations seen in experienced e-cigarette users over time (Farsalinos, Romagna, Tsiapras, Kyrzopoulos, & Voudris, 2013a; Lechner, Tackett, et al., 2015b; Polosa et al., 2015) is suggestive of a lesser important role of nicotine concentrations as a unique variable at least over time. Counterintuitively, reducing nicotine concentrations in e-liquid do not necessarily lead to a reduction in nicotine exposure. In fact, whilst experienced users gradually reduce their nicotine concentrations in liquid over time, they do by increasing their nicotine liquid consumption, which may form part of the mechanism by which they maintain their cotinine to steady levels (Etter, 2016; Soar, Kimber, McRobbie, & Dawkins, 2018). The increase in nicotine liquid consumption and constant cotinine levels suggest that users self-titrate and adjust their puffing patterns in response to changes in nicotine exposure; and they do so to maintain satisfactory nicotine levels in their blood. Thus, although nicotine concentrations is critical to nicotine delivery, its interweaved relationship with device characteristics and puffing patterns makes it difficult to treat it in isolation.

Effects of e-cigarettes use on craving, withdrawal and subjective effects

Many studies have suggested that e-cigarettes can be effective at alleviating craving and withdrawal symptoms related to smoking (Bullen et al., 2010; Dawkins &

Corcoran, 2014; Dawkins et al., 2015; Dawkins, Turner, & Crowe, 2013; Dawkins et al., 2012; Etter, 2015; Foulds et al., 2015; Lechner, Meier, et al., 2015; Spindle et al., 2015; Vansickel, Weaver, & Eissenberg, 2012; Vansickel et al., 2010). That nicotine intake has a direct and linear effect on craving reduction has been suggested in previous clinical studies which found IPI to be inversely related to serum nicotine and cotinine levels as well as craving reduction scores (Williams et al., 2011). This suggests that puffing topography and nicotine delivery influence craving reduction, and highlights the importance of efficient nicotine delivery given the strong negative reinforcement potential of craving on smoking and vulnerability to relapse (Adriaens, Van Gucht, & Baeyens, 2018; Pokhrel et al., 2015). Crucially, research suggests that the inability of e-cigarettes to deal with craving is one of the chief reasons for discontinued use (ASH, 2017). It is important therefore to further the understanding of the influence of nicotine concentration and puffing patterns on craving alleviation in order to increase the product acceptability to smokers and capitalise on the potential of e-cigarettes.

In Study 1, although plasma nicotine levels in the low nicotine concentration condition failed to equate those in the high nicotine concentration, such attempts to self-titrate appeared to be effective in alleviating subjective urge to vape and withdrawal discomfort. The e-cigarette with both high and low nicotine concentrations were able to raise plasma nicotine to satisfactory levels, and were sufficient to satisfy craving and alleviate withdrawal symptoms. These findings are inconsistent with previous studies suggesting that higher plasma nicotine levels are associated with greater craving reduction and higher '*satisfaction'* (Farsalinos, Spyrou, Tsimopoulou et al., 2014). That craving was reduced to the same magnitude in the low compared to the high condition may be largely due to the more intensive puffing regimen exerted in the low nicotine concentrations. Thus, the use of a standard puffing regimen by Farsalinos et al' study

(2014) unlike Study 1 may explain these conflicting results. Moreover, it is worth noting that the small sample size could be a contributing factor for the lack of statistically significant differences between conditions since the study was powered to detect an effect of puffing topography not subjective effects. Nonetheless, that e-cigarettes can reduce craving to the same extent than tobacco cigarettes has been observed in previous studies also, in which five minutes of ad libitum e-cigarette use was sufficient to alleviate craving symptoms to the same levels obtained after the smoking of a tobacco cigarette (Adriaens et al., 2014). In a more recent experiment, ad libitum use of 36mg/mL nicotine containing e-cigarettes led to greater craving reduction compared to no nicotine e-cigarettes which strongly suggests a nicotine concentrations effect (Van Heel et al., 2017). Interestingly, in the same study, conditioning cues, such as hand to mouth gestures, elicited a significant craving reduction regardless of the presence of nicotine. This suggests that whilst nicotine has reinforcement value, conditioning cues that form part of the rituals of smoking, can also be reinforcing; this may explain the appeal of e-cigarettes, particularly tanks, which are associated with effective nicotine delivery.

In Study 2, following the ad libitum vaping baseline session, craving reduction scores were greater in the Tank 18 compared to the Tank 6. Craving reduction scores in the cigalike condition fell mid-way between Tank 18 and 6 but did not differ statistically from either. Across all 3 sessions (baseline, 1 week, 2 weeks) and consistent with the baseline session, participants in the Tank 18 tended to report greater craving reduction than those in the Tank 6; which suggests a nicotine concentration rather than a device type effect and that higher nicotine concentrations were more effective at reducing craving. The nicotine concentration effect in craving scores is in

line with previous studies which suggests that greater craving reduction is associated with higher nicotine concentrations (Etter, 2015).

Conversely, in the same study, that cigalikes did not differ from Tank 18 conflict with previous studies which found that newer devices (Farsalinos, Spyrou, et al., 2014) and higher nicotine concentrations are more effective at reducing craving than cigalikes, and are capable of inducing greater satisfaction and nicotine dependence (Foulds et al., 2015). Given that nicotine exposure was not measured in Study 2, such discrepancies between findings may be attributed to the variance in nicotine delivery of cigalikes, since chemical analyses have demonstrated that this may vary widely within e-cigarette categories (Goniewicz, Kuma, Gawron, Knysak, & Kosmider, 2013). Furthermore, others found that cigalikes equally reduced craving in e-cigarette naïve smokers to the same magnitude as did a second penlike generation over the short term and concluded that the visual similarities to a cigarette may have been partly responsible (Dawkins, Kimber, Puwanesarasa, & Soar, 2015). In Study 1, that the lower nicotine concentration condition was associated with lower scores on craving agrees well with previous studies which found that nicotine-containing e-cigarettes elicit greater levels of craving reduction compared to placebo e-cigarettes (Dawkins, Turner, Hasna, & Soar, 2012), although placebo e-cigarettes have shown to reduce craving in smokers who were told they contained nicotine (Copp et al., 2015); thus since the experiment was not blinded whether knowledge of the nicotine concentrations administered influenced responses is unclear.

An alternative explanation may be that craving alleviation may also be influenced by non-pharmacological cues such as the visual similarity with a tobacco cigarette. In a lab experiment, a white cigalike (visually similar to a cigarette) elicited greater craving reduction in naïve e-cigarette smokers compared to those using the same cigalike in a

red colour (visually dissimilar to a cigarette) (Dawkins, Munafò, Christoforou, Olumegbon, & Soar, 2015); this is in good agreement with earlier e-cigarette studies which found non-nicotine e-cigarettes to be associated with craving alleviation (Dawkins et al., 2012) as well as tobacco studies which found that nicotine's influence on negative and positive affect is negligible (Perkins, Karelitz, Conklin, et al., 2010). This evidence suggests that these sensorimotor cues may be important and have reinforcing properties, at least in naive e-cigarette smokers. However, findings from the diary data in study 2, are inconsistent with the lab-based findings and suggest that both Tank 18 and Tank 6 were associated with greater scores on craving reduction compared to the cigalike. The fact that these self-recorded data were noted at the end of each day across 7 days in settings where the experimenter had no control over accuracy, may explain the conflicting findings, but also draws attention to the fluctuating nature of e-cigarettes impact on smoking related symptoms such as craving. On the other hand, that the data were obtained outside of the contrived lab environment, in the 'real world' lends more credibility to the findings based on the diary. The real-world data better reflects the chronic aspect (seven time points over a week) rather than acute aspect of the experience with the device. The fact that the cigalikes were just as effective for craving reduction in the lab may represent non-pharmacological, short-term cue effects which over time may dissipate whilst other factors (such as the ease of drawing the aerosol, smoothness and tastes of the aerosol) become more important.

The findings of Study 3 are novel; to date, no studies have explored the ability of craving reduction to predict cessation in a sample of smokers who have been newly introduced to e-cigarette. Findings of Study 3 suggest that the first encounter with e-cigarettes may have some predictive value for smoking cessation. Whilst, the predictive ability of craving reduction fell short of significance at 6-months and was not significant

at 1 month, craving reduction experienced at baseline was found to be a significant predictor of cessation at 3 months follow-up. Those who experienced greater craving reduction following e-cigarette use at baseline were more likely to quit at 3 months. That higher nicotine concentrations and the use of tanks elicit greater craving reduction compared to lower nicotine concentrations and cigalikes respectively (see Study 2, Chapter III), provides a good explanation for the greater probability of success in quitting using tanks over cigalikes in Study 3. These findings are important and suggest that measures of craving reduction at e-cigarettes initiation have some predictive utility that can be fostered to inform smoking cessation programmes and vape shops (for example, initial craving reduction can be monitored in order to tailor devices to smokers). Future research could focus on disentangling the effects of craving reduction, the factors and mechanisms that mediate smoking cessation, and to what extent initial craving reduction can predict cessation.

In Study 1, in relation to withdrawal symptoms, there were no statistically significant difference between High (24 mg/mL) and Low (6 mg/mL) nicotine concentrations; '*depressed mood*', '*irritability*', '*anxiety*', '*drowsiness*', '*restlessness*', '*hunger*' and '*inability to concentrate*'. There were no statistically significant differences in subjective effects also, although means in hit and satisfaction were numerically higher in the high compared with the low condition. These results reiterate that although self-titration was incomplete with significantly lower plasma levels in the low condition, compensatory puffing was sufficient to achieve subjective satisfaction at least in the short term. It is unclear whether a longer ad libitum session or a larger sample could have yielded differences between conditions. Likewise, there were no statistically significant differences in adverse effects.

In study 2, based on both in lab reporting and diary data, withdrawal symptoms ameliorated across the 2 weeks post initiation of use, there was an increase in alleviation at week 1 and further increase at week 2. Unlike craving, withdrawal symptoms scores did not differ across conditions. This is inconsistent with a previous study which demonstrated that a second generation e-cigarette (with the same nicotine concentration) was more effective than a cigalike for attenuating withdrawal symptoms (Lechner, Meier, et al., 2015).

The steady increase in withdrawal relief reflects the changes in puffing topography, and may be synonymous of an increment in learning to use the e-cigarette more effectively. These findings add to the current literature which suggests that e-cigarettes are capable of alleviating withdrawal symptoms not only in acute (Dawkins & Corcoran, 2014; Farsalinos, Spyrou, Tsimopoulou, et al., 2015; Nides et al., 2014) but also in real word conditions following a period in which users have had the opportunity to accustom themselves with the device.

Subjective effects

As suggested in Chapter 1, the dosage and rapidity of nicotine are highly important for smokers' satisfaction, craving reduction and other subjective effects. These subjective effects (Carpenter et al., 2007; Rose et al., 2000, 2010) are likely to influence e-cigarette product acceptability and promote sustained use. Several studies have documented the ability of e-cigarettes to elicit positive subjective effects (such as satisfaction, 'pleasant' and 'taste good') (Bullen et al., 2010; Spindle et al., 2015; Vansickel et al., 2012; Vansickel et al., 2010; Vansickel & Eissenberg, 2013), however not always to the same extent as tobacco cigarettes (Martínez-Sánchez et al., 2014; Norton et al., 2014); whilst others report varying abilities between device types to induce these positive effects (Dawkins et al., 2015; Farsalinos, Spyrou, et al., 2014). In

line with the latter, Tank 18 and Tank 6 were rated as more satisfying compared to cigalikes, suggesting that tank systems are preferred in terms of satisfaction. This is consistent with previous studies comparing tanks and cigalikes. For example, among experienced e-cigarette users, Farsalinos' group reported that use of a tank versus a cigalike was more satisfying (Farsalinos, Spyrou, et al., 2014).

Interestingly and against expectations, in Study 2, when asked about the device similarities with that of tobacco cigarettes ("Does it feel like…") the cigalike did not differ from the tank. Thus, the close visual resemblance of cigalikes to tobacco cigarettes does not necessarily translate into a closer approximation of tobacco cigarettes in respect of tanks. The weight of the cigalike in relation to a tobacco cigarette is likely to have played a part as anecdotally noted by some participants in study 2 and in previous studies (Van Heel et al., 2017). In Study 2, other anecdotal reports by those using the cigalike comprised mentions of the unpleasant sensation and taste of the aerosol, which may have also contributed to these findings.

In Study 2, scores on the overall scale of all positive effects decreased from Baseline to week 2. Whilst overall scores on positive effects remained fairly constant for the Tank 18, scores for the Cigalikes decreased considerably from Baseline to week 2, suggesting a marked decline in satisfaction levels, which is reflective in the higher drop-out rate in the Cigalike condition.

In Study 2, there were no changes over time in the overall adverse effect scores and no differences between Cigalikes, Tank 18 and 6. Amongst all adverse effects, throat irritation was the most exacerbated symptom in each condition for the cohort of e-cigarette-naïve smokers as well as in the sample of experienced users. Farsalinos et al (2014) found that the use of the tank induced greater 'throat hit' compared to the

cigalikes, which corroborates the findings of Study 2. However, documenting throat-related sensory effects present its challenges. Whilst throat hit can be a desired effect sought after by smokers (Dawkins, Turner, Roberts, et al., 2013; Farsalinos, Romagna, Tsiapras, Kyrzopoulos, Spyrou, et al., 2013), it can equally be synonymous of adverse effects; thus, it seems that an optimal and individualistic throat hit is required, that is sufficiently strong to be discernible and satisfying to the user but at an optimal level not to induce harsh and unpleasant effects (Dautzenberg et al., 2016). Here, in this thesis, throat irritation was measured as opposed to throat hit, although this makes direct comparisons with previous studies challenging, that both concepts capture similar sensory effects are of worthy mention given the dependent relationship with nicotine concentrations and device characteristics. Interestingly, previous studies found that for an optimum throat hit, a specific nicotine concentration and voltage combination is required to better support the switch to e-cigarettes in smokers (Dautzenberg et al., 2016), which, given that cigalike devices do not allow voltage adjustment, renders tanks more compatible in providing an optimal throat hit. Given the link between satisfaction and the latter, the findings of Study 2 suggesting a reduced satisfaction in the cigalike condition aligns well with this.

Smoking Reduction and Cigarette Dependence

Smoking behaviour

The findings of this thesis add to the literature which suggests that e-cigarettes can be an effective aid to support smoking reduction for those who do not intend to quit or are unable to do so. In study 2, a two-week experimentation period led to a reduction in smoking and cigarette dependence in e-cigarette-naïve smokers. However, complete cessation was modest at 1 and, 3 months but encouraging at 6 months. Studies have demonstrated little health benefits of smoking reduction using e-cigarettes as indicated by biomarkers in dual users (Shahab et al., 2018), thus the quit rate obtained in the

current study may be too negligible to translate into real public health benefit. Nonetheless, consistent with the findings of Study 2, a number of studies suggest an increased likelihood of reducing cigarette dependence over time (Etter & Eissenberg, 2015; Farsalinos, Romagna, Tsiapras, Kyrzopoulos, & Voudris, 2013a; Foulds et al., 2015) thereby increasing their e-cigarette use to daily use and eventually achieving complete cessation (Biener & Hargraves, 2015).

In Study 2, both e-cigarette types, the tank and the cigalike as well as the 6 and 18 mg/mL nicotine concentrations led to a reduction in smoking. Although it is worth noting that there was differential drop-out rates across conditions with 20.8% in the cigalike compared to 13% in both the Tank 6 and 18. The greater drop-out rate in the cigalike compared to the tank conditions may suggest that those who used the Cigalikes and successfully maintained use of the device and reduced their smoking may have had greater motivation to do so compared to those who dropped out. This corroborates the prediction model also, suggesting that participants in the cigalikes condition were less likely to have quit at 6 months. That both tanks 18 and 6 were more likely to induce satisfaction compared to cigalikes during the early stage of the cessation attempt (see Study 2, chapter III) fits with the increased likelihood of quitting using a tank.

The reduction in smoking found over the two-week period in Study 2 (Chapter III), is consistent with previous findings (Adriaens et al., 2014). In an 8-month RCT, in 48 smokers who successfully reduced their smoking over time, the initial decrease in salivary cotinine during the early stage post e-cigarette use initiation was soon followed by an increase in later months (Adriaens et al., 2014). This reflects the learning period process characterised by an adjustment in puffing practices to equate the blood nicotine levels that they are accustomed to as smokers. This shares similarity with the increase in puffing duration seen in Study 2. The stability in cotinine levels over time in the

transitional phase from smoker to dual user to exclusive e-cigarette user has been reported by others. In an 8-month pilot study, 34 smokers unwilling to quit received medical assistance training as a way to enhance learning. At each follow-up session (1, 4 and 8-month) cotinine levels remained unchanged whilst CO and CPD reduced significantly. Although these two aforementioned studies do not report puffing topography, a successful adjustment in puffing regimen combined with the use of effective nicotine delivery e-cigarettes are likely to have facilitated the stability in cotinine levels despite the reduction in smoking. This further supports the titration theory of e-cigarette use and accords with empirical evidence that more intensive users typically achieve greater smoking reduction (Biener & Hargraves, 2015; Biener, Hargraves, & Hargraves, 2015; Brose et al., 2015; Hitchman et al., 2015; Manzoli et al., 2015).

Cigarette dependence

By virtue of its reinforcing properties from which dosage, mode (i.e. inhalation) and speed of delivery are key determinants of its dependence liability (Benowitz, 1990b; Benowitz, 2010), it is anticipated that nicotine delivered via e-cigarettes will have the potential to reduce cigarette dependence by acting as substitutes to combustible cigarettes.

Consistent with previous studies (Baldassarri et al., 2018; Rohsenow et al., 2018a), cigarette dependence reduced significantly over the two-week period of the early cessation attempt in Study 2, and surprisingly, the decrease did not differ between conditions. Similarly, regardless of the device type or nicotine concentration, at the second session, participants' habitual first cigarette of the day was reported to be smoked at a later time compared to the time reported in the baseline session. The reduction in cigarette dependence concurs with the reduction in smoking and suggests

that e-cigarettes offered an effective coping mechanism in the absence (or reduction) of smoking at least at the initiation stage.

Given the influence of nicotine delivery on dependence potential, the lack of difference in cigarette dependence scores between those in the tank 18, tank 6 and cigalikes conditions was unexpected. Many studies have associated cigalikes with modest nicotine delivery (Bullen et al., 2010; Farsalinos, Spyrou, et al., 2014; Rüther et al., 2017; Vansickel et al., 2010) and significantly lower dependence potential in comparison to tank systems specifically when used with nicotine concentrations deemed sufficient to raise blood nicotine levels and induce satisfaction (Foulds et al., 2015). The short duration of the e-cigarette experimentation (two-week period) may have been insufficient to allow for differences to arise between conditions given the chronic and fluctuating nature of cigarette dependence within which lapses are likely to occur even after cessation (Baldassarri et al., 2018; Notley et al., 2017).

The link between cigarette dependence and cessation is well founded, several studies suggest dependence levels as a predictor of cessation (Hagimoto et al., 2010; Hughes et al., 2014; Rohsenow et al., 2018b; Vangeli et al., 2011). However, findings are contradictory, and although this may be due to the use of varying methods and indexes (e.g. Cigarette Dependence Scale, Heaviness of Smoking Index) (Zawertailo, Voci, & Selby, 2018), it remains the case that cigarette dependence has its limitations in predicting cessation certainly where e-cigarettes are involved. In study 3, whilst cigarette dependence predicted cessation at 1 month, it did not at subsequent follow-ups 3 and 6 months. A reasonable hypothesis may be that as the smoker transitions from smoking to e-cigarette use, reducing smoking whilst increasing e-cigarette use, his/her dependence on cigarettes slowly transfers onto the e-cigarette, thereby becoming less dependent on cigarettes. This proposition is consistent with previous reports

documenting lower cigarette dependence in experienced e-cigarette users in comparison to smokers (Etter & Eissenberg, 2015; Farsalinos, Romagna, Tsiapras, Kyrzopoulos, & Voudris, 2013a; Foulds et al., 2015). Thus, it appears that quitting smoking with the aid of e-cigarettes differs from quitting via other means (e.g. cold turkey or with NRT), and that cigarette dependence is not as important when transitioning to e-cigarettes use as the dependence can be transferred, which is not the case when quitting cold turkey for example. This might explain why cigarette dependence only predicted cessation at 1 month; whether the dependence transference is an important mechanism which facilitates complete cessation would merit further exploration.

The role of motivation

Motivation did not predict cessation at any of the follow-up points despite a moderately motivated sample at baseline; this may suggest that motivation alone is not sufficient to achieve complete cessation. Although motivation to quit measured at baseline has proven a robust measure to predict quit (Borland, Yong, Balmford, et al., 2010b; Hummel, Brown, Willemsen, West, & Kotz, 2016; Kotz, Brown, & West, 2013; Vangeli, Stapleton, Smit, Borland, & West, 2011), it falsely suggests motivation as a static process (Hughes et al., 2014). Rather, past studies suggest like cigarette dependence, since motivation fluctuates over time, it is not sufficient to measure motivation at one time, rather its consistency over time should be taken into account in any predictive model (Perski et al., 2018). Further considerations are that unlike quitting with more conventional cessation aids (i.e. NRT) or cold turkey, when quitting with e-cigarettes motivation may be a less salient factor. For instance, previous studies have consistently demonstrated high levels of successful cessation outcomes even in smokers unmotivated to quit (Caponnetto et al., 2014; Polosa et al., 2015); such success may have been '*accidental*' indeed facilitated by the use of e-cigarettes (Notley, Ward,

Dawkins, & Holland, 2018). This certainly highlights the differences between quitting with e-cigarettes versus quitting with other methods.

Limitations and strengths: E-cigarette technology a changing landscape

In addition to the aforementioned limitations specific to each study, there are several more general issues and limitations to consider. Firstly, one of the primary aims of this thesis was to gain further understanding of the impact of high versus low nicotine concentrations on puffing topography. However, the accuracy of nicotine exposure could not be determined given that nicotine concentrations contained in the e-liquid were not chemically measured. That said, newer chemical studies suggest little discrepancy between labels and content (Etter et al., 2013; Farsalinos, Spyrou, et al., 2014; Goniewicz, Hajek, & McRobbie, 2014) compared to earlier manufactured products (Cameron et al., 2014; Trehy et al., 2011); suggesting some improvement in manufacturing practices.

Another possible limitation is the possible lack of statistical power to detect differences between conditions. Study 2 and 3 suffered a large drop-out rate in follow-up sessions which considerably affected the sample size. Likewise Study 1 also used a very modest sample size, powered on puffing topography but which may have led to some non-significant findings on other measures.

The overreliance on self-reported data may also be considered a limitation, specifically in Study 2, for the measure of CPD. In Study 3, cessation was not biologically verified as it was not always possible to collect CO, and salivary cotinine was collected at baseline but could not always be collected at self-reported quit to assess nicotine exposure after quitting. More frequent face-to-face follow-ups would have allowed better monitoring of daily e-cigarette use behaviours, including greater understanding of the interactions between device characteristics and users' responses

which would have allowed for a better understanding of how e-cigarettes promote smoking reduction and cessation. Instead, participants were contacted every 2 months for 6 months which could have resulted in recall bias. That said, such an approach (telephone contacts) continues to be standard practise in many large scale surveys and has contributed greatly to the literature on predictors of cessation (Bauld et al., 2017; Maddison et al., 2010). Additionally, that previous studies found self-report measures of puffing patterns to have good reliability (Shahab et al., 2008), combined with the strong correlation found between the biochemical measure of CO levels and self-report CPD in Study 2, provides a great deal of confidence in these findings.

Furthermore, given the apparent differences in gender and between ethnic groups (Benowitz, Hukkanen, & Jacob III, 2009) in the tobacco (Perkins et al., 1999) and the e-cigarette literature (Dawkins et al., 2012; Jorenby et al., 2017), another limitation is the sample constitution of each study within this thesis; all suffering a gender imbalance, more so study 1 with 100% Caucasian and 92% male, whilst study 2 included a sample consisting of 63% females at baseline, which is not a true reflection of the smoking population. Unlike study 1, studies 2 and 3 suffer a lack of representation of lower education smokers, however it is worth noting that the university campus (UEL) wherein all studies took place as an inclusive institution made up of a large black and ethnic minority groups from varied socio-economic status, and this was reflected in the samples.

Finally, the lengthy process of the running of studies involving human participants (ethical approval, completion of data collection and so on) combined with the rapid evolving nature of this field of research in terms of technological advancement, reinforce the point that any findings in this area of studies are often already outdated at point of publication, and thus must be interpreted cautiously. At the

time study 2 and 3 were designed, the prevalence of cigalikes was greater and little data had been published in relation to the factors influencing puffing topography. Since data collection for this thesis, due to significant technology advancement, the e-cigarettes landscape has evolved rapidly shaping users' behaviours and preferences. The emergence of subohming has led to in an increase in the use of lower nicotine concentrations perhaps by users who would have otherwise opted for higher concentrations or by high nicotine dependent smokers with needs for higher concentrations. Thus, cigalikes have slowly fallen in disfavour for better nicotine and aerosols delivery tank systems that are associated with more enhanced experience (Tackett et al., 2015). Further changes in the e-cigarettes landscape has been the recent introduction of JUUL devices in the UK and growing prevalence in the US. Its visual similarity with cigalikes in size and purported effective nicotine delivery combined with its appeal in the US, suggest that the findings of the current study continue to be relevant. Given their close similarity to cigalikes, the high popularity of the JUUL devices suggests the need for effective nicotine delivery products and for a 'vaping' experience that closely approximates smoking still remain.

One of the strengths of this work was contrasting two valid methods (video recordings and the use of the eVic™) to measure puffing topography, both of which have been previously used and proven to yield reliable results (e.g. Farsalinos et al., 2013, 2014, 2015). The use of video recordings allowed to counteract the issues of pre-puff phenomenon which refers to pressing the activation button prior to the start of a puff, whilst bringing the device to the lips, or once in the mouth (Farsalinos et al., 2013b), in order to warm the atomiser and allow a stronger throat hit (Behar et al., 2015). In such instances, the time frame was captured only when the device was clearly seen in the mouth with both lips closed. A further strength of the ad libitum paradigm,

is its pragmatic design which allowed to capture e-cigarette-naïve smokers' puffing adjustments as they learned to use the e-cigarette over time. That said, the instructions of a modest one-hour abstinence in Study 2 may have been insufficient for the low dependent (light) smokers for whom their habitual routines may have been to smoke very few cigarettes in the day and felt satiated at the start of the session.

Implications

The findings of this thesis carry several implications. The first implication to consider is compensatory puffing behaviours which carry greater financial expense (consuming a greater quantity of e-liquid) and potential health risks. Whilst studies suggest that e-cigarette emissions carry only five percent of the health risks of tobacco cigarettes (McNeill et al., 2015), more intensive puffing regimens as documented with the use of low nicotine concentrations in this thesis, may lead to increased toxicant exposure and adverse long term health effects (Dawkins et al., 2018; Kośmider, Kimber, Kurek, Corcoran, & Dawkins, 2017). Secondly, whilst most experienced e-cigarettes users gradually reduce their nicotine concentration over time (as a result of either more advanced products with greater nicotine delivery or regulatory restrictions) (Farsalinos, et al, 2013a; Soar et al., 2018); on the other hand, e-cigarette naïve smokers are likely to require an increase in their nicotine e-liquid concentration in order to achieve complete smoking abstinence in the early stages of a cessation attempt (Farsalinos et al., 2013a; Polosa, Caponnetto, Cibella, & Le-Houezec, 2015). This is in line with the observed increase in puffing intensity found here. Thus, a sensible strategy would be to recommend higher nicotine concentrations to smokers that closely approximate that of their tobacco cigarettes to optimise acceptability, promote sustained use and eventually smoking cessation. Thirdly, in line with the literature, Study 2 and Study 3 findings from both suggest the use of tanks or products that deliver a more

enhanced experienced and are associated with better nicotine delivery may be more advisable for cessation. A fourth implication is the craving reduction findings in Study 3. As noted earlier, if craving reduction associated with early e-cigarettes use can predict cessation, this could be harnessed for advice given in vape shops and smoking cessation programmes, but also this can be used in a cost and time effective approach to assess likely effectiveness of devices without the need for lengthy studies; although these findings are in need of replication.

Future Directions for Research

Using a low and a high nicotine concentration (6 and 24 mg/mL) in a double-blind within-participants design, Study I provided direct evidence for compensatory puffing behaviours. However, such dramatic drop in nicotine concentrations does not reflect the gradual reduction in nicotine concentrations use seen in most experienced users overtime (Soar et al., 2018). As highlighted in the limitations of Study 1, it is unclear whether the use of a closer range of concentrations for example 18 and 24 mg/mL (or 6 and 12 mg/mL) would have allowed participants to self-titrate fully and achieve matching plasma nicotine levels. Likewise, despite significantly lower puff number and duration as found in Study 1, plasma nicotine levels were significantly higher in the high nicotine concentration condition. It is unclear whether the use of a more prolonged ad libitum session would have led to further increase in plasma nicotine levels in the lower nicotine concentration. Future work may focus on more naturalistic settings to obtain more realistic puffing topography data and biomarkers whilst including a greater range of nicotine concentrations and more intermediary ones.

In the same light, in the smoking literature, great attention has been given to exploring whether, aside nicotine, other chemicals are responsible for self-titration.

Since in Study 1 all variables were held constant, and that fewer chemicals are present in e-liquid compared to in tobacco cigarettes, there is a great deal of certainty that nicotine played the key role in driving compensatory behaviours. However, given the continued rise in the use of low nicotine concentrations and emerging research on the influence of flavours on puffing topography (St Helen et al., 2018), future compensatory work can focus on exploring the possible role of other agents aside nicotine on driving titration.

In Study 2, cigalikes and 18 mg/mL containing tanks did not differ and led to similar levels in craving reduction, whilst the 6 mg/mL nicotine containing tank performed the poorest. That the 18 mg/mL containing tank did not differ from the cigalike in reducing craving, points to the important role of high nicotine concentrations to reduce craving. Future research could focus in exploring whether there is a nicotine concentration threshold to help dealing with cigarette craving and explore its underpinning mechanisms and associated users' characteristics (e.g. nicotine/cigarette dependence).

Furthermore, Study 3 found craving reduction experienced by e-cigarette naïve smokers at first use a significant predictor at 3 months follow-up. It could be useful to investigate the predictive value of craving reduction further, so this could be applied in e-cigarettes smoking cessation programmes. Future research could focus on disentangling the effects of craving reduction, the factors and mechanisms that mediate smoking cessation, and to what extent initial craving reduction can predict cessation.

In addition, the 18 and 6 mg/mL containing tanks elicited greater levels of satisfaction whilst cigalikes were associated with poor satisfaction rates; this reduced satisfaction was echoed in a greater drop-out rates in the cigalike condition. That the

low nicotine concentrations tank did not reduce craving to the same extent, whilst providing equal levels of satisfaction compared to Tank 18 is suggestive of different pathways by which e-cigarette users experience craving and satisfaction. These differences could be due to greater levels of pleasantness and more favourable taste and/or higher aerosol visibility associated with tank systems compared to cigalikes (Vena, Howe, Cao & King, 2019), and this may account for the greater satisfaction levels in tanks. Equally important, this thesis draws attention to the differences between satisfaction and craving and suggests a lesser important role for nicotine concentrations for providing satisfaction. Future work should turn their focus towards delineating the subtlety between satisfaction and craving, and furthering the understanding of the various facets of satisfaction.

Summary and conclusion

The aims of this thesis were primarily to explore the inter-relationships between e-cigarette puffing topography, nicotine concentrations and e-cigarette characteristics. Specific objectives were to: i) determine the effects of varying nicotine concentrations (high vs. low) in e-cigarettes on users' puffing topography, ii) explore the effects of device types (tanks vs. cigalikes) on users' puffing topography and smoking related effects and behaviours, iii) document how e-cigarette puffing topography evolves over time and differs in response to device types and nicotine concentrations and, iv) further the understanding of the complementary roles of subjective effects namely satisfaction, the alleviation of craving and withdrawal symptoms. Additional objectives were to explore v) the factors (including puffing topography) that best predict smoking

cessation at 1, 3 and 6 months in a sample of 70 e-cigarette-naïve smokers using different device types and varied nicotine concentrations.

Study 1 was the first to directly explore self-titration by allowing experienced e-cigarette users, customarily using high nicotine concentration e-liquid, to use an e-cigarette device ad libitum in the lab. In fact, the findings provide direct empirical evidence of a clear attempt to self-titrate; when given a lower nicotine concentration e-liquid, users increased their puff frequency and duration and consumed more e-liquid. The findings herein suggest that, similar to smokers, experienced e-cigarette users have a tendency to attempt to self-titrate by altering their puffing patterns when switching to lower nicotine concentrations. Similar to previous tobacco cigarette studies which suggest that upregulation is more commonly achieved compared to downregulation REF, titration was only partial; despite significantly lower puff number and duration, plasma nicotine levels were significantly higher in the high nicotine concentration condition. However, that users reported equivalent reduction in craving and withdrawal symptoms between conditions, suggests that very high nicotine content liquids may not be an absolute necessity for alleviation of craving or withdrawal, at least for experienced vapers in acute laboratory conditions. Study 1 has demonstrated that experienced users using a tank system e-cigarette filled with 24 mg/mL nicotine concentrations e-liquid can achieve high blood nicotine levels very quickly, matching and even exceeding those reported in tobacco smokers; this is indicative of a remarkable improvement in e-cigarette nicotine delivery. Such patterns of nicotine delivery, may bolster cessation rates but may raise concerns over nicotine dependence. Whilst previous studies have used standardised puffing protocols either exclusively or preceding an ad lib session, here it is argued that the use of prescribed puffing regimes do not reflect real-life device use and will affect nicotine delivery and absorption. Thus,

although they are suggestive of compensatory puffing behaviours, they do not provide clear evidence of titration.

Although Study 2 was not designed to allow for any direct conclusions on compensatory puffing, it did provide useful data in support of the titration theory. Indeed, the increase in puffing duration a week post e-cigarette initiation suggests a need to optimise nicotine delivery and bring nicotine blood to habitual levels. Study 2 adds to the literature by suggesting that a week seems to be sufficient for e-cigarette naïve smokers to develop an awareness for the need to adjust their puffing from smoking to e-cigarette use. Likewise, differences in puffing intensity between smokers using cigalikes versus those using tanks concur with the observed adjustment in puffing topography, and reiterate the proposition of a more erratic or intense puffing profile associated in the cigalikes compared to the tank systems. Consistent with the idea that the vacuum mechanism of cigalikes may be harder and require a stronger draw than tanks, the cigalike was associated with longer puff duration and more frequent puffs. That they require stronger suction may have contributed to their inability to elicit satisfaction to the same magnitude than did the tanks. The significant decrease in the number of puffs over the two-week period which, combined with a significant increase in puff duration strongly suggest a dramatic change in puffing behaviour characterised by a shift to slower, longer more paced puffs from a more erratic puffing style at initial use, again this is in good agreement with the differences between smokers' and experienced users' puffing profiles.

Cigalikes and 18 mg/mL nicotine concentrations containing tanks did not differ in the ability to reduce craving, whilst the 6 mg/mL nicotine containing tank performed the poorest. In addition, the 18 mg/mL containing tank elicited greater levels of satisfaction. Given that craving and satisfaction are chief motives for e-cigarette use

discontinuation, these findings suggest that higher nicotine concentrations may be more effective in the relief of craving at least during initiation of e-cigarette use.

Taken together, these findings confirm previous observations that puffing patterns differ across device types and change over a one-week period as the user learns to use the device. Across all subjective variables, the 18 mg/mL nicotine concentrations containing tank performed the strongest and, although the cigalike was associated with some promising results, these need to be considered in the context of the high drop-out rate in this condition.

In a sample of 70 e-cigarette naïve smokers willing to quit (Study 3), predictors of smoking cessation were assessed at 1, 3 and 6 months after e-cigarette initiation. These findings suggest that lower cigarette dependent smokers were more likely to quit at 1 month, whilst those with higher craving reduction associated with e-cigarette use at baseline had greater odds of successfully quitting at 3 months. At 6 months, only device type at follow-up and craving reduction following e-cigarette use at baseline were significant predictors. Thus, participants using a tank device were at greater odds of quitting smoking compared to those using a cigalike. The evidence presented herein add to the literature and suggest i) the superiority of tanks over cigalikes in nicotine delivery (e.g Farsalinos et al., 2014; Lechner et al., 2015) and ii) the importance to consider nicotine concentrations certainly during the initiating phase of e-cigarette use. Thus, in order to maintain the appeal of e-cigarettes in allowing them to rival with, and act as an effective substitution to tobacco cigarettes, the findings of this thesis support previous recommendations that adopting higher nicotine concentrations may be more sensible (Dawkins et al., 2016).

REFERENCES

Action on Smoking and Health. (2015). *Key dates in the history of anti-tobacco campaigning*. *Action on Smoking and Health*. Retrieved from http://www.ash.org.uk/files/documents/ASH_741.pdf

Adkison, S. E., O'Connor, R. J., Bansal-Travers, M., Hyland, A., Borland, R., Yong, H. H., … Fong, G. T. (2013). Electronic nicotine delivery systems: International Tobacco Control Four-Country Survey. *American Journal of Preventive Medicine*. http://doi.org/10.1016/j.amepre.2012.10.018

Adriaens, K., Van Gucht, D., & Baeyens, F. (2017). Differences between Dual Users and Switchers Center around Vaping Behavior and Its Experiences Rather than Beliefs and Attitudes. *International Journal of Environmental Research and Public Health*, *15*(1), 12. http://doi.org/10.3390/ijerph15010012

Adriaens, K., Van Gucht, D., & Baeyens, F. (2018). Differences between dual users and switchers center around vaping behavior and its experiences rather than beliefs and attitudes. *International Journal of Environmental Research and Public Health*, *15*(1). http://doi.org/10.3390/ijerph15010012

Adriaens, K., Van Gucht, D., Declerck, P., & Baeyens, F. (2014). Effectiveness of the electronic cigarette: An eight-week flemish study with six-month follow-up on smoking reduction, craving and experienced benefits and complaints. *International Journal of Environmental Research and Public Health*, *11*(11), 11220–11248. http://doi.org/10.3390/ijerph111111220

Al-Safi, S. A. (2005). Does Smoking Affect Blood Pressure and Heart Rate? *European Journal of Cardiovascular Nursing*, *4*(4), 286–289. http://doi.org/10.1016/j.ejcnurse.2005.03.004

Allem, J.-P., Unger, J. B., Garcia, R., Baezconde-Garbanati, L., & Sussman, S. (2015). Tobacco Attitudes and Behaviors of Vape Shop Retailers in Los Angeles. *American Journal of Health Behavior*, *39*(6), 794–798. http://doi.org/10.5993/AJHB.39.6.7

Ambrose, J. A., & Barua, R. S. (2004). The pathophysiology of cigarette smoking and cardiovascular disease: An update. *Journal of the American College of Cardiology*, *43*(10), 1731–1737. http://doi.org/10.1016/j.jacc.2003.12.047

Andy Field. (2013). Logistic Regression. In *Discovering Statistics Using IBM SPSS Statistics* (pp. 760–814). London: SAGE Publications.

ASH. (2016). *Use of electronic cigarettes (vapourisers) among adults in Great Britain*.

ASH. (2017). *Use of electronic cigarettes (vapourisers) among adults in Great Britain*.

Ashton, H., Stepney, R., Thompson, J. W., & Rawbone, R. G. (1979). Self-titration by cigarette smokers. *British Medical Journal*, *2*(6192), 731–732. Retrieved from http://www.scopus.com/inward/record.url?eid=2-s2.0-0018798575&partnerID=40&md5=30a018ac8dc2ec73d13e404f9d49c4e0

Ashton, H., Watson, D. W., Marsh, R., & Sadler, J. (1970). Puffing frequency and nicotine intake in cigarette smokers. *The British Medical Journal*, *3*(5724), 679–681. http://doi.org/10.1136/bmj.3.5724.679

Baker, T. B., Ph, D., Piper, M. E., Ph, D., Mccarthy, D. E., Ph, D., … Ph, D. (2010). Time to first cigarette in the morning as an index of ability to quit smoking: Implications for nicotine dependence. *Nicotine & Tobacco Research*, *9*(Suppl 4). http://doi.org/10.1080/14622200701673480.Time

Baldassarri, S. R., Bernstein, S. L., Chupp, G. L., Slade, M. D., Fucito, L. M., & Toll, B. A. (2018). Electronic cigarettes for adults with tobacco dependence enrolled in a tobacco treatment program: A pilot study. *Addictive Behaviors*, *80*, 1–5. http://doi.org/10.1016/j.addbeh.2017.11.033

Balfour, D. J. (2002). The neurobiology of nicotine dependence and depression: common pathways and mechanisms. *European Psychiatry*, *17*, 80. http://doi.org/http://dx.doi.org/10.1016/S0924-9338(02)80365-0

Balfour, D. J. K. (2004). The neurobiology of tobacco dependence: A preclinical perspective on the role of the dopamine projections to the nucleus. *Nicotine & Tobacco Research*, *6*(6), 899–912. http://doi.org/10.1080/14622200412331324965

Balfour, D. J. K., & Fagerström, K. O. (1996). Pharmacology of nicotine and its therapeutic use in smoking cessation and neurodegenerative disorders. *Pharmacology and Therapeutics*, *72*(1), 51–81. http://doi.org/10.1016/S0163-7258(96)00099-X

Balfour, D. J. K., Wright, A. E., Benwell, M. E. M., & Birrell, C. E. (2000). The putative role of extra-synaptic mesolimbic dopamine in the neurobiology of nicotine dependence. *Behavioural Brain Research*, *113*(1–2), 73–83. http://doi.org/10.1016/S0166-4328(00)00202-3

Balmford, J., & Borland, R. (2008). What does it mean to want to quit? *Drug and Alcohol Review*, *27*(1), 21–27. http://doi.org/10.1080/09595230701710829

Barrett, S. P. (2010). The effects of nicotine, denicotinized tobacco, and nicotine-containing tobacco on cigarette craving, withdrawal, and self-administration in male and female smokers. *Behavioural Pharmacology*, *21*(2), 144–152.

Bauld, L., Mackintosh, A. M., Eastwood, B., Ford, A., Moore, G., Dockrell, M., … McNeill, A. (2017). Young people's use of e-cigarettes across the united kingdom: Findings from five surveys 2015–2017. *International Journal of Environmental Research and Public Health*, *14*(9). http://doi.org/10.3390/ijerph14090973

Bauld, L., MacKintosh, A. M., Ford, A., & McNeill, A. (2016). E-cigarette uptake amongst UK youth: Experimentation, but little or no regular use in nonsmokers. *Nicotine and Tobacco Research*. http://doi.org/10.1093/ntr/ntv132

Baweja, R., Curci, K. M., Yingst, J., Veldheer, S., Hrabovsky, S., Wilson, S. J., … Foulds, J. (2016). Views of experienced electronic cigarette users. *Addiction Research & Theory*, *24*(1), 80–88. http://doi.org/10.3109/16066359.2015.1077947

Beard, E., Brown, J., McNeill, A., Michie, S., & West, R. (2015). Has growth in electronic cigarette use by smokers been responsible for the decline in use of licensed nicotine products? Findings from repeated cross-sectional surveys. *Thorax*, *70*(10), 974–978. http://doi.org/10.1136/thoraxjnl-2015-206801

Beard, E., Bruguera, C., McNeill, A., Brown, J., & West, R. (2015). Association of amount and duration of NRT use in smokers with cigarette consumption and motivation to stop smoking: A national survey of smokers in England. *Addict Behav*, *40*(0), 33–38. http://doi.org/http://dx.doi.org/10.1016/j.addbeh.2014.08.008

Beard, E., West, R., Michie, S., & Brown, J. (2016). Association between electronic cigarette use and changes in quit attempts , success of quit attempts , use of smoking cessation pharmacotherapy , and use of stop smoking services in England : time series analysis of population trends. *Bmj*, *354*(i4645). http://doi.org/10.1136/bmj.i4645

Beard, E., West, R., Michie, S., & Brown, J. (2016). Cigarette use and changes in quit attempts, success of quit attempts, use of smoking cessation pharmacotherapy, and use of stop smoking services in England …. *Bmj*. Retrieved from http://www.bmj.com/content/354/bmj.i4645.long

Bechara, A., Dolan, S., Denburg, N., Hindes, A., Anderson, S. W., & Nathan, P. E. (2001). Decision-making deficits, linked to a dysfunctional ventromedial prefrontal cortex, revealed in alcohol and stimulant abusers. *Neuropsychologia*, *39*, 376–389. Retrieved from www.elsevier.com/locate/neuropsychologia

Bechara, A., Dolan, S., & Hindes, A. (2002). Decision-making and addiction (part I): Impaired activation of somatic states in substance dependent individuals when pondering decisions with negative future consequences. *Neuropsychologia*, *40*(10), 1675–1689. http://doi.org/10.1016/S0028-3932(02)00015-5

Behar, R. Z., Hua, M., & Talbot, P. (2015). Puffing topography and nicotine intake of electronic cigarette users. *PloS One*, *10*(2), e0117222. http://doi.org/10.1371/journal.pone.0117222

Behar, R. Z., Luo, W., Lin, S. C., Wang, Y., Valle, J., Pankow, J. F., & Talbot, P. (2016). Distribution, quantification and toxicity of cinnamaldehyde in electronic cigarette refill fluids and aerosols. *Tobacco Control*, tobaccocontrol-2016-053224. http://doi.org/10.1136/tobaccocontrol-2016-053224

Bell, K., & Keane, H. (2012). Nicotine control: E-cigarettes, smoking and addiction. *International Journal of Drug Policy*, *23*(3), 242–247. http://doi.org/10.1016/j.drugpo.2012.01.006

Bell, K., & Keane, H. (2014). All gates lead to smoking: The 'gateway theory', e-cigarettes and the remaking of nicotine. *Social Science & Medicine*, *119*, 45–52. http://doi.org/http://dx.doi.org/10.1016/j.socscimed.2014.08.016

Benowitz, N. L. (1988). Pharmacologic Aspects of Cigarette Smoking and Nicotine Addiction. *New England Journal of Medicine*, *319*(20), 1318–1330. http://doi.org/10.1056/NEJM198811173192005

Benowitz, N. L. (1990a). Clinical pharmacology of inhaled drugs of abuse: implications in understanding nicotine dependence. *NIDA Research Monograph*, *99*, 12–29.

Benowitz, N. L. (1990b). Pharmacokinetic considerations in understanding nicotine dependence. *Ciba Foundation Symposium*, *152*, 186–189.

Benowitz, N. L. (1999, September). Nicotine addiction. *Primary Care - Clinics in Office Practice*. http://doi.org/10.1016/S0095-4543(05)70120-2

Benowitz, N. L. (2001). Compensatory smoking of low-yield cigarettes. *National Cancer Institute. Risks Associated with Smoking Cigarettes with Low Machine Yields of Tar and Nicotine. Smoking and Tobacco Control Monograph No*, *13*, 39–63.

Benowitz, N. L. (2008). Neurobiology of Nicotine Addiction: Implications for Smoking Cessation Treatment. *American Journal of Medicine*, *121*(4 SUPPL.), S3–S10. Retrieved from http://www.scopus.com/inward/record.url?eid=2-s2.0-40849141241&partnerID=40&md5=5e1e4b38c090ed3582cc161b11170ae0

Benowitz, N. L. (2009). Pharmacology of nicotine: Addiction, smoking-induced disease, and therapeutics. *Annual Review of Pharmacology and Toxicology*. Retrieved from http://www.scopus.com/inward/record.url?eid=2-s2.0-65249154248&partnerID=40&md5=7a6323e45380f7f9844ad42705cdd29c

Benowitz, N. L. (2010). Nicotine addiction. *New England Journal of Medicine*, *362*(24), 2295–2303+2250. Retrieved from http://www.scopus.com/inward/record.url?eid=2-s2.0-77953688842&partnerID=40&md5=9e40462fa67f83a7a700a7b355b492b9

Benowitz, N. L. (2010). Nicotine Addiction. *New England Journal of Medicine*, *362*(24), 2295–2303. http://doi.org/10.1056/NEJMra0809890

Benowitz, N. L., Hall, S. M., Herning, R. I., Jacob, P., Jones, R. T., & Osman, A.-L. (1983). Smokers of Low-Yield Cigarettes Do Not Consume Less Nicotine. *New England Journal of Medicine*, *309*(3), 139–142. http://doi.org/10.1056/NEJM198307213090303

Benowitz, N. L., Hukkanen, J., & Jacob Iii, P. (2009). Nicotine chemistry, metabolism, kinetics and biomarkers. *Handbook of Experimental Pharmacology*. Retrieved

from http://www.scopus.com/inward/record.url?eid=2-s2.0-63849147368&partnerID=40&md5=a7493b52dfbb152ae5f04f4f68aa8008
Benowitz, N. L., & Jacob, P. (1984a). Daily intake of nicotine during cigarette smoking. *Clinical Pharmacology and Therapeutics*, *35*(4), 499–504. http://doi.org/10.1038/clpt.1984.67
Benowitz, N. L., & Jacob, P. (1984b). Daily intake of nicotine during cigarette smoking. *Clinical Pharmacology & Therapeutics*, *35*(4), 499–504. http://doi.org/10.1038/clpt.1984.67
Benowitz, N. L., & Jacob, P. (1985). Nicotine renal excretion rate influences nicotine intake during cigarette smoking. *Journal of Pharmacology and Experimental Therapeutics*, *234*(1), 153 LP-155. Retrieved from http://jpet.aspetjournals.org/content/234/1/153.abstract
Benowitz, N. L., & Jacob, P. (1994). Metabolism of nicotine to cotinine studied by a dual stable isotope method. *Clinical Pharmacology & Therapeutics*, *56*(5), 483–493. http://doi.org/10.1038/clpt.1994.169
Benowitz, N. L., Kuyt, F., & Jacob, P. (1982). Circadian blood nicotine concentrations during cigarette smoking. *Clinical Pharmacology and Therapeutics*, *32*(6), 758–764. http://doi.org/10.1038/clpt.1982.233
Benowitz, N. L., Porchet, H., Sheiner, L., & Jacob, P. (1988). Nicotine absorption and cardiovascular effects with smokeless tobacco use: comparison with cigarettes and nicotine gum. *Clinical Pharmacology and Therapeutics*, *44*(1), 23–28. http://doi.org/10.1038/clpt.1988.107
Benowitz, N. L., Swan, G. E., JacobIII, P., Lessovschlaggar, C., Tyndale, R. F., Jacob, P., … Tyndale, R. F. (2006). CYP2A6 genotype and the metabolism and disposition kinetics of nicotine. *Clinical Pharmacology & Therapeutics*, *80*(5), 457–467. http://doi.org/10.1016/j.clpt.2006.08.011
Benowitz, N. L., Zevin, S., & Jacob Iii, P. (1998). Suppression of nicotine intake during ad libitum cigarette smoking by high-dose transdermal nicotine. *Journal of Pharmacology and Experimental Therapeutics*, *287*(3), 958–962. Retrieved from http://www.scopus.com/inward/record.url?eid=2-s2.0-0032253783&partnerID=40&md5=3c28a1b40d2e9ad57e74bb646bff47ac
Benwell, M. E., Balfour, D. J., & Anderson, J. M. (1988). Evidence that tobacco smoking increases the density of (-)-[3H]nicotine binding sites in human brain. *Journal of Neurochemistry*, *50*(4), 1243–1247.
Berg, C. J., Haardoerfer, R., Escoffery, C., Zheng, P., & Kegler, M. (2015). Cigarette users' interest in using or switching to electronic nicotine delivery systems for smokeless tobacco for harm reduction, cessation, or novelty: a cross-sectional survey of US adults. *Nicotine Tob Res*, *17*(2), 245–255. http://doi.org/10.1093/ntr/ntu103
Berridge, M. S., Apana, S. M., Nagano, K. K., Berridge, C. E., Leisure, G. P., & Boswell, M. V. (2010). Smoking produces rapid rise of [11C]nicotine in human brain. *Psychopharmacology*, *209*(4), 383–394. http://doi.org/10.1007/s00213-010-1809-8
Berry, J. N., Engle, S. E., Mcintosh, J. M., & Drenan, R. M. (2016). α6-Containing Nicotinic Acetylcholine Receptors in Midbrain Dopamine Neurons are Poised to Govern Dopamine-Mediated Behaviors and Synaptic Plasticity. *Neuropsychopharmacology : Official Publication of the American College of Neuropsychopharmacology*, *304*, 161–175. http://doi.org/10.1016/j.neuroscience.2015.07.052.
Berry, K. M., Reynolds, L. M., Collins, J. M., Siegel, M. B., Fetterman, J. L., Hamburg,

N. M., … Stokes, A. (2018). E-cigarette initiation and associated changes in smoking cessation and reduction: the Population Assessment of Tobacco and Health Study, 2013-2015. *Tobacco Control*, tobaccocontrol-2017-054108. http://doi.org/10.1136/tobaccocontrol-2017-054108

Biener, L., & Hargraves, J. L. (2015). A Longitudinal Study of Electronic Cigarette Use Among a Population-Based Sample of Adult Smokers: Association With Smoking Cessation and Motivation to Quit. *Nicotine & Tobacco Research*, *17*(2), 127–133. http://doi.org/10.1093/ntr/ntu200

Billieux, J., Gay, P., Rochat, L., Khazaal, Y., Zullino, D., & Van der Linden, M. (2010). Lack of inhibitory control predicts cigarette smoking dependence: Evidence from a non-deprived sample of light to moderate smokers. *Drug and Alcohol Dependence*, *112*(1–2), 164–167. http://doi.org/10.1016/j.drugalcdep.2010.06.006

Blank, M. D., Disharoon, S., & Eissenberg, T. (2009). Comparison of methods for measurement of smoking behavior: Mouthpiece-based computerized devices versus direct observation. *Nicotine & Tobacco Research*, *11*(7), 896–903. Retrieved from http://10.0.4.69/ntr/ntp083

Borland, R., Yong, H.-H., Balmford, J., Cooper, J., Cummings, K. M., O'Connor, R. J., … Fong, G. T. (2010). Motivational factors predict quit attempts but not maintenance of smoking cessation: Findings from the International Tobacco Control Four country project. *Nicotine & Tobacco Research*, *12*(Supplement 1), S4–S11. http://doi.org/10.1093/ntr/ntq050

Borland, R., Yong, H. H., O'Connor, R. J., Hyland, A., & Thompson, M. E. (2010). The reliability and predictive validity of the heaviness of smoking index and its two components: Findings from the International Tobacco Control Four Country Study. *Nicotine and Tobacco Research*, *12*(SUPPL. 1), 45–50. http://doi.org/10.1093/ntr/ntq038

Bozarth, M. A., Pudiak, C. M., & KuoLee, R. (1998). Effect of chronic nicotine on brain stimulation reward. II. An escalating dose regimen. *Behavioural Brain Research*, *96*(1–2), 189–194. http://doi.org/10.1016/S0166-4328(98)00013-8

Brandt, A. M. (1990). The Cigarette, Risk, and American Culture. *Daedalus*, *119*(4), 155–176. Retrieved from http://dash.harvard.edu/handle/1/3372904

Breese, C. R., Marks, M. J., Logel, J., Adams, C. E., Sullivan, B., Collins, A. C., & Leonard, S. (1997). Effect of smoking history on [3H]nicotine binding in human postmortem brain. *The Journal of Pharmacology and Experimental Therapeutics*, *282*(1), 7–13.

Brody, A. L., Mandelkern, M. A., Costello, M. R., Abrams, A. L., Scheibal, D., Farahi, J., … Mukhin, A. G. (2009). Brain nicotinic acetylcholine receptor occupancy: effect of smoking a denicotinized cigarette. *The International Journal of Neuropsychopharmacology*, *12*(3), 305–16. http://doi.org/10.1017/S146114570800922X

Brody, A., Mandelkern, M., London, E., Olmstead, R. E., Farahi, J., Scheibal, D., … Mukhin, A. G. (2006). Cigarette smoking saturates brain α4β2 nicotinic acetylcholine receptors. *Archives of General Psychiatry*, *63*(8), 907–914. Retrieved from http://dx.doi.org/10.1001/archpsyc.63.8.907

Brose, L. S., Hitchman, S. C., Brown, J., West, R., & McNeill, A. (2015). Is the use of electronic cigarettes while smoking associated with smoking cessation attempts, cessation and reduced cigarette consumption? A survey with a 1-year follow-up. *Addiction*, *110*(7), 1160–1168. http://doi.org/10.1111/add.12917

Brown, C. J., & Cheng, J. M. (2014). Electronic cigarettes: Product characterization and design considerations. *Tobacco Control*, *23*(SUPPL. 2), ii4-ii10. Retrieved from

http://www.scopus.com/inward/record.url?eid=2-s2.0-84899653963&partnerID=40&md5=5e32004cb8364be7c7f22e73857347d7

Brown, J., Beard, E., Kotz, D., Michie, S., & West, R. (2014). Real-world effectiveness of e-cigarettes when used to aid smoking cessation: a cross-sectional population study. *Addiction*, *109*(9), 1531–1540. Retrieved from http://search.ebscohost.com/login.aspx?direct=true&db=s3h&AN=97445181&site=ehost-live

Brown, J., West, R., Beard, E., Michie, S., Shahab, L., & McNeill, A. (2014). Prevalence and characteristics of e-cigarette users in Great Britain: Findings from a general population survey of smokers. *Addict Behav*, *39*(6), 1120–1125. Retrieved from http://www.scopus.com/inward/record.url?eid=2-s2.0-84897569974&partnerID=40&md5=2fb170c113ff5dacaf643e43b44059e8

Brunzell, D. H., Mcintosh, J. M., & Papke, R. L. (2014). Diverse strategies targeting α7 homomeric and α6β2* heteromeric nicotinic acetylcholine receptors for smoking cessation. *Annals of the New York Academy of Sciences*, *1327*(1), 27–45. http://doi.org/10.1111/nyas.12421

Buchhalter, A. R., Acosta, M. C., Evans, S. E., Breland, A. B., & Eissenberg, T. (2005). Tobacco abstinence symptom suppression: The role played by the smoking-related stimuli that are delivered by denicotinized cigarettes. *Addiction*, *100*(4), 550–559. Retrieved from http://www.scopus.com/inward/record.url?eid=2-s2.0-17244380405&partnerID=40&md5=a6da287420d014e93a2d7906441aa094

Buisson, B., & Bertrand, D. (2001). Chronic exposure to nicotine upregulates the human (alpha)4((beta)2 nicotinic acetylcholine receptor function. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *21*(6), 1819–1829. http://doi.org/21/6/1819 [pii]

Bullen, C., Howe, C., & Laugesen, M. (2014). Electronic Cigarettes for Smoking Cessation: A Randomised Controlled Trial. *Journal of Vascular Surgery*, *59*(3). http://doi.org/10.1016/j.jvs.2014.01.028

Bullen, C., Howe, C., Laugesen, M., McRobbie, H., Parag, V., Williman, J., & Walker, N. (2013). Electronic cigarettes for smoking cessation: A randomised controlled trial. *The Lancet*, *382*(9905), 1629–1637. Retrieved from http://www.scopus.com/inward/record.url?eid=2-s2.0-84887680599&partnerID=40&md5=338270106f8bace7060840680a5ab6c0

Bullen, C., McRobbie, H., Thornley, S., Glover, M., Lin, R., & Laugesen, M. (2010). Effect of an electronic nicotine delivery device (e cigarette) on desire to smoke and withdrawal, user preferences and nicotine delivery: randomised cross-over trial. *Tobacco Control*, *19*(2), 98–103.

Burns, D. M., Dybing, E., Gray, N., Hecht, S., Anderson, C., Sanner, T., … Straif, K. (2008). Mandated lowering of toxicants in cigarette smoke: a description of the World Health Organization TobReg proposal. *Tobacco Control*, *17*(2), 132–41. http://doi.org/10.1136/tc.2007.024158

Buu, A., Hu, Y.-H., Piper, M. E., & Lin, H.-C. (2018). The association between e-cigarette use characteristics and combustible cigarette consumption and dependence symptoms: Results from a national longitudinal study. *Addictive Behaviors*, *84*(March), 69–74. http://doi.org/10.1016/j.addbeh.2018.03.035

Cadoni, C., & Di Chiara, G. (2000). Differential changes in accumbens shell and core dopamine in behavioral sensitization to nicotine. *European Journal of Pharmacology*, *387*(3), R23–R25. http://doi.org/10.1016/S0014-2999(99)00843-2

Caggiula, A. R., Donny, E. C., Chaudhri, N., Perkins, K. A., Evans-Martin, F. F., & Sved, A. F. (2002a). Importance of nonpharmacological factors in nicotine self-

administration. *Physiology and Behavior*, *77*(4–5), 683–687. http://doi.org/10.1016/S0031-9384(02)00918-6

Caldwell, B. O., & Crane, J. (2016). Combination Nicotine Metered Dose Inhaler and Nicotine Patch for Smoking Cessation: A Randomized Controlled Trial. *Nicotine & Tobacco Research*, *18*(10), 1944–1951. Retrieved from http://dx.doi.org/10.1093/ntr/ntw093

Cameron, J. M., Howell, D. N., White, J. R., Andrenyak, D. M., Layton, M. E., & Roll, J. M. (2014). Variable and potentially fatal amounts of nicotine in e-cigarette nicotine solutions. *Tobacco Control*, *23*(1), 77–78. http://doi.org/10.1136/tobaccocontrol-2012-050604

Cancer Research UK. (2014). Tobacco statistics | Cancer Research UK. Retrieved July 9, 2017, from http://www.cancerresearchuk.org/health-professional/cancer-statistics/risk/tobacco#ref-6

Caponnetto, P., Auditore, R., Russo, C., Cappello, G. C., & Polosa, R. (2013). Impact of an electronic cigarette on smoking reduction and cessation in schizophrenic smokers: A prospective 12-month pilot study. *International Journal of Environmental Research and Public Health*, *10*(2), 446–461. http://doi.org/10.3390/ijerph10020446

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://doi.org/10.1371/journal.pone.0066317

Caponnetto, P., Maglia, M., Cannella, M. C., Inguscio, L., Buonocore, M., Scoglio, C., … Vinci, V. (2017). Impact of different e-cigarette generation and models on cognitive performances, craving and gesture: A randomized cross-over trial (CogEcig). *Frontiers in Psychology*, *8*(MAR). http://doi.org/10.3389/fpsyg.2017.00127

Caponnetto, P., Polosa, R., Auditore, R., Minutolo, G., Signorelli, M., Maglia, M., … Aguglia, E. (2014). Smoking cessation and reduction in schizophrenia (SCARIS) with e-cigarette: study protocol for a randomized control trial. *Trials*, *15*. http://doi.org/10.1186/1745-6215-15-88

Caponnetto, P., Polosa, R., Morjaria, J., Caruso, M., Strano, S., & Russo, C. (2015). Evidence for Harm Reversal in Asthmatic Smokers Who Switched to Regular Electronic Cigarettes Use. *Journal of Allergy and Clinical Immunology*, *135*(2, Supplement), AB157. http://doi.org/http://dx.doi.org/10.1016/j.jaci.2014.12.1454

Carpenter, C. M., Wayne, G. F., & Connolly, G. N. (2007). The role of sensory perception in the development and targeting of tobacco products. *Addiction*, *102*(1), 136–147. http://doi.org/10.1111/j.1360-0443.2006.01649.x

Celada, P., Bel, N., & Artigas, F. (1994). The effects of brofaromine, a reversible MAO-A inhibitor, on extracellular serotonin in the raphe nuclei and frontal cortex of freely moving rats. *J Neural Transm Suppl*, *41*, 357–363. Retrieved from http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=7931251

Center for Disease COntrol and Prevention. (2010). How Tobacco Smoke Causes Disease. *How Tobacco Smoke Causes Disease: The Biology and Behavioral Basis for Smoking-Attributable Disease: A Report of the Surgeon General*, 1–16. http://doi.org/Dec 1 2014

Chait, L. D., & Griffiths, R. R. (1982). Smoking behavior and tobacco smoke intake: response of smokers to shortened cigarettes. *Clin Pharmacol Ther*, *32*(1), 90–97.

http://doi.org/10.1038/clpt.1982.131
Charlton, A. (2004). Medicinal uses of tobacco in history. *J R Soc Med*, *97*, 292–296. http://doi.org/10.1258/jrsm.97.6.292
Chen, C., Zhuang, Y. L., & Zhu, S. H. (2016). E-Cigarette Design Preference and Smoking Cessation: A U.S. Population Study. *American Journal of Preventive Medicine*, *51*(3), 356–363. http://doi.org/10.1016/j.amepre.2016.02.002
Clarke, P. B. S. (1990). Dopaminergic mechanisms in the locomotor stimulant effects of nicotine. *Biochemical Pharmacology*, *40*(7), 1427–1432. http://doi.org/10.1016/0006-2952(90)90436-O
Cohen, L. M., McCarthy, D. M., Brown, S. a, & Myers, M. G. (2002). Negative affect combines with smoking outcome expectancies to predict smoking behavior over time. *Psychology of Addictive Behaviors : Journal of the Society of Psychologists in Addictive Behaviors*, *16*(2), 91–97. http://doi.org/10.1037/0893-164X.16.2.91
Collins, C. C., Epstein, D. H., Parzynski, C. S., Zimmerman, D., Moolchan, E. T., & Heishman, S. J. (2010). Puffing behavior during the smoking of a single cigarette in tobacco-dependent adolescents. *Nicotine & Tobacco Research*, *12*(2), 164–167. http://doi.org/10.1093/ntr/ntp176
Cooper, M., Harrell, M. B., & Perry, C. L. (2016). A qualitative approach to understanding real-world electronic cigarette use: Implications for measurement and regulation. *Preventing Chronic Disease*, *13*, 150502. http://doi.org/10.5888/pcd13.150502
Copp, S. R., Collins, J. L., Dar, R., & Barrett, S. P. (2015). The effects of nicotine stimulus and response expectancies on male and female smokers' responses to nicotine-free electronic cigarettes. *Addictive Behaviors*, *40*, 144–147. http://doi.org/10.1016/j.addbeh.2014.09.013
Corrigall, W. A., & Coen, K. M. (1991). Selective dopamine antagonists reduce nicotine self-administration. *Psychopharmacology*, *104*(2), 171–6. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/1876661
Corrigall, W. A., Coen, K. M., & Adamson, K. L. (1994). Self-administered nicotine activates the mesolimbic dopamine system through the ventral tegmental area. *Brain Research*, *653*(1–2), 278–284. http://doi.org/10.1016/0006-8993(94)90401-4
Cox, S., Kośmider, L., McRobbie, H., Goniewicz, M., Kimber, C., Doig, M., & Dawkins, L. (2016). E-cigarette puffing patterns associated with high and low nicotine e-liquid strength: Effects on toxicant and carcinogen exposure. *BMC Public Health*, *16*(1), 999. http://doi.org/10.1186/s12889-016-3653-1
Cunningham, A., Slayford, S., Vas, C., Gee, J., Costigan, S., & Prasad, K. (2016). Development, validation and application of a device to measure e-cigarette users' puffing topography. *Scientific Reports*, *6*(June), 1–7. http://doi.org/10.1038/srep35071
D'Ruiz, C. D., Graff, D. W., Yan, X. S., Sherwin Yan, X., & 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 Public Health*, *15*(1), 991. http://doi.org/10.1186/s12889-015-2349-2
Dani, J. A., & Balfour, D. J. K. (2011). Historical and current perspective on tobacco use and nicotine addiction. *Trends in Neurosciences*, *34*(7), 383–392. http://doi.org/10.1016/j.tins.2011.05.001
Dani, J. A., & De Biasi, M. (2001). Cellular mechanisms of nicotine addiction. *Pharmacology Biochemistry and Behavior*, *70*(4), 439–446. http://doi.org/http://dx.doi.org/10.1016/S0091-3057(01)00652-9
Dani, J. A., & Heinemann, S. (1996). Molecular and cellular aspects of nicotine abuse.

Neuron, *16*(5), 905–908. http://doi.org/10.1016/S0896-6273(00)80112-9
Dar, R., & Frenk, H. (2002). Nicotine self-administration in animals: A reevaluation. *Addiction Research and Theory*, *10*(6), 545–579. http://doi.org/10.1080/1606635021000041753
Dar, R., & Frenk, H. (2004). Do smokers self-administer pure nicotine? A review of the evidence. *Psychopharmacology*, *173*(1), 18–26. http://doi.org/10.1007/s00213-004-1781-2
Dautzenberg, B., & Bricard, D. (2015). Real-Time Characterization of E-Cigarettes Use: The 1 Million Puffs Study. Journal of Addiction Research & Therapy, 06(02), 3–8. http://doi.org/10.4172/2155-6105.1000229
Dautzenberg, B., Scheck, A., Garelik, D., Kayal, C., & Dominique, M. (2016). Satisfactory throat-hit is needed to switch from tobacco to e-cigarettes : a lesson from an e-liquid blind test. *Tobacco Prevention & Cessation*, *52*(2), 1–5. http://doi.org/10.18332/tpc/62918
David, S. P., Munafò, M. R., Johansen-Berg, H., Smith, S. M., Rogers, R. D., Matthews, P. M., & Walton, R. T. (2007). Ventral Striatum/Nucleus Accumbens Activation to SmokingRelated Pictorial Cues in Smokers and Nonsmokers: A Functional Magnetic Resonance Imaging Study. *Biol Psychiatry*, *58*(6), 488–494. http://doi.org/10.1136/jnnp.2006.097345
Dawkins, L., & Corcoran, O. (2014). Acute electronic cigarette use: Nicotine delivery and subjective effects in regular users. *Psychopharmacology (Berl)*, *231*(2), 401–407. http://doi.org/10.1007/s00213-013-3249-8
Dawkins, L. E., Cox, S., Goniewicz, M. L., McRobbie, H., Kimber, C. F., Doig, M., & Kosmider, L. (2018). 'Real-world' compensatory behaviour with low nicotine concentration e-liquid: subjective effects and nicotine, acrolein and formaldehyde exposure. *Addiction*. http://doi.org/DOI:10.1111/add.14271
Dawkins, L. E., Kimber, C. F., Doig, M., Feyerabend, C., & Corcoran, O. (2016). Self-titration by experienced e-cigarette users: blood nicotine delivery and subjective effects. *Psychopharmacology*, *233*(15–16), 2933–2941. http://doi.org/10.1007/s00213-016-4338-2
Dawkins, L. E., Kimber, C., Puwanesarasa, Y., & Soar, K. (2015). First- versus second-generation electronic cigarettes: predictors of choice and effects on urge to smoke and withdrawal symptoms. *Addiction*, *110*(4). http://doi.org/10.1111/add.12807
Dawkins, L. E., Munafò, M., Christoforou, G., Olumegbon, N., & Soar, K. (2015). The Effects of E-Cigarette Visual Appearance on Craving and Withdrawal Symptoms in Abstinent Smokers. *Psychology of Addictive Behaviors*. http://doi.org/10.1037/adb0000112
Dawkins, L., Turner, J., & Crowe, E. (2013). Nicotine derived from the electronic cigarette improves time-based prospective memory in abstinent smokers. *Psychopharmacology (Berl)*, *227*(3), 377–384. Retrieved from http://search.ebscohost.com/login.aspx?direct=true&db=s3h&AN=87609508&site=ehost-live
Dawkins, L., Turner, J., Hasna, S., & Soar, K. (2012). The electronic-cigarette: Effects on desire to smoke, withdrawal symptoms and cognition. *Addict Behav*, *37*(8), 970–973. http://doi.org/10.1016/j.addbeh.2012.03.004
Dawkins, L., Turner, J., Roberts, A., & Soar, K. (2013). "Vaping" profiles and preferences: An online survey of electronic cigarette users. *Addiction*, *108*(6), 1115–1125. Retrieved from http://search.ebscohost.com/login.aspx?direct=true&db=psyh&AN=2013-16822-018&site=ehost-live

de Lacy, E., Fletcher, A., Hewitt, G., Murphy, S., & Moore, G. (2017). Cross-sectional study examining the prevalence, correlates and sequencing of electronic cigarette and tobacco use among 11–16-year olds in schools in Wales. *BMJ Open*, *7*(2), e012784. http://doi.org/10.1136/bmjopen-2016-012784

Delnevo, C. D., Giovenco, D. P., Steinberg, M. B., Villanti, A. C., Pearson, J. L., Niaura, R. S., & Abrams, D. B. (2015). Patterns of Electronic Cigarette Use Among Adults in the United States. *Nicotine & Tobacco Research*. http://doi.org/10.1093/ntr/ntv237

Dempsey, D., Tutka, P., Jacob, P., Allen, F., Schoedel, K., Tyndale, R. F., & Benowitz, N. L. (2004). Nicotine metabolite ratio as an index of cytochrome P450 2A6 metabolic activity. *Clinical Pharmacology & Therapeutics*, *76*(1), 64–72. http://doi.org/10.1016/j.clpt.2004.02.011

Department of Health. (2017). *Towards a Smokefree Generation*.

Department of Health, U., Services, H., for Disease Control, C., Center for Chronic Disease Prevention, N., Promotion, H., & on Smoking, O. (2016). *Chapter 4. Activities of the E-Cigarette Companies (in E-Cigarette Use Among Youth and Young Adults: A Report of the Surgeon General)*. Retrieved from https://www.cdc.gov/tobacco/data_statistics/sgr/e-cigarettes/pdfs/2016_SGR_Chapter_4_non-508.pdf

Di Chiara, G. (2000). Behavioural pharmacology and neurobiology of nicotine reward and dependence. *Neuronal Nicotinic Receptors*, 603–750. http://doi.org/10.1007/978-3-642-57079-7_24

Di Chiara, G. (2000). Role of dopamine in the behavioural actions of nicotine related to addiction. *European Journal of Pharmacology*, *393*(1–3), 295–314. http://doi.org/10.1016/S0014-2999(00)00122-9

Digard, H., Proctor, C., Kulasekaran, A., Malmqvist, U., & Richter, A. (2013). Determination of nicotine absorption from multiple tobacco products and nicotine gum. *Nicotine Tob Res*, *15*(1), 255–261. http://doi.org/10.1093/ntr/nts123

Doll, R. (1999). Tobacco: a Medical History. *Journal of Urban Health*, *76*(3), 289–313.

Doll, R., & Hill, A. B. (1950). Smoking and Carcinoma of the Lung. *BMJ*, *2*(4682), 739–748. http://doi.org/10.1136/bmj.2.4682.739

Doll, R., Peto, R., Boreham, J., & Sutherland, I. (2004). Mortality in relation to smoking: 50 years' observations on male British doctors. *BMJ (Clinical Research Ed.)*, *328*(7455), 1519. http://doi.org/10.1136/bmj.38142.554479.AE

Donny, E. C., Chaudhri, N., Caggiula, A. R., Evans-Martin, F. F., Booth, S., Gharib, M. A., … Sved, A. F. (2003). Operant responding for a visual reinforcer in rats is enhanced by noncontingent nicotine: implications for nicotine self-administration and reinforcement. *Psychopharmacology*, *169*(1), 68–76. http://doi.org/10.1007/s00213-003-1473-3

Doran, N., Brikmanis, K., Petersen, A., Delucchi, K., Al-Delaimy, W. K., Luczak, S., … Strong, D. (2017). Does e-cigarette use predict cigarette escalation? A longitudinal study of young adult non-daily smokers. *Preventive Medicine*, *100*, 279–284. http://doi.org/10.1016/j.ypmed.2017.03.023

Dutra, L. M., & Glantz, S. A. (2014). Electronic cigarettes and conventional cigarette use among US adolescents: a cross-sectional study. *JAMA Pediatrics*.

Ebert, R. V, McNabb, M. E., & Snow, S. L. (1984). Effect of nicotine chewing gum on plasma nicotine levels of cigarette smokers. *Clinical Pharmacology Therapy*, *35*, 495–498.

Eissenberg, T. (2010). Electronic nicotine delivery devices: ineffective nicotine delivery and craving suppression after acute administration. *Tobacco Control*, *19*(1), 87–88.

Retrieved from http://search.ebscohost.com/login.aspx?direct=true&db=a9h&AN=48126177&site=ehost-live

Elferink, J. G. R. (1983). The narcotic and hallucinogenic use of tobacco in Pre-Columbian Central America. *Journal of Ethnopharmacology*, *7*(1), 111–122. http://doi.org/10.1016/0378-8741(83)90084-3

Elliott, R., Friston, K. J., & Dolan, R. J. (2000). Dissociable neural responses in human reward systems. *The Journal of Neuroscience*, *20*(16), 6159–6165. http://doi.org/20/16/6159 [pii]

Etter, J.-F. (2005). A comparison of the content-, construct- and predictive validity of the cigarette dependence scale and the Fagerström test for nicotine dependence. *Drug and Alcohol Dependence*, *77*(3), 259–68. http://doi.org/10.1016/j.drugalcdep.2004.08.015

Etter, J.-F. (2015). Explaining the effects of electronic cigarettes on craving for tobacco in recent quitters. *Drug Alcohol Depend*, *148*(0), 102–108. http://doi.org/10.1016/j.drugalcdep.2014.12.030

Etter, J.-F., & Bullen, C. (2011). Electronic cigarette: users profile, utilization, satisfaction and perceived efficacy. *Addiction*, *106*(11), 2017–2028. http://doi.org/10.1111/j.1360-0443.2011.03505.x

Etter, J.-F., & Bullen, C. (2014). A longitudinal study of electronic cigarette users. *Addict Behav*, *39*(2), 491–494. http://doi.org/10.1016/j.addbeh.2013.10.028

Etter, J.-F., & Stapleton, J. A. (2006). Nicotine replacement therapy for long-term smoking cessation: a meta-analysis. *Tobacco Control*, *15*(4), 280–5. http://doi.org/10.1136/tc.2005.015487

Etter, J. F. (2015). Explaining the effects of electronic cigarettes on craving for tobacco in recent quitters. *Drug Alcohol Depend*, *148*, 102–108. http://doi.org/10.1016/j.drugalcdep.2014.12.030

Etter, J. F. (2016). A longitudinal study of cotinine in long-term daily users of e-cigarettes. *Drug and Alcohol Dependence*, *160*, 218–221. http://doi.org/10.1016/j.drugalcdep.2016.01.003

Etter, J. F., & Eissenberg, T. (2015). Dependence levels in users of electronic cigarettes, nicotine gums and tobacco cigarettes. *Drug Alcohol Depend*, *147*, 68–75. http://doi.org/10.1016/j.drugalcdep.2014.12.007

Etter, J., Zather, E., Svensson, S., Zäther, E., Svensson, S., Zather, E., & Svensson, S. (2013). Analysis of refill liquids for electronic cigarettes. *Addiction*, *108*(9), 1671–1679. http://doi.org/10.1111/add.12235

European Union. EU-Tobacco Products Directive, 127 Official Journal of the European Union 19 (2014). Retrieved from http://ec.europa.eu/health/tobacco/docs/dir_201440_en.pdf

Fagerström, K. (2003). Time to first cigarette; The best single indicator of tobacco dependence? *Monaldi Archives for Chest Disease - Pulmonary Series*, *59*(1), 91–94.

Fagerström, K. (2012). Determinants of tobacco use and renaming the FTND to the Fagerström Test for Cigarette Dependence. *Nicotine & Tobacco Research*, *14*(1), 75–78. Retrieved from http://search.ebscohost.com/login.aspx?direct=true&db=psyh&AN=2011-30003-008&site=ehost-live

Fan, J., Flombaum, J. I., McCandliss, B. D., Thomas, K. M., & Posner, M. I. (2003). Cognitive and Brain Consequences of Conflict. *NeuroImage*, *18*(1), 42–57. http://doi.org/10.1006/nimg.2002.1319

Fant, R. V, Henningfield, J. E., Shiffman, S., Strahs, K. R., & Reitberg, D. P. (2000). A pharmacokinetic crossover study to compare the absorption characteristics of three transdermal nicotine patches. *Pharmacology Biochemistry and Behavior*, *67*(3), 479–482. http://doi.org/10.1016/S0091-3057(00)00399-3

Farsalinos, K. E., 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. *Internal and Emergency Medicine*, *11*(1), 85–94. http://doi.org/10.1007/s11739-015-1361-y

Farsalinos, K. E., Kistler, K. A., Pennington, A., Spyrou, A., Kouretas, D., & Gillman, G. (2017). Aldehyde levels in e-cigarette aerosol: Findings from a replication study and from use of a new-generation device. *Food and Chemical Toxicology*. http://doi.org/10.1016/j.fct.2017.11.002

Farsalinos, K. E., & Polosa, R. (2014). Safety evaluation and risk assessment of electronic cigarettes as tobacco cigarette substitutes: A systematic review. *Therapeutic Advances in Drug Safety*, *5*(2), 67–86. http://doi.org/10.1177/2042098614524430

Farsalinos, K. E., Poulas, K., & Voudris, V. (2017). Changes in puffing topography and nicotine consumption depending on the power setting of electronic cigarettes. Nicotine & Tobacco Research. http://doi.org/10.1093/ntr/ntx219/4318827/Changes-in-puffing-topography-and-nicotine

Farsalinos, K. E., Poulas, K., Voudris, V., & Le Houezec, J. (2016). Electronic cigarette use in the European Union: analysis of a representative sample of 27 460 Europeans from 28 countries. *Addiction*, *111*(11), 2032–2040. http://doi.org/10.1111/add.13506

Farsalinos, K. E., Romagna, G., Tsiapras, D., Kyrzopoulos, S., Spyrou, A., & Voudris, V. (2013). Impact of flavour variability on electronic cigarette use experience: An internet survey. *Int J Environ Res Public Health*, *10*(12), 7272–7282. Retrieved from http://www.scopus.com/inward/record.url?eid=2-s2.0-84890504368&partnerID=40&md5=177ac1d5dcc31636c64309a83db11a84

Farsalinos, K. E., Romagna, G., Tsiapras, D., Kyrzopoulos, S., & Voudris, V. (2013a). Evaluating nicotine levels selection and patterns of electronic cigarette use in a group of "vapers" who had achieved complete substitution of smoking. *Substance Abuse: Research and Treatment*, *7*, 139.

Farsalinos, K. E., Romagna, G., Tsiapras, D., Kyrzopoulos, S., & Voudris, V. (2013b). Evaluation of electronic cigarette use (Vaping) topography and estimation of liquid consumption: Implications for research protocol standards definition and for public health authorities' regulation. *International Journal of Environmental Research and Public Health*, *10*(6), 2500–2514. http://doi.org/10.3390/ijerph10062500

Farsalinos, K. E., Romagna, G., Tsiapras, D., Kyrzopoulos, S., & Voudris, V. (2014). Characteristics, perceived side effects and benefits of electronic cigarette use: a worldwide survey of more than 19,000 consumers. *Int J Environ Res Public Health*, *11*(4), 4356–4373. http://doi.org/10.3390/ijerph110404356

Farsalinos, K. E., Romagna, G., & Voudris, V. (2015). Factors associated with dual use of tobacco and electronic cigarettes: A case control study. *Int J Drug Policy*, *26*(6), 595–600. http://doi.org/10.1016/j.drugpo.2015.01.006

Farsalinos, K. E., Spyrou, A., Stefopoulos, C., Tsimopoulou, K., Kourkoveli, P., Tsiapras, D., … Voudris, V. (2015). Nicotine absorption from electronic cigarette use: comparison between experienced consumers (vapers) and naïve users (smokers). *Scientific Reports*, *5*(April), 13506. http://doi.org/10.1038/srep11269

Farsalinos, K. E., Spyrou, A., Tsimopoulou, K., Stefopoulos, C., Romagna, G., & Voudris, V. (2014). Nicotine absorption from electronic cigarette use: comparison between first and new-generation devices. *Scientific Reports*, *4*, 4133. http://doi.org/10.1038/srep04133

Farsalinos, K. E., Spyrou, A., Tsimopoulou, K., Stefopoulos, C., Romagna, G., & Voudris, V. (2015). Nicotine absorption from electronic cigarette use: comparison between first and new-generation devices. *Scientific Reports*, *4*(1), 4133. http://doi.org/10.1038/srep04133

Farsalinos, K. E., Voudris, V., & Poulas, K. (2015). E-cigarettes generate high levels of aldehydes only in 'dry puff'conditions. *Addiction*, *110*(8), 1352–1356. Retrieved from http://onlinelibrary.wiley.com/doi/10.1111/add.12942/abstract

Farsalinos, K. E., Voudris, V., Spyrou, A., & Poulas, K. (2017). E-cigarettes emit very high formaldehyde levels only in conditions that are aversive to users: A replication study under verified realistic use conditions. *Food and Chemical Toxicology*, *109*, 90–94. http://doi.org/10.1016/j.fct.2017.08.044

Farsalinos, K. E., Yannovits, N., Sarri, T., Voudris, V., & Poulas, K. (2016). Protocol proposal for, and evaluation of, consistency in nicotine delivery from the liquid to the aerosol of electronic cigarettes atomizers: Regulatory implications. *Addiction*, *111*(6), 1069–1076. http://doi.org/10.1111/add.13299

FDA. (2012). Harmful and potentially harmful constituents in tobacco products and tobacco smoke; established list. *Federal Register*, *77*(64), 20034–20037. Retrieved from http://scholar.google.com/scholar?hl=en&btnG=Search&q=intitle:Harmful+and+Potentially+Harmful+Constituents+in+Tobacco+Products+and+Tobacco+Smoke;+Established+List#0

Fearon, I. M., Eldridge, A., Gale, N., Shepperd, C. J., McEwan, M., Camacho, O. M., … Proctor, C. J. (2017). E-cigarette Nicotine Delivery: Data and Learnings from Pharmacokinetic Studies. *American Journal of Health Behavior*, *41*(1), 16–32. http://doi.org/10.5993/AJHB.41.1.2

Ferguson, J. A., Patten, C. A., Schroeder, D. R., Offord, K. P., Eberman, K. M., & Hurt, R. D. (2003). Predictors of 6-month tobacco abstinence among 1224 cigarette smokers treated for nicotine dependence. *Addictive Behaviors*, *28*(7), 1203–18. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/12915164

Flora, J. W., Meruva, N., Huang, C. B., Wilkinson, C. T., Ballentine, R., Smith, D. C., … McKinney, W. J. (2016). Characterization of potential impurities and degradation products in electronic cigarette formulations and aerosols. *Regulatory Toxicology and Pharmacology*, *74*, 1–11. http://doi.org/10.1016/j.yrtph.2015.11.009

Foulds, J., Veldheer, S., Yingst, J., Hrabovsky, S., Wilson, S. J., Nichols, T. T., & Eissenberg, T. (2015). Development of a questionnaire for assessing dependence on electronic cigarettes among a large sample of ex-smoking e-cigarette users. *Nicotine and Tobacco Research*, *17*(2), 186–192. http://doi.org/10.1093/ntr/ntu204

Fowler, J. S., Volkow, N. D., Wang, G. J., Pappas, N., Logan, J., Shea, C., … Wolf, A. P. (1996). Brain monoamine oxidase A inhibition in cigarette smokers. *Proceedings of the National Academy of Sciences of the United States of America*, *93*(24), 14065–9. http://doi.org/10.1073/pnas.93.24.14065

Fowles, J., Bates, M., & Noiton, D. (2000). The Chemical Constituents in Cigarettes and Cigarette Smoke : Priorities for Harm Reduction. *Epidemiology*, (March), 65.

Fowles, J., & Dybing, E. (2003). Chemical Constituents of Cigarette Smoke, 424–431.

Friedman, A. S. (2015). How does electronic cigarette access affect adolescent

smoking? *Journal of Health Economics*, *44*, 300–308. http://doi.org/10.1016/j.jhealeco.2015.10.003

Fucile, S. (2004). Ca2+ permeability of nicotinic acetylcholine receptors. *Cell Calcium*, *35*(1), 1–8. http://doi.org/10.1016/j.ceca.2003.08.006

Gallus, S., Lugo, A., Pacifici, R., Pichini, S., Colombo, P., Garattini, S., & La Vecchia, C. (2014). E-cigarette awareness, use, and harm perceptions in Italy: a national representative survey. *Nicotine & Tobacco Research : Official Journal of the Society for Research on Nicotine and Tobacco*, *16*(12), 1541–1548. http://doi.org/10.1093/ntr/ntu124

García-Vázquez, E., Marcos, M. A., Mensa, J., De Roux, A., Puig, J., Font, C., … Torres, A. (2004). Assessment of the usefulness of sputum culture for diagnosis of community-acquired pneumonia using the PORT predictive scoring system. *Archives of Internal Medicine*, *164*(16), 1807–1811. http://doi.org/10.1001/archinte.164.16.1807

Geiss, O., Bianchi, I., & Barrero-Moreno, J. (2016). Correlation of volatile carbonyl yields emitted by e-cigarettes with the temperature of the heating coil and the perceived sensorial quality of the generated vapours. *International Journal of Hygiene and Environmental Health*, *219*(3), 268–277. http://doi.org/10.1016/j.ijheh.2016.01.004

Gentry, C. L., & Lukas, R. J. (2002). Regulation of nicotinic acetylcholine receptor numbers and function by chronic nicotine exposure. *Current Drug Targets. CNS and Neurological Disorders*, *1*(4), 359–385.

Gillman, I. G., Kistler, K. A., Stewart, E. W., & Paolantonio, A. R. (2016). Effect of variable power levels on the yield of total aerosol mass and formation of aldehydes in e-cigarette aerosols. *Regulatory Toxicology and Pharmacology*, *75*, 58–65. http://doi.org/10.1016/J.YRTPH.2015.12.019

Giovenco, D. P., & Delnevo, C. D. (2018). Prevalence of population smoking cessation by electronic cigarette use status in a national sample of recent smokers. *Addictive Behaviors*, *76*(April 2017), 129–134. http://doi.org/10.1016/j.addbeh.2017.08.002

Glasser, A. M., Collins, L., Pearson, J. L., Abudayyeh, H., Niaura, R. S., Abrams, D. B., & Villanti, A. C. (2016). Overview of Electronic Nicotine Delivery Systems: A Systematic Review. *American Journal of Preventive Medicine*, 1–34. http://doi.org/10.1016/j.amepre.2016.10.036

Goniewicz, M. L., Gawron, M., Smith, D. M., Peng, M., Jacob, 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 and Tobacco Research*, *19*(2), 160–167. http://doi.org/10.1093/ntr/ntw160

Goniewicz, M. L., Hajek, P., & McRobbie, H. (2014). Nicotine content of electronic cigarettes, its release in vapour and its consistency across batches: regulatory implications. *Addiction*, *109*(3), 500–507. http://doi.org/10.1111/add.12410

Goniewicz, M. L., Knysak, J., Gawron, M., Kosmider, L., Sobczak, A., Kurek, J., … Benowitz, N. (2014). Levels of selected carcinogens and toxicants in vapour from electronic cigarettes. *Tobacco Control*, *23*(2), 133–139. http://doi.org/10.1136/tobaccocontrol-2012-050859

Goniewicz, M. L., Kuma, T., Gawron, M., Knysak, J., & Kosmider, L. (2013). Nicotine Levels in Electronic Cigarettes. *Nicotine and Tobacco Research*, *15*(1), 158–166. Retrieved from http://search.ebscohost.com/login.aspx?direct=true&db=a9h&AN=84556692&site=ehost-live

Goniewicz, M. L., Lingas, E. O., & Hajek, P. (2013). Patterns of electronic cigarette use and user beliefs about their safety and benefits: An Internet survey. *Drug and Alcohol Review*, *32*(2), 133–140. Retrieved from http://www.scopus.com/inward/record.url?eid=2-s2.0-84874942955&partnerID=40&md5=b14e87beeda7dd4bce2cf690931d43c5

Grainge, M. J., Shahab, L., Hammond, D., O'Connor, R. J., & McNeill, A. (2009). First cigarette on waking and time of day as predictors of puffing behaviour in UK adult smokers. *Drug and Alcohol Dependence*, *101*(3), 191–195. http://doi.org/10.1016/j.drugalcdep.2009.01.013

Grana, R., Benowitz, N., & Glantz, S. A. (2014). E-cigarettes: A scientific review. *Circulation*, *129*(19), 1972–1986. http://doi.org/10.1161/CIRCULATIONAHA.114.007667

Grassi, G., Seravalle, G., Calhoun, D. A., Bolla, G.B., Giannattasio, C. G., … Mansia, G. (1994). Mechanisms responsible for sympathetic activation by cigarret smoking in humans; *Circulation*, *90*(1), 248–253.

Greenhill, R., Dawkins, L., Notley, C., Finn, M. D., & Turner, J. J. D. (2016). Adolescent Awareness and Use of Electronic Cigarettes: A Review of Emerging Trends and Findings. *Journal of Adolescent Health*, *59*(6), 612–619. http://doi.org/10.1016/j.jadohealth.2016.08.005

Grilli, M., Parodi, M., Raiteri, M., & Marchi, M. (2005). Chronic nicotine differentially affects the function of nicotinic receptor subtypes regulating neurotransmitter release. *Journal of Neurochemistry*, *93*(5), 1353–1360. http://doi.org/10.1111/j.1471-4159.2005.03126.x

Gritz, E. R., Rose, J. E., & Jarvik, M. E. (1983). Regulation of tobacco smoke intake with paced cigarette presentation. *Pharmacology, Biochemistry and Behavior*, *18*(3), 457–462. http://doi.org/10.1016/0091-3057(83)90469-0

Gust, S. W., Pickens, R. W., & Pechacek, T. F. (1983). Relation of puff volume to other topographical measures of smoking. *Addictive Behaviors*, *8*(2), 115–119. http://doi.org/10.1016/0306-4603(83)90004-7

Hagimoto, A., Nakamura, M., Morita, T., Masui, S., & Oshima, A. (2010). Smoking cessation patterns and predictors of quitting smoking among the Japanese general population: A 1-year follow-up study. *Addiction*, *105*(1), 164–173. http://doi.org/10.1111/j.1360-0443.2009.02735.x

Hajdu, S. I., & Vadmal, M. S. (2010). A Note from History: The use of tobacco. *Annals of Clinical and Laboratory Science*, *40*(2), 178–81. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/20421631

Hajek, P., Etter, J.-F., Benowitz, N., Eissenberg, T., & McRobbie, H. (2014). Electronic cigarettes: review of use, content, safety, effects on smokers and potential for harm and benefit. *Addiction*, *109*(11), 1801–1810. http://doi.org/10.1111/add.12659

Hajek, P., Goniewicz, M. L., Phillips, A., Smith, K. M., West, O., & McRobbie, H. (2015). Nicotine intake from electronic cigarettes on initial use and after 4 weeks of regular use. *Nicotine and Tobacco Research*, *17*(2), 175–179. http://doi.org/10.1093/ntr/ntu153

Hajek, P., Przulj, D., Phillips-Waller, A., Anderson, R., & McRobbie, H. (2018). Initial ratings of different types of e-cigarettes and relationships between product appeal and nicotine delivery. *Psychopharmacology*, 1–10. http://doi.org/10.1007/s00213-017-4826-z

Hajek, P., Przulj, D., Phillips, A., Anderson, R., & McRobbie, H. (2017). Nicotine delivery to users from cigarettes and from different types of e-cigarettes. *Psychopharmacology*, 773–779. http://doi.org/10.1007/s00213-016-4512-6

Hall, J., Parkinson, J. A., Connor, T. M., Dickinson, A., & Everitt, B. J. (2001). Involvement of the central nucleus of the amygdala and nucleus accumbens core in mediating Pavlovian influences on instrumental behaviour. *European Journal of Neuroscience*, *13*(10), 1984–1992. http://doi.org/10.1046/j.0953-816x.2001.01577.x

Hammond, D., Fong, G. T., Cummings, K. M., & Hyland, A. (2005). Smoking topography, brand switching, and nicotine delivery: Results from an in vivo study. *Cancer Epidemiology Biomarkers and Prevention*, *14*(6), 1370–1375. http://doi.org/10.1158/1055-9965.EPI-04-0498

Hammond, E. C., & Horn, D. (1958). Smoking and death rates: report on forty-four months of follow-up of 187,783 men. 2. Death rates by cause. *Journal of the American Medical Association*, *166*(11), 1294–1308.

Harrell, P. T., Naqvi, S. M. H., Plunk, A. D., Ji, M., & Martins, S. S. (2017). Patterns of youth tobacco and polytobacco usage: The shift to alternative tobacco products. *American Journal of Drug and Alcohol Abuse*, *43*(6), 694–702. http://doi.org/10.1080/00952990.2016.1225072

Harvey, D. M., Yasar, S., Heishman, S. J., Panlilio, L. V, Henningfield, J. E., & Goldberg, S. M. (2004). Nicotine serves as an effective reinforcer of intravenous drug-taking behavior in human cigarette smokers. *Psychopharmacology (Berl)*, *175*(2), 134–142. http://doi.org/10.1007/s00213-004-1818-6

Heatherton, T. F., Kozlowski, L. T., Frecker, R. C., Rickert, W. S., & Robinson, J. C. (1989). Measuring the Heaviness of Smoking: using self-reported time to the first cigarette of the day and number of cigarettes smoked per day. *British Journal of Addiction*, *84*(7), 791–800. http://doi.org/10.1111/j.1360-0443.1989.tb03059.x

Hellman, R., Cummings, K. M., Haughey, B. P., Zielezny, M. A., & O'Shea, R. M. (1991). Predictors of attempting and succeeding at smoking cessation. *Health Education Research*, *6*(1), 77–86. http://doi.org/10.1093/her/6.1.77

Henningfield, J. E., & Keenan, R. M. (1993). Nicotine delivery kinetics and abuse liability. *Journal of Consulting and Clinical Psychology*, *61*(5), 743–50. http://doi.org/10.1037/0022-006X.61.5.743

Henningfield, J. E., Stapleton, J. M., Benowitz, N. L., Grayson, R. F., & London, E. D. (1993). Higher levels of nicotine in arterial than in venous blood after cigarette smoking. *Drug and Alcohol Dependence*, *33*(1), 23–9. http://doi.org/10.1016/0376-8716(93)90030-T

Herning, R. I., Jones, R. T., Bachman, J., & Mines, A. H. (1981). Puff volume increases when low-nicotine cigarettes are smoked. *British Medical Journal (Clinical Research Edition)*, *283*(6285), 187–189. Retrieved from http://search.ebscohost.com/login.aspx?direct=true&db=a9h&AN=4949103&site=ehost-live

Hiler, M. M. (2016a). *Electronic Cigarette User Plasma Nicotine Concentration, Puff Topography, Heart Rate, and Subjective Effects: Influence of Liquid Nicotine Concentration and User Experience*. *Experimental and Clinical Psychopharmacology*. Retrieved from https://scholarscompass.vcu.edu/etd/4613/

Hiler, M. M. (2016b). *Electronic Cigarette User Plasma Nicotine Concentration and Puff Topography: Influence of Liquid Nicotine Concentration and User Experience*. Retrieved from https://oatd.org/oatd/record?record=oai%5C:scholarscompass.vcu.edu%5C:etd-5675&q=%28%22electronic cigarette%22%29 AND pub_dt%3A%5B2016-01-01T00%3A00%3A00Z TO 2017-01-01T00%3A00%3A00Z%5D

Hitchman, S. C., Brose, L. S., Brown, J., Robson, D., & McNeill, A. (2015).

Associations Between E-Cigarette Type, Frequency of Use, and Quitting Smoking: Findings From a Longitudinal Online Panel Survey in Great Britain. *Nicotine & Tobacco Research*. http://doi.org/10.1093/ntr/ntv078

Hoek, J., Thrul, J., & Ling, P. (2017). Qualitative analysis of young adult ENDS users' expectations and experiences. *BMJ Open*, *7*(3), 26–28. http://doi.org/10.1136/bmjopen-2016-014990

Hoffman, F. L. L. D. (1931). Cancer and Smoking Habits, 50–67.

Hoffmann, D., & Hoffmann, I. (1997). The changing cigarette, 1950-1995. *Journal of Toxicology and Environmental Health*, *50*(4), 307–64. http://doi.org/10.1080/009841097160393

Holm, H., Jarvis, M. J., Russell, M. A., & Feyerabend, C. (1992). Nicotine intake and dependence in Swedish snuff takers. *Psychopharmacology*, *108*(4), 507–11. http://doi.org/10.1007/BF02247429

Hua, M., Yip, H., & Talbot, P. (2011). Mining data on usage of electronic nicotine delivery systems (ENDS) from YouTube videos. *Tobacco Control*, *22*(November), 103–106. http://doi.org/10.1136/tobaccocontrol-2011-050226

Hua, M., Yip, H., & Talbot, P. (2013). Mining data on usage of electronic nicotine delivery systems (ENDS) from YouTube videos. *Tobacco Control*, *22*(2), 103–106. http://doi.org/10.1136/tobaccocontrol-2011-050226

Hughes, J. R., & Hatsukami, D. (1986). Signs and symptoms of tobacco withdrawal. *Archives of General Psychiatry*, *43*(3), 289–294. Retrieved from http://www.scopus.com/inward/record.url?eid=2-s2.0-0022451897&partnerID=40&md5=358c2ff5f950eceb2408e3874769fc93

Hughes, J. R., Keely, J., & Naud, S. (2004). REVIEW Shape of the relapse curve and long-term abstinence among untreated smokers. *Addiction*, *99*(1), 29–38. http://doi.org/10.1111/j.1360-0443.2004.00540.x

Hughes, J. R., Lesmes, G. R., Hatsukami, D. K., Richmond, R. L., Lichtenstein, E., Jorenby, D. E., … Heatley, S. A. (1999). Are higher doses of nicotine replacement more effective for smoking cessation? *Nicotine & Tobacco Research : Official Journal of the Society for Research on Nicotine and Tobacco*, *1*(2), 169–74. http://doi.org/10.1080/14622299050011281

Hughes, J. R., Solomon, L. J., Naud, S., Fingar, J. R., Helzer, J. E., & Callas, P. W. (2014). Natural history of attempts to stop smoking. *Nicotine and Tobacco Research*, *16*(9), 1190–1198. http://doi.org/10.1093/ntr/ntu052

Hukkanen, J., Jacob, P., & Benowitz, N. L. (2005). Metabolism and Disposition Kinetics of Nicotine. *Pharmacological Reviews*, *57*(1), 79–115. http://doi.org/10.1124/pr.57.1.3

Hummel, K., Hoving, C., Nagelhout, G. E., de Vries, H., van den Putte, B., Candel, M. J. J. M., … Willemsen, M. C. (2015). Prevalence and reasons for use of electronic cigarettes among smokers: Findings from the International Tobacco Control (ITC) Netherlands Survey. *International Journal of Drug Policy*, *26*(6), 601–608. http://doi.org/10.1016/j.drugpo.2014.12.009

Inoue-Choi, M., Liao, L. M., Reyes-Guzman, C., Hartge, P., Caporaso, N., & Freedman, N. D. (2017). Association of Long-term, Low-Intensity Smoking With All-Cause and Cause-Specific Mortality in the National Institutes of Health-AARP Diet and Health Study. *JAMA Internal Medicine*, *177*(1), 87–95. http://doi.org/10.1001/jamainternmed.2016.7511

Iyaniwura, T. T., Wright, A. E., & Balfour, D. J. K. (2001). Evidence that mesoaccumbens dopamine and locomotor responses to nicotine in the rat are influenced by pretreatment dose and strain. *Psychopharmacology*, *158*(1), 73–79.

http://doi.org/10.1007/s002130100852
Jamal, A., Gentzke, A., Hu, S. S., Cullen, K. A., Apelberg, B. J., Homa, D. M., & King, B. A. (2017). Tobacco Use Among Middle and High School Students — United States, 2011–2016. *MMWR. Morbidity and Mortality Weekly Report*, *66*(23), 597–603. http://doi.org/10.15585/mmwr.mm6623a1
Jarvik, M. E., Madsen, D. C., Olmstead, R. E., Iwamoto-Schaap, P. N., Elins, J. L., & Benowitz, N. L. (2000). Nicotine blood levels and subjective craving for cigarettes. *Pharmacology Biochemistry and Behavior*, *66*(3), 553–558. http://doi.org/10.1016/S0091-3057(00)00261-6
Jensen, R. P., Luo, W., Pankow, J. F., Strongin, R. M., & Peyton, D. H. (2015). Hidden Formaldehyde in E-Cigarette Aerosols. *New England Journal of Medicine*, *372*(4), 392–394. http://doi.org/10.1056/NEJMc1413069
Ji, D., Lape, R., & Dani, J. A. (2001). Timing and location of nicotinic activity enhances or depresses hippocampal synaptic plasticity. *Neuron*, *31*(1), 131–141. http://doi.org/10.1016/S0896-6273(01)00332-4
Jorenby, D. E., Smith, S. S., Fiore, M. C., & Baker, T. B. (2017). Nicotine levels, withdrawal symptoms, and smoking reduction success in real world use: A comparison of cigarette smokers and dual users of both cigarettes and E-cigarettes. *Drug and Alcohol Dependence*, *170*, 93–101. http://doi.org/10.1016/j.drugalcdep.2016.10.041
June, K. M., Norton, K. J., Rees, V. W., & O'Connor, R. J. (2012). Influence of measurement setting and home smoking policy on smoking topography. *Addictive Behaviors*, *37*(1), 42–46. http://doi.org/10.1016/j.addbeh.2011.07.039
Kalkhoran, S., & Glantz, S. A. (2016). E-cigarettes and smoking cessation in real-world and clinical settings: A systematic review and meta-analysis. *The Lancet Respiratory Medicine*, *4*(2), 116–128. http://doi.org/10.1016/S2213-2600(15)00521-4
Keithly, L., Ferris Wayne, G., Cullen, D., & Connolly, G. (2005). Industry research on the use and effects of levulinic acid: A case study in cigarette additives. *Nicotine & Tobacco Research*, *7*(5), 761–771. http://doi.org/10.1080/14622200500259820
King, B. A., Patel, R., Nguyen, K. H., & Dube, S. R. (2015). Trends in awareness and use of electronic cigarettes among US adults, 2010-2013. *Nicotine Tob Res*, *17*(2), 219–227. http://doi.org/10.1093/ntr/ntu191
Kjeldsen, K., Thomsen, H. K., & Astrup, P. (1974). Effects of Carbon Monoxide on Myocardium. *Circulation Research*, *34*(3), 339 LP-348. Retrieved from http://circres.ahajournals.org/content/34/3/339.abstract
Kolonen, S., Tuomisto, J., Puustinen, P., & Airaksinen, M. M. (1991). Smoking behavior in low-yield cigarette smokers and switchers in the natural environment. *Pharmacology, Biochemistry and Behavior*, *40*(1), 177–180. http://doi.org/10.1016/0091-3057(91)90341-X
Kolonen, S., Tuomisto, J., Puustinen, P., & Airaksinen, M. M. (1992). Effects of smoking abstinence and chain-smoking on puffing topography and diurnal nicotine exposure. *Pharmacology, Biochemistry and Behavior*, *42*(2), 327–332. Retrieved from http://search.ebscohost.com/login.aspx?direct=true&db=psyh&AN=1993-12815-001&site=ehost-live
Kośmider, L., Kimber, C. F., Kurek, J., Corcoran, O., & Dawkins, L. E. (2017). Compensatory Puffing with Lower Nicotine Concentration E-liquids Increases Carbonyl Exposure in E-cigarette Aerosols. *Nicotine & Tobacco Research,* 1–6. http://doi.org/10.1093/ntr/ntx162.
Kośmider, L., Madej, D., Gawron, M., & Sobczak, A. (2016). Influence of electronic

cigarettes puffing regimes on amount of vaporized liquid*. *Przegląd Lekarski*, *73*(10), 699–703. Retrieved from http://www.wple.net/plek/numery_2016/numer-10-2016/699-703.pdf

Kośmider, L., Sobczak, A., Fik, M., Knysak, J., Zaciera, M., Kurek, J., & Goniewicz, M. L. (2014). Carbonyl compounds in electronic cigarette vapors: Effects of nicotine solvent and battery output voltage. *Nicotine and Tobacco Research*, *16*(10), 1319–1326. http://doi.org/10.1093/ntr/ntu078. Retrieved from http://search.ebscohost.com/login.aspx?direct=true&db=psyh&AN=2014-37086-005&site=ehost-live

Kotz, D., Brown, J., & West, R. (2013). Predictive validity of the Motivation To Stop Scale (MTSS): A single-item measure of motivation to stop smoking. *Drug and Alcohol Dependence*, *128*(1–2), 15–19. http://doi.org/10.1016/j.drugalcdep.2012.07.012

Kotz, D., Brown, J., & West, R. (2014). "Real-world" effectiveness of smoking cessation treatments: A population study. *Addiction*, *109*(3), 491–499. http://doi.org/10.1111/add.12429

Kotz, D., Fidler, J., & West, R. (2012). Very low rate and light smokers: smoking patterns and cessation-related behaviour in England, 2006–11. *Addiction*, *107*(5), 995–1002. http://doi.org/10.1111/j.1360-0443.2011.03739.x

Kozlowski, L. T., Director, J., & Harford, M. A. (1981). Tobacco dependence, restraint and time to the first cigarette of the day. *Addictive Behaviors*, *6*(4), 307–312. http://doi.org/10.1016/0306-4603(81)90044-7

Kozlowski, L. T., Lynn Homish, D., Homish, G. G., Homish, D. L., & Homish, G. G. (2017). Daily users compared to less frequent users find vape as or more satisfying and less dangerous than cigarettes, and are likelier to use non-cig-alike vaping products. *Preventive Medicine Reports*, *6*, 111–114. http://doi.org/10.1016/j.pmedr.2017.02.026

Kozlowski, L. T., Pillitteri, J. L., & Sweeney, C. T. (1994). Misuse of "light" cigarettes by means of vent blocking. *Journal of Substance Abuse*, *6*(3), 333–336. http://doi.org/10.1016/S0899-3289(94)90524-X

Kozlowski, L. T., Pope, M. A., & Lux, J. (1988). Prevalence of the Misuse of Ultra-Low-Tar Cigarettes by Blocking Filter Vents. *American Journal of Public Health*, *78*(6), 1987–1988.

Kozlowski, L. T., Porter, C. Q., Orleans, C. T., Pope, M. A., & Heatherton, T. (1994). Predicting smoking cessation with self-reported measures of nicotine dependence: FTQ, FTND, and HSI. *Drug and Alcohol Dependence*, *34*(3), 211–216. http://doi.org/10.1016/0376-8716(94)90158-9

Kozlowski, L. T., & Warner, K. E. (2017). Adolescents and e-cigarettes: Objects of concern may appear larger than they are. *Drug and Alcohol Dependence*, *174*(1 May 2017), 209–214. http://doi.org/10.1016/j.drugalcdep.2017.01.001

Krebs, N. M., Chen, A., Zhu, J., Sun, D., Liao, J., Stennett, A. L., & Muscat, J. E. (2016). Comparison of Puff Volume With Cigarettes per Day in Predicting Nicotine Uptake Among Daily Smokers. *American Journal of Epidemiology*, *184*(1), 48–57. Retrieved from http://10.0.4.69/aje/kwv341

Lamb, R. J., Preston, K. L., Schindler, C. W., Meisch, R. A., Davis, F., Katz, J. L., … Goldberg, S. R. (1991). The reinforcing and subjective effects of morphine in post-addicts: a dose-response study. *The Journal of Pharmacology and Experimental Therapeutics*, *259*(3), 1165–1173.

Lancet, T. (2015). E-cigarettes: Public Health England's evidence-based confusion. *The Lancet*, *386*(9996), 829. http://doi.org/10.1016/S0140-6736(15)00042-2

Le Foll, B., & Goldberg, S. R. (2005). Control of the reinforcing effects of nicotine by associated environmental stimuli in animals and humans. *Trends in Pharmacological Sciences*, *26*(6), 287–293. http://doi.org/10.1016/J.TIPS.2005.04.005

Le Foll, B., Wertheim, C., & Goldberg, S. R. (2007). High reinforcing efficacy of nicotine in non-human primates. *PLoS ONE*, *2*(2). http://doi.org/10.1371/journal.pone.0000230

Le Houezec, J. (2003). Role of nicotine pharmacokinetics in nicotine addiction and nicotine replacement therapy: A review. *International Journal of Tuberculosis and Lung Disease*, *7*(9), 811–819.

Lechner, W. V., Meier, E., Wiener, J. L., Grant, D. M., Gilmore, J., Judah, M. R., … Wagener, T. L. (2015). The comparative efficacy of first- versus second-generation electronic cigarettes in reducing symptoms of nicotine withdrawal. *Addiction*, *110*(5), 862–867. http://doi.org/10.1111/add.12870

Lechner, W. V., Tackett, A. P., Grant, D. M., Tahirkheli, N. N., Driskill, L. M., & Wagener, T. L. (2015a). Effects of duration of electronic cigarette use. *Nicotine and Tobacco Research*, *17*(2), 180–185. http://doi.org/10.1093/ntr/ntu061

Lee, Y. H., Gawron, M., & Goniewicz, M. L. (2015). Changes in puffing behavior among smokers who switched from tobacco to electronic cigarettes. *Addictive Behaviors*, *48*, 1–4. http://doi.org/10.1002/aur.1474.Replication

Leigh, N. J., Lawton, R. I., Hershberger, P. A., & Goniewicz, M. L. (2016). Flavourings significantly affect inhalation toxicity of aerosol generated from electronic nicotine delivery systems (ENDS). *Tobacco Control*, *0*, 1–7. http://doi.org/10.1136/tobaccocontrol-2016-053205

Leshner, A. I., & Koob, G. F. (2015). Drugs of abuse and the brain. *Proceedings of the Association of American Physicians*, *111*(2), 99–108. http://doi.org/10.1046/j.1525-1381.1999.09218.x

Leventhal, A. M., Strong, D. R., Kirkpatrick, M. G., Unger, J. B., Sussman, S., Riggs, N. R., … Audrain-McGovern, J. (2015). Association of Electronic Cigarette Use With Initiation of Combustible Tobacco Product Smoking in Early Adolescence. *Journal of American Medical Association Pediatrics*, *314*(7), 700. http://doi.org/10.1001/jama.2015.8950

Levi, M., Dempsey, D. A., Benowitz, N. L., & Sheiner, L. B. (2007). Prediction methods for nicotine clearance using cotinine and 3-hydroxy-cotinine spot saliva samples II. Model application. *Journal of Pharmacokinetics and Pharmacodynamics*, *34*(1), 23–34. http://doi.org/10.1007/s10928-006-9026-0

Lewis, A., Miller, J. H., & Lea, R. A. (2007). Monoamine oxidase and tobacco dependence. *NeuroToxicology*, *28*(1), 182–195. http://doi.org/10.1016/j.neuro.2006.05.019

Li, J., Newcombe, R., & Walton, D. (2015). The prevalence, correlates and reasons for using electronic cigarettes among New Zealand adults. *Addict Behav*, *45*(0), 245–251. http://doi.org/10.1016/j.addbeh.2015.02.006

Litt, M. D., Duffy, V., & Oncken, C. (2016). Cigarette smoking and electronic cigarette vaping patterns as a function of e-cigarette flavourings. *Tobacco Control*, *25*, ii67-ii72. http://doi.org/10.1136/tobaccocontrol-2016-053223

Liu, G., Wasserman, E., Kong, L., & Foulds, J. (2017). A comparison of nicotine dependence among exclusive E-cigarette and cigarette users in the PATH study. *Preventive Medicine*, 6–11. http://doi.org/10.1016/j.ypmed.2017.04.001

Lopez, A. A., & Eissenberg, T. (2015). Science and the evolving electronic cigarette. *Preventive Medicine*, *80*, 101–106. http://doi.org/10.1016/j.ypmed.2015.07.006

Lopez, A. A., Hiler, M. M., Soule, E. K., Ramôa, C. P., Karaoghlanian, N. V., Lipato, T., … Eissenberg, T. (2016). Effects of electronic cigarette liquid nicotine concentration on plasma nicotine and puff topography in tobacco cigarette smokers: A preliminary report. *Nicotine and Tobacco Research*, *18*(5), 720–723. http://doi.org/10.1093/ntr/ntv182

Lubman, D. I., Yücel, M., & Pantelis, C. (2004). Addiction, a condition of compulsive behaviour? Neuroimaging and neuropsychological evidence of inhibitory dysregulation. *Addiction*, *99*(12), 1491–1502. http://doi.org/10.1111/j.1360-0443.2004.00808.x

Maddison, R., Roberts, V., Bullen, C., McRobbie, H., Jiang, Y., Prapavessis, H., … Brown, P. (2010). Design and conduct of a pragmatic randomized controlled trial to enhance smoking-cessation outcomes with exercise: The Fit2Quit study. *Mental Health and Physical Activity*, *3*(2), 92–101. http://doi.org/10.1016/j.mhpa.2010.09.003

Mantey, D. S., Cooper, M. R., Loukas, A., & Perry, C. L. (2017). E-cigarette use and cigarette smoking cessation among Texas college students. *American Journal of Health Behavior*, *41*(6), 750–759. http://doi.org/10.5993/AJHB.41.6.9

Manzoli, L., Flacco, M. E., Fiore, M., La Vecchia, C., Marzuillo, C., Gualano, M. R., … Villari, P. (2015). Electronic cigarettes efficacy and safety at 12 months: Cohort study. *PLoS ONE*. http://doi.org/10.1371/journal.pone.0129443

Marshall, D. L., Redfern, P. H., & Wonnacott, S. (1997). Presynaptic nicotinic modulation of dopamine release in the three ascending pathways studied by in vivo microdialysis: comparison of naive and chronic nicotine-treated rats. *Journal of Neurochemistry*, *68*(4), 1511–9. http://doi.org/10.1046/j.1471-4159.1997.68041511.x

Martínez-Sánchez, J. M., Ballbè, M., Fu, M., Martín-Sánchez, J. C., Saltó, E., Gottlieb, M., … Fernández, E. (2014). Electronic cigarette use among adult population: A cross-sectional study in Barcelona, Spain (2013-2014). *BMJ Open*, *4*(8), 1–7. http://doi.org/10.1136/bmjopen-2014-005894

Maskos, U., Molles, B. E., Pons, S., Besson, M., Guiard, B. P., Guilloux, J.-P., … Changeux, J.-P. (2005). Nicotine reinforcement and cognition restored by targeted expression of nicotinic receptors. *Nature*, *436*(7047), 103–107. Retrieved from http://dx.doi.org/10.1038/nature03694

McAuley, T. R., Hopke, P. K., Zhao, J., & Babaian, S. (2012). Comparison of the effects of e-cigarette vapor and cigarette smoke on indoor air quality. *Inhalation Toxicology*, *24*(12), 850–857.

McAuliffe, W. E. (1982). A Test of Wikler's Theory of Relapse: The Frequency of Relapse Due to Conditioned Withdrawal Sickness. *International Journal of the Addictions*, *17*(1), 19–33. http://doi.org/10.3109/10826088209054607

McKay, B. E., Placzek, A. N., & Dani, J. A. (2007). Regulation of synaptic transmission and plasticity by neuronal nicotinic acetylcholine receptors. *Biochemical Pharmacology*, *74*(8), 1120–1133. http://doi.org/10.1016/j.bcp.2007.07.001

McKee, M., & Capewell, S. (2015). Evidence about electronic cigarettes: a foundation built on rock or sand? *BMJ*, h4863. http://doi.org/10.1136/bmj.h4863

Mckeganey, N., & Dickson, T. (2017). Why Don't More Smokers Switch to Using E-Cigarettes: The Views of Confirmed Smokers. *International Journal of Environmental Research and Public Health*, *14*(6), 647. http://doi.org/10.3390/ijerph14060647

McMorrow, M. J., & Foxx, R. M. (1983). Nicotine's role in smoking: An analysis of

nicotine regulation. *Psychological Bulletin*, *93*(2), 302–327. http://doi.org/10.1037/0033-2909.93.2.302
McNabb, M. E. (1984). Chewing nicotine gum for 3 months: What happens to plasma nicotine levels? *Canadian Medical Association Journal*, *131*(6), 589–592.
McNeill, A., Brose, L. S., Calder, R., Hitchman, S. C., Hajek, P., & H, M. (2015). E-cigarettes: an evidence update A report commissioned by Public Health England. *Public Health England*, 111. Retrieved from www.gov.uk/government/uploads/system/uploads/attachment_data/file/454516/Ecigarettes_an_evidence_update_A_report_commissioned_by_Public_Health_England.pdf
McNeill, A., Brose, L. S., Calder R, Hitchman, S. C., Hajek, P., & McRobbie, H. (2015). Evidence about e-cigarettes: They are much safer than cigarettes and do not lure children to smoking. *BMJ*, *351*(h:4863). Retrieved from http://www.bmj.com/content/351/bmj.h4863/rr
McNeill, A., Brose, L. S., Calder R, Hitchman SC, Hajek P, & McRobbie H. (2015). *E-cigarettes: an evidence update A report commissioned by Public Health England*.
McNeill, A., & Hajek, P. (2014). Underpinning evidence for the estimate that e-cigarette use is around 95 % safer than smoking : authors ' note. *Public Health England*, 1–2.
McNeill, A., & Robson, D. (2017). A man before his time: Russell's insights into nicotine, smoking, treatment and curbing the smoking problem. *Addiction*, 1–5. http://doi.org/10.1111/add.14043
McQueen, A., Tower, S., & Sumner, W. (2011). Interviews With "Vapers": Implications for Future Research With Electronic Cigarettes. *Nicotine & Tobacco Research*, *13*(9), 860–867. Retrieved from http://search.ebscohost.com/login.aspx?direct=true&db=a9h&AN=65456814&site=ehost-live
McRobbie, H., Bullen, C., Hartmann-Boyce, J., & Hajek, P. (2014). Electronic cigarettes for smoking cessation and reduction. *Cochrane Database Syst Rev*, *12*. http://doi.org/10.1002/14651858.CD010216.pub2
McRobbie, H., Bullen, C., Hartmann-Boyce, J., & Hajek, P. (2014). *Electronic cigarettes for smoking cessation and reduction (Review) Cochrane ecig review*.
Meier, E., Tackett, A. P., & Wagener, T. L. (2013). Effectiveness of Electronic Aids for Smoking Cessation. *Current Cardiovascular Risk Reports*, *7*(6), 464–472. http://doi.org/10.1007/s12170-013-0343-8
Millar, N. S., & Gotti, C. (2009). Diversity of vertebrate nicotinic acetylcholine receptors. *Neuropharmacology*, *56*(1), 237–246. http://doi.org/10.1016/j.neuropharm.2008.07.041
Millar, N. S., & Harkness, P. C. (2008). Assembly and trafficking of nicotinic acetylcholine receptors (Review). *Molecular Membrane Biology*, *25*(4), 279–292. http://doi.org/10.1080/09687680802035675
Monterosso, J. R., Aron, A. R., Cordova, X., Xu, J., & London, E. D. (2005). Deficits in response inhibition associated with chronic methamphetamine abuse. *Drug and Alcohol Dependence*, *79*(2), 273–277. http://doi.org/10.1016/j.drugalcdep.2005.02.002
Moore, D., Aveyard, P., Connock, M., Wang, D., Fry-Smith, A., & Barton, P. (2009). Effectiveness and safety of nicotine replacement therapy assisted reduction to stop smoking: systematic review and meta-analysis. *Bmj*, *338*(apr02 3), b1024–b1024. http://doi.org/10.1136/bmj.b1024
Morrison, C. F., & Stephenson, J. A. (1969). Nicotine injections as the conditioned

stimulus in discrimination learning. *Psychopharmacologia*, *15*(5), 351–360. http://doi.org/10.1007/BF00403710

Muhammad-Kah, R. S., Hayden, A. D., Liang, Q., Frost-Pineda, K., & Sarkar, M. (2011). The relationship between nicotine dependence scores and biomarkers of exposure in adult cigarette smokers. *Regulatory Toxicology and Pharmacology*, *60*(1). http://doi.org/10.1016/j.yrtph.2011.02.008

Nakajima, M., Yamamoto, T., Nunoya, K., Yokoi, T., Nagashima, K., Inoue, K., … Kuroiwa, Y. (1996). IN C-OXIDATION OF NICOTINE. *Drug Metabolism & Disposition*, *24*(11). Retrieved from http://citeseerx.ist.psu.edu/viewdoc/download?doi=10.1.1.976.4054&rep=rep1&type=pdf

Naqvi, N., & Bechara, A. (2005). The airway sensory impact of nicotine contributes to the conditioned reinforcing effects of individual puffs from cigarettes. *Pharmacology Biochemistry and Behavior*, *81*(4), 821–829. http://doi.org/10.1002/ana.22528.Toll-like

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://doi.org/http://dx.doi.org/10.1016/j.drugalcdep.2015.05.005

New Zealand Ministry of Health. Ministry of Health position statement – E-cigarettes (2017). Retrieved from https://www.health.govt.nz/our-work/preventative-health-wellness/tobacco-control/e-cigarettes

NHS, Smoke Free (n.d.). Retrieved from https://www.nhs.uk/smokefree/help-and-advice/e-cigarettes

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://doi.org/10.5993/AJHB.38.2.12

Norton, K. J., June, K. M., & O'Connor, R. J. (2014). Initial puffing behaviors and subjective responses differ between an electronic nicotine delivery system and traditional cigarettes. *Tobacco Induced Diseases*, *12*(1). http://doi.org/10.1186/1617-9625-12-17

Notley, C., Dawkins, L., Holland, R., Jakes, S., & Ward, E. (2017). *Redefining understanding of tobacco smoking lapse in the context of vaping*.

Notley, C., Ward, E., Dawkins, L., & Holland, R. (2018). The unique contribution of e-cigarettes for tobacco harm reduction in supporting smoking relapse prevention, 1–12. http://doi.org/10.1186/s12954-018-0237-7

Office for National Statistics. (2017). *Adult smoking habits in the UK: 2016*. http://doi.org/10.2105/AJPH.76.11.1337

Olale, F., Gerzanich, V., Kuryatov, A., Wang, F., & Lindstrom, J. (1997). Chronic nicotine exposure differentially affects the function of human ?3, ?4, and ?7 neuronal nicotinic receptor subtypes. *Journal of Pharmacology and Experimental Therapeutics*, *283*(2), 675–683.

Palmatier, M. I., Evans-Martin, F. F., Hoffman, A., Caggiula, A. R., Chaudhri, N., Donny, E. C., … Sved, A. F. (2006). Dissociating the primary reinforcing and reinforcement-enhancing effects of nicotine using a rat self-administration paradigm with concurrently available drug and environmental reinforcers. *Psychopharmacology*, *184*(3–4), 391–400. http://doi.org/10.1007/s00213-005-0183-4

Pankow, J. F. (2001). A consideration of the role of gas/particle partitioning in the

deposition of nicotine and other tobacco smoke compounds in the respiratory tract. *Chemical Research in Toxicology*, *14*(11), 1465–1481.

Pankow, J. F., Kim, K., McWhirter, K. J., Luo, W., Escobedo, J. O., Strongin, R. M., … Peyton, D. H. (2017). Benzene formation in electronic cigarettes. *Plos One*, *12*(3), e0173055. http://doi.org/10.1371/journal.pone.0173055

Panlilio, L. V., Yasar, S., Nemeth-Coslett, R., Katz, J. L., Henningfield, J. E., Solinas, M., … Goldberg, S. R. (2005). Human cocaine-seeking behavior and its control by drug-associated stimuli in the laboratory. *Neuropsychopharmacology*, *30*(2), 433–443. http://doi.org/10.1038/sj.npp.1300599

Parrott, A. C., & Craig, D. (1995). Psychological functions served by nicotine chewing gum. *Addict Behav*, *20*(3), 271–278. Retrieved from http://search.ebscohost.com/login.aspx?direct=true&db=psyh&AN=1996-06213-001&site=ehost-live

Parzynski, C. S., Jaszyna-Gasior, M., Franken, F. H., & Moolchan, E. T. (2008). Measuring nicotine intake among highly-dependent adolescent smokers: Comparability of saliva and plasma cotinine concentrations. *Pharmacology, Biochemistry & Behavior*, *89*(2), 145–149. http://doi.org/10.1016/j.pbb.2007.12.003

Patterson, F., Benowitz, N., Shields, P., Kaufmann, V., Jepson, C., & Wileyto, P. (2003). Individual Differences in Nicotine Intake per Cigarette. *Prevention*, *12*(May), 468–471.

Pechacek, T. F., Nayak, P., Slovic, P., Weaver, S. R., Huang, J., & Eriksen, M. P. (2017). Reassessing the importance of 'lost pleasure' associated with smoking cessation: implications for social welfare and policy. *Tobacco Control*, tobaccocontrol-2017-053734. http://doi.org/10.1136/tobaccocontrol-2017-053734

Peng, C., Engle, S. E., Yan, Y., Weera, M. M., Berry, J. N., Arvin, C., … Drenan, R. M. (2017). Altered nicotine reward-associated behavior following α 4 nAChR subunit deletion in ventral midbrain, *2*, 1–23. http://doi.org/10.1371/journal.pone.0182142

Perkins, K. A., Donny, E., Caggiula, A. R., & Perkins, Kenneth A., Donny Eric, C. A. R. (1999). Sex differences in nicotine effects and self-administration: review of human and animal evidence. *Nicotine & Tobacco Research*, *1*(4), 301–315. Retrieved from http://search.ebscohost.com/login.aspx?direct=true&db=a9h&AN=6060844&site=ehost-live

Perkins, K. A., Gerlach, D., Vender, J., Grobe, J., Meeker, J., & Hutchison, S. (2001). Sex differences in the subjective and reinforcing effects of visual and olfactory cigarette smoke stimuli. *Nicotine & Tobacco Research*, *3*(2), 141–150. http://doi.org/10.1080/14622200110043059

Perkins, K. A., Karelitz, J. L., Conklin, C. A., Sayette, M. A., & Giedgowd, G. E. (2010). Acute negative affect relief from smoking depends on the affect situation and measure but not on nicotine. *Biological Psychiatry*, *67*(8), 707–714. Retrieved from http://ac.els-cdn.com/S0006322309014784/1-s2.0-S0006322309014784-main.pdf?_tid=c250b35a-613b-11e6-a225-00000aab0f02&acdnat=1471082177_449c4496c634d284afb465db73d3adb5

Perkins, K. A., Karelitz, J. L., Giedgowd, G. E., Conklin, C. A., & Sayette, M. A. (2010). Differences in negative mood-induced smoking reinforcement due to distress tolerance, anxiety sensitivity, and depression history. *Psychopharmacology*, *210*(1), 25–34. http://doi.org/10.1007/s00213-010-1811-1.Differences

Perkins, K. A., Kunkle, N., & Karelitz, J. L. (2017). Threshold dose for behavioral

discrimination of cigarette nicotine content in menthol vs. non-menthol smokers. *Psychopharmacology*, *234*(8), 1255–1265. http://doi.org/10.1007/s00213-017-4563-3

Perkins, K. A., Kunkle, N., Michael, V. C., Karelitz, J. L., & Donny, E. C. (2016). Assessing Discrimination of Nicotine in Humans Via Cigarette Smoking. *Nicotine & Tobacco Research*, *18*(9), 1830–1836. http://doi.org/10.1093/ntr/ntw082

Perski, O., Herd, N., Brown, J., & West, R. (2018). Does consistent motivation to stop smoking improve the explanation of recent quit attempts beyond current motivation? A cross-sectional study. *Addictive Behaviors*, *81*(October 2017), 12–16. http://doi.org/10.1016/j.addbeh.2018.01.037

Pesko, M. F., Hughes, J. M., & Faisal, F. S. (2016). The influence of electronic cigarette age purchasing restrictions on adolescent tobacco and marijuana use. *Preventive Medicine*, *87*, 207–212. http://doi.org/10.1016/J.YPMED.2016.02.001

Picciotto, M. R., Zoli, M., Rimondini, R., Lena, C., Marubio, L. M., Pich, E. M., … Changeux, J.-P. (1998). Acetylcholine receptors containing the [beta]2 subunit are involved in the reinforcing properties of nicotine. *Nature*, *391*(6663), 173–177. Retrieved from http://dx.doi.org/10.1038/34413

Pidoplichko, V. I., DeBiasi, M., Williams, J. T., & Dani, J. A. (1997). Nicotine activates and desensitizes midbrain dopamine neurons. *Nature*, *390*(6658), 401–404. Retrieved from http://dx.doi.org/10.1038/37120

Piñeiro, B., López-Durán, A., Martínez-Vispo, C., Fernández del Río, E., Martínez, Ú., Rodríguez-Cano, R., … Becoña, E. (2017). Smoking relapse situations among a community-recruited sample of Spanish daily smokers. *Addictive Behaviors*, *75*, 152–158. http://doi.org/10.1016/j.addbeh.2017.07.022

Piper, M. E., McCarthy, D. E., & Baker, T. B. (2006). Assessing tobacco dependence: a guide to measure evaluation and selection. *Nicotine & Tobacco Research : Official Journal of the Society for Research on Nicotine and Tobacco*, *8*(3), 339–51. http://doi.org/10.1080/14622200600672765

Pokhrel, P., Herzog, T. A., Muranaka, N., Regmi, S., & Fagan, P. (2015). Contexts of cigarette and e-cigarette use among dual users: a qualitative study. *BMC Public Health*. http://doi.org/10.1186/s12889-015-2198-z

Polosa, R., Caponnetto, P., Cibella, F., & Le-Houezec, J. (2015). Quit and smoking reduction rates in vape shop consumers: a prospective 12-month survey. *Int J Environ Res Public Health*, *12*(4), 3428–3438. http://doi.org/10.3390/ijerph120403428

Polosa, R., Caponnetto, P., Maglia, M., Morjaria, J. B., & Russo, C. (2014). Success rates with nicotine personal vaporizers: a prospective 6-month pilot study of smokers not intending to quit. *BMC Public Health*, *14*(1), 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 Public Health*, *11*. Retrieved from http://www.scopus.com/inward/record.url?eid=2-s2.0-80053630191&partnerID=40&md5=acf61351331de6cdf617cb436dcf5dd7

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

Polosa, R., Morjaria, J. B., Caponnetto, P., Prosperini, U., Russo, C., Pennisi, A., & Bruno, C. M. (2016). Evidence for harm reduction in COPD smokers who switch to electronic cigarettes. *Respiratory Research*, *17*(1), 166.

http://doi.org/10.1186/s12931-016-0481-x
Polosa, R., Morjaria, J., Caponnetto, P., Caruso, M., Strano, S., Battaglia, E., & Russo, C. (2014). Effect of smoking abstinence and reduction in asthmatic smokers switching to electronic cigarettes: evidence for harm reversal. *Int J Environ Res Public Health*, *11*(5), 4965–4977. http://doi.org/10.3390/ijerph110504965
Polosa, R., Russell, C., Nitzkin, J., & Farsalinos, K. E. (2017). A critique of the US Surgeon General's conclusions regarding e-cigarette use among youth and young adults in the United States of America. *Harm Reduction Journal*, *14*(1), 61. http://doi.org/10.1186/s12954-017-0187-5
Pons, S., Fattore, L., Cossu, G., Tolu, S., Porcu, E., McIntosh, J. M., … Fratta, W. (2008). Crucial Role of alpha 4 and alpha 6 Nicotinic Acetylcholine Receptor Subunits from Ventral Tegmental Area in Systemic Nicotine Self-Administration. *Journal of Neuroscience*, *28*(47), 12318–12327. http://doi.org/10.1523/JNEUROSCI.3918-08.2008
Pritchard, W. S., Robinson, J. H., Guy, T. D., Davis, R. A., & Stiles, M. F. (1996a). Assessing the sensory role of nicotine in cigarette smoking: Erratum. *Psychopharmacology*, *128*(4), 432. http://doi.org/10.1007/s002130050154
Pritchard, W. S., Robinson, J. H., Guy, T. D., Davis, R. A., & Stiles, M. F. (1996b). Assessing the sensory role of nicotine in cigarette smoking. *Psychopharmacology*, *127*(1), 55–62. http://doi.org/10.1007/BF02805975
Pryor, W. A., Stone, K., Zang, L.-Y., & Bermúdez, E. (1998). Fractionation of Aqueous Cigarette Tar Extracts: Fractions That Contain the Tar Radical Cause DNA Damage. *Chemical Research in Toxicology*, *11*(5), 441–448. http://doi.org/10.1021/tx970159y
Puustinen, P., Olkkonen, H., Kolonen, S., & Tuomisto, J. (1987). Microcomputer-aided measurement of puff parameters during smoking of low- and medium-tar cigarettes. Scandinavian Journal of Clinical and Laboratory Investigation, 47(7), 655–660. http://doi.org/10.1080/00365518709168925
Quick, M. W., & Lester, R. A. J. (2002). Desensitization of neuronal nicotinic receptors. *Journal of Neurobiology*, *53*(4), 457–478. http://doi.org/10.1002/neu.10109
Ramôa, C. P., Hiler, M. M., Spindle, T. R., Lopez, A. A., Karaoghlanian, N., Lipato, T., … Eissenberg, T. (2015). Electronic cigarette nicotine delivery can exceed that of combustible cigarettes: a preliminary report. *Tobacco Control*. http://doi.org/10.1136/tobaccocontrol-2015-052447
Reid, R. T., Lloyd, G. K., & Rao, T. S. (1999). Pharmacological characterization of nicotine-induced acetylcholine release in the rat hippocampus in vivo: evidence for a permissive dopamine synapse. *British Journal of Pharmacology*, *127*(6), 1486–94. http://doi.org/10.1038/sj.bjp.0702683
Reidel, B., Radicioni, G., Clapp, P., Ford, A. A., Abdelwahab, S., Rebuli, M. E., … Kesimer, M. (2017). E-Cigarette Use Causes a Unique Innate Immune Response in the Lung, Involving Increased Neutrophilic Activation and Altered Mucin Secretion. *American Journal of Respiratory and Critical Care Medicine*. http://doi.org/DOI: 10.1164/rccm.201708-1590OC
Rennard, S. I., Glover, E. D., Leischow, S., Daughton, D. M., Glover, P. N., Muramoto, M., … Westin, A. (2006). Efficacy of the nicotine inhaler in smoking reduction: A double-blind, randomized trial. *Nicotine & Tobacco Research : Official Journal of the Society for Research on Nicotine and Tobacco*, *8*(4), 555–564. http://doi.org/10.1080/14622200600789916
Reynolds, B., Patak, M., Shroff, P., Penfold, R. B., Melanko, S., & Duhig, A. M. (2007). Laboratory and self-report assessments of impulsive behavior in adolescent

daily smokers and nonsmokers. *Experimental and Clinical Psychopharmacology*, *15*(3), 264–271. http://doi.org/10.1037/1064-1297.15.3.264
Robertson, L., Hoek, J., Blank, M.-L., Richards, R., Ling, P., & Popova, L. (2018). Dual use of electronic nicotine delivery systems (ENDS) and smoked tobacco: a qualitative analysis. *Tobacco Control*, tobaccocontrol-2017-054070. http://doi.org/10.1136/tobaccocontrol-2017-054070
Robinson, R. J., Hensel, E. C., Morabito, P. N., & Roundtree, K. A. (2015). Electronic Cigarette Topography in the Natural Environment. *PLoS One*, *10*(6), 1–14. http://doi.org/10.1371/journal.pone.0129296
Robinson, T. E., & Berridge, K. C. (1993). The neural basis of drug craving: An incentive-sensitization theory of addiction. *Brain Research Reviews*, *18*(3), 247–291. http://doi.org/10.1016/0165-0173(93)90013-P
Robinson, T. E., & Berridge, K. C. (2008). The incentive sensitization theory of addiction: some current issues. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *363*(1507), 3137–3146. http://doi.org/10.1098/rstb.2008.0093
Rodu, B. (2017). Tobacco Truth: 2016 CDC Data Shows E-Cigarette Use Declines Again. http://doi.org/http://www.webcitation.org/6zA4LgMUv
Rodu, B., & Plurphanswat, N. (2017a). E-cigarette Use Among US Adults: Population Assessment of Tobacco and Health (PATH) Study. *Nicotine & Tobacco Research*, (November), 1–9. http://doi.org/10.1093/ntr/ntx194
Rodu, B., & Plurphanswat, N. (2017b). *E-cigarette Use Among US Adults: Population Assessment of Tobacco and Health (PATH) Study*. *Nicotine & Tobacco Research*. http://doi.org/10.1093/ntr/ntx194
Rohsenow, D. J., Tidey, J. W., Martin, R. A., Colby, S. M., & Eissenberg, T. (2018a). Effects of six weeks of electronic cigarette use on smoking rate, CO, cigarette dependence, and motivation to quit smoking: A pilot study. *Addictive Behaviors*, *80*, 65–70. http://doi.org/10.1016/j.addbeh.2018.01.012
Romanelli, L., Öhman, B., Adem, A., & Nordberg, A. (1988). Subchronic treatment of rats with nicotine: Interconversion of nicotinic receptor subtypes in brain. *European Journal of Pharmacology*, *148*(2), 289–291. http://doi.org/10.1016/0014-2999(88)90577-8
Rose, J. E. (2006). Nicotine and nonnicotine factors in cigarette addiction. *Psychopharmacology*, *184*(3–4), 274–285. http://doi.org/10.1007/s00213-005-0250-x
Rose, J. E., Behm, F. M., & Levin, E. D. (1993). Role of nicotine dose and sensory cues in the regulation of smoke intake. *Pharmacology Biochemistry and Behavior*, *44*(4), 891–900. http://doi.org/10.1016/0091-3057(93)90021-K
Rose, J. E., Behm, F. M., & Westman, E. C. (2001). Acute effects of nicotine and mecamylamine on tobacco withdrawal symptoms, cigarette reward and ad lib smoking. *Pharmacology Biochemistry and Behavior*, *68*(2), 187–197. http://doi.org/10.1016/S0091-3057(00)00465-2
Rose, J. E., Behm, F. M., Westman, E. C., Bates, J. E., & Salley, A. (2003). Pharmacologic and sensorimotor components of satiation in cigarette smoking. *Pharmacology Biochemistry and Behavior*, *76*(2), 243–250. http://doi.org/10.1016/j.pbb.2003.07.002
Rose, J. E., Behm, F. M., Westman, E. C., & Coleman, R. E. (1999). Arterial nicotine kinetics during cigarette smoking and intravenous nicotine administration: Implications for addiction. *Drug and Alcohol Dependence*, *56*(2), 99–107. http://doi.org/10.1016/S0376-8716(99)00025-3
Rose, J. E., Behm, F. M., Westman, E. C., & Johnson, M. (2000). Dissociating nicotine

and nonnicotine components of cigarette smoking. *Pharmacology Biochemistry and Behavior*, *67*(1), 71–81. http://doi.org/10.1016/S0091-3057(00)00301-4

Rose, J. E., Salley, A., Behm, F. M., Bates, J. E., & Westman, E. C. (2010). Reinforcing effects of nicotine and non-nicotine components of cigarette smoke. *Psychopharmacology (Berl)*, *210*(1), 1–12.

Rose, J. E., Tashkin, D. P., Ertle, A., Zinser, M. C., & Lafer, R. (1985). Sensory blockade of smoking satisfaction. *Pharmacology, Biochemistry and Behavior*, *23*(2), 289–293. http://doi.org/10.1016/0091-3057(85)90572-6

Rose, J. E., Westman, E. C., Behm, F. M., Johnson, M. P., & Goldberg, J. S. (1999). Blockade of smoking satisfaction using the peripheral nicotinic antagonist trimethaphan. *Pharmacology Biochemistry and Behavior*, *62*(1), 165–172. http://doi.org/10.1016/S0091-3057(98)00153-1

Rose, J., Lokitz, S., Garg, S., Turkington, T., & Minton, R. (2006). Highly dependent cigarette smokers show slower brain uptake of nicotine than less dependent smokers. *Proceedings of the 36th Annual Meeting of the Society for Neuroscience*. Retrieved from https://scholar.google.co.uk/scholar?hl=en&q=Highly+dependent+cigarette+smokers+show+slower+brain+uptake+of+nicotine+than+less+dependent+smokers&btnG=&as_sdt=1%2C5&as_sdtp=

Ross, K. C., & Juliano, L. M. (2016). Smoking Through a Topography Device Diminishes Some of the Acute Rewarding Effects of Smoking. *Nicotine & Tobacco Research*, *18*(5), 564–571. Retrieved from http://10.0.4.69/ntr/ntv159

Royal College of Physicians, & Group, T. A. (2016). *Nicotine without smoke Tobacco harm reduction*. Retrieved from https://www.rcplondon.ac.uk/projects/outputs/nicotine-without-smoke-tobacco-harm-reduction-0

Royal College of Physicians of London. (1962). Smoking and health. Summary of a report of the Royal College of Physicians of London on smoking in relation to cancer of the lung and other diseases. *Pitman Medical Publishing*. http://doi.org/http://www.rcplondon.ac.uk/sites/default/files/documents/smoking-and-health-1962_1.pdf

Russell, M. A. (1976). Low-tar medium-nicotine cigarettes: a new approach to safer smoking. *British Medical Journal*, *1*(6023), 1430–3. http://doi.org/10.1136/bmj.1.6023.1430

Russell, M. A. H. (1980). Nicotine intake and its regulation. *Journal of Psychosomatic Research*, *24*(5), 253–264. http://doi.org/10.1016/0022-3999(80)90015-X

Russell, M. A. H., & Feyerabend, C. (1978). Cigarette Smoking: A Dependence on High-Nicotine Boli. *Drug Metabolism Reviews*, *8*(1), 29–57. http://doi.org/10.3109/03602537808993776

Russell, M. A. H., Jarvis, M., Iyer, R., & Feyerabend, C. (1980). Relation of nicotine yield of cigarettes to blood nicotine concentrations in smokers. *British Medical Journal*, *280*(6219), 972–976. http://doi.org/10.1136/bmj.280.6219.972

Russell, M. A. H., Jarvis, M. J., Devitt, G., & Feyerabend, C. (1981). Nicotine intake by snuff users. *British Medical Journal*, *283*(6295), 814–817. Retrieved from http://www.scopus.com/inward/record.url?eid=2-s2.0-0019384265&partnerID=40&md5=68bf71881647e6f8e6a75c49ab83e78b

Russell, M. A. H., Sutton, S. R., Iyer, R., Feyerabend, C., & Vesey, C. J. (1982). Long-Term Switching to Low-Tar Low-Nicotine Cigarettes. *British Journal of Addiction*, *77*(2), 145–158. Retrieved from http://search.ebscohost.com/login.aspx?direct=true&db=a9h&AN=6272769&site=

ehost-live
Russell, M. A., Wilson, C., Patel, U. A., Feyerabend, C., & Cole, P. V. (1975). Plasma nicotine levels after smoking cigarettes with high, medium, and low nicotine yields. *British Medical Journal*, *2*(5968), 414–416. Retrieved from http://www.ncbi.nlm.nih.gov/pmc/articles/PMC1681802/
Russell, M. a, Wilson, C., Patel, U. a, Cole, P. V, & Feyerabend, C. (1973). Comparison of effect on tobacco consumption and carbon monoxide absorption of changing to high and low nicotine cigarettes. *British Medical Journal*, *4*(5891), 512–6. Retrieved from http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=1587479&tool=pmcentrez&rendertype=abstract
Russell, P. O., Epstein, L. H., & Dickson, B. E. (1983). Behavioral and physiological effects of low-nicotine cigarettes during rapid smoking. *Journal of Consulting and Clinical Psychology*, *51*(2), 312. http://doi.org/10.1037/0022-006X.51.2.312
Russo, P., Nastrucci, C., Alzetta, G., & Szalai, C. (2011). Tobacco Habit historical, cultural, neurobiological, and genetic features of people's relationship with an addictive drug. In *Project MUSE* (pp. 557–577).
Rüther, T., Hagedorn, D., Schiela, K., Schettgen, T., Osiander-Fuchs, H., & Schober, W. (2017). Nicotine delivery efficiency of first- and second-generation e-cigarettes and its impact on relief of craving during the acute phase of use. *International Journal of Hygiene and Environmental Health*, (August), 0–1. http://doi.org/10.1016/j.ijheh.2017.10.012
Salminen, O., Drapeau, J. A., McIntosh, J. M., Collins, A. C., Marks, M. J., & Grady, S. R. (2007). Pharmacology of -Conotoxin MII-Sensitive Subtypes of Nicotinic Acetylcholine Receptors Isolated by Breeding of Null Mutant Mice. *Molecular Pharmacology*, *71*(6), 1563–1571. http://doi.org/10.1124/mol.106.031492.calcium
Scherer, G. (1999). Smoking behaviour and compensation: a review of the literature. *Psychopharmacology (Berl)*, *145*(1), 1–20. Retrieved from http://search.ebscohost.com/login.aspx?direct=true&db=a9h&AN=4694245&site=ehost-live
Scherer, G., & Lee, P. N. (2014). Smoking behaviour and compensation: A review of the literature with meta-analysis. *Regulatory Toxicology and Pharmacology*, *70*(3), 615–628. http://doi.org/10.1016/j.yrtph.2014.09.008
Schmeltz, I., & Hoffmann, D. (1977). Nitrogen-containing compounds in tobacco and tobacco smoke. *Chemical Reviews*, *77*(3), 295–311. http://doi.org/10.1021/cr60307a001
Schneider, N. G., Olmstead, R. E., Franzon, M. A., & Lunell, E. (2001). The Nicotine Inhaler. *Clinical Pharmacokinetics*, *40*(9), 661–684. http://doi.org/10.2165/00003088-200140090-00003
Schnoll, R. A., Goren, A., Annunziata, K., & Suaya, J. A. (2013). The prevalence, predictors and associated health outcomes of high nicotine dependence using three measures among US smokers. *Addiction*. http://doi.org/10.1111/add.12285
Schoedel, K. a, Hoffmann, E. B., Rao, Y., Sellers, E. M., & Tyndale, R. F. (2004). Ethnic variation in CYP2A6 and association of genetically slow nicotine metabolism and smoking in adult Caucasians. *Pharmacogenetics*, *14*(9), 615–626. http://doi.org/10.1097/00008571-200409000-00006
Schripp, T., Markewitz, D., Uhde, E., & Salthammer, T. (2013). Does e-cigarette consumption cause passive vaping? *Indoor Air*, *23*(1), 25–31. Retrieved from http://www.scopus.com/inward/record.url?eid=2-s2.0-84872398546&partnerID=40&md5=f9c5ed4f12c040ea064c649154e541bc

Schuh, K. J., & Stitzer, M. L. (1995). Desire to smoke during spaced smoking intervals. *Psychopharmacology*, *120*(3), 289–295.

Scungio, M., Stabile, L., & Buonanno, G. (2018). Measurements of electronic cigarette-generated particles for the evaluation of lung cancer risk of active and passive users. *Journal of Aerosol Science*, *115*(March 2017), 1–11. http://doi.org/10.1016/j.jaerosci.2017.10.006

Selman, J. E., & Selman, J. E. (n.d.). *NEW DEAL FOR COMMUNITIES YOUTH INCLUSION PROGRAMME : EARLY OUTCOMES IN WEST HAM & Centre for Institutional Studies*.

Senate Government Bill. BILL S-5 An Act to amend the Tobacco Act and the Non-smokers' Health Act and to make consequential amendments to other Acts (2017). Retrieved from http://www.parl.ca/LegisInfo/BillDetails.aspx?Bill=S5&Language=E&Mode=1&Parl=42&Ses=1

Shahab, L., Goniewicz, M. L., Blount, B. C., Brown, J., McNeill, A., Alwis, K. U., … Al, E. (2017). Nicotine, carcinogen, and toxin exposure in long-term e-cigarette and nicotine replacement therapy users: A cross-sectional study. *Annals of Internal Medicine*, *24*(18), 442–8. http://doi.org/10.7326/M16-1107

Shahab, L., Hammond, D., O'Connor, R. J., Cummings, K. M., Borland, R., King, B., & McNeill, A. (2008). The reliability and validity of self-reported puffing behavior: Evidence from a cross-national study. *Nicotine & Tobacco Research*, *10*(5), 867–874. http://doi.org/10.1080/14622200802027156

Shields, P. G. (2011). Long-term nicotine replacement therapy: cancer risk in context. *Cancer Prevention Research (Philadelphia, Pa.)*, *4*(11), 1719–23. http://doi.org/10.1158/1940-6207.CAPR-11-0453

Shiffman, S., Sembower, M., & Kim, M. (2018). USE PATTERNS AND TOBACCO USE HISTORIES AMONG USERS OF DIFFERENT VAPOR PRODUCTS: CIGALIKES, TANKS, AND OTHER VAPOR PRODUCTS.

Shihadeh, A., & Eissenberg, T. (2015). Electronic Cigarette Effectiveness and Abuse Liability: Predicting and Regulating Nicotine Flux. *Nicotine & Tobacco Research*, *17*(2), 158–162. Retrieved from http://search.ebscohost.com/login.aspx?direct=true&db=a9h&AN=101033880&site=ehost-live

Siegel, M. B., Tanwar, K. L., & Wood, K. S. (2011). Electronic Cigarettes As a Smoking-Cessation Tool: Results from an Online Survey. *American Journal of Preventive Medicine*, *40*(4), 472–475. http://doi.org/10.1016/j.amepre.2010.12.006

Simonavicius, E., McNeill, A., Arnott, D., & Brose, L. S. (2017). What factors are associated with current smokers using or stopping e-cigarette use? *Drug and Alcohol Dependence*, *173*, 139–143. http://doi.org/10.1016/j.drugalcdep.2017.01.002

Smith, C., & Fisher, T. (2001). Particulate and vapor phase constituents of cigarette mainstream smoke and risk of myocardial infarction. *Atherosclerosis*, *158*(2), 257–267. http://doi.org/10.1016/S0021-9150(01)00570-6

Soar, K., Kimber, C. F., McRobbie, H., & Dawkins, L. E. (2018). *Nicotine absorption from E-cigarette over 12 months*. *Addictive Behaviors*. Retrieved from https://doi.org/10.1016/j.addbeh.2018.07.019

Sofuoglu, M., Yoo, S., Hill, K. P., & Mooney, M. (2008). Self-Administration of Intravenous Nicotine in Male and Female Cigarette Smokers. *Neuropsychopharmacology*, *33*(4), 715–720. http://doi.org/10.1038/sj.npp.1301460

Solinas, M., Panlilio, L. V, Justinova, Z., Yasar, S., & Goldberg, S. R. (2006). Using drug-discrimination techniques to study the abuse-related effects of psychoactive drugs in rats. *Nat Protoc*, *1*(3), 1194–1206. http://doi.org/10.1038/nprot.2006.167

Spindle, T. R. (2015). *Examination of Electronic Cigarette User Puff Topography: The Effect of a Mouthpiece-based Topography Measurement Device on Plasma nicotine and Subjective Effects.* http://doi.org/10.1093/ntr/ntu186

Spindle, T. R., Breland, A. B., Karaoghlanian, N. V., Shihadeh, A. L., & Eissenberg, T. (2015). Preliminary results of an examination of electronic cigarette user puff topography: The effect of a mouthpiece-based topography measurement device on plasma nicotine and subjective effects. *Nicotine and Tobacco Research*, *17*(2), 142–149. http://doi.org/10.1093/ntr/ntu186

St.Helen, G., Shahid, M., Chu, S., & Benowitz, N. L. (2018). Impact of e-liquid flavors on e-cigarette vaping behavior. *Drug and Alcohol Dependence*, *189*, 42–48. http://doi.org/10.1016/j.drugalcdep.2018.04.032

St Helen, G., Havel, C., Dempsey, D., Jacob 3rd, P., & Benowitz, N. L. (2015). Nicotine delivery, retention, and pharmacokinetics from various electronic cigarettes. *Addiction*. http://doi.org/10.1111/add.13183

Stanhope, B.-J., Burdette, W. J., Cochran, W. G., Farber, E., Fieser, L. F., Furth, J., … Seevers, M. H. (1964). Report of the Advisory Committee to the Surgeon General of the Public Health Service. *... , Education and Welfare, Public Health Service ...*, 1–386. Retrieved from http://profiles.nlm.nih.gov/NN/B/B/M/Q/_/nnbbmq.ocr

Stead, L. F., Perera, R., Bullen, C., Mant, D., Hartmann-Boyce, J., Cahill, K., & Lancaster, T. (2012). Nicotine replacement therapy for smoking cessation. *Cochrane Database of Systematic Reviews (Online)*, *11*. Retrieved from http://www.scopus.com/inward/record.url?eid=2-s2.0-84872173821&partnerID=40&md5=b26694d9defa6685b4426c6be7c2b47f

Stephens, W. E. (2017). Comparing the cancer potencies of emissions from vapourised nicotine products including e-cigarettes with those of tobacco smoke. *Tobacco Control*. Retrieved from http://tobaccocontrol.bmj.com/content/early/2017/08/04/tobaccocontrol-2017-053808.abstract

Stewart, G. G. (1967). A history of the medicinal use of tobacco: 1492-1860. *Medical History*, *11*(3), 228–268.

Stewart, J. (1984). Reinstatement of heroin and cocaine self-administration behavior in the rat by intracerebral application of morphine in the ventral tegmental area. *Pharmacology Biochemistry and Behavior*, *20*(6), 917–923. http://doi.org/10.1016/0091-3057(84)90017-0

Stolerman, I. P., Goldfarb, T., Fink, R., & Jarvik, M. E. (1973). Influencing cigarette smoking with nicotine antagonists. *Psychopharmacologia*, *28*(3), 247–259. http://doi.org/10.1007/BF00429305

Strasser, A. A., Benowitz, N. L., Pinto, A. G., Tang, K. Z., Hecht, S. S., Carmella, S. G., … Lerman, C. E. (2011). Nicotine metabolite ratio predicts smoking topography and carcinogen biomarker level. *Cancer Epidemiology Biomarkers and Prevention*, *20*(2), 234–238. http://doi.org/10.1158/1055-9965.EPI-10-0674

Strasser, A. A., Lerman, C., Sanborn, P. M., Pickworth, W. B., & Feldman, E. A. (2007). New lower nicotine cigarettes can produce compensatory smoking and increased carbon monoxide exposure. *Drug & Alcohol Dependence*, *86*(2/3), 294–300. http://doi.org/10.1016/j.drugalcdep.2006.06.017

Strasser, A. a, Malaiyandi, V., Hoffmann, E., Tyndale, R. F., & Lerman, C. (2007). An association of CYP2A6 genotype and smoking topography. *Nicotine & Tobacco*

Research : Official Journal of the Society for Research on Nicotine and Tobacco, *9*(4). http://doi.org/10.1080/14622200701239605

Sutherland, G., Stapleton, J. ., Russell, M. A. ., Jarvis, M. ., Hajek, P., Belcher, M., & Feyerabend, C. (1992). Randomised controlled trial of nasal nicotine spray in smoking cessation. *The Lancet*, *340*(8815), 324–329. http://doi.org/10.1016/0140-6736(92)91403-U

Sutton, S. R., Russell, M. A. H., Iyer, R., Feyerabend, C., & Saloojee, Y. (1982). Relationship between cigarette yields, puffing patterns, and smoke intake: evidence for tar compensation? *British Medical Journal (Clinical Research Edition)*, *285*(6342), 600–603. Retrieved from http://search.ebscohost.com/login.aspx?direct=true&db=a9h&AN=4937613&site=ehost-live

Tackett, A. P., Lechner, W. V., Meier, E., Grant, D. M., Driskill, L. M., Tahirkheli, N. N., & Wagener, T. L. (2015). Biochemically verified smoking cessation and vaping beliefs among vape store customers. *Addiction*, *110*(5), 868–874. http://doi.org/10.1111/add.12878

Talhout, R., Schulz, T., Florek, E., van Benthem, J., Wester, P., & Opperhuizen, A. (2011). Hazardous compounds in tobacco smoke. *International Journal of Environmental Research and Public Health*, *8*(2), 613–628. http://doi.org/10.3390/ijerph8020613

Talih, S., Balhas, Z., Eissenberg, T., Salman, R., Karaoghlanian, N., El Hellani, A., … Shihadeh, A. (2014). Effects of User Puff Topography, Device Voltage, and Liquid Nicotine Concentration on Electronic Cigarette Nicotine Yield: Measurements and Model Predictions. *Nicotine and Tobacco Research*, *17*(2), 150–157. http://doi.org/10.1093/ntr/ntu174

Talih, S., Balhas, Z., Salman, R., Karaoghlanian, N., & Shihadeh, A. (2016). "Direct dripping": A high-temperature, high- formaldehyde emission electronic cigarette use method. *Nicotine and Tobacco Research*, *18*(4), 453–459. http://doi.org/10.1093/ntr/ntv080

Talih, S., Salman, R., Karaoghlanian, N., El-Hellani, A., Saliba, N., Eissenberg, T., & Shihadeh, A. (2017). "Juice Monsters": Sub-Ohm Vaping and Toxic Volatile Aldehyde Emissions. *Chemical Research in Toxicology*. http://doi.org/10.1021/acs.chemrestox.7b00212

Tanner, J.-A., Chenoweth, M. J., & Tyndale, R. F. (2015). The Neurobiology and Genetics of Nicotine and Tobacco. *Current Topics in Behavioral Neurosciences*, *23*(July 2016), 37–86. http://doi.org/10.1007/978-3-319-13665-3

Tapper, A. R., McKinney, S. L., Nashmi, R., Schwarz, J., Deshpande, P., Labarca, C., … Lester, H. A. (2004). Nicotine Activation of α4* Receptors: Sufficient for Reward, Tolerance, and Sensitization. *Science*, *306*(5698), 1029 LP-1032. Retrieved from http://science.sciencemag.org/content/306/5698/1029.abstract

Tayyarah, R., & Long, G. A. (2014). Comparison of select analytes in aerosol from e-cigarettes with smoke from conventional cigarettes and with ambient air. *Regulatory Toxicology and Pharmacology*, *70*(3), 704–710. http://doi.org/http://dx.doi.org/10.1016/j.yrtph.2014.10.010

Teneggi, V., Tiffany, S. T., Squassante, L., Milleri, S., Ziviani, L., & Bye, A. (2005). Effect of sustained-release (SR) bupropion on craving and withdrawal in smokers deprived of cigarettes for 72 h. *Psychopharmacology*, *183*(1), 1–12. http://doi.org/10.1007/s00213-005-0145-x

The Tobacco Atlas. (2010). Smoking Death's Toll. Retrieved from http://www.tobaccoatlas.org/topic/smokings-death-toll/

TIRC, T. I. R. C. (1954). A FRANK STATEMENT TO CIGARETTE SMOKERS - Truth Tobacco Industry Documents. Retrieved July 20, 2017, from http://archive.tobacco.org/History/540104frank.html

Tonnesen, P., Paoletti, P., Gustavsson, G., Russell, M. A., Saracci, R., Gulsvik, A., … Sawe, U. (1999). Higher dosage nicotine patches increase one-year smoking cessation rates: Results from the European CEASE trial. *European Respiratory Journal*, *13*(2), 238–246. http://doi.org/10.1034/j.1399-3003.1999.13b04.x

Trehy, M. L., Ye, W., Hadwiger, M. E., Moore, T. W., Allgire, J. F., Woodruff, J. T., … Westenberger, B. J. (2011). Analysis of Electronic Cigarette Cartridges, Refill Solutions, and Smoke for Nicotine and Nicotine Related Impurities. *Journal of Liquid Chromatography & Related Technologies*, *34*(14), 1442–1458. http://doi.org/10.1080/10826076.2011.572213

Tricker, A. R. (2003). Nicotine metabolism, human drug metabolism polymorphisms, and smoking behaviour. *Toxicology*, *183*(1–3), 151–173. http://doi.org/10.1016/S0300-483X(02)00513-9

Troisi II, J. R., Bryant, E., & Kane, J. (2012). Extinction of the discriminative stimulus effects of nicotine with a devalued reinforcer: Recovery following revaluation. *Psychological Record*, *62*(4), 707–718. Retrieved from https://www.scopus.com/inward/record.uri?eid=2-s2.0-84869193650&partnerID=40&md5=931c9faeceb83bb8880aeaaa260756a6

Trtchounian, A., Williams, M., & Talbot, P. (2010). Conventional and electronic cigarettes (e-cigarettes) have different smoking characteristics. *Nicotine & Tobacco Research*, *12*(9), 905–912. http://doi.org/10.1093/ntr/ntq114

Tseng, T. Y., Ostroff, J. S., Campo, A., Gerard, M., Kirchner, T., Rotrosen, J., & Shelley, D. (2016). A Randomized Trial Comparing the Effect of Nicotine Versus Placebo Electronic Cigarettes on Smoking Reduction Among Young Adult Smokers. *Nicotine Tob Res*. http://doi.org/10.1093/ntr/ntw017

U.S. Department of Health and Human Services. (2004). The Health Consequences of Smoking: A Report of the Surgeon General. *U.S. Department of Health and Human Services*. Retrieved from https://scholar.google.com/scholar?cluster=15142520776977378 13&hl=en&as_sdt=2005&sciodt=0,5

Ucar, E. Y., Araz, O., Yilmaz, N., Akgun, M., Meral, M., Kaynar, H., & Saglam, L. (2014). Effectiveness of pharmacologic therapies on smoking cessation success: Three years results of a smoking cessation clinic. *Multidisciplinary Respiratory Medicine*, *9*(1). Retrieved from http://www.scopus.com/inward/record.url?eid=2-s2.0-84901912357&partnerID=40&md5=5a84c67408fae8596d37566c17592b00

Ulrich, Y. M., Hargreaves, K. M., & Flores, C. M. (1997). A comparison of multiple injections versus continuous infusion of nicotine for producing up-regulation of neuronal [3H]-epibatidine binding sites. *Neuropharmacology*, *36*(8), 1119–1125. http://doi.org/10.1016/S0028-3908(97)00107-X

United States Department of Health and Human Services. (2014). *The Health Consequences of Smoking—50 Years of Progress A Report of the Surgeon General*. *A Report of the Surgeon General*.

Van Gucht, D., Adriaens, K., & Baeyens, F. (2017). Online vape shop customers who use e-cigarettes report abstinence from smoking and improved quality of life, but a substantial minority still have vaping-related health concerns. *International Journal of Environmental Research and Public Health*, *14*(7), 798. http://doi.org/10.3390/ijerph14070798

Van Heel, M., Van Gucht, D., Vanbrabant, K., & Baeyens, F. (2017). The importance

of conditioned stimuli in cigarette and e-cigarette craving reduction by e-cigarettes. *International Journal of Environmental Research and Public Health*, *14*(2). http://doi.org/10.3390/ijerph14020193

Van Overmeire, I. P. I., De Smedt, T., Dendale, P., Nackaerts, K., Vanacker, H., Vanoeteren, J. F. A., … De Cremer, K. A. J. (2016). Nicotine Dependence and Urinary Nicotine, Cotinine and Hydroxycotinine Levels in Daily Smokers. *Nicotine & Tobacco Research*, *18*(9), 1813–1819. Retrieved from http://dx.doi.org/10.1093/ntr/ntw099

Vandrevala, T., Coyle, A., Walker, V., Cabrera Torres, J., Ordoña, I., & Rahman, P. (2017). 'A good method of quitting smoking' or 'just an alternative to smoking'? Comparative evaluations of e-cigarette and traditional cigarette usage by dual users. *Health Psychology Open*, *4*(1), 205510291668464. http://doi.org/10.1177/2055102916684648

Vangeli, E., Stapleton, J., Smit, E. S., Borland, R., & West, R. (2011). Predictors of attempts to stop smoking and their success in adult general population samples: a systematic review. *Addiction*, *106*(12), 2110–2121. http://doi.org/10.1111/j.1360-0443.2011.03565.x

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. *Cancer Epidemiology Biomarkers & Prevention*, *19*(8), 1945–1953.

Vansickel, A. R., & Eissenberg, T. (2013). Electronic cigarettes: Effective nicotine delivery after acute administration. *Nicotine and Tobacco Research*, *15*(1), 267–270. http://doi.org/10.1093/ntr/ntr316

Vansickel, A. R., Weaver, M. F., & Eissenberg, T. (2012). Clinical laboratory assessment of the abuse liability of an electronic cigarette. *Addiction*, *107*(8), 1493–1500. http://doi.org/10.1111/j.1360-0443.2012.03791.x

Vena, A., Howe, M., Cao, D., & King, A. (2019). The role of E-liquid vegetable glycerin and exhaled aerosol on cue reactivity to tank-based electronic nicotine delivery systems (ENDS). Psychopharmacology. http://doi.org/10.1007/s00213-019-05202-6

Vickerman, K. A., Carpenter, K. M., Altman, T., Nash, C. M., & Zbikowski, S. M. (2013). Use of electronic cigarettes among state tobacco cessation quitline callers. *Nicotine and Tobacco Research*, *15*(10), 1787–1791. http://doi.org/10.1093/ntr/ntt061

Vickerman, K. A., Schauer, G. L., Malarcher, A. M., Zhang, L., Mowery, P., & Nash, C. M. (2017). Reasons for Electronic Nicotine Delivery System use and smoking abstinence at 6 months: a descriptive study of callers to employer and health plan-sponsored quitlines. *Tobacco Control*, *26*(2), 126–134. http://doi.org/10.1136/tobaccocontrol-2015-052734

Villégier, A.-S., Blanc, G., Glowinski, J., & Tassin, J. P. (2003). Transient behavioral sensitization to nicotine becomes long-lasting with monoamine oxidases inhibitors. *Pharmacology Biochemistry and Behavior*, *76*(2), 267–274. http://doi.org/10.1016/S0091-3057(03)00223-5

Villégier, A.-S., Salomon, L., Granon, S., Changeux, J.-P., Belluzzi, J. D., Leslie, F. M., & Tassin, J.-P. (2006). Monoamine Oxidase Inhibitors Allow Locomotor and Rewarding Responses to Nicotine. *Neuropsychopharmacology*, *31*(8), 1704–1713. http://doi.org/10.1038/sj.npp.1300987

Wadsworth, E., Neale, J., Mcneill, A., & Hitchman, S. C. (2016). How and Why Do Smokers Start Using E-Cigarettes? Qualitative Study of Vapers in London, UK.

http://doi.org/10.3390/ijerph13070661
Wagener, T. L., Floyd, E. L., Stepanov, I., Driskill, L. M., Frank, S. G., Meier, E., … Queimado, L. (2016). Have combustible cigarettes met their match? The nicotine delivery profiles and harmful constituent exposures of second-generation and third-generation electronic cigarette users. *Tobacco Control*. http://doi.org/10.1136/tobaccocontrol-2016-053041
Wagener, T. L., Meier, E., Hale, J. J., Oliver, E. R., Warner, M. L., Driskill, L. M., … Foster, S. (2014). Pilot investigation of changes in readiness and confidence to quit smoking after E-cigarette experimentation and 1 week of use. *Nicotine and Tobacco Research*, *16*(1), 108–114. http://doi.org/10.1093/ntr/ntt138
Ward, E., Cox, S., Dawkins, L., Jakes, S., Holland, R., & Notley, C. (2018). A Qualitative Exploration of the Role of Vape Shop Environments in Supporting Smoking Abstinence. *International Journal of Environmental Research and Public Health*, *15*(2), 297. http://doi.org/10.3390/ijerph15020297
Wayne, G. F., & Carpenter, C. M. (2009). Tobacco Industry Manipulation of Nicotine Dosing BT - Nicotine Psychopharmacology. In J. E. Henningfield, E. D. London, & S. Pogun (Eds.) (pp. 457–485). Berlin, Heidelberg: Springer Berlin Heidelberg. http://doi.org/10.1007/978-3-540-69248-5_16
Weinberg, M. A., & Segelnick, S. L. (2011). A profile of electronic cigarettes. *U.S. Pharmacist*, *36*(7). Retrieved from http://www.scopus.com/inward/record.url?eid=2-s2.0-79960859117&partnerID=40&md5=3f952706f460a1609adf741d14b69297
West, R. (2006). Defining and Assessing Nicotine Dependence in Humans. In *Understanding nicotine and tobacco addiction* (pp. 36–58). Chichester, UK: John Wiley & Sons, Ltd. Retrieved from https://books.google.co.uk/books?hl=en&lr=&id=PeJlVCBXMqMC&oi=fnd&pg=PA36&dq=Dependence+loss+of+control+Robert+West&ots=QAk81o3AU-&sig=bUt3RT36osafjJCR6mxrwaUvaYs&redir_esc=y#v=onepage&q=Dependence loss of control Robert West&f=false
West, R. (2007). The PRIME Theory of motivation as a possible foundation for addiction treatment. *In: Drug Addiction Treatment in the 21st Century: Science and Policy Issues. John's Hopkins University Press: Baltimore. (2007)*. Retrieved from http://discovery.ucl.ac.uk/862346/
West, R., Beard, E., & Brown, J. (2017). The Smoking Toolkit Survey - Trends in electronic cigarette use in England. Retrieved December 20, 2017, from http://www.smokinginengland.info/latest-statistics/
West, R., Beard, E., & Brown, J. (2018). Smoking Toolkit Study Trends in e-cigarette use in England Jan 2018.
West, R., & Brown, J. (2013). *Theory of Addiction*. *Alcohol and Alcoholism* (Addiction, Vol. 42). Wiley Blackwell. http://doi.org/10.1093/alcalc/agl107
West, R., & Brown, J. (2018). Latest Statistics - Smoking In England. Retrieved February 8, 2018, from http://www.smokinginengland.info/latest-statistics/
West, R., & Hajek, P. (2004). Evaluation of the mood and physical symptoms scale (MPSS) to assess cigarette withdrawal. *Psychopharmacology (Berl)*, *177*(1/2), 195–199. http://doi.org/10.1007/s00213-004-1923-6
West, R., Hajek, P., & Belcher, M. (1989). Severity of withdrawal symptoms as a predictor of outcome of an attempt to quit smoking. *Psychological Medicine*, *19*(4), 981–985. http://doi.org/10.1017/S0033291700005705
West, R., McEwen, a, Bolling, K., & Owen, L. (2001). Smoking cessation and smoking patterns in the general population: a 1- year follow-up. *Addiction*, *96*(6), 891–902.

http://doi.org/10.1080/09652140020051013
West, R., Russell, M. A. H., Jarvis, M. J., & Feyerabend, C. (1984). Does switching to an ultra-low nicotine cigarette induce nicotine withdrawal effects? *Psychopharmacology (Berl)*, *84*(1), 120–123. http://doi.org/10.1007/bf00432039
West, R., Shahab, L., & Brown, J. (2016). 'Estimating the population impact of e-cigarettes on smoking cessation in England.' *Addiction*, *111*(9), 1684. http://doi.org/10.1111/add.13405
Westman, E. C., Behm, F. M., & Rose, J. E. (1996). Dissociating the nicotine and airway sensory effects of smoking. *Pharmacology Biochemistry and Behavior*, *53*(2), 309–315. http://doi.org/10.1016/0091-3057(95)02027-6
Willemse, B. W. M., ten Hacken, N. H. T., Rutgers, B., Lesman-Leegte, I. G. A. T., Postma, D. S., & Timens, W. (2005). Effect of 1-year smoking cessation on airway inflammation in COPD and asymptomatic smokers. *European Respiratory Journal*, *26*(5), 835–845. http://doi.org/10.1183/09031936.05.00108904
Willett, J. G., Bennett, M., Hair, E. C., Xiao, H., Greenberg, M. S., Harvey, E., … Vallone, D. (2018). Recognition, use and perceptions of JUUL among youth and young adults. *Tobacco Control*, tobaccocontrol-2018-054273. http://doi.org/10.1136/tobaccocontrol-2018-054273
Williams, J. M., Gandhi, K. K., Lu, S. E., Kumar, S., Steinberg, M. L., Cottler, B., & Benowitz, N. L. (2011). Shorter interpuff interval is associated with higher nicotine intake in smokers with schizophrenia. *Drug Alcohol Depend*, *118*(2–3), 313–319. http://doi.org/10.1016/j.drugalcdep.2011.04.009
Williams, M., Ghai, S., & Talbot, P. (2015). Disposable electronic cigarettes and electronic hookahs: Evaluation of performance. *Nicotine and Tobacco Research*, *17*(2), 201–208. http://doi.org/10.1093/ntr/ntu118
Wonnacott, S., Sidhpura, N., & Balfour, D. J. K. (2005). Nicotine: From molecular mechanisms to behaviour. *Current Opinion in Pharmacology*, *5*(1), 53–59. http://doi.org/10.1016/j.coph.2004.12.002
Woodward, M., & Tunstall-Pedoe, H. (1993). Self-titration of nicotine: evidence from the Scottish Heart Health Study. *Addiction*, *88*(6), 821–830. Retrieved from http://search.ebscohost.com/login.aspx?direct=true&db=a9h&AN=6617591&site=ehost-live
Yan, X. S., & D'Ruiz, C. (2015). Effects of using electronic cigarettes on nicotine delivery and cardiovascular function in comparison with regular cigarettes. *Regulatory Toxicology & Pharmacology: RTP*, *71*(1), 24–34. http://doi.org/10.1016/j.yrtph.2014.11.004
Yang, X., Criswell, H. E., & Breese, G. R. (1996). Nicotine-induced inhibition in medial septum involves activation of presynaptic nicotinic cholinergic receptors on gamma-aminobutyric acid-containing neurons. *Journal of Pharmacology and Experimental Therapeutics*, *276*(2), 482 LP-489. Retrieved from http://jpet.aspetjournals.org/content/276/2/482.abstract
Yingst, J. M., Veldheer, S., Hrabovsky, S., Nichols, T. T., Wilson, S. J., & Foulds, J. (2015). Factors associated with electronic cigarette users' device preferences and transition from first generation to advanced generation devices. *Nicotine and Tobacco Research*, *17*(10), 1242–1246. http://doi.org/10.1093/ntr/ntv052
Yong, H.-H., Hitchman, S. C., Cummings, K. M., Borland, R., Gravely, S. M. L., McNeill, A., & Fong, G. T. (2017). Does the Regulatory Environment for E-Cigarettes Influence the Effectiveness of E-Cigarettes for Smoking Cessation?: Longitudinal Findings From the ITC Four Country Survey. *Nicotine & Tobacco Research*, (April). http://doi.org/10.1093/ntr/ntx056

Zacny, J. P., & Stitzer, M. L. (1996). Human smoking patterns. *Smoking and Tobacco Control Monograph No. 7*, 151–160. Retrieved from http://cancercontrol.cancer.gov/brp/tcrb/monographs/7/m7_11.pdf

Zawertailo, L., Voci, S., & Selby, P. (2018). The Cigarette Dependence Scale and Heaviness of Smoking Index as predictors of smoking cessation after 10 weeks of nicotine replacement therapy and at 6-month follow-up. *Addictive Behaviors*, *78*(November 2017), 223–227. http://doi.org/10.1016/j.addbeh.2017.11.036

Zham, D. S., & Brog, J. S. (1992). On the Significance of Subterritories in the "Accumbens" Part of the Rat Ventral Striatum. *Neuroscience*, *50*(4), 751–767.

Zhang, X. Y., Chen, D. C., Xiu, M. H., Hui, L., Liu, H., Luo, X., … Kosten, T. R. (2012). Association of functional dopamine-beta-hydroxylase (DBH) 19bp insertion/deletion polymorphism with smoking severity in male schizophrenic smokers. *Schizophrenia Research*, *141*(1), 48–53. http://doi.org/10.1016/j.schres.2012.07.011

Zhou, X., Nonnemaker, J., Sherrill, B., Gilsenan, A. W., Coste, F., & West, R. (2009). Attempts to quit smoking and relapse: Factors associated with success or failure from the ATTEMPT cohort study. *Addictive Behaviors*, *34*(4), 365–373. http://doi.org/10.1016/j.addbeh.2008.11.013

Zhu, S.-H., Zhuang, Y.-L., Wong, S., Cummins, S. E., & Tedeschi, G. J. (2017). E-cigarette use and associated changes in population smoking cessation: evidence from US current population surveys. *Bmj*, j3262. http://doi.org/10.1136/bmj.j3262

Zhuang, Y.-L., Cummins, S. E., Y Sun, J., & Zhu, S.-H. (2016). Long-term e-cigarette use and smoking cessation: a longitudinal study with US population. *Tobacco Control*, *25*(Suppl 1), i90–i95. http://doi.org/10.1136/tobaccocontrol-2016-053096

APPENDICES

APPENDIX 1 – IMAGES OF VARIOUS E-CIGARETTE MODELS AND THEIR COMPONENTS

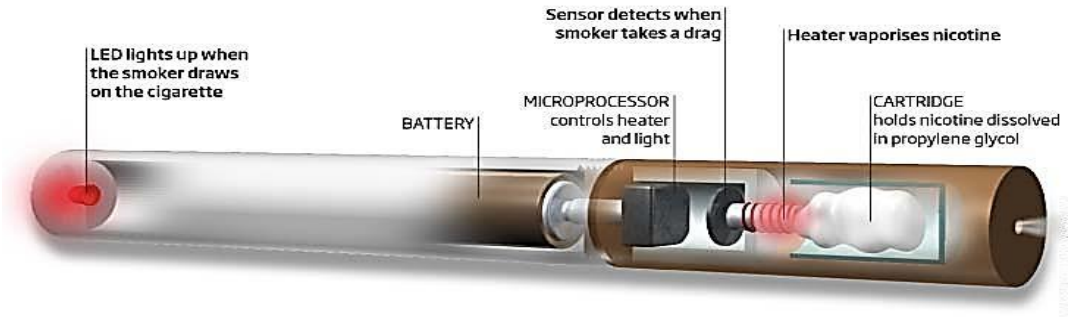


Figure 1.1 Typical components of an e-cigarette

Figure 1.2. Main components of a typical cigalike model

Figure 1.3. Typical penlike e-cigarette (2nd generation e-cigarette)

Figure 1.4. Typical clearomiser (2nd generation e-cigarette)

Figure 1.5. Examples of third generation tank system e-cigarettes

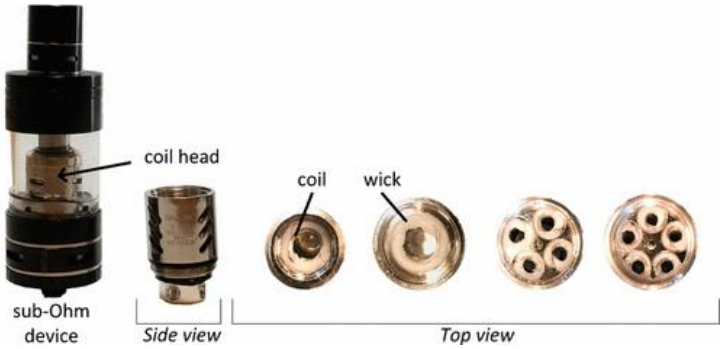


Figure 1.6. Sub-ohm tank, side and top view of the atomiser (Talih et al., 2017)

APPENDIX 2 – IMAGES OF E-CIGARETTE EQUIPMENT USED IN STUDY 1 AND PILOT STUDY (CHAPTER II)

Figure 2.1 The 'Joyetech eVic™ Supreme' e-cigarette and its components

Figure 2.2 The 'Aspire Nautilus' tank and its components

Figure 2.3 The 'bottom vertical coil (BVC) Aspire' atomiser (1.8 ohm resistance)

APPENDIX 3 - PILOT STUDY ETHICS APPLICATION (INCLUDE AMENDMENTS, RISK ASSESSMENT) AND APPROVAL LETTER

Application for ethical review of research involving human participants, human data or human material

This application should be completed by **members of staff and postgraduate research degree students (i.e. MRes, MPhil, PhD and Professional Doctorate)** undertaking research which involves human participants, sensitive human data (personal or otherwise) and human material (including human tissue, embryos, foetuses and bodily fluids, from living or deceased participants).

No form of contact with potential participants for the proposed research should occur until written approval has been received from University Research Ethics Committee (UREC). Where a member of staff or student is found to have breached this expectation, they may be subject to disciplinary action.

This application should be submitted alongside copies of any supporting documentation which will be handed to participants, including a participant information sheet, consent form, self-completion survey or questionnaire.

For further guidance please contact researchethics@uel.ac.uk or refer to the guidance at http://www.uel.ac.uk/qa/research/index.htm. Only those applications received by the submission deadline date shown on the University's Research Ethics web page will be considered at the next meeting. Where a form is submitted and sections are incomplete, the form will not be considered by UREC and will be returned to the applicant for completion.

PROJECT DETAILS

Current project title	E-cigarette puffing behaviour with different nicotine strength e-liquids
Is this project externally funded?	**YES** ☐ **NO** ☒ **NOT APPLICABLE** ☐
Does the project require UREC approval before consideration by the funding body?	**YES** ☐ **NO** ☐ **NOT APPLICABLE** ☒ If **YES**, please indicate funding body deadline below:
If externally funded, please provide details of funding body.	N/A
How will participants be informed of the source of funding? N/A **PARTICIPANT INFORMATION SHEET** ☐ **CONSENT FORM** ☐ **OTHER** ☐ If **OTHER**, please specify further below:	

Proposed project start date	22/9/14	**Anticipated project end date**	December 2014

APPLICANT DETAILS

Name of Principal Investigator (PI) (For research degree students, the Director of Studies)	Catherine Kimber
School	Psychology
Status (please tick relevant box)	**UEL STAFF ☒ RESEARCH DEGREE STUDENT ☐**
Email address	l.e.dawkins@uel.ac.uk
Contact telephone number	020 8223 4421
Name of co-researchers	Catherine Kimber (PhD UEL) Olivia Corcoran (HS&B, UEL)
Will parts of the proposed research or research administration be carried out by independent contractors or partner institutions, domestic or international?	**YES ☐ NO ☒** If **YES**, please provide a brief explanation of who the contractor or partner is, what their role will be and how their contribution will be monitored. Please note, responsibility for proper conduct of all parties involved in the research resides with the Principal Investigator.

CONFLICTS OF INTEREST

Will any of the researchers or their institutions receive any other benefits or incentives for taking part in this research over and above their normal salary package or the costs of undertaking the research? **YES ☐ NO ☒** If **YES**, please detail below:
Is there any further possibility for conflict of interest? YES ☐ NO ☒ If **YES**, please detail below:

FOR ALL APPLICANTS

Has external ethics approval been sought for this research? **(i.e. submission via Integrated Research Application System (IRAS) to the Health Research Authority (HRA) or other external research ethics committee)**	**YES ☐ NO ☒**

If **YES**, please supply details below:

DEAN OF SCHOOL OR ASSOCIATE DEAN • Does the proposed research as detailed herein have your support and endorsement to proceed? **YES** ☒ **NO** ☐	
Signed	
Date	

APPLICANT DECLARATION I confirm that: • The information contained in this application is, to the best of my knowledge, correct and up to date. • I have attempted to identify all risks related to the research. • I acknowledge my obligations and commitment to upholding our University's Code of Practice for ethical research and observing the rights of the participants. • I am aware that cases of proven misconduct, in line with our University's policies, may result in formal disciplinary proceedings and/or the cancellation of the proposed research.	
Applicant	Lynne Dawkins
Signed	L. Dawkins
Date	1/8/14

FOR RESEARCH DEGREE STUDENT APPLICANTS ONLY

Name and School of Director of Studies	
Qualification for which research is being undertaken	

Director of Studies (DoS) – • Does the student have the necessary skills to carry out the research? **YES** ☐ **NO** ☐ ▪ Is the participant information sheet, consent form and any other documentation appropriate? **YES** ☐ **NO** ☐ ▪ Are the procedures for recruitment of participants and obtaining informed consent suitable and sufficient? **YES** ☐ **NO** ☐ ▪ Where required, do all members of the research team have current Criminal Records Bureau (CRB) clearance? **YES** ☐ **NO** ☐	
Signed	
Date	

DETAILS OF THE PROPOSED RESEARCH

1. Provide a brief description of the proposed research, including the requirements of participants. This must be in lay terms and free from technical or discipline specific terminology or jargon. If such terms are required, please ensure they are adequately explained (Do not exceed 500 words)

This pilot study aims to explore the puffing patterns (puff frequency, puff duration, puff strength) of 5-10 regular e-cigarette users under two conditions: a 'usual strength' condition and '2 strengths lower' condition (e.g. 24mg/ml nicotine and 12mg/ml nicotine e-liquids). Evidence from cigarette smokers indicates that when regular smokers switch to 'light' (i.e. lower nicotine content) cigarettes, they alter their puffing patterns (i.e. take more, longer & harder puffs) in order to maintain a fairly constant blood nicotine level (self-titration). It is likely that the same happens with e-cigarette users although this has not yet been explored empirically. If e-cigarette users do take more intensive puffs when switching to a lower strength e-liquid, this is likely to lead to higher atomiser temperature, evaporation and increased exposure to toxins and carcinogens (see plans for full grant application to MRC under section 2 below).

In this proposed pilot study, regular e-cigarette users using second or third generation devices and e-liquid of 18mg/ml or higher will be recruited. They will be asked to refrain from all nicotine use overnight (10-12 hours) before visiting our lab in the School of Psychology, Stratford Campus, UEL (or another pre-arranged location) on 2 consecutive mornings. Participants will use the eVik e-cigarette from Joyetech with a Nautilus Tank System (which collects information on puff frequency; see further details in section 3 below) and will be asked to use the device for 45 minutes whilst the researcher video-records their puffing behaviour. On one occasion, participants will use their own e-liquid and on the other occasion, a liquid (matched for flavour) two strengths lower will be used (order counterbalanced). Data on puff frequency, puff duration and inhalation time will be gathered from video recordings and from eVik software.

2. Provide a statement on the aims and significance of the proposed research, including potential impact to knowledge and understanding in the field (where appropriate, indicate the associated hypothesis which will be tested). This should be a clear justification of the proposed research, why it should proceed and a statement on any anticipated benefits to the community. (Do not exceed 700 words)

Electronic cigarettes are becoming increasingly popular and are currently used by approximately 2 million smokers in the UK. The recent EU Tobacco Products Directive (TPD) stipulates that all nicotine containing fluids above a 20mg/ml will be banned from 2017 unless they are licensed as medicines (UK only). Since an estimated 1/3 of e-cigarette users use strengths higher than this, they will be forced to switch to a lower strength fluid. We plan to submit an application to the MRC to assess the behavioural and health outcomes associated with the use of different strength nicotine containing e-liquid and in order to do so, we require pilot data from 5-10 participants. Evidence from the tobacco smoking literature demonstrates that regular smokers compensate (by taking larger puff volumes, more frequent puffs and holding the puff for longer) when they switch to lower strength cigarettes to gain similar levels of nicotine and it is likely that the same happens with e-cigarette users although this has not yet been explored. Although studies of e-cigarette vapour have reported very low levels of toxicants and carcinogens, given that a more intensive puffing pattern is associated with a higher atomiser temperature, a high amount of inhaled vapour, and potentially greater exposure to toxins and carcinogens, any

compensatory puffing behaviour might subject the user to increased health risks (albeit, still far lower than from conventional tobacco cigarettes). This pilot study will provide essential information on whether, and how, puffing patterns change when a higher strength nicotine containing e-liquid is replaced with a lower one. This will then help to inform the design and sample size for the larger scale application to the MRC. If e-cigarette users do adapt the way they puff when switching to a lower strength nicotine e-liquid, and this increases exposure to toxins, although the risks are likely to be low compared to smoking, e-cigarette users might be inadvertently increasing their risks of cancer and respiratory disease. The results of the larger scale study will have implications for those making decisions about the strength of nicotine e-liquid including smokers, stop smoking service advisors and policy makers.

3. Provide an outline of the methodology for the proposed research, including proposed method of data collection, tasks assigned to participants of the research and the proposed method and duration of data analysis. If the proposed research makes use of pre-established and generally accepted techniques, please make this clear. (Do not exceed 500 words)

Design:
Within-subjects design with two conditions: 'usual strength' and 'low strength' e-liquid.

Equipment:

Carbon Monoxide (CO) Breatherlyser (Bedfont Smokerlyser) to measure exhaled CO levels

eVic™ electronic cigarette from Joyetech. Joyetech is the world's leading electronic cigarette manufacturer and the company has been at the forefront of the industry with top quality products and excellent reviews. We are using this product because it has downloadable software allowing us to collect data on puff duration and frequency from the product itself. See: http://www.joyetech.co.uk/electronic-cigarettes/eVic™-supreme#.U9uFRKNeLpd

Nautilus Tank System. This top of the range tank system is large enough to hold 5ml of fluid which means we will not have to re-fill it during the 45 minute ad lib vaping period. It also comes with a measuring gauge and airflow control system which makes it ideal for our purposes. See: http://www.ukecigstore.com/nautilus-tank-system-by-aspire.html

Nicotine e-liquid: participants usual e-liquid will be used as well as a lower strength e-liquid purchased from the same provider.

Video recorder

Precision scales

Procedure:

Potential volunteers will be given an invitation letter and consent form (see appendices) and if agreeable to participate, a date for testing will be set. Participants will be asked to remain abstinent from e-cigarette use, smoking and any other nicotine products overnight (10-12 hours). All testing will take place in the morning to avoid discomfort associated with not using nicotine.

On the morning of testing, after going through the information sheet and ensuring that the participant understands what is required of him/her, the researcher will ask the participant to sign the consent form. Participants will then be asked to breath into the smokerlyser to verify their abstinence from smoking.

Participants will then complete a demographic and smoking/e-cigarette related questionnaire and a craving and mood scale. S/he will then be invited to use the e-cigarette ad lib for 45 minutes whilst being video-recorded and finally will complete a subjective effects scale and the craving/mood scale for the second time (see appendices for all questionnaires). The whole procedure will last approximately one hour and will be repeated the following day with the other strength nicotine e-liquid (order counterbalanced).

Participants will be informed at the beginning of the session that they are free to leave at any point either for a comfort break or permanently should they wish to do so. After 20 minutes, participants will be reminded that they can take a break at any point.

On each occasion the researcher will weigh the e-cigarette tank before and after use in order to more accurately calculate the amount of fluid used and cross reference this will the measuring gauge on the side of the tank.

PARTICIPANT DETAILS

4. Provide an explanation detailing how you will identify, approach and recruit the participants for the proposed research, including clarification on sample size and location. Please provide justification for the exclusion/inclusion criteria for this

study (i.e. who will be allowed to / not allowed to participate) and explain briefly, in lay terms, why this criteria is in place. (Do not exceed 500 words)

This study aims to test 5 – 10 non-smoking, regular e-cigarette users (vapers). This sample size is sufficient for this proof of principle pilot study and will help to inform sample size, design and procedural aspects of the larger scale study. We will directly approach e-cigarette users known to us via e-mail, twitter or facebook. Testing will take place in the School of Psychology, Stratford Campus wherever possible, but if participants are unable to travel to UEL, we will consider travelling to participants' homes (where participants are known to us and after informing a third party).

Inclusion Criteria: Ex-smoker, Daily e-cigarette user, using a second or third generation device and at least 18mg/ml nicotine e-liquid. Aged 18 or over. Fluent English speakers.

Exclusion Criteria: Smokers, non-daily vapers, pregnancy, breast-feeding, neurological or heart condition, under 18 years.

5. Will the participants be from any of the following groups?*(Tick as appropriate)*

☒ Students or staff of this University (i.e. recruitment on-site at University of East London).

☒ Adults (over the age of 18 years with mental capacity to give consent to participate in the research).

☐ Young people between 16 and 18 years (please note parental consent may still be required for s006Fme research).☐ Children or legal minors (anyone under the age of 16 years)[1]

☐ Adults who are unconscious, severely ill or have a terminal illness.

☐ Adults who may lose mental capacity to consent during the course of the research.

☐ Adults in emergency situations.

☐ Adults[2] with mental illness - particularly those detained under the Mental Health Act (1983 & 2007).

☐ Participants who may lack capacity to consent to participate in the research under the research requirements of the Mental Capacity Act (2005).

☐ Prisoners, where ethical approval may be required from the National Offender Management Service (NOMS).

☐ Young Offenders, where ethical approval may be required from the National Offender Management Service (NOMS).

☐ Healthy volunteers (in high risk intervention studies).

☐ Participants who may be considered to have a pre-existing and potentially dependent[3] relationship with the investigator (e.g. those in care homes, students, colleagues, service-users, patients).

☐ Other vulnerable groups (see Question 6).

☐ Adults who are in custody, custodial care, or for whom a court has assumed responsibility.

☐ Participants who are members of the Armed Forces.

[1]If the proposed research involves children or adults who meet the Police Act (1997) definition of vulnerability[3], any researchers who will have contact with participants must have current enhanced Disclosure and Barring Service check (was previously called Criminal Records Bureau, or CRB,

clearance). [2] 'Adults with a learning or physical disability, a physical or mental illness, or a reduction in physical or mental capacity, and living in a care home or home for people with learning difficulties or receiving care in their own home, or receiving hospital or social care services.' (Police Act, 1997)
[3] Proposed research involving participants with whom the investigator or researcher(s) shares a dependent or unequal relationships (e.g. teacher/student, clinical therapist/service-user) may compromise the ability to give informed consent which is free from any form of pressure (real or implied) arising from this relationship. UREC recommends that, wherever practicable, investigators choose participants with whom they have no dependent relationship. Following due scrutiny, if the investigator is confident that the research involving participants in dependent relationships is vital and defensible, UREC will require additional information setting out the case and detailing how risks inherent in the dependent relationship will be managed. UREC will also need to be reassured that refusal to participate will not result in any discrimination or penalty.

6. Will the study involve participants who are vulnerable? YES ☐ NO ☒

For the purposes of research, 'vulnerable' participants may be adults whose ability to protect their own interests are impaired or reduced in comparison to that of the broader population. Vulnerability may arise from the participant's personal characteristics (e.g. mental or physical impairment) or from their social environment, context and/or disadvantage (e.g. socio-economic mobility, educational attainment, resources, substance dependence, displacement or homelessness). Where prospective participants are at high risk of consenting under duress, or as a result of manipulation or coercion, they must also be considered as vulnerable.

Adults lacking mental capacity to consent to participate in research and children are automatically presumed to be vulnerable. Studies involving adults (over the age of 16) who lack mental capacity to consent in research must be submitted to a REC approved for that purpose.

6.1. **If YES,** a Disclosure and Barring Service (DBS) check within the last three years is required.

Please provide details of the "clear disclosure":

Date of disclosure:
Type of disclosure:
Organisation that requested disclosure:
DBS certificate number:

(NOTE: information concerning activities which require DBS checks can be found via https://www.gov.uk/government/publications/dbs-check-eligible-positions-guidance)

6.2.If YES, what special arrangements are in place to protect vulnerable participants' interests?

7. Do you propose to make any form of payment or incentive available to participants of the research? YES ☐ NO ☒

If **YES**, please provide details taking into account that any payment or incentive should be representative of reasonable remuneration for participation and may not be of a value that could be coercive or exerting undue influence on potential participants' decision to take part in the research. Wherever possible, remuneration in a monetary form should be avoided and substituted with vouchers, coupons or equivalent. Any payment made to research participants may have benefit or HMRC implications and participants should be

alerted to this in the participant information sheet as they may wish to choose to decline payment.

8. What special arrangements are in place for eliciting informed consent from participants who may not adequately understand verbal explanations or written information provided in English; where participants have special communication needs; where participants have limited literacy; or where children are involved in the research? (Do not exceed 200 words)

For this pilot study, all participants will be known to the research team and will be native English speakers. However, the researcher will go through the information sheet with every participant and check that they understand what is involved prior to their consent. Information sheets will also be e-mailed to participants before the date of testing so that participants have time to process and digest the information. No children are involved in the research.

RISK ASSESSMENT AND RISK MANAGEMENT

9. Does the proposed research involve any of the following? *(Tick as appropriate)*

☒ use of a questionnaire, self-completion survey or data-collection instrument (attach copy)
☐ use of emails or the internet as a means of data collection
☐ use of written or computerised tests
☐ interviews (attach interview questions)
☐ diaries (attach diary record form)
☒ participant observation
☐ participant observation (in a non-public place) without their knowledge / covert research
☐ audio-recording interviewees or events
☒ video-recording interviewees or events
☐ access to personal and/or sensitive data (i.e. student, patient, client or service-user data) without the participant's informed consent for use of these data for research purposes
☐ administration of any questions, tasks, investigations, procedures or stimuli which may be experienced by participants as physically or mentally painful, stressful or unpleasant during or after the research process
☐ performance of any acts which might diminish the self-esteem of participants or cause them to experience discomfiture, regret or any other adverse emotional or psychological reaction
☐ investigation of participants involved in illegal or illicit activities (e.g. use of illegal drugs)
☐ procedures that involve the deception of participants
☒ administration of any substance or agent **(yes, but NOTE: only what participants would use themselves anyway on a daily basis).**
☐ use of non-treatment of placebo control conditions
☐ collection of body tissues or fluid samples
☐ collection and/or testing of DNA samples
☐ collection and/or testing of gametes or embryo tissue
☐ participation in a clinical trial
☐ administration of ionising radiation to participants
☒ research undertaken at an off-campus location (risk assessment attached)
☐ research overseas (copy of VCG overseas travel approval attached)

10. Does the proposed research involve any specific or anticipated risks (e.g. physical, psychological, social, legal or economic) to participants that are greater than those encountered in everyday life? YES ☐ NO ☒
If **YES**, please describe below including details of precautionary measures.

Participants will all be regular e-cigarette users and will be using their own strength e-liquid as well as a lower strength e-liquid, both *ad libitum* (i.e. as much or as little as they want) Therefore the risks are no greater than those encountered in everyday life through participants' usual vaping habits. Adverse effects of nicotine include nausea and headache and e-cigarettes are sometime associated with a dry mouth although these effects are rarely experienced in regular e-cigarette users. Water or any drink will be available at all times and the participants will be reminded that they are free to leave at any time either for a comfort break or permanently. The investigator and researcher are experienced in e-cigarette and smoking research and any participant experiencing adverse effects will be advised to stop using the device and allowed to rest in a quiet location with the researcher present should they wish to do so. Adverse effects usually subside within an hour. A taxi home will be also offered to anyone experiencing ill effects.

11. Where the procedures involve potential hazards and/or discomfort or distress for participants, please state what previous experience the investigator or researcher(s) have had in conducting this type of research.

As mentioned above, it is unlikely that there are any hazards associated with taking part in this research although water will be available throughout the session and participants will be reminded that they are under no obligation to use the product and are free to leave at any time. Participants may experience slight discomfort associated with not using nicotine for 10-12 hours although all testing sessions will be held in the morning in order to minimise any discomfort.

The principle investigator (Lynne Dawkins) has had over 15 years of experience of working with smokers in a research capacity and 4 years experience of working with electronic cigarettes. She is generally regarded as one of the UK's leading authorities on e-cigarettes. The researcher (Catherine Kimber) has recently conducted her undergraduate project on e-cigarette use and choice in smokers with Dr Dawkins so has on years experience of working with smokers and e-cigarettes.

12. Provide an explanation of any potential benefits to participants. Please ensure this is framed within the overall contribution of the proposed research to knowledge or practice. (Do not exceed 400 words)
NOTE: Where the proposed research involves students of our University, they should be assured that accepting the offer to participate or choosing to decline will have no impact on their assessments or learning experience. Similarly, it should be made clear to participants who are patients, service-users and/or receiving any form of treatment or medication that they are not invited to participate in the belief that participation in the research will result in some relief or improvement in their condition.

There are no direct benefits to the participants themselves although the findings of the study will help to inform a larger scale study that will benefit the wider clinical, academic and regulatory community by providing information about e-cigarette puffing behaviour and effects on toxin and carcinogen production. Participants will be informed that whilst their involvement in the study will not benefit them directly, the findings will help to add to the knowledge base on e-cigarettes.

No UEL students will take part.

Although most of the participants taking part will be known to the researcher, none are in a dependent relationship with the researcher and all have previously expressed interest in taking part in research studies after hearing of her work.

13. Provide an outline of any measures you have in place in the event of adverse or unexpected outcomes and the potential impact this may have on participants involved in the proposed research. (Do not exceed 300 words)

No adverse effects are expected as all participants will be regular e-cigarette users using their own e-liquid or a lower strength.

Participants will however, be using a new device which they are not familiar with, and may experience difficulty with at first. We have factored in sufficient time (45 mins) in order to demonstrate use and for participants to become familiar with the device.

In the unexpected event of the device malfunctioning, participants will be permitted to use their own device in order to avoid re-scheduling.

In relation to the study findings, it is possible that we find no changes in puffing behaviours with the two strength e-liquids. This will be informative in itself and will help to shape our future funding applications.

14. Provide an outline of your debriefing, support and feedback protocol for participants involved in the proposed research. This should include, for example, where participants may feel the need to discuss thoughts or feelings brought about following their participation in the research. This may involve referral to an external support or counseling service, where participation in the research has caused specific issues for participants. Where medical aftercare may be necessary, this should include details of the treatment available to participants. Debriefing may involve the disclosure of further information on the aims of the research, the participant's performance and/or the results of the research. (Do not exceed 500 words)

At the end of the study, the researcher will allow 5-10 minutes in order to answer any questions that the participant may have about their participation and e-cigarette use. Participants will be verbally informed of the aim of the study – i.e. to look at any changes in puffing patterns that occur when users switch to a lower strength e-liquid. They will also be told that the findings will help to inform a larger scale study looking at effects of different puffing techniques on toxicants in the vapour. Participants will also be given the principle investigator's e-mail address and telephone number should they feel the need to discuss any aspect of their participation at a later date.

PARTICIPANT CONSENT AND WITHDRAWAL

15. Have you attached a copy of your participant information sheet (this should be in *plain English*)? Where the research involves non-English speaking participants, please include translated materials. YES☒ NO ☐

If **NO**, please indicate what alternative arrangements are in place below:

16. Have you attached a copy of your participant consent form (this should be in *plain English*)? Where the research involves non-English speaking participants, please include translated materials.

YES ☒ NO ☐

If **NO**, please indicate what alternative arrangements are in place below:

17. The following is a <u>participant information sheet</u> checklist covering the various points that should be included in this document.

☒ Clear identification of UEL as the sponsor for the research, the schools(s) involved, the project title, the Principal Investigator and other researchers along with relevant contact details.

☒Details of what involvement in the proposed research will require (e.g., participation in interviews, completion of questionnaire, audio/video-recording of events), estimated time commitment and any risks involved.

☒A statement confirming that the research has received formal approval from UREC.

☒ If the sample size is small, advice to participants that this may have implications for confidentiality / anonymity.

N/A A clear statement that where participants are in a dependent relationship with any of the researchers that participation in the research will have no impact on assessment / treatment / service-use or support.

☒ Assurance that involvement in the project is voluntary and that participants are free to withdraw consent at any time, and to withdraw any unprocessed data previously supplied.

☒Advice as to arrangements to be made to protect confidentiality of data, including that confidentiality of information provided is subject to legal limitations.

☒A statement that the data generated in the course of the research will be retained in accordance with the University's Data Protection Policy.

☒Advice that if participants have any concerns about the conduct of the investigator, researcher(s) or any other aspect of this research project, they should contact <u>researchethics@uel.ac.uk</u>.

☒Confirmation on any limitations in confidentiality where disclosure of imminent harm to self and/or others may occur.

N/A. For research involving under 16 s or vulnerable groups, where true, a statement has been included on all information sheets that the investigators have passed appropriate Disclosure and Barring Service checks.

18. The following is a <u>consent form</u> checklist covering the various points that should be included in this document.

☒University of East London letterhead or logo.

☒Title of the project (with research degree projects this need not necessarily be the title of the thesis) and names of investigators.

☒Confirmation that the project is research.

☒Confirmation that involvement in the project is voluntary and that participants are free to withdraw at any time, or to withdraw any unprocessed data previously supplied.

☒Confirmation of particular requirements of participants, including for example whether interviews are to be audio-/video-recorded, whether anonymised quotes will be used in publications advice of legal limitations to data confidentiality.

☒ If the sample size is small, confirmation that this may have implications for anonymity any other relevant information.

☒The proposed method of publication or dissemination of the research findings.

N/A. Details of any external contractors or partner institutions involved in the research.

N/A. Details of any funding bodies or research councils supporting the research.

N/A Confirmation on any limitations in confidentiality where disclosure of imminent harm to self and/or others may occur.

N/A. Separate forms and information sheets requesting parental/guardian consent for research involving under age children have been included / attached.

N/A. Letters requesting consent for the research to be conducted at external sites (e.g. Managers/ owners/ head teachers /etc.) where the research will be carried out have been included / attached.

☒Where research is to be published (it is envisaged that this is true in most cases) it has been made clear in the information sheet and, or form (preferably both) that both consent to take part and to publication is being obtained.

<u>CONFIDENTIALITY AND ANONYMITY</u>

19. Below is a checklist covering key points relating to the confidentiality and anonymity of participants. Please indicate where relevant to the proposed research.

☐ Participants will be completely anonymised and their identity will not be known by the investigator or researcher(s) (i.e. the participants are part of an anonymous randomised sample and return responses with no form of personal identification)?

☐ The responses are anonymised or are an anonymised sample (i.e. a permanent process of coding has been carried out whereby direct and indirect identifiers have been removed from data and replaced by a code, with <u>no</u> record retained of how the code relates to the identifiers).

☒The samples and data are de-indentified (i.e. direct and indirect identifiers have been removed and replaced by a code. The investigator or researchers <u>are</u> able to link the code to the original identifiers and isolate the participant to whom the sample or data relates).

☐ Participants have the option of being identified in a publication that will arise from the research.

☐ Participants will be pseudo-anonymised in a publication that will arise from the research.

☐ The proposed research will make use of personal sensitive data.

☐ Participants consent to be identified in the study and subsequent dissemination of research findings and/or publication.

20. Participants must be made aware that the confidentiality of the information they provide is subject to legal limitations in data confidentiality (i.e. the data may be subject to a subpoena, a freedom of information request or mandated reporting by some professions). This only applies to named or de-identified data. If your participants are named or de-identified, please confirm that you will specifically state these limitations.

YES ☒ **NO** ☐

If **NO**, please indicate why this is the case below:

NOTE: WHERE THE PROPOSED RESEARCH INVOLVES A SMALL SAMPLE OR FOCUS GROUP, PARTICIPANTS SHOULD BE ADVISED THAT THERE WILL BE DISTINCT LIMITATIONS IN THE LEVEL OF ANONYMITY THEY CAN BE AFFORDED.

DATA ACCESS, SECURITY AND MANAGEMENT

21. Will the Principal Investigator be responsible for the security of all data collected in connection with the proposed research? YES ☒ **NO** ☐

If **NO**, please indicate what alternative arrangements are in place below:

22. In line with the 5th principle of the Data Protection Act (1998), which states that personal data shall not be kept for longer than is necessary for that purpose or those purposes for which it was collected; please state how long data will be retained for.

☒ 1-2 years ☐ 3-5 years ☐ 6-10 years ☐ 10> years

NOTE: Research Councils UK (RCUK) guidance currently states that data should normally be preserved and accessible for 10 years, but for projects of clinical or major social, environmental or heritage importance, for 20 years or longer. (http://www.rcuk.ac.uk/documents/reviews/grc/grcpoldraft.pdf)

23. Below is a checklist which relates to the management, storage and secure destruction of data for the purposes of the proposed research. Please indicate where relevant to your proposed arrangements.

☒Research data, codes and all identifying information to be kept in separate locked filing cabinets.

☒Access to computer files to be available to research team by password only.

☐ Access to computer files to be available to individuals outside the research team by password only (See **23.1**).

☐ Research data will be encrypted and transferred electronically within the European Economic Area (EEA).

☐ Research data will be encrypted and transferred electronically outside of the European Economic Area (EEA). (See **23.2**).

NOTE: Transfer of research data via third party commercial file sharing services, such as Google Docs and YouSendIt are not necessarily secure or permanent. These systems may also be located overseas and not covered by UK law. If the system is located outside the European Economic Area (EEA) or territories deemed to have sufficient standards of data protection, transfer may also breach the Data Protection Act (1998).

☒Use of personal addresses, postcodes, faxes, e-mails or telephone numbers.

☒Use of personal data in the form of audio or video recordings.

☐ Primary data gathered on encrypted mobile devices (i.e. laptops). **NOTE:** This should be transferred to secure UEL servers at the first opportunity.

☒All electronic data will undergo secure disposal.

NOTE: For hard drives and magnetic storage devices (HDD or SSD), deleting files does not permanently erase the data on most systems, but only deletes the reference to the file. Files can be restored when deleted in this way. Research files must be overwritten to ensure they are completely irretrievable. Software is available for the secure erasing of files from hard drives which meet recognised standards to securely scramble sensitive data. Examples of this software are BC Wipe, Wipe File, DeleteOnClick and Eraser for Windows platforms. Mac users can use the standard 'secure empty trash' option; an alternative is Permanent eraser software.

☒All hardcopy data will undergo secure disposal.

NOTE: For shredding research data stored in hardcopy (i.e. paper), adopting DIN 3 ensures files are cut into 2mm strips or confetti like cross-cut particles of 4x40mm. The UK government requires a minimum standard of DIN 4 for its material, which ensures cross cut particles of at least 2x15mm.

23.1. Please provide details of individuals outside the research team who will be given password protected access to encrypted data for the proposed research.

N/A

23.2. Please provide details on the regions and territories where research data will be electronically transferred that are external to the European Economic Area (EEA).

N/A

OVERSEAS TRAVEL FOR RESEARCH

24. Does the proposed research involve travel outside of the UK? YES ☐ NO ☒

24.1. Have you consulted the Foreign and Commonwealth Office website for guidance/travel advice? http://www.fco.gov.uk/en/travel-and-living-abroad/ **YES** ☐ **NO** ☐

24.2. If you are a non-UK national, have you sought travel advice/guidance from the Foreign Office (or equivalent body) of your country? **YES** ☐ **NO** ☐ **NOT APPLICABLE** ☐

24.3. Have you completed the overseas travel approval process and enclosed a copy of the document with this application? **YES** ☐ **NO** ☐

Details on this process are available here http://www.uel.ac.uk/qa/research/fieldwork.htm

24.4. Is the research covered by our University's insurance and indemnity provision?

YES ☐ **NO** ☐

(Please seek confirmation via researchethics@uel.ac.uk)

NOTE: Where research is undertaken at an off-campus location within the UK or overseas, the Risk Assessment policy must be consulted: http://dl-cfs-01.uel.ac.uk/hrservices/documents/hshandbook/risk_assess_policy.pdf. The Dean of School or Director of Service has overall responsibility for risk assessment regarding the health and safety of staff or students conducting research where UEL is the sponsor.

24.5. Please evidence how compliance with all local research ethics and research governance requirements have been assessed for the country(ies) in which the research is taking place.

24.6. Will this research be financially supported by the United States Department of Health and Human Services or any of its divisions, agencies or programs? **YES** ☐ **NO** ☐

PUBLICATION AND DISSEMINATION OF RESEARCH FINDINGS

25. How will the results of the research be reported and disseminated? ***(Select all that apply)***

- ☒ Peer reviewed journal
- ☒ Conference presentation
- ☐ Internal report
- ☒ Dissertation/Thesis
- ☐ Other publication
- ☐ Written feedback to research participants
- ☒ Presentation to participants or relevant community groups
- ☐ Other (Please specify below)

OTHER ETHICAL ISSUES

26. Are there any other ethical issues that have not been addressed which you would wish to bring to the attention of University Research Ethics Committee (UREC)?

No

CHECKLIST FOR ATTACHED DOCUMENTS

27. Please check that the following documents are attached to your application.

☐ Recruitment advertisement
☒ Participant information sheets (including easy-read where relevant)
☒ Consent forms (including easy-read where relevant)
☐ Assent form for children (where relevant)
☐ Evidence of any external approvals needed
☒ Questionnaire
☐ Interview Schedule or topic guide
☒ Risk assessment (where applicable)
☐ Overseas travel approval (where applicable)

27.1. Where it is not possible to attach the above materials, please provide an explanation below.

Recruitment will be via direct e-mail invitation to e-cigarette users known to us, or twitter/facebook if insufficient numbers are recruited via personal e-mail contacts.

E-mail example: *Dear X, we are conducting a pilot study of e-cigarette users to explore patterns of puffing behaviour with different strength e-liquids and we wondered whether you would be able to take part? It would involve 2 visits to UEL (or we could come to your home if that's easier) on 2 consecutive days and you would complete one questionnaire and then be video-recorded using an e-cigarette for about 45 minutes. I have attached an information sheet which provides further information. I look forward to hearing from you... Best wishes....*

RISK ASSESSMENT FOR THE PILOT STUDY

University of East London
School of Psychology

Risk assessment for testing participants away from UEL.
E-cigarette puffing behaviour with different strength e-liquids

	Testing Location(s)	Possible Risk	Severity of hazard (H, M, L)	Likelihood of hazard (H, M, L)	Risk (H, M, L)	Mitigating Activity
1.	Most testing will take place at UEL (Stratford campus) although at times it may be necessary to test participants in their own homes or at their place of work in order to maximise recruitment.	The researcher may be exposed to risk if she is alone with a participant in an unfamiliar environment.	Low	Low	Low	All the participants will be known to the research team so we do not expect there to be any risks. However, the researcher will always keep the PI or another colleague informed of her whereabouts if testing participants in locations other than UEL.
2.	UEL/participants home	Participants experience adverse effect of nicotine	Low	Low	Low	Very unlikely as participants will be given their OWN STRENGTH and a LOWER strength nicotine e-liquid and using *ad libitum*. However, should a participant feel any adverse effects, s/he will be advised to stop using the e-cigarette immediately and the research will stay with the participant until s/he feels better. Effects usually wear off within an hour.

3.	UEL/participants own home	E-liquid spill	Low	Low	Low	The device we have chosen is very easy to re-fill and the investigator and researcher have experience using e-liquid refills. We will only use 10ml of liquid at a time. In the case of accidental spillage or contact with the skin, the participant/researcher will use the sink in the laboratory to wash their hands immediately. No reports of adverse effects relating to exposure of this concentration and amount of e-liquid to the skin have been reported.

Principle Investigator: L. Dawkins

NOTE THAT TO AVOID REPETITIONS, QUESTIONNAIRES HAVE BEEN REMOVED FROM THE ETHICS APPLICATION AND CAN BE FOUND IN SUBSEQUENT APPENDICES

APPENDIX 4 - PARTICIPANT INVITATION LETTER (PILOT STUDY)

UNIVERSITY OF EAST LONDON

School of Psychology
Stratford Campus
Water Lane
London E15 4LZ

The Principal Investigator(s)
Dr. Lynne Dawkins; E-mail: l.e.dawkins@uel.ac.uk
Catherine Kimber; E-mail: C.Kimber@uel.ac.uk

Consent to Participate in a Research Study

The purpose of this letter is to provide you with the information that you need to consider in deciding whether to participate in a research study being conducted at the University of East London.

Project Title

E-cigarette puffing behaviour with different strength nicotine e-liquids

Project Description

This study aims to compare e-cigarette puffing behaviour with two different strength e-liquids – your own, and another lower strength liquid matched to your preferred flavour and from the same supplier. If you decide to take part, we would like to see you on two consecutive days for about 1 hour each time. You will be asked to refrain from e-cigarette and nicotine use overnight (for 10-12 hours) prior to each session. Testing sessions will take place in the mornings in order to minimize any discomfort associated with not using nicotine. We will provide the e-cigarette (an eVic™ third generation device) for you to use.

During the study, you will first be asked to use a smokerlyser to check your carbon monoxide levels to show that you have not smoked. This is a simple, non-invasive breath test which simply requires you to hold your breath and then breath slowly into a tube. You will then be asked to provide some background information about yourself and your e-cigarette use via a questionnaire and complete a craving and mood scale. We would then like to video-record you using the e-cigarette for about 45 minutes, after which, you will complete another questionnaire about your mood and vaping experience. Please note, you are free to take comfort breaks during this time and you will be reminded after 20 minutes that you are free to take a break.

Confidentiality of the Data

All data generated in the course of the research will be treated confidentially and will be retained in accordance with the University's Data Protection Policy. All data will be

numerically coded although we plan to collect data from only 5-10 people so it is possible that complete anonymity may not be achievable. Nevertheless, consent forms will be detached and separated from the numerically-coded data and your questionnaire data and video recordings will be stored in a locked filing cabinet at UEL. Your video recordings will be analysed by two independent researchers at UEL who will look at the number and length of puffs you took. The e-Vic e-cigarette also records puff number and length and we will compare this information with our video recordings. Once this information is entered into a computerised dataset, we will reset the eVic™ to delete this information and will dispose of the original video recordings securely. The anonymised data will be used for the current study and it is expected that the findings will be written up for publication in a peer reviewed journal. Should any data be used in subsequent studies or for further analysis, the data will still be kept anonymous and confidential. Nevertheless, please be aware that the confidentiality of the information provided is subject to legal limitations in data confidentiality (i.e. the data may be subject to a subpoena, a freedom of information request or mandated reporting by some professions).

Location

The study is likely to be carried out at the University East London, Stratford Campus. However, should it be required, the study may be carried out at a place more convenient to you such as your own home.

Remuneration

There is no payment for taking part in this study, nor will your involvement in this study benefit you directly although the findings will help to add to the scientific knowledge base on e-cigarettes.

Disclaimer

You are not obliged to take part in this study and should not feel coerced. You are free to withdraw at any time. Should you choose to withdraw from the study you may do so without disadvantage to yourself and without any obligation to give a reason. Your right to withdraw from the study includes the right to request the destruction of any of your data obtained from the study though you must memorise your personal participant number in order for the experimenter to delete your data. You can email us at any point prior stating your participation number, and we will remove your data for you. Following completion of the study, none of your contact information will be retained without your specific permission.

Please feel free to ask any questions. If you are happy to continue you will be asked to sign a consent form prior to your participation. Please retain this invitation letter for reference.

This study has received full approval from the University of East London's Ethical Committee. If you have any questions or concerns about how the study has been conducted, please contact the study's Principle Investigator:

Dr. Lynne Dawkins, School of Psychology, University of East London, Water Lane, London E15 4LZ. +44 (0)20 8223 4421. E-mail: l.e.dawkins@uel.ac.uk

Or

Catherine Fieulleteau, Ethics Integrity Manager, Graduate School, EB 1.43
University of East London, Docklands Campus, London E16 2RD
(Telephone: 020 8223 6683, Email: researchethics@uel.ac.uk).

APPENDIX 5 - CONSENT FORM (PILOT STUDY)

UNIVERSITY OF EAST LONDON

Consent to participate in a research study

Title

E-cigarette puffing behaviour with different strength nicotine e-liquids

I have the read the information sheet relating to the above research study and have been given a copy to keep. The nature and purpose of the research has been explained to me, and I have had the opportunity to discuss the details and ask questions about this information. I understand what is being proposed and the procedures in which I will be involved have been explained to me. I understand that I am able to take breaks whenever I want and that I am free to leave at any time.

I understand that my involvement in this study, and particular data from this research, will remain strictly confidential. Only the researcher(s) involved in the study will have access to identifying data. I understand that the findings from this research project may be written up, submitted for publication and presented at conferences. I understand that all collected data will remain confidential during this process. It has been explained to me what will happen once the research study has been completed.

I hereby freely and fully consent to participate in the study which has been fully explained to me. Having given this consent I understand that I have the right to withdraw from the study at any time without disadvantage to myself and without being obliged to give any reason.

Participant's Name (BLOCK CAPITALS)

…….

Participant's Signature

……..

Researcher's Name (BLOCK CAPITALS)

……..

Researcher's Signature

……

Date: ……..…….

APPENDIX 6 - PILOT STUDY BASELINE DEMOGRAPHIC DATA QUESTIONNAIRE

1. Which type of device do you currently use most (please circle one):

    a. A disposable e-cigarette (non-rechargeable)
    b. A rechargeable e-cigarette (sometimes called 'stick-like', 'cigarette-like' or 'cartomizers' without fluid)
    c. A rechargeable e-cigarette ('stick-like/cigarette-like/cartomizer' without fluid) starter kit
    d. A rechargeable e-cigarette (non cigarette-like with fluid)
    e. A rechargeable e-cigarette (non cigarette-like with fluid) starter kit
    f. A modular system (your own combination of separate parts: battery, atomizer, fluid etc).
    g. E-cigar

2. Which brand and model of e-cigarette are you currently using most:?

    Brand (please state) _____
    Model (please state)_____

3. Which strength(s) of nicotine fluid/cartridge are you currently using? (circle all that apply).
    a. 0mg
    b. 4 mg
    c. 6mg
    d. 8mg
    e. 10 mg
    f. 11mg
    g. 15 mg
    h. 16mg
    i. 18mg
    j. 24mg
    k. 36mg
    l. Higher than 36mg
    m. I don't know

4. If you have circled more than one strength, please state which strength you use most: _____

5. Please try to estimate the amount you use per day:
    a. In puffs _____
    b. In cartridges _____
    c. In ml _____

6. Which is your preferred flavour? (please circle one)
    a. Tobacco
    b. Mint/menthol
    c. Fruit (various)
    d. Coffee
    e. Vanilla
    f. RY4
    g. Chocolate
    h. Cinnamon
    i. Tea
    j. Alcohol-related
    k. Other (please state)

7. How soon after you wake up do you use your electronic cigarette? (circle one answer)
    a. Within 5 minutes
    b. 6 – 30 minutes
    c. 31 – 60 minutes
    d. After 60 minutes

APPENDIX 7 – MPSS (MOOD AND PHYSICAL SYMPTOMS SCALE)

How strong is your urge to vape <u>right now?</u> *(Circle one number)*

Not at all strong			Moderately strong			Extremely strong
1	2	3	4	5	6	7

Please indicate how you feel <u>right now</u> by placing a tick in the appropriate box for each of the descriptions below

	Not at all	Slightly	Somewhat	Very	Extremely
Depressed					
Irritable					
Anxious					
Drowsy					
Restless					
Hungry					
Unable to concentrate					

APPENDIX 8 – SUBJECTIVE EFFECTS (POSITIVE AND ADVERSE)

Subjective Effects:

Please indicate, by marking the line, the extent to which you agree with each of the following statements *based on how you feel right now*

I feel a definite hit from the e-cigarette

Not at all ***Extremely***

The e-cigarette is satisfying

Not at all ***Extremely***

The e-cigarette is pleasant

Not at all ***Extremely***

The e-cigarette tastes good

Not at all ***Extremely***

The e-cigarette has reduced my craving for nicotine

Not at all ***Extremely***

The e-cigarette has helped my concentration

Not at all ***Extremely***

The e-cigarette has made me feel calmer

Not at all ***Extremely***

The e-cigarette has made me feel more awake

Not at all ***Extremely***

The e-cigarette has reduced my hunger

Not at all ***Extremely***

The e-cigarette tastes like my usual brand/model

Not at all ***Extremely***

The e-cigarette feels like using my usual brand/model

Not at all ***Extremely***

Subjective Effects

Please indicate, by marking the line, the extent to which you feel each of the following based on how you feel *right now*

Confused

Not at all ***Extremely***

Dizzy

Not at all ***Extremely***

Headache

Not at all ***Extremely***

Pounding Heart

Not at all ***Extremely***

Light-headed

Not at all ***Extremely***

Nausea/Feeling sick

Not at all ***Extremely***

Nervous

Not at all ***Extremely***

Salivation

Not at all ***Extremely***

Sweaty

Not at all ***Extremely***

Weak

Not at all ***Extremely***

Mouth irritation

Not at all ***Extremely***

Throat irritation

Not at all ***Extremely***

Aching jaws

Not at all ***Extremely***

Vomiting

Not at all ***Extremely***

Flatulence/Bloating

Not at all ***Extremely***

Stomach ache

Not at all ***Extremely***

Heartburn

Not at all ***Extremely***

Diarrhoea

Not at all ***Extremely***

Hiccups

Not at all ***Extremely***

Cold hands/feet

Not at all ***Extremely***

Palpitations

Not at all ***Extremely***

APPENDIX 9 – ETHICS APPLICATION AND APPROVAL FOR STUDY 1 (INCLUDE AMENDMENTS AND RISK ASSESSMENT)

APPLICATION FOR ETHICAL REVIEW OF RESEARCH INVOLVING HUMAN PARTICIPANTS, HUMAN DATA OR HUMAN MATERIAL

This application should be completed by **members of staff and postgraduate research degree students (i.e. MRes, MPhil, PhD and Professional Doctorate)** undertaking research which involves human participants, sensitive human data (personal or otherwise) and human material (including human tissue, embryos, foetuses and bodily fluids, from living or deceased participants).

No form of contact with potential participants for the proposed research should occur until written approval has been received from University Research Ethics Committee (UREC). Where a member of staff or student is found to have breached this expectation, they may be subject to disciplinary action.

This application should be submitted alongside copies of any supporting documentation which will be handed to participants, including a participant information sheet, consent form, self-completion survey or questionnaire.

For further guidance please contact researchethics@uel.ac.uk or refer to the guidance at http://www.uel.ac.uk/qa/research/index.htm. Only those applications received by the submission deadline date shown on the University's Research Ethics web page will be considered at the next meeting. Where a form is submitted and sections are incomplete, the form will not be considered by UREC and will be returned to the applicant for completion.

PROJECT DETAILS

Current project title	Puffing Patterns in Electronic Cigarettes: Self-titration of Nicotine		
Is this project externally funded?	**YES** ☐ **NO** ☒ **NOT APPLICABLE** ☐		
Does the project require UREC approval before consideration by the funding body?	**YES** ☐ **NO** ☐ **NOT APPLICABLE** ☒ If **YES**, please indicate funding body deadline below:		
If externally funded, please provide details of funding body.	N/A		
How will participants be informed of the source of funding? N/A **PARTICIPANT INFORMATION SHEET** ☐ **CONSENT FORM** ☐ **OTHER** ☐ If **OTHER**, please specify further below:			
Proposed project start date	22/03/15	**Anticipated project end date**	December 2015

APPLICANT DETAILS

Name of Principal Investigator (PI) (For research degree students, the Director of Studies)	Dr Lynne Dawkins (DoS, School of Psychology) on behalf of Catherine Kimber (PhD student)
School	Psychology
Status (please tick relevant box)	**UEL STAFF ☒ RESEARCH DEGREE STUDENT ☐**
Email address	L.e.dawkins@uel.ac.uk
Contact telephone number	0208 223 2241
Name of co-researchers	Catherine Kimber (PhD student) Prof. Olivia Corcoran (Second supervisor from HSB school)
Will parts of the proposed research or research administration be carried out by independent contractors or partner institutions, domestic or international?	**YES ☐ NO ☒** If **YES**, please provide a brief explanation of who the contractor or partner is, what their role will be and how their contribution will be monitored. Please note, responsibility for proper conduct of all parties involved in the research resides with the Principal Investigator.

CONFLICTS OF INTEREST

Will any of the researchers or their institutions receive any other benefits or incentives for taking part in this research over and above their normal salary package or the costs of undertaking the research? **YES ☐ NO ☒** If **YES**, please detail below:
Is there any further possibility for conflict of interest? YES ☐ NO ☒ If **YES**, please detail below:

FOR ALL APPLICANTS

Has external ethics approval been sought for this research? **(i.e. submission via Integrated Research Application System (IRAS) to the Health Research Authority (HRA) or other external research ethics committee)**	**YES ☐ NO ☒**
If **YES**, please supply details below:	

DEAN OF SCHOOL OR ASSOCIATE DEAN

- Does the proposed research as detailed herein have your support and endorsement to proceed?

  YES ☒ NO ☐

Signed	
Date	

APPLICANT DECLARATION

I confirm that:

- The information contained in this application is, to the best of my knowledge, correct and up to date.
- I have attempted to identify all risks related to the research.
- I acknowledge my obligations and commitment to upholding our University's Code of Practice for ethical research and observing the rights of the participants.
- I am aware that cases of proven misconduct, in line with our University's policies, may result in formal disciplinary proceedings and/or the cancellation of the proposed research.

Applicant	Lynne Dawkins (for Catherine Kimber)
Signed	L.Dawkins
Date	15/12/14

FOR RESEARCH DEGREE STUDENT APPLICANTS ONLY

Name and School of Director of Studies	Dr Lynne Dawkins, School of Psychology
Qualification for which research is being undertaken	PhD via MPhil

Director of Studies (DoS) –

- Does the student have the necessary skills to carry out the research?

  YES ☒ NO ☐

- Is the participant information sheet, consent form and any other documentation appropriate?

  YES ☒ NO ☐

- Are the procedures for recruitment of participants and obtaining informed consent suitable and sufficient?

  YES ☒ NO ☐

- Where required, do all members of the research team have current Criminal Records Bureau (CRB) clearance?

  YES ☐ NO ☐ N/A

Signed	
Date	

DETAILS OF THE PROPOSED RESEARCH

28. Provide a brief description of the proposed research, including the requirements of participants. This must be in lay terms and free from technical or discipline specific terminology or jargon. If such terms are required, please ensure they are adequately explained (Do not exceed 500 words)

This application is essentially combining two previous studies conducted at UEL by exploring effects of e-cigarette puffing patterns on blood nicotine delivery. A previously published study by Dawkins and Corcoran ('An evaluation of the SKYCIG electronic cigarette; UREC approval gained 7/11/12 and amendment approved 14/1/13 by Merlin Harries) which explored nicotine delivery in e-cigarette users and a pilot study entitled "E-cigarette puffing behaviour with different nicotine strength e-liquids" (ethic approval UREC_1415_02, 8/10/14).

The proposed study aims to explore puffing patterns (puff frequency, duration and interval between puffs) and blood nicotine delivery in experienced electronic cigarette (hereafter referred to as e-cigarettes) users under two conditions: a 'high nicotine strength' and a 'low nicotine strength' condition (i.e.: 24mg/ml nicotine and 6mg/ml nicotine e-liquids). Evidence from tobacco cigarette smokers suggests that when given lower nicotine content cigarettes, smokers tend to alter their puffing patterns by increasing the duration, numbers and intensity of their puffs in order to maintain a constant blood nicotine level. This phenomenon referred to as self-titration has been extensively explored in tobacco smoking. Although findings from studies on nicotine delivery from e-cigarettes seem to indicate that self-titration might occur, this is yet to be properly explored in the laboratory. The eventuality that e-cigarette users take more intensive puffs when switching to a lower nicotine concentration, presents the risk of increasing temperature of the heating element of the device, the atomiser and eventually formation and exposure to toxins and carcinogens.

The study will be carried at the UEL clinical education building at the Stratford campus. In this proposed study, recruited participants will be experienced e-cigarette users with a history of at least 3 months use, currently using the newest re-fillable (second or third generation) e-cigarette devices and nicotine concentration of 24mg/ml or higher will be recruited. Following an initial saliva screening session, participants will attend two sessions a week apart to allow for a sufficient rest period, where they will be given a third generation device with a high nicotine concentration on one day and a lower nicotine concentration on the other (the order of presentation will be counterbalanced). Third generation devices are latest models customisable and more sophisticated than earlier models, they have a rechargeable battery and adjustable power and can be mounted with a refillable tank system. E-liquid flavours will be

selected based on participants' preferences as this worked well in our pilot study. Participants will be asked to refrain from all nicotine use overnight (10-12 hours) prior to each session.

Demographic and vaping/smoking history will be collected using questionnaires. Blood samples will be collected from participants at a maximum of six time-points (baseline, five, ten, twenty, thirty and sixty minutes) and a questionnaire measuring 'urge to vape' and 'withdrawal symptoms' will be administered at each point. Participants will be presented with the e-Vic e-cigarette from 'Joyetech' with a Nautilus Tank System (which collects data on puff frequency; see further details in section 3 below) and asked to use the device for 60 minutes whilst the puffing behaviour is being video-recorded. Data on puff frequency, puff duration and inhalation time will be gathered from video recordings as well as from e-Vic Joyetech software for back up purposes.

29. Provide a statement on the aims and significance of the proposed research, including potential impact to knowledge and understanding in the field (where appropriate, indicate the associated hypothesis which will be tested). This should be a clear justification of the proposed research, why it should proceed and a statement on any anticipated benefits to the community. (Do not exceed 700 words)

Smoking is the most preventable cause of premature mortality. Each year, in the UK alone, approximately 100 000 of premature death is attributed to smoking. Although, the ultimate goal remains that smokers should cease any use of tobacco and nicotine products totally and permanently, there is a consensus which support regulated harm reduction approaches to help smokers unable or unwilling to quit. While some studies indicate that nicotine replacement therapies (hereafter referred to as NRTs) help in achieving abstinence (Ucar et al., 2014), fewer than 20% of users remain abstinent after 12 months (Stead et al., 2012). It could be because NRTs fall short of providing the psycho-behavioural cues associated with the rituals of smoking or because of the slow nicotine delivery pace.

E-cigarettes (also known as electronic nicotine delivery systems or personal vaporisers) are battery-operated devices which deliver nicotine via a mist-produced mechanism. Given there is no tar and combustion they provide a safer alternative to traditional smoking. Since their introduction, e-cigarettes are becoming increasingly popular, with the latest figures suggesting 2.1 million users in the UK. Because they closely mimic the appearance and use of traditional tobacco smoking, e-cigarettes hold the promise to significantly reduce the prevalence of smoking compared to NRTs. Studies show they can be more effective than NRTs (Brown, Beard, Kotz, Michie, & West, 2014) and effective at helping smokers to achieve abstinence and quit tobacco smoking (Etter and Bullen, 2014).

E-cigarettes are currently regulated as consumer products and there is no mandatory quality standard control in place to ensure a standard of quality across manufacturers. That said, in May 2016, once the European Commission Tobacco Product Directive comes into effect, all e-cigarette products which contain a nicotine concentration of 20 mg/ml or above will require authorisation by the Medicines and Healthcare Products and Regulatory Agency (MHRA) thus be classified as medicinal products. The implications are that for users who requires nicotine concentration of 20mg/ml or above, access and availability will be severely restricted, thus these users will be forced to switching to lower nicotine concentration. If e-cigarettes are to be successful in reducing tobacco cigarette consumption, it is crucial that smokers are given the nicotine concentration that they need. Indeed, evidence in the literature found that some users had to increase their nicotine concentration in order to sustain tobacco smoking abstinence (Farsalinos, Romagna, Tsiapras, Kyrzopoulos, & Voudris, 2013a).

Moreover, empirical evidence from tobacco studies show that smokers will compensate to achieve a desired (habitual) blood nicotine level, when given lighter cigarettes (lower nicotine concentration) by increasing the puff frequency, taking longer and more intensive puffs (Strasser et al., 2006), thereby increasing their exposure to toxicants and carcinogens and risk of cancer. Although, toxic substances and carcinogens reported in e-cigarettes vapour are at only at trace levels (Hajek et al., 2014), a harsher puffing pattern can lead to an increase to over-heating the atomiser and results in greater amount of e-liquid mass per unit of air volume and

lead to an alteration of particle sizes (Tucker & Jordan, 2013); thereby increasing the risk of production and exposure to toxins and carcinogens. Self-titration has been extensively investigated in tobacco smokers, however has not been explored empirically in e-cigarette use, despite findings on pharmacokinetics effects of nicotine via e-cigarettes indicating that users might self-titrate. Besides, data gathered from our recent pilot study (previously approved by UREC; reference 1415_02, 8th October 2014) conducted in five e-cigarette users, indicates that users do change their puffing patterns when given a lower nicotine concentration e-liquid. However, this pilot study did not measure blood nicotine levels, thus it is unknown whether altered puffing patterns were effective for increasing blood nicotine delivery in the lower condition.

This study aims are to explore puffing topography in e-cigarette users and evaluate whether users self-titrate (compensate) to maintain their plasma nicotine levels when given an acute administration of a lower nicotine concentration than their habitual strength by increasing their puff number and duration. Additional aims are to explore whether altered puffing patterns lead to increases in blood nicotine levels.

Findings of this study will be informative to regulatory bodies and those making decisions vis-à-vis nicotine concentration of e-liquids but also to smokers, e-cigarette users and smoking cessation service advisors.

30. Provide an outline of the methodology for the proposed research, including proposed method of data collection, tasks assigned to participants of the research and the proposed method and duration of data analysis. If the proposed research makes use of pre-established and generally accepted techniques, please make this clear. (Do not exceed 500 words)

Design:

Double-blind within-participants design with two conditions: 'high strength' and 'low strength' e-liquid.

The order of presentation will be counterbalanced.

Equipment:

- For blood collection:
- Pre-filled pharmaceutical grade saline syringes for flushing
- 22 gauge needles and peripheral IV cannula with injection port
- BDQ Stand Alone 385100
- BD Veca-C IV dressings
- Lithium Heparin vacutainers
- Personal protective equipment (surgical gloves, lab coat, laboratory safety spectacles)
- (All purchased from AB Medicals; http://www.bd.com/uk/)

To render blood samples acellular for storage:

- Centrifuge
- Gas chromatography – mass spectrometer (Agilent technologies, 5975a series, GC/MSD with triple-axis detector; Agilent, Atlanta, GA) in UH006/7 chemistry laboratory UEL.
- Freezer designated for storage of biological samples (Norhof biological storage freezer, model DPF30, Norhof, Holland) in UH006/7 chemistry laboratory UEL.

For the vaping session:

- Carbon Monoxide (CO) Breatherlyser (Bedfont Smokerlyser) to measure exhaled CO levels.
- 'Salivette' to measure saliva cotinine concentration at the screening phase (provided by ABS labs)

- eVic™ e-cigarette from 'Joyetech' (as used in our pilot study; UREC_1415_02, 8th October 2014) has a downloadable software which collects data such as puff duration and frequency. See: http://www.joyetech.co.uk/electronic-cigarettes/eVic™-supreme#.U9uFRKNeLpd
- Nautilus Tank System (as used in our pilot study; UREC_1415_02, 8th October 2014) which has a large capacity of 5ml of fluid which is beneficial for research purposes we will not have to re-fill it during the 45 minute ad lib vaping period. It also comes with a measuring gauge and airflow control system which makes it ideal for our purposes. See: http://www.ukecigstore.com/nautilus-tank-system-by-aspire.html
- Nicotine e-liquid: a 6mg/ml and 24mg/ml nicotine concentration. Sampling will be based on the most popular brands available in the market. This information will be accessed via market research databases (eg. MINTEL). To avoid biases, the manufacturer or provider will be chosen using a blinding process, where several of the most popular providers will be selected in the first instance by one researcher, then one provider will be selected at random by a colleague researcher. The research team intends to seek advice with regard to manufacturer liability through the UEL legal advisor. In addition, prior to the current study and as part of a PhD research project, a study will be conducted to confirm that the nicotine concentration present in each e-liquid bottles is accurate. These concentrations have been selected in the context of the proposed regulations in the European Tobacco Product Directive (hereafter referred to as TPD) which will come into effect in May 2016 stipulating that all e-cigarette products containing nicotine e-liquid above 20mg/mL will require to undergo an application process through the Medicines and Healthcare Products Regulatory Agency (MHRA) (Action on Smoking and Health, 2014) and be classified as medicines.
- Video recorder
- Precision scales for weighing tank before and after use.

Questionnaires / Measures:

The questionnaires used will be the same as in our pilot study and will comprise:

- Baseline demographics questionnaire (Appendix C)
- The Fagerström Test of Nicotine Dependence (Fagerström, 2012) will be modified to measure nicotine dependence, vaping history and e-cigarette use (Appendix D). Examples of items include "*How soon after you wake up, you use your e-cigarette?*". The available option '*within 5 minutes*' corresponds to the highest score, whilst the option '*after 60 minutes'* scores the lowest. High scores signify high dependence.
- A modified version of the multidimensional Mood and Physical Symptoms Scale (MPSS) questionnaire (West & Hajek, 2004) to measure 'Urge to vape' and withdrawal symptoms (Appendix E). The former is measured on a seven-point rating scale (scores range from 1 to 7, "*Not at all strong*" to "*Extremely strong*"); the latter is divided as follow 'depressed mood', 'irritability', 'anxiety', 'restlessness', 'hunger' and 'inability to concentrate', each measured using a five-point rating scale (scores range from 1 to 5 "*Not at all*" to "*Extremely*").
- Subjective effects to nicotine: 'Positive' effects will be measured using an 11-item visual analogue scale derived from Blank et al., (2008) (Appendix F). Participants are required to rate each statement by drawing a cross or a small line adjacent to the 20cm-line with the far left representing 'not at all' and the opposite right end 'extremely'. Examples include 'I feel a definite hit from the e-cigarette', 'The e-cigarette is satisfying?', 'The e-cigarette is pleasant'. Scoring is obtained by measuring from the far left corner to the drawn cross or line in millimetres then this is halved to translate the score in percentage. A higher score is indicative of more positive effect.
- Subjective effects: Adverse effects will be measured with a 21-item visual analogue scale. This has been used in previous work (Vansickel & Eissenberg, 2013) (Appendix G). Examples of items include 'confused', 'dizzy', 'nausea', 'headache', 'salivation', 'sweaty'. Participants are required to place a cross through a 20cm-line.

<u>Procedure:</u>

Participants will be screened via phone interviews to ensure all inclusion criteria are met and an information sheet will be provided via emails or post. Thereafter, pre-screening analysis will be conducted by sending via post a 'Salivette' kit measuring salivary cotinine in order to ensure that participants are daily users and accustomed to the nicotine concentration specified in the inclusion criteria (cut-off point for cotinine ≤ 100mg/mL). Participants will be informed that cotinine analysis will only be used for the purposes of screening for this study then destroyed.

If participants are eligible to take part, they will be asked to abstain from any tobacco and nicotine products including e-cigarette use for a period of 12 hours prior to the testing sessions. Smoking abstinence will be verified by measuring carbon monoxide in exhaled breath levels and nicotine abstinence from a baseline blood sample.

Upon arrival, the researcher will go through the information sheet with the participants and check that they understand all aspects of the procedure. Participants will be asked to provide written informed consent (Appendices A and B) where they will be invited to ask further questions. The researcher will ensure that they fully understand the nature and purpose of their participation. Participants will be invited to have a break and reminded of their right to withdraw at the beginning and during sessions.

Baseline questionnaires (Appendix C) will be administered before a qualified phlebotomist nurse inserts a venous cannula into the participant's forearm and collects a baseline blood sample. The cannula will be secured and remain in place for the duration of the study (maximum 1.5 hr) as in our previous pharmacokinetic study of nicotine delivery via e-cigarettes (UREC approval gained on 7/11/12 and amendment on 14/1/13 from Merlin Harries. Participants' blood samples will be taken (by the phlebotomy nurse only) and MPSS questionnaires (Appendix D) completed at baseline, at five, ten, twenty, thirty and sixty minutes. Similar protocols have been successfully used in previous studies (Dawkins & Corcoran, 2014; Farsalinos et al., 2014; Vansickel & Eissenberg, 2013). After baseline questionnaires have been completed and a blood sample has been taken, participants will be

presented with the e-Vic and ask to use it *ad libitum* for an hour (for hygiene purposes, a new dismountable drip-tip mouth-piece will be attached to the device for each participant). During this time, the whole session will be video-recorded to measure puffing patterns. The venous cannula will be removed following the last blood sample collection, before completion of the final subjective effects (positive and adverse) questionnaires. Finally, participant will be thanked, offered refreshments and reimbursed for their time.

The next session will take place a week later using the other nicotine concentration.

Blood Nicotine Analysis:

Blood will be stored on ice then centrifuged within 4 hours of collection to render samples acellular before storage in the HSB forensic lab at minus 20 Degrees centigrade, awaiting transportation at an offsite laboratory, the Advanced Bioanalytical Service (ABS) Laboratories Ltd., Welwyn Garden City, UK. Blood samples will be transported in one full batch in designated and appropriate containers via taxi. All blood nicotine analysis will be conducted by ABS labs and disposed of in accordance with the MHRA, GLP (Good Laboratory Practise) and GCP (Good Clinical Practise) accreditation protocols. A certificate of destruction will be issued and provided by ABS laboratories Ltd.

Data protection:

All data will be coded and treated confidentially and will be retained in accordance with the University's Data Protection Policy, stored for a limited period of time on password protected hard drives. Hard copies of questionnaires will be stored in a locked filing cabinet in a locked room then destroyed in accordance with the university's data protection policies. Signed consent forms will be detached and stored separately from questionnaire and video data, accessible to only the principal investigator and the researchers. To facilitate any withdrawal, each participant will be given a participant number which they will need to memorise in order to later request that their data is withdrawn.

Video recordings will be analysed (by 2 members of the team) and then entered into a computerised dataset before being disposed of securely.

PARTICIPANT DETAILS

31. Provide an explanation detailing how you will identify, approach and recruit the participants for the proposed research, including clarification on sample size and location. Please provide justification for the exclusion/inclusion criteria for this study (i.e. who will be allowed to / not allowed to participate) and explain briefly, in lay terms, why this criteria is in place. (Do not exceed 500 words)

Participants:

All testing sessions will take place in a specialist laboratory, the UEL clinical education building at the Stratford campus with secure access to develop the study.

Fifteen participants will be recruited. A similar study by Dawkins and Corcoran (2014) demonstrated that this sample size is sufficient to generate enough statistical power and statistically significant results. Moreover, such sample size would allow for some degree of drop out (failure to attend second testing session). All will be e-cigarette users (ex-smokers) with a history of 3 months use minimum. Empirical evidence shows that a three-month practise is sufficient for users to develop effective puffing techniques and achieve tobacco abstinence (Hajek et al., 2014). Given that Dawkins & Corcoran (2014) previously found it more challenging to collect blood samples from females, all participants will be male as to reduce the risk of unnecessary physical discomfort.

Recruitment will be initiated via advertisements on social media (Twitter and Facebook), diverse e-cigarette forums, leaflet distribution on and outside UEL, posters display at UEL's permitted points and at e-cigarette retail outlets and vaping cafes. Thus, participants may include UEL staff and students as well as members of the general public. However, students will be made aware that their learning, assessments or experience at UEL will not be impacted by their participation,

withdrawal or non-participation. This will be clearly outlined in the information sheet (see appendices).

The inclusion criteria are as follow: 1) must be male, 2) must no longer be a smoker, 3) regular / daily e-cigarette users, 4) currently using a second or third generation e-cigarette 5) being accustomed to (using or having used in recent past) 24 mg/mL nicotine concentration, 6) aged 18 years or above, 7) fluent in English, 8) be willing to abstain from any nicotine including e-cigarette use for a period of at least 12 hours before testing session. Exclusion criteria are that participants must not be females, current smokers, non-daily e-cigarette users, under the age of 18, have neurobiological or heart conditions or previous history of fainting during blood collection procedures and have levels of cotinine under 100mg/mL during pre-screening phase.

32. Will the participants be from any of the following groups?*(Tick as appropriate)*

☒ Students or staff of this University (i.e. recruitment on-site at University of East London).
☒ Adults (over the age of 18 years with mental capacity to give consent to participate in the research).
☐ Young people between 16 and 18 years (please note parental consent may still be required for s006Fme research).
☐ Children or legal minors (anyone under the age of 16 years)[1]
☐ Adults who are unconscious, severely ill or have a terminal illness.
☐ Adults who may lose mental capacity to consent during the course of the research.
☐ Adults in emergency situations.
☐ Adults[2] with mental illness - particularly those detained under the Mental Health Act (1983 & 2007).
☐ Participants who may lack capacity to consent to participate in the research under the research requirements of the Mental Capacity Act (2005).
☐ Prisoners, where ethical approval may be required from the National Offender Management Service (NOMS).
☐ Young Offenders, where ethical approval may be required from the National Offender Management Service (NOMS).
☐ Healthy volunteers (in high risk intervention studies).
☐ Participants who may be considered to have a pre-existing and potentially dependent[3] relationship with the investigator (e.g. those in care homes, students, colleagues, service-users, patients).
☐ Other vulnerable groups (see Question 6).
☐ Adults who are in custody, custodial care, or for whom a court has assumed responsibility.
☐ Participants who are members of the Armed Forces.

[1]If the proposed research involves children or adults who meet the Police Act (1997) definition of vulnerability[3], any researchers who will have contact with participants must have current enhanced Disclosure and Barring Service check (was previously called Criminal Records Bureau, or CRB, clearance). [2] 'Adults with a learning or physical disability, a physical or mental illness, or a reduction in physical or mental capacity, and living in a care home or home for people with learning difficulties or receiving care in their own home, or receiving hospital or social care services.' (Police Act, 1997)

[3] *Proposed research involving participants with whom the investigator or researcher(s) shares a dependent or unequal relationships (e.g. teacher/student, clinical therapist/service-user) may compromise the ability to give informed consent which is free from any form of pressure (real or implied) arising from this relationship. UREC recommends that, wherever practicable, investigators choose participants with whom they have no dependent relationship. Following due scrutiny, if the investigator is confident that the research involving participants in dependent relationships is vital and defensible, UREC will require additional information setting out the case and detailing how risks inherent in the dependent relationship will be managed. UREC will also need to be reassured that refusal to participate will not result in any discrimination or penalty.*

33. Will the study involve participants who are vulnerable? YES ☐ NO ☒

For the purposes of research, 'vulnerable' participants may be adults whose ability to protect their own interests are impaired or reduced in comparison to that of the broader population. Vulnerability may arise from the participant's personal characteristics (e.g. mental or physical impairment) or from their social environment, context and/or disadvantage (e.g. socio-economic mobility, educational attainment, resources, substance dependence, displacement or homelessness). Where prospective participants are at high risk of consenting under duress, or as a result of manipulation or coercion, they must also be considered as vulnerable.

Adults lacking mental capacity to consent to participate in research and children are automatically presumed to be vulnerable. Studies involving adults (over the age of 16) who lack mental capacity to consent in research must be submitted to a REC approved for that purpose.

6.1. **If YES,** a Disclosure and Barring Service (DBS) check within the last three years is required.

Please provide details of the "clear disclosure":

Date of disclosure:
Type of disclosure:
Organisation that requested disclosure:
DBS certificate number:

(NOTE: information concerning activities which require DBS checks can be found via https://www.gov.uk/government/publications/dbs-check-eligible-positions-guidance)

6.2.If YES, what special arrangements are in place to protect vulnerable participants' interests?

34. Do you propose to make any form of payment or incentive available to participants of the research? YES ☒ NO ☐

If **YES**, please provide details taking into account that any payment or incentive should be representative of reasonable remuneration for participation and may not be of a value that could be coercive or exerting undue influence on potential participants' decision to take part in the research. Wherever possible, remuneration in a monetary form should be avoided and substituted with vouchers, coupons or equivalent. Any payment made to research participants may have benefit or HMRC implications and participants should be alerted to this in the participant information sheet as they may wish to choose to decline payment.

A remuneration of £50 will be offered to participants at the end of their second testing session: firstly, in compensation for their time (up to 4 hours in total), secondly to cover travel expenses and thirdly because the study involves an invasive procedure involving collection of blood samples. The remuneration will be offered at the end of the second testing session.

The remuneration will be clearly outlined in the information sheet. However, to avoid coercion, this will not be advertised and will only be disclosed once potential participants have expressed their interest in taking part and have asked for further information.

35. What special arrangements are in place for eliciting informed consent from participants who may not adequately understand verbal explanations or written information provided in English; where participants have special communication needs; where participants have limited literacy; or where children are involved in the research? (Do not exceed 200 words)

Part of the inclusion criteria are to be fluent in English. Nonetheless, the researcher will ensure that participants are fully aware and understand what the study involves and what their participation entails. Each participant will be invited to ask questions before giving informed consent. Participants will be provided with a copy of the information sheet via e-mail or post, to allow time for participants to understand and absorb the content prior to testing sessions.
There will be no children or under 18s involved in the research.

RISK ASSESSMENT AND RISK MANAGEMENT

36. Does the proposed research involve any of the following? *(Tick as appropriate)*

☒ use of a questionnaire, self-completion survey or data-collection instrument (attach copy)
☐ use of emails or the internet as a means of data collection
☐ use of written or computerised tests
☐ interviews (attach interview questions)
☐ diaries (attach diary record form)
☒ participant observation
☐ participant observation (in a non-public place) without their knowledge / covert research
☐ audio-recording interviewees or events
☒ video-recording interviewees or events
☐ access to personal and/or sensitive data (i.e. student, patient, client or service-user data) without the participant's informed consent for use of these data for research purposes
☒ administration of any questions, tasks, investigations, procedures or stimuli which may be experienced by participants as physically or mentally painful, stressful or unpleasant during or after the research process **(initial insertion of the catherter might induce slight discomfort, however, note this will be done by a qualified and experienced phlebotomist nurse)**
☐ performance of any acts which might diminish the self-esteem of participants or cause them to experience discomfiture, regret or any other adverse emotional or psychological reaction
☐ investigation of participants involved in illegal or illicit activities (e.g. use of illegal drugs)
☐ procedures that involve the deception of participants
☒ administration of any substance or agent **(yes, but NOTE: all participants will control their own level of nicotine intake and will be regular nicotine products users who are therefore exposed to nicotine on a daily basis so are fully aware and tolerant of any potential adverse effects).**
☐ use of non-treatment of placebo control conditions
☒ collection of body tissues or fluid samples
☐ collection and/or testing of DNA samples
☐ collection and/or testing of gametes or embryo tissue
☐ participation in a clinical trial
☐ administration of ionising radiation to participants
☐ research undertaken at an off-campus location (risk assessment attached)
☐ research overseas (copy of VCG overseas travel approval attached)

37. Does the proposed research involve any specific or anticipated risks (e.g. physical, psychological, social, legal or economic) to participants that are greater than those encountered in everyday life? YES ☒ NO ☐
If **YES**, please describe below including details of precautionary measures.

Potential risks and hazards:

- The current study involves blood samples collection, so unavoidably participants will be exposed to invasive venal puncture equipment under controlled conditions.
- Participants might feel slight discomfort during the insertion of the needle and cannula.
- There is a minimal risk of infection and cross-infection associated with the use of needles in blood sampling.

- There is a minimal risk of syncope (fainting) during blood sampling

Precautions which will be taken:

- All researchers handling blood samples will be fully trained and vaccinated against Hepatitis B.
- An insured, qualified and experienced phlebotomist nurse will be employed to collect all blood samples. Only she will be carrying out blood samples collection.
- Blood sampling will be carried in a sterile and safe environment.
- Proper procedures will be adhered to, to minimise risks associated with hazards of handling certain types of equipment and samples. All researchers will be issued with and instructed on proper usage of personal protective equipment including lab coat, surgical gloves and laboratory safety spectacles. For transfer of the biological samples a surgical mask will be worn where appropriate as in direct exposure to the blood samples.
- Prior to sessions (approximately two weeks), a full walk through of the laboratory with all equipment in place will be carried out with the technical lab manager.
- In the information letter and initial screening stage, participants will be advised that if they have a history of fainting or feeling faint when providing blood samples, they should not take part.
- To reduce the likelihood of fainting occurring, all participants will be recommended to have a substantial breakfast before the commencement of the study.
- Participants will be pre-screened (via cotinine analysis in a saliva sample) to ensure they are daily users and accustomed to the nicotine concentration specified in the inclusion criteria.

Given that vapers are characterised by their dependency on nicotine, participants may experience slight discomfort induced by the required 12 hours or overnight abstinence. To minimise discomfort, testing sessions will be held in the morning.

As with any nicotine product, there is a small risk of side effects associated with e-cigarette use. This includes nausea, headache, dizziness, and mouth/throat irritation. Refreshment and a glass of water will be offered to participants upon arrival and throughout the entire duration of the experiment. However, given that all participants will be regular users and be given nicotine concentrations that are below or equal to their habitual ones, adverse effects are unlikely to occur. Note, as part of the study's procedures, participants will be asked to use the device ad libitum (i.e. as much as they desire), thus potential risks associated with adverse effects are not greater than those encountered in their everyday lives. In the event that adverse effects do occur, it is likely that they will be short-lived. In the unlikely event that ill-effects are reported, participants will be strongly recommended to stop using the device, the cannula will be removed immediately, and they can rest in a calm and quiet location with the researcher and nurse by his side should they wish to do so. If required, a taxi home will be offered when the participant has been assessed to be well enough to leave the premises.

38. Where the procedures involve potential hazards and/or discomfort or distress for participants, please state what previous experience the investigator or researcher(s) have had in conducting this type of research.

As stated above, in the event of hazards and/or discomfort, participants will be advised to stop using the device and be reminded that they are not obliged to continue with the study.

Professor Olivia Corcoran is experienced in biological fluids handling and plasma extraction. She has previously worked in partnership with Dr Lynne Dawkins on a similar project, the first study on the pharmacokinetics effects of nicotine via e-cigarettes to be published in Europe. Participants were required to use an e-cigarette whilst providing blood samples (Dawkins & Corcoran, 2014).

An insured, qualified phlebotomist nurse with extensive experience taking blood samples will be employed to collect all blood samples.

Dr Lynne Dawkins, the principle investigator and Director of Studies has over seventeen years of experience of working with smokers in a research capacity and six years of experience of working with electronic cigarettes. She has published extensively in various peer-reviewed journals work on smoking addiction and electronic cigarettes, and is regarded as one of the UK's leading authorities on e-cigarettes. Dr Lynne Dawkins has been awarded the Good Clinical Practise certificate.

The PhD student and researcher Catherine Kimber has recently completed and passed the UEL Ethic and Research Integrity course. She has conducted her undergraduate project on e-cigarette use and choice in smokers with Dr Dawkins, which has been recently published in a peer-reviewed journal article (Dawkins, Kimber, Puwanesarasa & Soar, in press). In addition, she has undertaken a research internship at UEL with Dr Lynne Dawkins, conducting a project on the effects of nicotine on smokers' cognitive functioning, thus involving smokers recruitment. More recently, she has conducted a pilot study which received full ethics approval by UREC, involving the recruitment of e-cigarette users. Thus, she currently has more than a year experience of working with smokers, vapers and e-cigarettes.

39. Provide an explanation of any potential benefits to participants. Please ensure this is framed within the overall contribution of the proposed research to knowledge or practice. (Do not exceed 400 words)
NOTE: Where the proposed research involves students of our University, they should be assured that accepting the offer to participate or choosing to decline will have no impact on their assessments or learning experience. Similarly, it should be made clear to participants who are patients, service-users and/or receiving any form of treatment or medication that they are not invited to participate in the belief that participation in the research will result in some relief or improvement in their condition.

Through the information sheet, participants will be made aware that there are no direct benefits, £50 will be given as compensation for their time, to cover their travel expenses and for the invasive procedure of blood sampling.

Participants will be informed that findings of the study will be informative and beneficial to the wider clinical, academic and regulatory community. If findings

indicate that e-cigarette users self-titrate by taking longer, harder and more puffs when given a lower nicotine concentration, this will enable future studies to replicate these conditions and mimic these altered puffing behaviours to assess if toxicants and carcinogens exposure is increased. Likewise, if findings indicate that e-cigarette users do not seem to self-titrate, this will be as equally informative for regulatory bodies and future regulations.
This study will add to the knowledge surrounding e-cigarette puffing behaviours and the pharmacokinetic effects of different nicotine concentration.

Participants will be informed that whilst their involvement in the study will not benefit them directly, the findings will help to add to the knowledge base on e-cigarettes. It will be clearly stated on the information sheet that where participant is a student, participation, non-participation and withdrawal will not impact their learning experience or assessments at UEL. It will be clearly stated that participants will be not obliged to take part. If they decide to do so, they may wish and have the right to withdraw at any time.
Although some of the participants taking part will be known to the researcher, most of these are external to UEL and none are in a dependent relationship with the researcher.

40. Provide an outline of any measures you have in place in the event of adverse or unexpected outcomes and the potential impact this may have on participants involved in the proposed research. (Do not exceed 300 words)

During blood sampling there is a minimal risk that participant might feel faint or faint. Note, all participants will be screened for previous history of feeling faint or fainting during blood samples collection. Participants will be asked to eat a substantial breakfast on the morning prior to each testing session. A telephone emergency services will be accessible and a nominated and trained first aider will be informed of the timings of our scheduled sessions and will be on call throughout the duration of the experiment to intervene if need be. Note also that the nurse will be present throughout the entire session.

Participants might feel a slight discomfort or pain during the insertion of the needle or cannula. All blood samples collection will be carried out by a qualified phlebotomist nurse who has experience and expertise in handling risks and hazards associated with blood sampling.
It is possible that blood samples could be spilt or dropped. All care will be taken to avoid this by ensuring that all the correct equipment is in place and is easily accessible. Only the qualified phlebotomy nurse and those with full Hepatitis B vaccination will handle blood samples.

If ill-effects are reported as a result of using the device, participants will be strongly recommended to stop and have a rest in a calm and quiet location with the researcher and nurse by his side should they wish to do so, and a taxi home will be offered when the participant has been assessed to be well enough to leave the premises.

Participants will be regular e-cigarette users with experience in using this type of tank system e-cigarettes, however, at first they might feel unfamiliarity with the device, sufficient time has been factored in to allow for familiarization. Also precautions will be taken to prepare and check the device is in working order prior to sessions. In the unforeseen event of malfunction, an alternative device will be available.

41. Provide an outline of your debriefing, support and feedback protocol for participants involved in the proposed research. This should include, for example, where participants may feel the need to discuss thoughts or feelings brought about following their participation in the research. This may involve referral to an external support or counseling service, where participation in the research has caused specific issues for participants. Where medical aftercare may be necessary, this should include details of the treatment available to participants. Debriefing may involve the disclosure of further information on the aims of the research, the participant's performance and/or the results of the research. (Do not exceed 500 words)

At the end of the study, participants will be thanked and fully debriefed verbally vis-à-vis the rationale, aims, hypotheses and the expected impacts of this research in the wider academic, clinical and regulatory communities. Participants will be invited to ask questions about their participation and other questions they may have regarding e-cigarette use. Participants will be verbally informed of the aims of the study – i.e. to look at any changes in puffing patterns and blood nicotine levels that may occur when users switch to a lower strength e-liquid. Participants will also be given the principle investigator's e-mail address and telephone number should they have any further queries at a later date.

PARTICIPANT CONSENT AND WITHDRAWAL

42. Have you attached a copy of your participant information sheet (this should be in *plain English*)? Where the research involves non-English speaking participants, please include translated materials. YES☒ NO ☐
If **NO**, please indicate what alternative arrangements are in place below:

43. Have you attached a copy of your participant consent form (this should be in *plain English*)? Where the research involves non-English speaking participants, please include translated materials.
YES ☒ NO ☐
If **NO**, please indicate what alternative arrangements are in place below:

44. The following is a participant information sheet checklist covering the various points that should be included in this document.

☒ Clear identification of UEL as the sponsor for the research, the schools(s) involved, the project title, the Principal Investigator and other researchers along with relevant contact details.

☒Details of what involvement in the proposed research will require (e.g., participation in interviews, completion of questionnaire, audio/video-recording of events), estimated time commitment and any risks involved.

☒A statement confirming that the research has received formal approval from UREC.

☒ If the sample size is small, advice to participants that this may have implications for confidentiality / anonymity.

N/A A clear statement that where participants are in a dependent relationship with any of the researchers that participation in the research will have no impact on assessment / treatment / service-use or support.

☒ Assurance that involvement in the project is voluntary and that participants are free to withdraw consent at any time, and to withdraw any unprocessed data previously supplied.

☒Advice as to arrangements to be made to protect confidentiality of data, including that confidentiality of information provided is subject to legal limitations.

☒A statement that the data generated in the course of the research will be retained in accordance with the University's Data Protection Policy.

☒Advice that if participants have any concerns about the conduct of the investigator, researcher(s) or any other aspect of this research project, they should contact researchethics@uel.ac.uk.

☒Confirmation on any limitations in confidentiality where disclosure of imminent harm to self and/or others may occur.

N/A. For research involving under 16 s or vulnerable groups, where true, a statement has been included on all information sheets that the investigators have passed appropriate Disclosure and Barring Service checks.

45. The following is a consent form checklist covering the various points that should be included in this document.

☒University of East London letterhead or logo.

☒Title of the project (with research degree projects this need not necessarily be the title of the thesis) and names of investigators.

☒Confirmation that the project is research.

☒Confirmation that involvement in the project is voluntary and that participants are free to withdraw at any time, or to withdraw any unprocessed data previously supplied.

☒Confirmation of particular requirements of participants, including for example whether interviews are to be audio-/video-recorded, whether anonymised quotes will be used in publications advice of legal limitations to data confidentiality.

☒ If the sample size is small, confirmation that this may have implications for anonymity any other relevant information.

☒The proposed method of publication or dissemination of the research findings.

N/A. Details of any external contractors or partner institutions involved in the research.

N/A. Details of any funding bodies or research councils supporting the research.

N/A Confirmation on any limitations in confidentiality where disclosure of imminent harm to self and/or others may occur.

N/A. Separate forms and information sheets requesting parental/guardian consent for research involving under age children have been included / attached.

N/A. Letters requesting consent for the research to be conducted at external sites (e.g. Managers/ owners/ head teachers /etc.) where the research will be carried out have been included / attached.

☒Where research is to be published (it is envisaged that this is true in most cases) it has been made clear in the information sheet and, or form (preferably both) that both consent to take part and to publication is being obtained.

CONFIDENTIALITY AND ANONYMITY

46. Below is a checklist covering key points relating to the confidentiality and anonymity of participants. Please indicate where relevant to the proposed research.

☐ Participants will be completely anonymised and their identity will not be known by the investigator or researcher(s) (i.e. the participants are part of an anonymous randomised sample and return responses with no form of personal identification)?
☐ The responses are anonymised or are an anonymised sample (i.e. a permanent process of coding has been carried out whereby direct and indirect identifiers have been removed from data and replaced by a code, with no record retained of how the code relates to the identifiers).
☒ The samples and data are de-indentified (i.e. direct and indirect identifiers have been removed and replaced by a code. The investigator or researchers are able to link the code to the original identifiers and isolate the participant to whom the sample or data relates).
☐ Participants have the option of being identified in a publication that will arise from the research.
☐ Participants will be pseudo-anonymised in a publication that will arise from the research.
☐ The proposed research will make use of personal sensitive data.
☐ Participants consent to be identified in the study and subsequent dissemination of research findings and/or publication.

47. Participants must be made aware that the confidentiality of the information they provide is subject to legal limitations in data confidentiality (i.e. the data may be subject to a subpoena, a freedom of information request or mandated reporting by some professions). This only applies to named or de-identified data. If your participants are named or de-identified, please confirm that you will specifically state these limitations.

YES ☒ **NO** ☐

If **NO**, please indicate why this is the case below:

NOTE: WHERE THE PROPOSED RESEARCH INVOLVES A SMALL SAMPLE OR FOCUS GROUP, PARTICIPANTS SHOULD BE ADVISED THAT THERE WILL BE DISTINCT LIMITATIONS IN THE LEVEL OF ANONYMITY THEY CAN BE AFFORDED.

DATA ACCESS, SECURITY AND MANAGEMENT

48. Will the Principal Investigator be responsible for the security of all data collected in connection with the proposed research? YES ☒ **NO** ☐
If **NO**, please indicate what alternative arrangements are in place below:

49. In line with the 5th principle of the Data Protection Act (1998), which states that personal data shall not be kept for longer than is necessary for that purpose or those purposes for which it was collected; please state how long data will be retained for.

☒ 1-2 years ☐ 3-5 years ☐ 6-10 years ☐ 10> years

NOTE: Research Councils UK (RCUK) guidance currently states that data should normally be preserved and accessible for 10 years, but for projects of clinical or major social, environmental or heritage importance, for 20 years or longer. (http://www.rcuk.ac.uk/documents/reviews/grc/grcpoldraft.pdf)

50. Below is a checklist which relates to the management, storage and secure destruction of data for the purposes of the proposed research. Please indicate where relevant to your proposed arrangements.

☒Research data, codes and all identifying information to be kept in separate locked filing cabinets.

☒Access to computer files to be available to research team by password only.

☐ Access to computer files to be available to individuals outside the research team by password only (See **23.1**).

☐ Research data will be encrypted and transferred electronically within the European Economic Area (EEA).

☐ Research data will be encrypted and transferred electronically outside of the European Economic Area (EEA). (See **23.2**).

NOTE: Transfer of research data via third party commercial file sharing services, such as Google Docs and YouSendIt are not necessarily secure or permanent. These systems may also be located overseas and not covered by UK law. If the system is located outside the European Economic Area (EEA) or territories deemed to have sufficient standards of data protection, transfer may also breach the Data Protection Act (1998).

☒Use of personal addresses, postcodes, faxes, e-mails or telephone numbers.

☒Use of personal data in the form of audio or video recordings.

☐ Primary data gathered on encrypted mobile devices (i.e. laptops). **NOTE:** This should be transferred to secure UEL servers at the first opportunity.

☒All electronic data will undergo secure disposal.

NOTE: For hard drives and magnetic storage devices (HDD or SSD), deleting files does not permanently erase the data on most systems, but only deletes the reference to the file. Files can be restored when deleted in this way. Research files must be overwritten to ensure they are completely irretrievable. Software is available for the secure erasing of files from hard drives which meet recognised standards to securely scramble sensitive data. Examples of this software are BC Wipe, Wipe File, DeleteOnClick and Eraser for Windows platforms. Mac users can use the standard 'secure empty trash' option; an alternative is Permanent eraser software.

☒All hardcopy data will undergo secure disposal.

NOTE: For shredding research data stored in hardcopy (i.e. paper), adopting DIN 3 ensures files are cut into 2mm strips or confetti like cross-cut particles of 4x40mm. The UK government requires a minimum standard of DIN 4 for its material, which ensures cross cut particles of at least 2x15mm.

50.1. Please provide details of individuals outside the research team who will be given password protected access to encrypted data for the proposed research.

N/A

50.2. Please provide details on the regions and territories where research data will be electronically transferred that are external to the European Economic Area (EEA).

N/A

OVERSEAS TRAVEL FOR RESEARCH

51. Does the proposed research involve travel outside of the UK? YES ☐ NO ☒

51.1. Have you consulted the Foreign and Commonwealth Office website for guidance/travel advice? http://www.fco.gov.uk/en/travel-and-living-abroad/ **YES** ☐ **NO** ☐

51.2. If you are a non-UK national, have you sought travel advice/guidance from the Foreign Office (or equivalent body) of your country? **YES** ☐ **NO** ☐ **NOT APPLICABLE** ☐

51.3. Have you completed the overseas travel approval process and enclosed a copy of the document with this application? **YES** ☐ **NO** ☐

Details on this process are available here http://www.uel.ac.uk/qa/research/fieldwork.htm

51.4. Is the research covered by our University's insurance and indemnity provision?

YES ☐ **NO** ☐

(Please seek confirmation via researchethics@uel.ac.uk)

NOTE: Where research is undertaken at an off-campus location within the UK or overseas, the Risk Assessment policy must be consulted: http://dl-cfs-01.uel.ac.uk/hrservices/documents/hshandbook/risk_assess_policy.pdf. The Dean of School or Director of Service has overall responsibility for risk assessment regarding the health and safety of staff or students conducting research where UEL is the sponsor.

51.5. Please evidence how compliance with all local research ethics and research governance requirements have been assessed for the country(ies) in which the research is taking place.

51.6. Will this research be financially supported by the United States Department of Health and Human Services or any of its divisions, agencies or programs? **YES** ☐ **NO** ☐

PUBLICATION AND DISSEMINATION OF RESEARCH FINDINGS

52. How will the results of the research be reported and disseminated? ***(Select all that apply)***

- ☒ Peer reviewed journal
- ☒ Conference presentation
- ☐ Internal report
- ☒ Dissertation/Thesis
- ☐ Other publication
- ☐ Written feedback to research participants
- ☒ Presentation to participants or relevant community groups
- ☐ Other (Please specify below)

OTHER ETHICAL ISSUES

53. Are there any other ethical issues that have not been addressed which you would wish to bring to the attention of University Research Ethics Committee (UREC)?

No

CHECKLIST FOR ATTACHED DOCUMENTS

54. Please check that the following documents are attached to your application.

☒ Recruitment advertisement
☒ Participant information sheets (including easy-read where relevant)
☒ Consent forms (including easy-read where relevant)
☐ Assent form for children (where relevant)
☐ Evidence of any external approvals needed
☒ Questionnaire
☐ Interview Schedule or topic guide
☒ Risk assessment (where applicable)
☐ Overseas travel approval (where applicable)

54.1. Where it is not possible to attach the above materials, please provide an explanation below.

Recruitment will be via e-mail invitations to e-cigarette users known to us, or twitter/facebook if insufficient numbers are recruited via personal e-mail contacts. In addition, flyers will be distributed at various e-cigarette retail outlets and vape café (e.g. Vape lab).

E-mail example: *Dear X, We are conducting a study exploring e-cigarette users' puffing patterns under two different nicotine concentrations and would like to ask if you would be interested in taking part.*
The study has received full ethical approval by the university's research committee and involves 2 visits to the Strafford campus of UEL a week apart. It is expected that each session will take approximately 2 hours.
You will be asked to provide a total of six blood samples via an intravenous cannula whilst you use an e-cigarette we provide for 45mins – 1 hour. . The entire session will be video-recorded to capture your puffing patterns.
All data generated in the course of the research will be treated confidentially and will be retained in accordance with the University's Data Protection Policy. Your video recordings will be analysed and entered into a computerised dataset. Finally, the video recordings will be disposed of securely. Should you require further details, I will send you the participation information sheet.

I look forward to hearing from you.

The recruitment advertisement materials will be based on the following text:

- Take part in a study exploring e-cigarette users' puffing patterns.
- The study involves 2 visits (2 hours each approx.) to the UEL Strafford campus one week apart.
- You will be asked to provide a total of six blood samples via an intravenous cannula whilst you use a third generation e-cigarette we provide for 45mins – 1 hour. Blood samples collection will be taken by a fully qualified and experienced phlebotomist nurse. The entire session will be video-recorded.
- To take part you must be:
    - 18 or over
    - Male
    - Non- or ex-smoker
    - Be willing to provide screening saliva sample for nicotine analysis

- Currently using a 2nd or 3rd generation e-cigarette
- Have used 24 mg/mL nicotine concentration e-liquid in recent past
- If interested contact the researcher:

- Catherine Kimber at u0951767@uel.ac.uk or C.kimber@uel.ac.uk; 020 8223 4592 and 078 9146 3562 leaving your name and contact details. Find me on Twitter at @Cat_kimber

22 July 2015

Dear Catherine,

Project Title:	**Puffing Patterns in Electronic Cigarettes: Self-titration of Nicotine**
Principal Investigator:	**Lynne Dawkins**
Researcher:	**Catherine Kimber**
Amendment ref no:	**AMD 1415 23**
Original UREC reference:	**UREC 1415 40**

I am writing to confirm that the application for an amendment to the aforementioned research study has now received ethical approval on behalf of University Research Ethics Committee (UREC).

Should any significant adverse events or considerable changes occur in connection with this research project that may consequently alter relevant ethical considerations, this must be reported immediately to UREC. Subsequent to such changes an Ethical Amendment Form should be completed and submitted to UREC.

Approved Research Site

I am pleased to confirm that the approval of the proposed research applies to the following research site.

Research Site	**Principal Investigator / Local Collaborator**
University of East London, Stratford campus	Lynne Dawkins

Approved revised documents

Document	***Version***	***Date***
UREC application form	2.0	01 July 2015
Information sheet	2.0	01 July 2015

Summary of Amendments
Study to include female participants, as well as the male participants originally proposed.

Ethical approval for the original study was granted on 04 January 2015.

Approval is given on the understanding that the UEL Code of Good Practice in Research is adhered to.

With the Committee's best wishes for the success of this project.

Please ensure you retain this letter, as in the future you may be asked to provide evidence of ethical approval for the changes made to your study.

Yours sincerely,

Rosalind Eccles
University Research Ethics Committee (UREC)
UREC Servicing Officer
Email: researchethics@uel.ac.uk

University of East London
School of Psychology

Risk assessment for testing participants
Puffing Patterns in Electronic Cigarettes: Self-titration of Nicotine

	Testing Location(s)	Possible Risk	Severity of hazard (H, M, L)	Likelihood of hazard (H, M, L)	Risk (H, M, L)	Mitigating Activity
1.	UEL clinical education building, Stratford campus	Participants experience adverse effect of nicotine	Low	Low	Low	Very unlikely as participants will be given their PREFERED STRENGTH and a LOWER strength nicotine e-liquid and using *ad libitum.* However, should a participant feel any adverse effects, s/he will be advised to stop using the e-cigarette immediately and the nurse and one of the researchers will stay with the participant until s/he feels better. Effects usually wear off within an hour. If needed, participant will be offered a taxi home. As a further precautionary measure, participants will be screened to ensure that they are regular users and accustomed to 24mg/mL, therefore risks are not greater than risks encountered in their everyday lives.
2.	UEL clinical education building,	It is possible that blood	Medium	Low	Low	

	Stratford campus	samples could be spilt or dropped.				All care will be taken to avoid this by ensuring that all the correct equipment and is in place and is easily accessible. A walk through of the protocol (without collection of blood samples) in the lab will be carried out with the technical lab manger prior to sessions. Only the qualified phlebotomy nurse and those with full Hepatitis B vaccination will handle blood samples. All researchers and the nurse will be issued with and instructed on proper usage of the personal protective equipment. All participants will be screened for previous history of feeling faint or fainting during blood samples collection. Participants will be asked to eat a substantial breakfast on the morning prior to each testing session. A telephone emergency services will be accessible and a qualified first aider will be on call throughout the duration of the experiment to intervene if need be.
4.	UEL clinical education building, Stratford campus	Participants might feel faint	Medium	Low	Low	
5.			Low	Low	Low	

	UEL clinical education building, Stratford campus	E-liquid spill				The device we have chosen is very easy to re-fill and the investigator and researcher have extensive experience in using e-liquid refills. We will only use 5ml of liquid at a time. In the case of accidental spillage or contact with the skin, the participant/researcher will use the sink in the laboratory to wash their hands immediately. No reports of adverse effects relating to exposure of this concentration and amount of e-liquid to the skin have been reported.

Principle Investigator: L. Dawkins

APPENDIX 10 - STUDY 1 PARTICIPANT INVITATION LETTER

UNIVERSITY OF EAST LONDON

School of Psychology
Stratford Campus
Water Lane
London E15 4LZ

The Principal Investigator(s)
Dr. Lynne Dawkins; E-mail: l.e.dawkins@uel.ac.uk ; tel: 020 8223 4421
Catherine Kimber; E-mail: C.Kimber@uel.ac.uk or u0951767@uel.ac.uk; tel: 020 8223 4592
Professor Olivia Corcoran; E-mail: o.corcoran@uel.ac.uk; tel: 020 8223 4034

Consent to Participate in a Research Study

The purpose of this letter is to provide you with the information that you need to consider in deciding whether to participate in a research study being conducted at the University of East London.

Project Title

Puffing Patterns in Electronic Cigarettes: Self-titration of Nicotine

Project Description

This study aims to compare e-cigarette puffing behaviour and blood nicotine levels under two different strength e-liquids – a strength that is similar to the one you are currently using, and another lower strength e-liquid from the same supplier. If you decide to take part, we would like to see you on two occasions a week apart for approximately 2 hours each time. You will be asked to refrain from e-cigarette and nicotine use overnight (for 10-12 hours) prior to each session. Since the study involves blood sampling, we strongly recommend that you have a large breakfast in the morning before attending the session.

Testing sessions will take place in the mornings in order to minimize any discomfort associated with not using nicotine. We will provide the e-cigarette (an e-Vic third generation device) for you to use. For hygiene purposes, a new removable drip-tip will be provided at each session although you are welcome to bring your own.

During the study, you will first be asked to use a smokerlyser which measures your carbon monoxide levels thus indicates whether you have smoked. This is a simple, non-invasive breath test which simply requires you to hold your breath and then breathe slowly into a tube. You will then be asked to provide some background information about yourself and your e-cigarette history of use then complete a brief questionnaire measuring craving, mood and physical symptoms. Thereafter, a fully qualified phlebotomist nurse will insert an intravenous catheter into your forearm before taking a

baseline blood sample. A total of six blood samples will be collected at 5, 10, 20, 30 and 60 minutes where you will be asked to complete the brief craving, mood and physical symptoms questionnaire. The entire session will be video-recorded to capture your puffing patterns (i.e. puff frequency and duration), after which, you will complete 2 questionnaires about your mood state and vaping experience. Please note, you are free to take comfort breaks during this time (although you will not be permitted to leave the building with an indwelling catheter) and you will be reminded after 20 minutes that you are free to take a break.

Study Eligibility and exclusion criteria

<u>To take part you must be</u>:

- male
- a regular e-cigarette user – that is you have been using the product for at least 3 months
- be a non- or ex-smoker
- accustomed to 24mg/ml nicotine concentration – that is you have used 24mg/ml nicotine concentration in the last 3 months.
- be willing to provide a saliva sample by post to check your nicotine levels.
- aged 18 or over
- physically fit and willing to provide blood samples
- available and willing to participate in two sessions at UEL, Stratford, each takes approximately two hours.
- willing to abstain from vaping and use of all nicotine-containing products for 12 hours prior to testing.

<u>You cannot participate, if you</u>:

- are female
- are under 18 years of age
- are a smoker or a dual user – that is you are still smoking whilst using an e-cigarette
- do not use your e-cigarette on a daily basis
- have previous history of fainting whilst providing blood samples
- have neurobiological or heart conditions

Confidentiality of the Data

All data generated in the course of the research will be treated confidentially and will be retained in accordance with the University's Data Protection Policy. All data will be numerically coded although we plan to collect data from only 15 people so it is possible that complete anonymity may not be achievable. Nevertheless, consent forms will be detached and separated from the numerically-coded data and your questionnaire data and video recordings will be stored in a locked filing cabinet at UEL, analysed by three independent researchers at UEL, entered into a dataset then discarded securely. Data

captured by the video-recording will be cross-referenced with data collected by the e-Vic e-cigarette which also records puff number and duration. The anonymised data will be used for the current study only. It is aimed that the findings will be written up for publication in a peer reviewed journal and might be presented at conferences. Should any data be used in subsequent studies or for further analysis, the data will still be kept anonymous and confidential. Nevertheless, please be aware that the confidentiality of the information provided is subject to legal limitations in data confidentiality (i.e. the data may be subject to a subpoena, a freedom of information request or mandated reporting by some professions).

Blood Samples

Providing blood can sometimes lead to feeling dizzy, nauseous or faint. If you have any history of this, please do not volunteer to participate in the study. To reduce the likelihood of this occurring, you should ensure you have a substantial breakfast before the start of the study.
You might feel slight discomfort during the insertion of the catheter in your forearm. However, all blood samples will be collected by an experience and qualified phlebotomist nurse. Blood samples will be stored at the University of East London before being transported to the Advanced Bioanalysis Service Laboratories Ltd., Welwyn Garden City, UK, for analysis of the nicotine content. Sampled blood will not be used for any other purposes or studies. Once blood nicotine levels from all samples have been determined and recorded electronically, all samples will be disposed of in accordance with the Human Tissue Authority's Code of Practice.

Location

The study will be conducted at the University East London, Stratford Campus in a clinical laboratory which offers safe and adequate facilities for blood samples collection.

Remuneration

Given the study involves an intrusive procedure blood samples collection, a remuneration of £50 will be offered as compensation and to cover your travel costs. However, there is no further direct benefit for taking part in the study, although the findings, thus your participation will help to add to the scientific knowledge base on e-cigarettes. Note, if you are a student, your participation, non-participation or later withdrawal will have no impact on your assessments (i.e.: no module credits involved) and learning experience at UEL.

Disclaimer

You are not obliged to take part in this study and should not feel coerced. You are free to withdraw at any time. Should you choose to withdraw from the study you may do so without disadvantage to yourself and without any obligation to give a reason. Your right to withdraw from the study includes the right to request the destruction of any of your

data obtained from the study though you must memorise your personal participant number in order for the experimenter to delete your data. You can email us at any point prior stating your participation number, and we will remove your data for you. Following completion of the study, none of your contact information will be retained without your specific permission.

Please feel free to ask any questions. If you are happy to continue you will be asked to sign a consent form prior to your participation. Please retain this invitation letter for reference.

This study has received full approval from the University of East London's Ethical Committee. If you have any questions or concerns about how the study has been conducted, please contact the study's Principle Investigator:

Dr. Lynne Dawkins, School of Psychology, University of East London, Water Lane, London E15 4LZ. +44 (0)20 8223 4421. E-mail: l.e.dawkins@uel.ac.uk

Or

Catherine Fieulleteau, Ethics Integrity Manager, Graduate School, EB 1.43
University of East London, Docklands Campus, London E16 2RD
(Telephone: 020 8223 6683, Email: researchethics@uel.ac.uk).

APPENDIX 11- CONSENT FORM

UNIVERSITY OF EAST LONDON

Consent to participate in a research study

Title

Puffing Patterns in Electronic Cigarettes: Self-titration of Nicotine

I have the read the information sheet relating to the above research study and have been given a copy to retain. The nature and purpose of the research has been explained to me, and I have had the opportunity to discuss the details and ask questions about this information. I understand what is being proposed and the procedures in which I will be involved have been explained to me. I understand that I am able to take breaks whenever I want and that I am free to leave at any time.

I understand that my involvement in this study, and particular data from this research, will remain strictly confidential. Only the researcher(s) involved in the study will have access to identifying data. I understand that the findings from this research project may be written up, submitted for publication and presented at conferences. I understand that all collected data will remain confidential during this process. It has been explained to me what will happen once the research study has been completed.

I hereby freely and fully consent to participate in the study which has been fully explained to me. Having given this consent, I understand that I have the right to withdraw from the study at any time without disadvantage to myself and without being obliged to give any reason.

Participant's Name (BLOCK CAPITALS)

…….

Participant's Signature

……..

Researcher's Name (BLOCK CAPITALS)

……..

Researcher's Signature

……

Date: ……..…….

APPENDIX 12 – MATERIALS AND APPARATUS FOR STUDY 1

For the blood collection, handling and storage, the materials used was as follows:

- Pre-filled pharmaceutical grade saline syringes for flushing
- 22 gauge needles and peripheral IV cannula with injection port
- BDQ Stand Alone 385100
- A non-latex tourniquet
- BD Veca-C IV dressings
- 4mL BD K2EDTA Lithium Heparin vacutainers (pre-labelled with participant number, times and dates)
- Personal protective equipment (surgical gloves, lab coat, laboratory safety spectacles)
- A disposable box (Designated yellow sharps needle bucket with cover firmly secured)
- A trolley at the foot of the bed to contain the blood collection equipment

For the plasma extraction:

- MSE Falcon 6/300R Centrifuge
- Pipette
- Pipette heads
- Blue roll Clinell to disinfect surfaces and to wipe spillages
- Soft tissue (to wipe vacutainer tubes to ensure accuracy during balancing
- Weighing scales to balance samples before centrifugation
- 10ml glass beaker to weigh vacutainer tubes
- 1.5ml Micro-vials to contain plasma after extraction
- Biohazard waste bags

- Freezer designated for storage of biological samples at minus 20 degrees Celsius (Norhof biological storage freezer, model DPF30, Norhof, Holland).
- Gas chromatography – mass spectrometer (Agilent technologies, 5975a series, GC/MSD with triple-axis detector; Agilent, Atlanta, GA) as in Dawkins and Corcoran's (2014) paper.

For the pre-screening of participants:

- 'Salivette' kits to measure saliva cotinine concentration and ensure that participants are regular users and habituated to 24mg/ml (cut-off point ≤ 100 ng/mL).

The equipment used for the vaping session was as follows:

- The Bedfont piCO Smokerlyzer® as used in the pilot study
- The 'e-Vic' e-cigarette was shown to be reliable and well tolerated in the pilot study. Findings from the pilot study, showed a strong correlation with the video recordings data also.
- Nicotine liquid bottles all, tobacco flavour, 6 and 24mg/mL nicotine concentration.

Other equipment included:

- Precision scales (Microbalance, readability .00001g)
- A 'Toshiba Camileo X400' video recorder. All sessions were video recorded for cross-referencing purposes and also as a backup in case 'e-Vic' data would undergo loss, be distorted or corrupted.

APPENDIX 13 - STUDY 1 - BASELINE CHARACTERISTICS QUESTIONNAIRE

The information provided will be treated as strictly confidential and anonymous, and will only be used in the interest of this study. Please complete or tick the most appropriate option.

Participant's unique number: ……..

Age (please state): ……..

Gender: ☐ Male ☐ Female

Ethnicity:

☐White ☐ Black Afro-Caribbean ☐ Mixed raced

☐ Asian (Indian/ Pakistani/Bangadleshi) ☐ North/South East Asian (Chinese)

☐Other

Occupational status:

☐ employed ☐ non-employed

☐ self-employed ☐ studying

What is your highest qualification to date?

☐ GCSEs or equivalent ☐ A-levels or equivalent ☐ Undergraduate study (level 5 to 6)

☐ Postgraduate study (level 7 and above)

APPENDIX 14 - STUDY 1 VAPING HISTORY AND CHARACTERISTICS (NAMED eFTND)

1. Which type of device do you currently use most (please circle one):

    e. A disposable e-cigarette (non-rechargeable)
    f. A rechargeable e-cigarette (sometimes called 'stick-like', 'cigarette-like' or 'cartomizers' without fluid)
    g. A rechargeable e-cigarette ('stick-like/cigarette-like/cartomizer' without fluid) starter kit
    h. A rechargeable e-cigarette (non-cigarette-like with fluid)
    i. A rechargeable e-cigarette (non-cigarette-like with fluid) starter kit
    j. A modular system (your own combination of separate parts: battery, atomizer, fluid etc).
    k. E-cigar

2. Which brand and model of e-cigarette are you currently using the most?

Brand (please state) _____
Model (please state)_____

3. Which strength(s) of nicotine fluid/cartridge are you currently using? (circle all that apply).
    a. 0mg
    b. 4 mg
    c. 6mg
    d. 8mg
    e. 10 mg
    f. 11mg
    g. 15 mg
    h. 16mg
    i. 18mg
    j. 24mg
    k. 36mg
    l. Higher than 36mg
    m. I don't know

4. If you have circled more than one strength, please state which strength you use most: _____

5. Please try to estimate the amount you use per day:
    a. In puffs _____
    b. In cartridges _____
    c. In ml _____

6. Which is your preferred flavour? (please circle one)
    a. Tobacco
    b. Mint/menthol
    c. Fruit (various)
    d. Coffee
    e. Vanilla
    f. RY4
    g. Chocolate
    h. Cinnamon
    i. Tea
    j. Alcohol-related
    k. Other (please state)

7. How soon after you wake up do you use your electronic cigarette?

    a. Within 5 minutes
    b. 6 – 30 minutes
    c. 31 – 60 minutes
    d. After 60 minutes

8. Do you find it difficult to refrain from using your electronic cigarette in places where it is forbidden (e.g. on the train, in a business meeting)

    a. Yes
    b. No

9. Which puffs on your e-cigarette would you hate most to give up?

    a. The first ones in the morning
    b. Any other one

10. Do you puff more frequently on your e-cigarette during the first hours after waking than during the rest of the day?

    a. Yes
    b. No

11. Do you use your e-cigarette if you are so ill that you are in bed most of the day?

    a. Yes
    b. No

12. Please rate your addiction to e-cigarettes on a scale of 0 to 100:_____

I am NOT addicted to cigarettes at all = 0
I am extremely addicted to cigarettes = 100

13. For you, stopping using the e-cigarette for good would be:

    a. Impossible
    b. Very difficult
    c. Fairly difficult
    d. Fairly easy
    e. Very easy

14. How much of the time have you felt the urge to ***vape (***i.e. use your e-cigarette) in the past week? *(Circle one number)*

All the time	Almost all the time	A lot of the time	Some of the time	A little of the time	Not at all
5	4	3	2	1	0

15. How strong have the urges been? *(Circle one number)*

Extremely strong	Very strong	Strong	Moderate	Slight	No urges
5	4	3	2	1	0

16. Since you started using the electronic cigarette, have you attempted to cut down the amount that you use it?

    a. Yes
    b. No

If YES: In your attempt to cut down your use of the electronic cigarette, how successful have you been?

a. Extremely successful: I have stopped using it completely now
b. Very successful: I only use it occasionally now
c. Quite successful: I use it much less that I did initially
d. Not very successful: I have only cut down slightly
e. Unsuccessful: My use has not changed
f. Very unsuccessful: I use it more than I did initially

17. Is it likely that in one month from now, you will have stopped using the electronic cigarette?

a. Very likely
b. Rather likely
c. Rather unlikely
d. Very unlikely

APPENDIX 15 – INDIVIDUAL PUFFING TOPOGRAPHY DATA (STUDY 1)

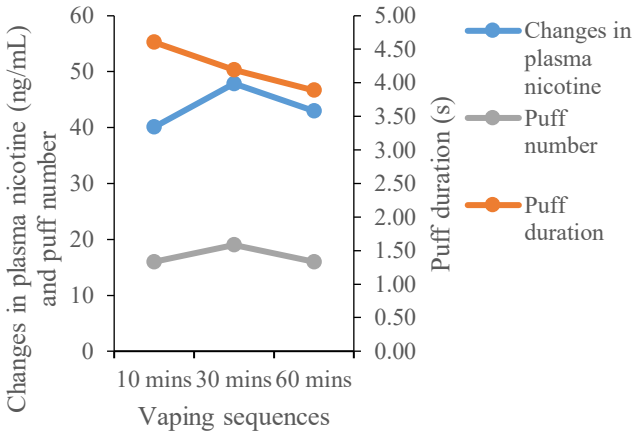


Figure 2.14 Participant 1 Puff number and duration against changes in plasma nicotine in the high condition

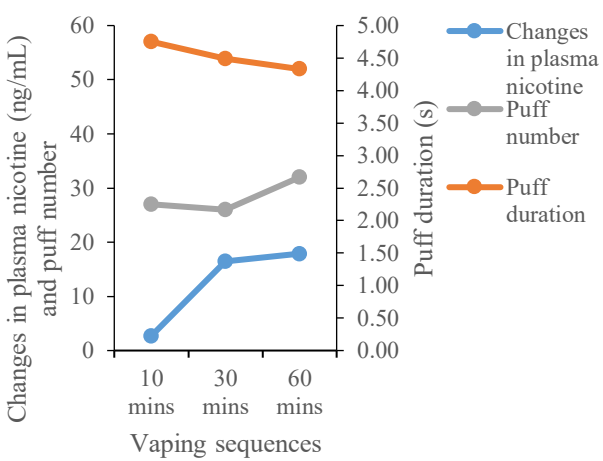


Figure 2.15 Participant 1 Puff number and duration against changes in plasma nicotine in the low condition

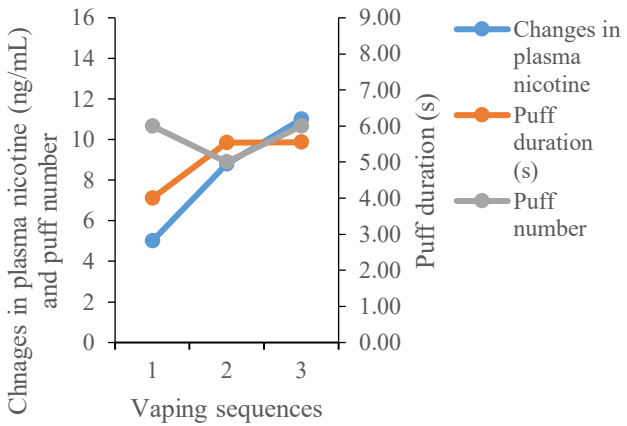


Figure 2.16 Participant 2 Puff number and duration against changes in plasma nicotine in the high condition

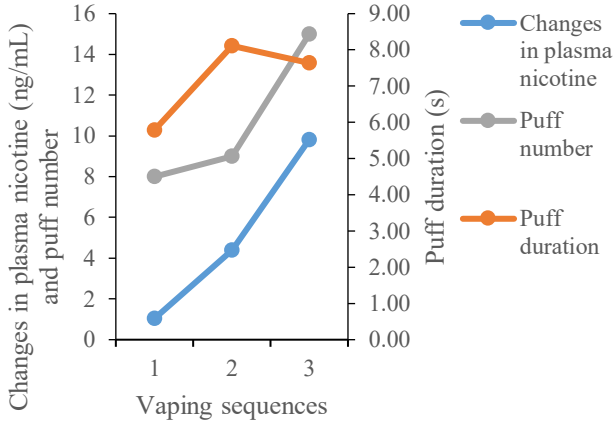


Figure 2.17 Participant 2 Puff number and duration against changes in plasma nicotine in the low condition

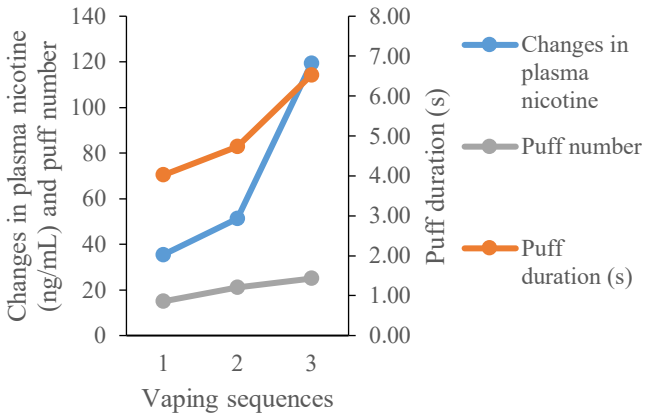


Figure 2.18 Participant 3 Puff number and duration against changes in plasma nicotine in the high condition

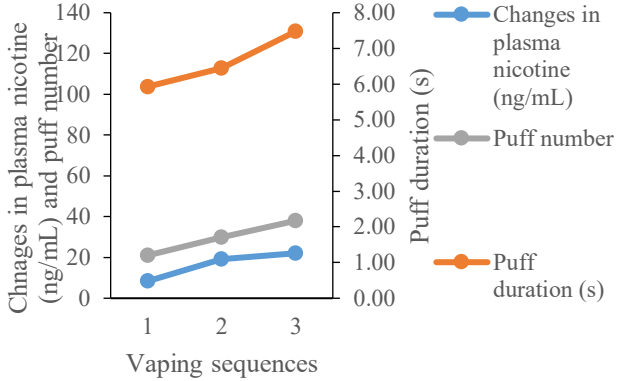


Figure 2.19 Participant 3 Puff number and duration against plasma nicotine in the low condition

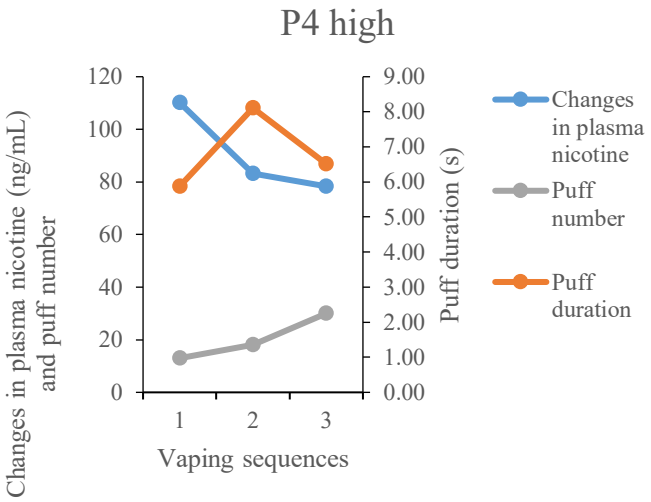


Figure 2.20 Participant 4 Puff number and duration against changes in plasma nicotine in the high condition

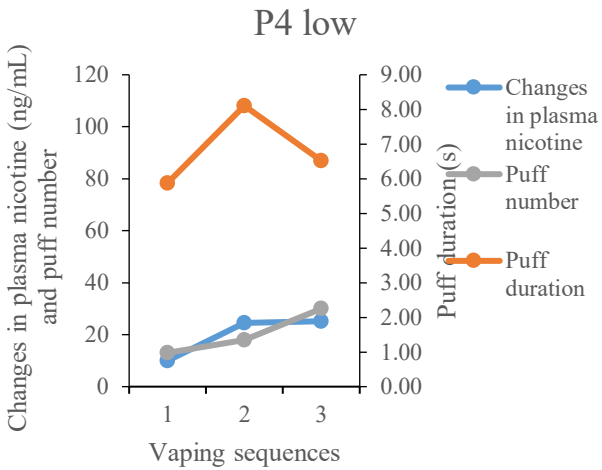


Figure 2.21 Participant 4 Puff number and duration against changes in plasma nicotine in the low condition

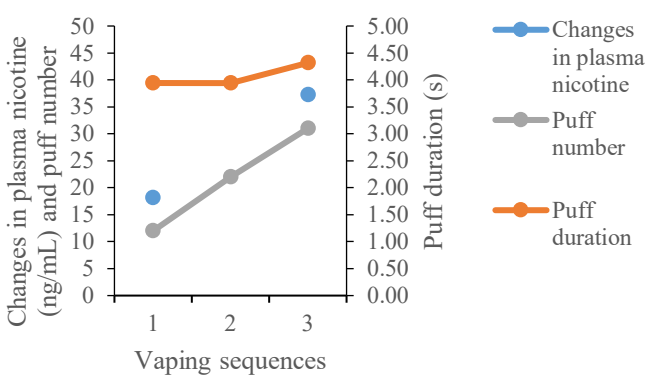


Figure 2.22 Participant 5 Puff number and duration against changes in plasma nicotine in the high condition

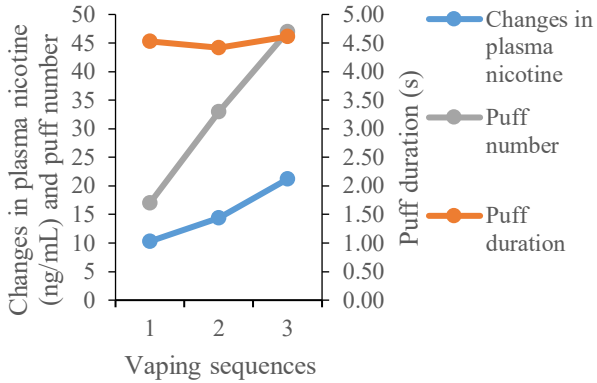


Figure 2.23 Participant 5 Puff number and duration against changes in plasma nicotine in the low condition

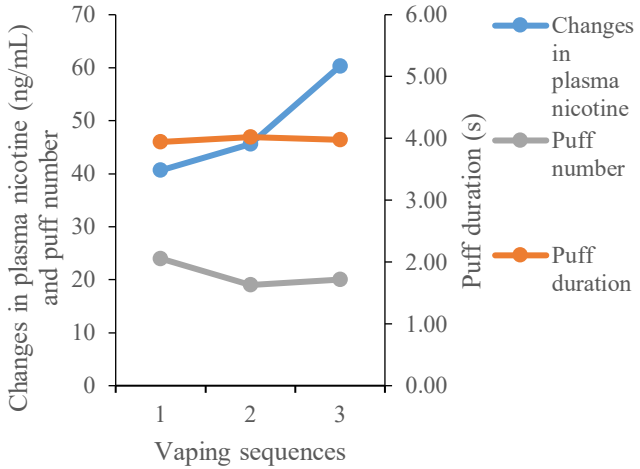


Figure 2.24 Participant 6 Puff number and duration against changes in plasma nicotine in the high condition

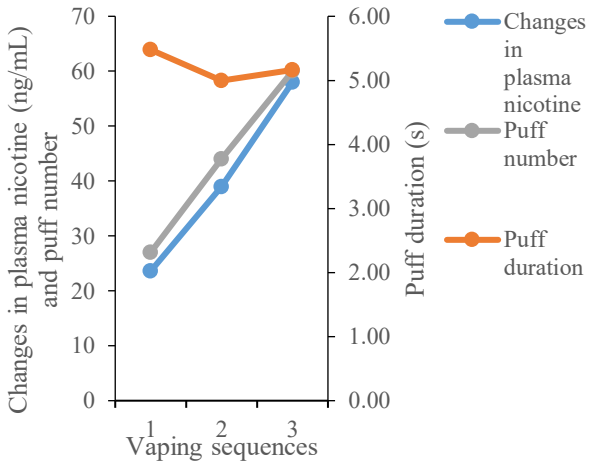


Figure 2.25 Participant 6 Puff number and duration against changes in plasma nicotine in the low condition

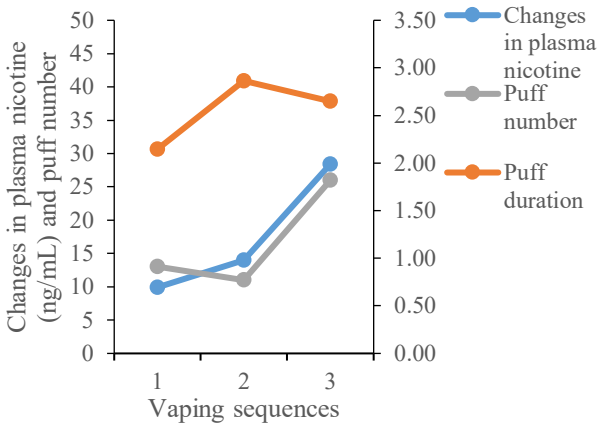


Figure 2.26 Participant 7 Puff number and duration against changes in plasma nicotine in the high condition

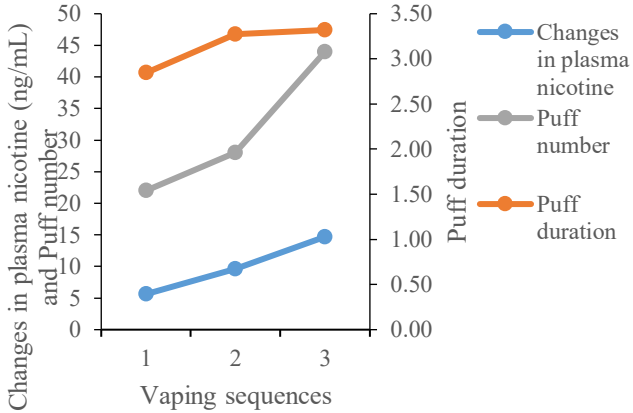


Figure 2.27 Participant 7 Puff number and duration against changes in plasma nicotine in the low condition

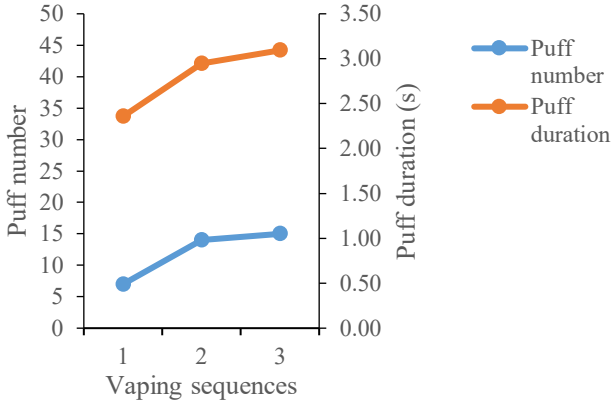


Figure 2.28 Participant 8 Puff number and duration in the high condition (plasma nicotine scores unavailable)

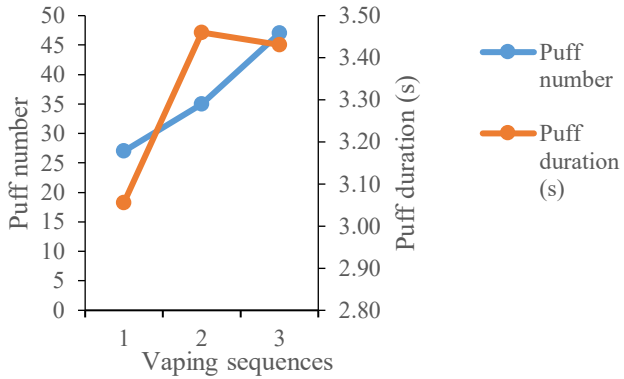


Figure 2.29 Participant 8 Puff number and duration in the low condition (plasma nicotine scores unavailable)

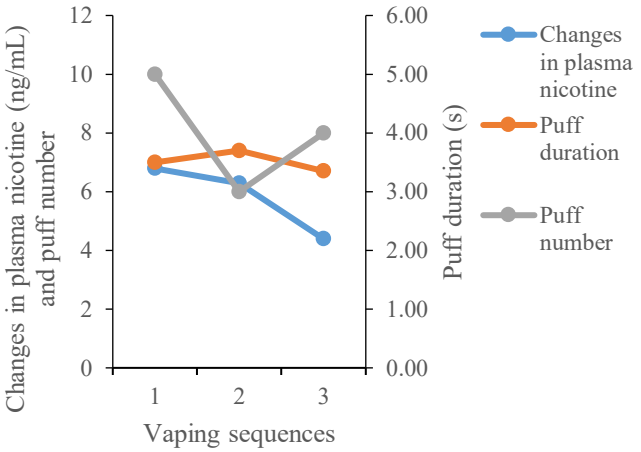


Figure 2.30 Participant 9 Puff number and duration against changes in plasma nicotine in the high condition

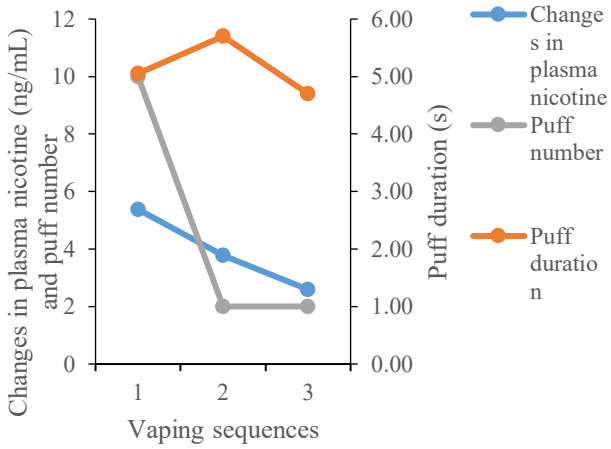


Figure 2.31 Participant 9 Puff number and duration against changes in plasma nicotine in the low condition

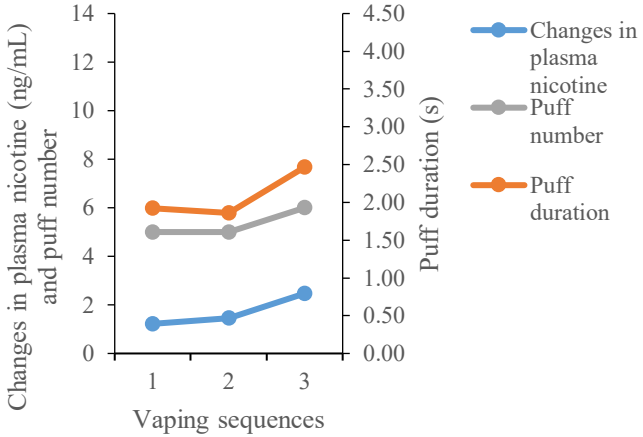


Figure 2.32 Participant 10 Puff number and duration against changes in plasma nicotine in the high condition

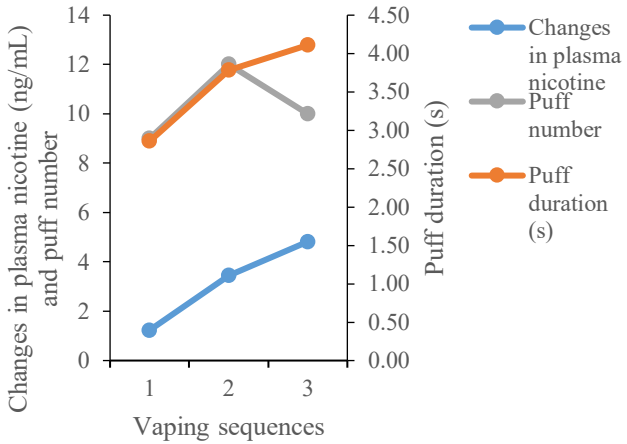


Figure 2.33 Participant 10 Puff number and duration against changes in plasma nicotine in the low condition

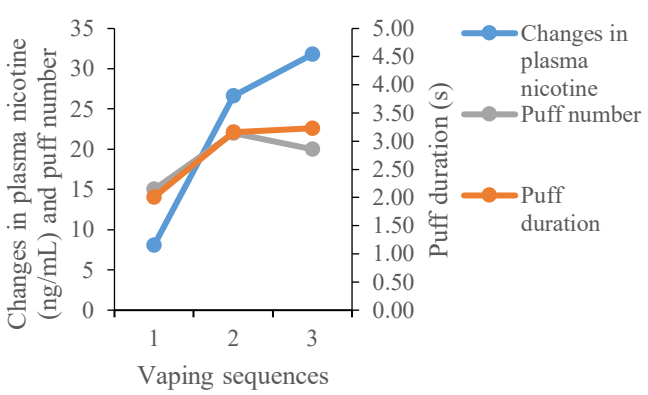


Figure 2.34 Participant 11 Puff number and duration against changes in plasma nicotine in the high condition

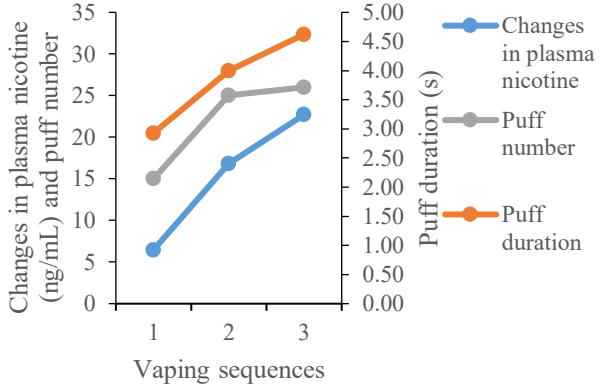


Figure 2.35 Participant 11 Puff number and duration against changes in plasma nicotine in the low condition

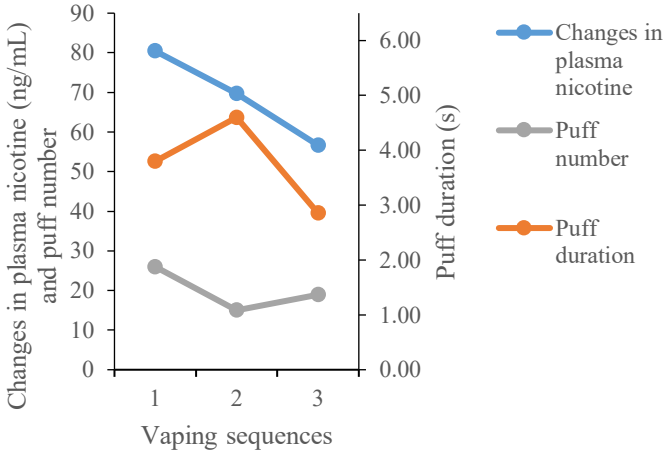


Figure 2.36 Participant 12 Puff number and duration against changes in plasma nicotine in the high condition

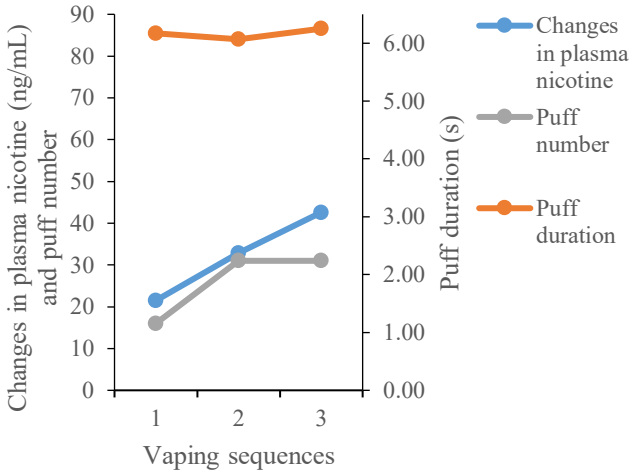


Figure 2.37 Participant 12 Puff number and duration against changes in plasma nicotine in the low condition

APPENDIX 16 – ETHICS APPLICATION AND APPROVAL LETTER FOR STUDY 2 (INCLUDE RISK ASSESSMENT)

APPLICATION FOR ETHICAL REVIEW OF RESEARCH INVOLVING HUMAN PARTICIPANTS, HUMAN DATA OR HUMAN MATERIAL

This application should be completed by **members of staff and postgraduate research degree students (i.e. MRes, MPhil, PhD and Professional Doctorate)** undertaking research which involves human participants, sensitive human data (personal or otherwise) and human material (including human tissue, embryos, foetuses and bodily fluids, from living or deceased participants).

No form of contact with potential participants for the proposed research should occur until written approval has been received from University Research Ethics Committee (UREC). Where a member of staff or student is found to have breached this expectation, they may be subject to disciplinary action.

This application should be submitted alongside copies of any supporting documentation which will be handed to participants, including a participant information sheet, consent form, self-completion survey or questionnaire.

For further guidance please contact researchethics@uel.ac.uk or refer to the guidance at http://www.uel.ac.uk/qa/research/index.htm. Only those applications received by the submission deadline date shown on the University's Research Ethics web page will be considered at the next meeting. Where a form is submitted and sections are incomplete, the form will not be considered by UREC and will be returned to the applicant for completion.

PROJECT DETAILS

Current project title	***Cigalikes Versus Tank systems: Effects of Users' experience, Device Characteristics and Nicotine Concentration During a Quit Attempt***
Is this project externally funded?	**YES** ☐ **NO** ☒ **NOT APPLICABLE** ☐

Does the project require UREC approval before consideration by the funding body?	**YES ☐ NO ☐ NOT APPLICABLE ☒** If **YES**, please indicate funding body deadline below:		
If externally funded, please provide details of funding body.	N/A		
How will participants be informed of the source of funding? N/A **PARTICIPANT INFORMATION SHEET ☐ CONSENT FORM ☐ OTHER ☐** If **OTHER**, please specify further below:			
Proposed project start date	28/09/15	**Anticipated project end date**	September 2017

APPLICANT DETAILS

Name of Principal Investigator (PI) (For research degree students, the Director of Studies)	Dr Lynne Dawkins (DoS, School of Psychology) on behalf of Catherine Kimber (PhD student)
School	Psychology

Status (please tick relevant box)	**UEL STAFF ☐ RESEARCH DEGREE STUDENT ☒**
Email address	L.e.dawkins@uel.ac.uk
Contact telephone number	0208 223 2241
Name of co-researchers	Catherine Kimber (PhD student) Prof. Olivia Corcoran (Second supervisor from HSB school)
Will parts of the proposed research or research administration be carried out by independent contractors or partner institutions, domestic or international?	**YES ☐ NO ☒** If **YES**, please provide a brief explanation of who the contractor or partner is, what their role will be and how their contribution will be monitored. Please note, responsibility for proper conduct of all parties involved in the research resides with the Principal Investigator.

CONFLICTS OF INTEREST

Will any of the researchers or their institutions receive any other benefits or incentives for taking part in this research over and above their normal salary package or the costs of undertaking the research? **YES ☐ NO ☒** If **YES**, please detail below:
Is there any further possibility for conflict of interest? YES ☐ NO ☒ If **YES**, please detail below:

FOR ALL APPLICANTS

Has external ethics approval been sought for this research?	**YES ☐ NO ☒**

(i.e. submission via Integrated Research Application System (IRAS) to the Health Research Authority (HRA) or other external research ethics committee)	
If **YES**, please supply details below:	

DEAN OF SCHOOL OR ASSOCIATE DEAN • Does the proposed research as detailed herein have your support and endorsement to proceed? **YES** ☒ **NO** ☐	
Signed	
Date	

APPLICANT DECLARATION I confirm that: • The information contained in this application is, to the best of my knowledge, correct and up to date. • I have attempted to identify all risks related to the research. • I acknowledge my obligations and commitment to upholding our University's Code of Practice for ethical research and observing the rights of the participants. • I am aware that cases of proven misconduct, in line with our University's policies, may result in formal disciplinary proceedings and/or the cancellation of the proposed research.	
Applicant	Lynne Dawkins (for Catherine Kimber)
Signed	
Date	

FOR RESEARCH DEGREE STUDENT APPLICANTS ONLY

Name and School of Director of Studies	Dr Lynne Dawkins, School of Psychology
Qualification for which research is being undertaken	PhD via MPhil

Director of Studies (DoS) –

- Does the student have the necessary skills to carry out the research?
  YES ☒ NO ☐
- Is the participant information sheet, consent form and any other documentation appropriate?
  YES ☒ NO ☐
- Are the procedures for recruitment of participants and obtaining informed consent suitable and sufficient?
  YES ☒ NO ☐
- Where required, do all members of the research team have current Criminal Records Bureau (CRB) clearance?
  YES ☐ NO ☐ N/A

Signed	
Date	

DETAILS OF THE PROPOSED RESEARCH

55. Provide a brief description of the proposed research, including the requirements of participants. This must be in lay terms and free from technical or discipline specific terminology or jargon. If such terms are required, please ensure they are adequately explained (Do not exceed 500 words)

This application is part of a PhD research project and a continuity of a precedent study which investigated compensatory behaviours for lower nicotine concentration e-liquid in regular electronic cigarette (hereafter referred to as e-cigarette) users over a one hour period. The proposed study will explore compensatory behaviours over a longer period of time alongside other factors which may also affect puffing topography and nicotine delivery. Puffing topography refers to number of puffs taken, duration of each puff and intervals between each puff (Inter-Puff Intervals hereafter referred to as IPI).

E-cigarettes are battery-operated devices which deliver nicotine via a mist-produced mechanism. They have now been endorsed as part of the tobacco harm reduction strategy by Public Health England (hereafter referred to as PHE) stating "The option of switching to electronic cigarettes as an alternative and much safer source of nicotine, as a personal lifestyle choice rather than medical service has enormous

potential to reach smokers currently refractory to existing approaches" (Britton & Bogdanovica, 2014).

When first introduced, e-cigarettes were designed to strongly resemble combustible cigarettes, hence their names 'cigalike'. Today, more sophisticated devices known as tank systems have been introduced. Studies suggest they are more popular with experienced users (Dawkins, Turner, Roberts, & Soar, 2013) and are thought to enhance users' experience.

Differences between cigalikes and tank systems initially emerged with the failure of the former to deliver nicotine with no to little increase in blood nicotine (Bullen et al., 2010; Eissenberg, 2010). However, later clinical studies using experienced e-cigarette users (Dawkins & Corcoran, 2014; Vansickel & Eissenberg, 2013) and tanks (Farsalinos et al., 2014) found significant increases in plasma nicotine levels. Additionally, studies found that practise could lead to significant increase in blood nicotine (Hajek et al., 2014); and the use of tanks to be associated with greater success quit rates in comparison to cigalikes (Hitchman, Brose, Brown, Robson, & McNeill, 2015; Polosa, Caponnetto, Maglia, Morjaria, & Russo, 2014).

Altogether, much evidence suggests users' experience, device characteristics and nicotine concentrations as likely factors influencing nicotine delivery and the way a device is used or puffed on (e.g. puff duration and frequency) (Talih et al., 2014). Given that nicotine is the primary reinforcer of smoking behaviour (Harvey et al., 2004), the implications are that models with poor nicotine delivery will preclude product acceptability, influence puffing behaviour and possibly put users at risk of relapsing to tobacco smoking.

Thus, this study will explore how puffing behaviours and nicotine delivery in naïve e-cigarette users (smokers) change over time (as a user becomes more experienced in vaping) and vary according to device type and nicotine concentration. It will also explore satisfaction, craving alleviation and product acceptability, and predictors of quitting behaviour.

A sample of 60 current smokers, willing to initiate a quit attempt, will be recruited ($\geq$ 5 cigarettes daily for > 1 year). Participants will be randomly (with the roll of a

dice) allocated to either condition: 1) given a cigalike, 2) given a tank with low nicotine concentration (6mg/ml), 3) given a tank with high nicotine concentration (18mg/ml). Participants will be invited to attend three sessions: at baseline, week-1 and week-2. They will be provided with an e-cigarette and refill cartridges/bottles (1-2 weeks supply depending on budgetary restraints) at baseline and told to substitute as many cigarettes as possible with the e-cigarette with the aim of quitting smoking completely. Each participant will be given a diary to record their daily e-cigarette and cigarette use and subjective effects associated with e-cigarettes use. Socio-demographic data, urge to smoke/vape and information on nicotine/smoking dependence will be collected.

After the 2-week session participants will be invited to keep the device and encouraged to continue use and stop smoking, however, there will be no reward or motivational incentives.

Follow-up interviews will be conducted at 4 weeks, 3 and 12 months to assess smoking status and e-cigarette use.

56. Provide a statement on the aims and significance of the proposed research, including potential impact to knowledge and understanding in the field (where appropriate, indicate the associated hypothesis which will be tested). This should be a clear justification of the proposed research, why it should proceed and a statement on any anticipated benefits to the community. (Do not exceed 700 words)

Each year, in the UK alone, approximately 100 000 of premature death is attributed to smoking (Peto et al, n.d.). Smoking still remains the most preventable cause of premature mortality. Although some commentators argue that the ultimate goal for smokers is to cease any use of tobacco and nicotine products, there is a consensus which support harm reduction approaches to help smokers unable or unwilling to quit. While some studies indicate that nicotine replacement therapies (hereafter referred to as NRTs) help in achieving abstinence (Ucar et al., 2014), fewer than 20% of users remain abstinent after 12 months (Stead et al., 2012). It could be because NRTs fall

short of providing the psycho-behavioural cues associated with the rituals of smoking or because the user has no control over the nicotine delivery pace.

Studies suggest that they are more effective than NRTs (Brown, Beard, Kotz, Michie, & West, 2014) and have helped smokers achieving abstinence and quit smoking (Etter & Bullen, 2014) even in smokers who were not interested to quit (Polosa et al., 2014). If e-cigarettes are to remain and help in alleviating the huge burden of smoking prevalence, it is important to further explore their potential as smoking cessation aids.

Although e-cigarettes research is in its infancy, in the past year there has been a surge in new studies depicting e-cigarettes as a potential smoking cessation aid (Caponnetto et al., 2013; Hajek et al., 2014; Polosa et al., 2014; Polosa et al., 2011). However, to date there is little evidence which help understand the dynamic interplay of the factors and mechanisms (e.g. puffing behaviour, nicotine concentration, device type) which influence nicotine delivery positively or negatively and result in success at quitting smoking. Previous studies, including some work from our group at UEL (Farsalinos' study, Dawkins & Corcoran, 2014 & the Dawkins, Kimber study 2015) suggest that different puffing behaviours may be associated with different blood nicotine delivery and that puffing behaviour may change over time and with different devices and nicotine strengths. Nevertheless how puffing behaviour and device characteristics influence smoking cessation success has not been explored.

Taking a more naturalistic approach, the aims of this study are threefold: 1) to explore how naïve users' puffing behaviours differ or change a) over time, b) between devices (cigalike vs. tank) and c) according to nicotine concentration (high vs. low); 2) to explore a) satisfaction, craving alleviation and product acceptability and b) nicotine delivery (via cotinine, a nicotine metabolite measured in saliva) between devices and according to nicotine concentration; and 3) to explore quit rates between groups and predictors of successful cessation.

The study is both novel and broad in its scope, generating a wealth of information on puffing topography and user behaviour with different e-cigarette devices/nicotine concentrations as well as information of successful predictors of smoking cessation. As such it will likely lead to a high impact publication (or two publications) and will be well cited in the academic literature. Outside of academia, the findings will be useful for policy makers, e-cigarette companies and health professionals, potentially enhancing smoking cessation programmes thus helping current smokers and ultimately alleviating the burden of smoking on the wider society.

Study 2

Main aims:

1- To explore how naïve users' puffing behaviours differ or change
   i. over time
   ii. between devices (cigalike vs. tank)
   iii. according to nicotine concentration (high vs. low)
2- To explore
   i. satisfaction, craving alleviation and product acceptability
   ii. nicotine delivery (via cotinine, a nicotine metabolite measured in saliva) between devices and according to nicotine concentration

Study 3

In a 4-week, 3 month and 12-month follow-up (Study 3), participants from the current study will be interviewed (via phone calls) to collect information vis-à-vis their smoking status (self-reported continuous abstinence, 7 day point prevalence abstinence and reduction in cigarette smoked), motivation to quit and e-cigarette use, to assess the efficacy of these devices as smoking cessation aids.

Primary aims:

1- To explore
   i. Quit rates (cigarette consumption reduction) between groups
   ii. Predictors of successful cessation

57. Provide an outline of the methodology for the proposed research, including proposed method of data collection, *tasks* assigned to participants of the research and the proposed method and duration of data analysis. If the proposed research makes use of pre-established and generally accepted techniques, please make this clear. (Do not exceed 500 words)

Design:

A mixed-participants design will be used, comprising 3 within-subjects factors for 'time':

Study 2	Study 3
➢ Baseline ➢ One-week ➢ Two-week	➢ 4 weeks ➢ 3 months ➢ 12 months

Participants will be randomly allocated to one of the following (between-subject factor):

1- cigalike - high nicotine concentration (18mg/ml)
2- tank - high nicotine concentration (18mg/ml)
3- tank - low nicotine concentration (6mg/ml) (Figure 1)

Outcome measures:

1- Puffing topography ('numbers of puffs', 'puff duration', 'IPI') and 'volume consumed'
2- Salivary cotinine at baseline and at complete cessation
3- Self-reported cigarette consumption/reduction
4- Subjective effects (e.g. 'urge to smoke' and 'withdrawal') and others (e.g. satisfaction, hit)

5- eCO (Exhaled Carbon Monoxide) levels at baseline, 1 and 2 weeks and at cessation

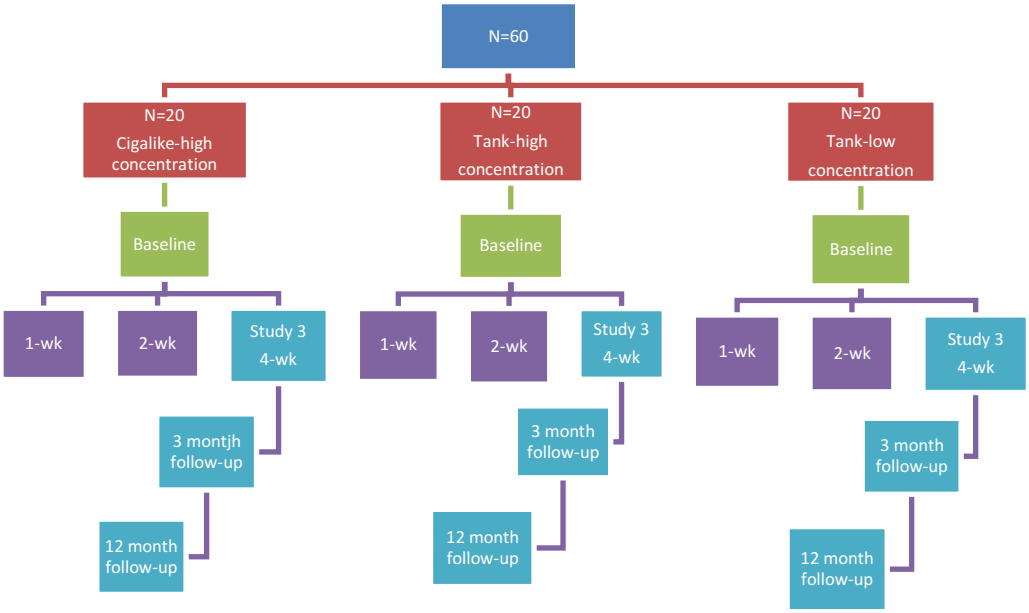


Figure 1. Diagram outline of study 2/3

Equipment:

- Carbon Monoxide (CO) Bedfont Smokerlyzer
- 'Salivette' to measure salivary cotinine
- Video recorder
- 'Go e-cig' Cigalike including a user manual, battery and charger
    - Tobacco flavour 'Go e-cig' Refill cartridges 1.8% nicotine concentration
- 'Curve Mini' Electronic cigarette by Totally Wicked with a tank and manual instructions
    - Refill bottles Tobacco flavour 'Totally Wicked's Red Label' 1.8% and 0.6% nicotine concentration

Note, the choice to purchase these products was based on price competitiveness and appropriateness in terms of design and features. All e-cigarette purchases were funded by ReDS (funds were allocated to the applicant's studentship). The e-

cigarette and nicotine e-liquid provider has no connection with this project or the applicant.

Questionnaires/Measures:

- Baseline socio-demographic questionnaire (Appendix C).
- Smoking history questionnaire (appendix D).
- The Fagerström Test of Nicotine Dependence (Fagerström, 2012) (Appendix E).
- The Mood and Physical Symptoms Scale (MPSS) questionnaire (West & Hajek, 2004) (Appendix F).
- A diary to record daily e-cigarette use, number of cigarettes smoked, etcetera. (Appendix G).
- Follow-up Interview questions for Study 3 (Appendix H)
- Subjective effects to nicotine (Appendix I and J)

Procedure:

The study will be advertised internally and externally, using social media (Twitter and Facebook), leaflets and posters. Respondents to the advert via emails or phone calls will be screened via phone interviews to ensure all inclusion criteria are met, and an information sheet provided. Participants will be invited to attend a baseline, 1 and 2-week individual sessions after a one-hour smoking abstinence.

Upon arrival, the researcher will go through the information sheet with participants and ensure all aspects of the procedure are understood. Participants will be asked to provide written informed consent (Appendices A and B), informed of their right to withdraw from the study and invited to ask further questions.

Salivary cotinine and Carbon monoxide breath tests will be administered before collecting baseline, socio-demographic data and information about their nicotine/cigarette dependence. Participants will be asked to place the test swab in

their mouth for 5 minutes until completely saturated, then place in a capped centrifugation tube on ice.

MPSS questionnaires will be completed at the start and end of each session. Participants will be allocated a condition, given the e-cigarette with instructions on how to use it and instructed to vape *ad libitum* for 30 minutes. Puffing topography will be measured by video-recording each session then using a frame by frame analysis of 29.97 fps (frame per second) to analyse timing measurements (using the 'Adobe Premiere Pro CS5' software).

At the end of the first session, participants will be told to keep a record of their e-cigarette use and number of cigarettes smoked in the diary and return all used and unused refills.

At week-2 post intervention initiation, supply of e-liquid/cartridges will cease. Participants will be invited to keep the device and encouraged to continue use, however, there will be no reward incentives.

Participants will be asked for consent to be contacted via phone calls for further follow-up phone interviews at 4 weeks, 3 and 12 months to provide information regarding their smoking status at these time points.

<u>Salivary cotinine analysis:</u>

Samples will be frozen (-20ºC) and stored in UH006/7 at UEL. Once collection of all samples is complete, they will be transported on dry ice in an appropriate container to the Advanced Bioanalytical Service (ABS) Laboratories Ltd., Welwyn Garden City, UK. All analysis will be conducted by ABS labs and disposed of in accordance with the MHRA, GLP (Good Laboratory Practise) and GCP (Good Clinical Practise) accreditation protocols. A certificate of destruction will be issued and provided by ABS laboratories Ltd.

PARTICIPANT DETAILS

58. Provide an explanation detailing how you will identify, approach and recruit the participants for the proposed research, including clarification on sample size and location. Please provide justification for the exclusion/inclusion criteria for this study (i.e. who will be allowed to / not allowed to participate) and explain briefly, in lay terms, why this criteria is in place. (Do not exceed 500 words)
Participants: All 60 participants will be current smokers (smoking 5 cigarettes or more per day for at least the preceding year), willing to make a quit attempt and not a current e-cigarette user (i.e. have not used an e-cigarette more than 5 times in the past and for no more than a week continuously). A sample size calculation was conducted based on data obtained from our pilot study (using 'G Power'): Puff duration variable: Effect size = 0.448, $p \leq 0.05$, CI = 0.95, N = 57 (sample size of 57) Puff number variable: Effect size = 2.97, $p \leq 0.05$, CI = 0.95, N = 6 (sample size of 6) Other inclusion criteria include aged 18 or over, fluent in English. Exclusion criteria are pregnant or lactating females, any known neurobiological or heart conditions. Participants will be included regardless of their gender, ethnicities and SES (collected via questionnaires). This information will be used in subsequent analysis. Recruitment will be initiated via advertisements on social media (Twitter and Facebook), leaflet distribution on and outside UEL, posters display at UEL's permitted points and at various supermarkets. Thus, participants are likely to include UEL staff and students as well as members of the general public. However, students

will be made aware that their learning, assessments or experience at UEL will not be impacted by their participation, withdrawal or non-participation. This will be clearly outlined in the information sheet (Appendix A).

59. Will the participants be from any of the following groups?*(Tick as appropriate)*

☒ Students or staff of this University (i.e. recruitment on-site at University of East London).

☒Adults (over the age of 18 years with mental capacity to give consent to participate in the research).

☐ Young people between 16 and 18 years (please note parental consent may still be required for s006Fme research).

☐ Children or legal minors (anyone under the age of 16 years)[1]

☐ Adults who are unconscious, severely ill or have a terminal illness.

☐ Adults who may lose mental capacity to consent during the course of the research.

☐ Adults in emergency situations.

☐ Adults[2] with mental illness - particularly those detained under the Mental Health Act (1983 & 2007).

☐ Participants who may lack capacity to consent to participate in the research under the research requirements of the Mental Capacity Act (2005).

☐ Prisoners, where ethical approval may be required from the **National Offender Management Service (NOMS).**

☐ Young Offenders, where ethical approval may be required from the National Offender Management Service (NOMS).

☐ Healthy volunteers (in high risk intervention studies).

☐ Participants who may be considered to have a pre-existing and potentially dependent[3] relationship with the investigator (e.g. those in care homes, students, colleagues, service-users, patients).

☐ Other vulnerable groups (see Question 6).

☐ Adults who are in custody, custodial care, or for whom a court has assumed responsibility.

☐ Participants who are members of the Armed Forces.

[1]If the proposed research involves children or adults who meet the Police Act (1997) definition of vulnerability[3], any researchers who will have contact with participants must have current enhanced Disclosure and Barring Service check (was previously called Criminal Records Bureau, or CRB, clearance). [2] 'Adults with a learning or physical disability, a physical or mental illness, or a reduction in physical or mental capacity, and living in a care home or home for people with learning difficulties or receiving care in their own home, or receiving hospital or social care services.' (Police Act, 1997)

[3] *Proposed research involving participants with whom the investigator or researcher(s) shares a dependent or unequal relationships (e.g. teacher/student, clinical therapist/service-user) may compromise the ability to give informed consent which is free from any form of pressure (real or implied) arising from this relationship. UREC recommends that, wherever practicable, investigators choose participants with whom they have no dependent relationship. Following due scrutiny, if the investigator is confident that the research involving participants in dependent relationships is vital and defensible, UREC will require additional information setting out the case and detailing how risks inherent in the dependent relationship will be managed. UREC will also need to be reassured that refusal to participate will not result in any discrimination or penalty.*

60. Will the study involve participants who are vulnerable? YES ☐ NO ☒

For the purposes of research, 'vulnerable' participants may be adults whose ability to protect their own interests are impaired or reduced in comparison to that of the broader population. Vulnerability may arise from the participant's personal characteristics (e.g. mental or physical impairment) or from their social environment, context and/or disadvantage (e.g. socio-economic mobility, educational attainment, resources, substance dependence, displacement or homelessness). Where prospective participants are at high risk of consenting under duress, or as a result of manipulation or coercion, they must also be considered as vulnerable.

Adults lacking mental capacity to consent to participate in research and children are automatically presumed to be vulnerable. Studies involving adults (over the age of 16) who lack mental capacity to consent in research must be submitted to a REC approved for that purpose.

6.1. **If YES,** a Disclosure and Barring Service (DBS) check within the last three years is required.

Please provide details of the "clear disclosure":

Date of disclosure:
Type of disclosure:
Organisation that requested disclosure:
DBS certificate number:

(NOTE: information concerning activities which require DBS checks can be found via https://www.gov.uk/government/publications/dbs-check-eligible-positions-guidance)

6.2.If YES, what special arrangements are in place to protect vulnerable participants' interests?

61. Do you propose to make any form of payment or incentive available to participants of the research? YES ☒ NO ☐

If **YES**, please provide details taking into account that any payment or incentive should be representative of reasonable remuneration for participation and may not be of a value that could be coercive or exerting undue influence on potential participants' decision to take part in the research. Wherever possible, remuneration in a monetary form should be avoided and substituted with vouchers, coupons or equivalent. Any payment made to research participants may have benefit or HMRC implications and participants should be alerted to this in the participant information sheet as they may wish to choose to decline payment.

There will be no monetary compensation for taking part in the study. However, all participants will be given an e-cigarette device accompanied with nicotine e-liquid refills (1-2 weeks supply) at the start of the study. Given the study's design, participants will be permitted to keep the device so their use and the effects on tobacco smoking can be followed over a period of time.

This will be clearly outlined in the information sheet but will not be used explicitly to coerce individuals into taking part.

62. What special arrangements are in place for eliciting informed consent from participants who may not adequately understand verbal explanations or written information provided in English; where participants have special communication needs; where participants have limited literacy; or where children are involved in the research? (Do not exceed 200 words)

Part of the inclusion criteria are to be fluent in English. Nonetheless, the researcher will ensure that participants are fully aware and understand what the study involves and what their participation entails. Each participant will be invited to ask questions before giving informed consent. Participants will be provided with a copy of the information sheet via e-mail or post, to allow time for participants to understand and absorb the content prior to testing sessions.

There will be no children or under 18s involved in the research.

RISK ASSESSMENT AND RISK MANAGEMENT

63. Does the proposed research involve any of the following? *(Tick as appropriate)*

☒ use of a questionnaire, self-completion survey or data-collection instrument (attach copy)

☐ use of emails or the internet as a means of data collection

☐ use of written or computerised tests

☒ interviews (attach interview questions)

☒ diaries (attach diary record form)

☒ participant observation

☐ participant observation (in a non-public place) without their knowledge / covert research

☐ audio-recording interviewees or events

☒ video-recording interviewees or events

☐ access to personal and/or sensitive data (i.e. student, patient, client or service-user data) without the participant's informed consent for use of these data for research purposes

☐ administration of any questions, tasks, investigations, procedures or stimuli which may be experienced by participants as physically or mentally painful, stressful or unpleasant during or after the research process

☐ performance of any acts which might diminish the self-esteem of participants or cause them to experience discomfiture, regret or any other adverse emotional or psychological reaction

☐ investigation of participants involved in illegal or illicit activities (e.g. use of illegal drugs)

☐ procedures that involve the deception of participants

☒ administration of any substance or agent **(yes, but NOTE: all participants will control their own level of nicotine intake and will be regular smokers who are therefore exposed to nicotine on a daily basis so are fully aware and tolerant of any potential adverse effects).**

☐ use of non-treatment of placebo control conditions

☒ collection of body tissues or fluid samples **(Non-invasive, non-painful salivary cotinine test to measure participants' cotinine (a by-product of nicotine) levels**

☐ collection and/or testing of DNA samples

☐ collection and/or testing of gametes or embryo tissue

☐ participation in a clinical trial

☐ administration of ionising radiation to participants

☒ research undertaken at an off-campus location (risk assessment attached)

☐ research overseas (copy of VCG overseas travel approval attached)

64. Does the proposed research involve any specific or anticipated risks (e.g. physical, psychological, social, legal or economic) to participants that are greater than those encountered in everyday life? YES ☒ NO ☐
If **YES**, please describe below including details of precautionary measures.

Potential risks and hazards:

- Given that saliva is a potentially hazardous source of infective hepatitis, there is a minimal risk that the researcher might get in contact with saliva samples.
- It is unlikely that participants experience any discomfort from the required period of abstinence, given it is only one hour.
- Small risk of side effects associated with nicotine delivery via e-cigarette use including, nausea, headache, dizziness, and mouth/throat irritation.

Precautions which will be taken:

- Gloves will be worn during handling of saliva samples. All saliva samples will be taken by participants themselves, these will be sealed then placed straight in a centrifugation tube then in a bag before being handed to the researcher.
- Refreshment and a glass of water will be offered to participants upon arrival and throughout the entire duration of the experiment. However, given that all participants will be smokers thus accustomed to the use of nicotine and be given nicotine concentrations that are below or equal to their habitual ones, adverse effects are unlikely to occur. Note, as part of the study's procedures, participants will be asked to use the device ad libitum (i.e. as much as they desire), thus potential risks associated with adverse effects are not greater than those encountered in their everyday lives. In the event that adverse effects do occur, it is likely that they will be short-lived. In the unlikely event that ill-effects are reported, participants will be strongly recommended to stop using the device, and they can rest in a calm and quiet location with the researcher by his/her side should they wish to do so. If required, a taxi home

will be offered when the participant has been assessed to be well enough to leave the premises.

65. Where the procedures involve potential hazards and/or discomfort or distress for participants, please state what previous experience the investigator or researcher(s) have had in conducting this type of research.

As stated above, in the event of hazards and/or discomfort, participants will be advised to stop using the device and be reminded that they are not obliged to continue with the study.

Dr Lynne Dawkins, the principle investigator and Director of Studies has over seventeen years of experience of working with smokers in a research capacity and six years of experience of working with electronic cigarettes. She has published extensively in various peer-reviewed journals work on smoking addiction and electronic cigarettes, and is regarded as one of the UK's leading authorities on e-cigarettes. Dr Lynne Dawkins has been awarded the Good Clinical Practise certificate.

The PhD student and researcher Catherine Kimber has recently passed the UEL Ethic and Research Integrity course and attended the Risk assessment and Laboratory Safety training session provided by the HSB school on 1st July 2015. She has conducted her undergraduate project on e-cigarette use and choice in smokers with Dr Dawkins, which was published in a peer-reviewed journal article (Dawkins, Kimber, Puwanesarasa & Soar, 2015). In addition, she completed a research internship at UEL with Dr Lynne Dawkins, conducting a project on the effects of nicotine on smokers' cognitive functioning, thus involving smokers recruitment. She conducted a pilot study which received full ethics approval by UREC, involving the recruitment of e-cigarette users. She also directed the recruitment of e-cigarette users and was involved in a study involving blood samples collection from e-cigarette users. Thus, she currently has 2 years of experience working with smokers and e-cigarette users.

The PhD student Catherine Kimber will be conducting the individual testing sessions with participants, thus will be at risk of exposure to the biohazardous fluids saliva samples. The researcher has received Hepatitis B vaccinations and will ensure to wear gloves at all times when handling samples.

66. Provide an explanation of any potential benefits to participants. Please ensure this is framed within the overall contribution of the proposed research to knowledge or practice. (Do not exceed 400 words)
NOTE: Where the proposed research involves students of our University, they should be assured that accepting the offer to participate or choosing to decline will have no impact on their assessments or learning experience. Similarly, it should be made clear to participants who are patients, service-users and/or receiving any form of treatment or medication that they are not invited to participate in the belief that participation in the research will result in some relief or improvement in their condition.

Taking part in this study could be greatly beneficial to participants. Indeed, evidence from observational studies and randomised control trials (Bullen et al., 2014; Bullen et al., 2013; Caponnetto et al., 2013; McRobbie et al., 2014) suggests that for many unable to quit with the use of NRTs, e-cigarettes have significantly helped reduce their cigarette consumption and achieve complete cessation (Brown et al., 2014; Bullen, Howe, & Laugesen, 2014; Caponnetto et al., 2015; McRobbie, Bullen, Hartmann-Boyce, & Hajek, 2014; Polosa et al., 2011). One study found that 22% of a sample of smokers (N = 477) using e-cigarettes had stopped smoking after one month and 46% (N = 367) following a year (Etter & Bullen, 2014). Others report significant improvement in asthmatic smokers' respiratory systems following a switch from cigarettes to e-cigarettes (Polosa et al., 2014). Moreover, a recent report by PHE suggests a) the decline in smoking prevalence to be attributed to the increase in e-cigarettes use and b) smokers unable to stop smoking should be encouraged to use e-cigarette (McNeill et al, 2015).

Altogether, the outcomes for each participant (cigarette reduction and/or stop smoking) could be greatly beneficial to their health. Indeed, as a consequence of taking part, it is likely that 20 to 40% of this sample will have quit smoking or at least halved their cigarette consumption thereby improving their overall health and potentially save lives. However, the information sheet states that if participants are patients, service-users and/or receiving any form of treatment or medication, they are

advised that although e-cigarette use may increase the likelihood of a positive outcome in terms of cigarette consumption reduction, they are not invited to hold the belief that participation in the research guarantees such an outcome. Equally, where participant is a student, participation, non-participation and withdrawal will not impact their learning experience or assessments at UEL.

The information sheet states participants are not obliged to take part. If they decide to do so, they have the right to withdraw at any time with no need to provide an explanation.

Although some of the participants taking part may be known to the researcher, most of these are external to UEL and none are in a dependent relationship with the researcher.

67. Provide an outline of any measures you have in place in the event of adverse or unexpected outcomes and the potential impact this may have on participants involved in the proposed research. (Do not exceed 300 words)

Participants will be submitted to salivary cotinine testing which involves a non-invasive procedure whereby the subject is instructed to gently place a swab in the mouth for a few minutes until saturated to take a saliva sample. Since there are potential risks of hepatitis associated with being in contact with infectious human tissue, gloves will be worn during the handling of the sample. Moreover, to further minimize risks, samples will be placed in a small bag before being handed to the researcher.

Potential but minimal effects that have been associated with the use of e-cigarettes are nausea, headache, dizziness, and mouth/throat irritation.

A glass of water will be offered to participants upon arrival and throughout the entire duration of the experiment. However, given that all participants will be smokers thus accustomed to the use of nicotine and be given nicotine concentrations that are below or equal to their habitual ones, adverse effects are unlikely to occur. Note, as part of the study's procedures, participants will be asked to use the device

ad libitum (i.e. as much as they desire), thus potential risks associated with adverse effects are not greater than those encountered in their everyday lives. In the event that adverse effects do occur, it is likely that they will be short-lived. In the unlikely event that ill-effects are reported, participants will be strongly recommended to stop using the device, and they can rest in a calm and quiet location with the researcher by his/her side should they wish to do so. If required, a taxi home will be offered when the participant has been assessed to be well enough to leave the premises.

Prior to the commencement of each testing sessions, checks will be run to minimize risks of equipment malfunction (E-cigarettes, batteries, Camera and Smokerlyzer). Participants will be given the e-cigarette with verbal and written instructions on how to use the device. Participants will be issued with contacts for technical support.

68. Provide an outline of your debriefing, support and feedback protocol for participants involved in the proposed research. This should include, for example, where participants may feel the need to discuss thoughts or feelings brought about following their participation in the research. This may involve referral to an external support or counseling service, where participation in the research has caused specific issues for participants. Where medical aftercare may be necessary, this should include details of the treatment available to participants. Debriefing may involve the disclosure of further information on the aims of the research, the participant's performance and/or the results of the research. (Do not exceed 500 words)

Given the behaviours we are observing in study 2 will be used as predictors of smoking cessation for study 3, we will not disclose the hypotheses of the study to participants until the very end of study 3 (12 month follow up). However, debriefing may be earlier for some participants if they have stopped using the e-cigarettes completely and relapsed to smoking. Debriefing will be done over the phone. Participants will be thanked and debriefed verbally vis-à-vis the rationale and the expected impacts of this research in the wider academic, clinical and regulatory communities. Participants will be verbally informed of the aims of the study – i.e. a) to look at any changes in puffing patterns and blood nicotine levels as the user acquires more experience using the device and how using a lower nicotine concentration and different types of device influence puffing patterns; b) to explore factors which influence quitting behaviour. Participants will be invited to ask questions about their participation and other questions they may have regarding their cigarette and e-cigarette use. Participants will also be given the principle

investigator's e-mail address and telephone number should they have any further queries at a later date.

PARTICIPANT CONSENT AND WITHDRAWAL

69. Have you attached a copy of your participant information sheet (this should be in *plain English*)? Where the research involves non-English speaking participants, please include translated materials. YES☒ NO ☐
If **NO**, please indicate what alternative arrangements are in place below:

70. Have you attached a copy of your participant consent form (this should be in *plain English*)? Where the research involves non-English speaking participants, please include translated materials.
YES ☒ NO ☐
If **NO**, please indicate what alternative arrangements are in place below:

71. The following is a <u>participant information sheet</u> checklist covering the various points that should be included in this document.

☒ Clear identification of UEL as the sponsor for the research, the schools(s) involved, the project title, the Principal Investigator and other researchers along with relevant contact details.

☒Details of what involvement in the proposed research will require (e.g., participation in interviews, completion of questionnaire, audio/video-recording of events), estimated time commitment and any risks involved.

☒A statement confirming that the research has received formal approval from UREC.

☒ If the sample size is small, advice to participants that this may have implications for confidentiality / anonymity.

N/A A clear statement that where participants are in a dependent relationship with any of the researchers that participation in the research will have no impact on assessment / treatment / service-use or support.

☒ Assurance that involvement in the project is voluntary and that participants are free to withdraw consent at any time, and to withdraw any unprocessed data previously supplied.

☒Advice as to arrangements to be made to protect confidentiality of data, including that confidentiality of information provided is subject to legal limitations.

☒A statement that the data generated in the course of the research will be retained in accordance with the University's Data Protection Policy.

☒Advice that if participants have any concerns about the conduct of the investigator, researcher(s) or any other aspect of this research project, they should contact researchethics@uel.ac.uk.

☒Confirmation on any limitations in confidentiality where disclosure of imminent harm to self and/or others may occur.

N/A. For research involving under 16 s or vulnerable groups, where true, a statement has been included on all information sheets that the investigators have passed appropriate Disclosure and Barring Service checks.

72. The following is a consent form checklist covering the various points that should be included in this document.

☒University of East London letterhead or logo.

☒Title of the project (with research degree projects this need not necessarily be the title of the thesis) and names of investigators.

☒Confirmation that the project is research.

☒Confirmation that involvement in the project is voluntary and that participants are free to withdraw at any time, or to withdraw any unprocessed data previously supplied.

☒Confirmation of particular requirements of participants, including for example whether interviews are to be audio-/video-recorded, whether anonymised quotes will be used in publications advice of legal limitations to data confidentiality.

☒ If the sample size is small, confirmation that this may have implications for anonymity any other relevant information.

☒The proposed method of publication or dissemination of the research findings.

N/A. Details of any external contractors or partner institutions involved in the research.

N/A. Details of any funding bodies or research councils supporting the research.

N/A Confirmation on any limitations in confidentiality where disclosure of imminent harm to self and/or others may occur.

N/A. Separate forms and information sheets requesting parental/guardian consent for research involving under age children have been included / attached.

N/A. Letters requesting consent for the research to be conducted at external sites (e.g. Managers/ owners/ head teachers /etc.) where the research will be carried out have been included / attached.

☒Where research is to be published (it is envisaged that this is true in most cases) it has been made clear in the information sheet and, or form (preferably both) that both consent to take part and to publication is being obtained.

CONFIDENTIALITY AND ANONYMITY

73. Below is a checklist covering key points relating to the confidentiality and anonymity of participants. Please indicate where relevant to the proposed research.

☐ Participants will be completely anonymised and their identity will not be known by the investigator or researcher(s) (i.e. the participants are part of an anonymous randomised sample and return responses with no form of personal identification)?

☐ The responses are anonymised or are an anonymised sample (i.e. a permanent process of coding has been carried out whereby direct and indirect identifiers have been removed from data and replaced by a code, with no record retained of how the code relates to the identifiers).

☒The samples and data are de-identified (i.e. direct and indirect identifiers have been removed and replaced by a code. The investigator or researchers are able to link the code to the original identifiers and isolate the participant to whom the sample or data relates).

☐ Participants have the option of being identified in a publication that will arise from the research.

☐ Participants will be pseudo-anonymised in a publication that will arise from the research.

☐ The proposed research will make use of personal sensitive data.

☐ Participants consent to be identified in the study and subsequent dissemination of research findings and/or publication.

74. Participants must be made aware that the confidentiality of the information they provide is subject to legal limitations in data confidentiality (i.e. the data may be subject to a subpoena, a freedom of information request or mandated reporting by some professions). This only applies to named or de-identified data. If your participants are named or de-identified, please confirm that you will specifically state these limitations.

YES ☒ **NO** ☐

If **NO**, please indicate why this is the case below:

NOTE: WHERE THE PROPOSED RESEARCH INVOLVES A SMALL SAMPLE OR FOCUS GROUP, PARTICIPANTS SHOULD BE ADVISED THAT THERE WILL BE DISTINCT LIMITATIONS IN THE LEVEL OF ANONYMITY THEY CAN BE AFFORDED.

DATA ACCESS, SECURITY AND MANAGEMENT

75. Will the Principal Investigator be responsible for the security of all data collected in connection with the proposed research? YES ☒ **NO** ☐

If **NO**, please indicate what alternative arrangements are in place below:

76. In line with the 5th principle of the Data Protection Act (1998), which states that personal data shall not be kept for longer than is necessary for that purpose or those purposes for which it was collected; please state how long data will be retained for.

☒ 1-2 years ☐ 3-5 years ☐ 6-10 years ☐ 10> years

NOTE: Research Councils UK (RCUK) guidance currently states that data should normally be preserved and accessible for 10 years, but for projects of clinical or major social, environmental or heritage importance, for 20 years or longer. (http://www.rcuk.ac.uk/documents/reviews/grc/grcpoldraft.pdf)

77. Below is a checklist which relates to the management, storage and secure destruction of data for the purposes of the proposed research. Please indicate where relevant to your proposed arrangements.

☒Research data, codes and all identifying information to be kept in separate locked filing cabinets.

☒Access to computer files to be available to research team by password only.

☐ Access to computer files to be available to individuals outside the research team by password only (See **23.1**).

☐ Research data will be encrypted and transferred electronically within the European Economic Area (EEA).

☐ Research data will be encrypted and transferred electronically outside of the European Economic Area (EEA). (See **23.2**).

NOTE: Transfer of research data via third party commercial file sharing services, such as Google Docs and YouSendIt are not necessarily secure or permanent. These systems may also be located overseas and not covered by UK law. If the system is located outside the European Economic Area (EEA) or territories deemed to have sufficient standards of data protection, transfer may also breach the Data Protection Act (1998).

☒Use of personal addresses, postcodes, faxes, e-mails or telephone numbers.

☒Use of personal data in the form of audio or video recordings.

☐ Primary data gathered on encrypted mobile devices (i.e. laptops). **NOTE:** This should be transferred to secure UEL servers at the first opportunity.

☒All electronic data will undergo secure disposal.

NOTE: For hard drives and magnetic storage devices (HDD or SSD), deleting files does not permanently erase the data on most systems, but only deletes the reference to the file. Files can be restored when deleted in this way. Research files must be overwritten to ensure they are completely irretrievable. Software is available for the secure erasing of files from hard drives which meet recognised standards to securely scramble sensitive data. Examples of this software are BC Wipe, Wipe File, DeleteOnClick and Eraser for Windows platforms. Mac users can use the standard 'secure empty trash' option; an alternative is Permanent eraser software.

☒All hardcopy data will undergo secure disposal.

NOTE: For shredding research data stored in hardcopy (i.e. paper), adopting DIN 3 ensures files are cut into 2mm strips or confetti like cross-cut particles of 4x40mm. The UK government requires a minimum standard of DIN 4 for its material, which ensures cross cut particles of at least 2x15mm.

77.1. Please provide details of individuals outside the research team who will be given password protected access to encrypted data for the proposed research.

N/A
77.2. Please provide details on the regions and territories where research data will be electronically transferred that are external to the European Economic Area (EEA).
N/A

OVERSEAS TRAVEL FOR RESEARCH

78. Does the proposed research involve travel outside of the UK? YES ☐ NO ☒ **78.1.** Have you consulted the Foreign and Commonwealth Office website for guidance/travel advice? http://www.fco.gov.uk/en/travel-and-living-abroad/ **YES** ☐ **NO** ☐ **78.2.** If you are a non-UK national, have you sought travel advice/guidance from the Foreign Office (or equivalent body) of your country? **YES** ☐ **NO** ☐ **NOT APPLICABLE** ☐ **78.3.** Have you completed the overseas travel approval process and enclosed a copy of the document with this application? **YES** ☐ **NO** ☐ Details on this process are available here http://www.uel.ac.uk/qa/research/fieldwork.htm

78.4. Is the research covered by our University's insurance and indemnity provision?

YES ☐ **NO** ☐

(Please seek confirmation via researchethics@uel.ac.uk)

NOTE: Where research is undertaken at an off-campus location within the UK or overseas, the Risk Assessment policy must be consulted:

http://dl-cfs-01.uel.ac.uk/hrservices/documents/hshandbook/risk_assess_policy.pdf.

The Dean of School or Director of Service has overall responsibility for risk assessment regarding the health and safety of staff or students conducting research where UEL is the sponsor.

78.5. Please evidence how compliance with all local research ethics and research governance requirements have been assessed for the country(ies) in which the research is taking place.

78.6. Will this research be financially supported by the United States Department of Health and Human Services or any of its divisions, agencies or programs? **YES** ☐ **NO** ☐

PUBLICATION AND DISSEMINATION OF RESEARCH FINDINGS

79. How will the results of the research be reported and disseminated? ***(Select all that apply)***

☒ Peer reviewed journal

☒ Conference presentation

☐ Internal report

☒ Dissertation/Thesis

☐ Other publication

☐ Written feedback to research participants

☒ Presentation to participants or relevant community groups

☐ Other (Please specify below)

OTHER ETHICAL ISSUES

80. Are there any other ethical issues that have not been addressed which you would wish to bring to the attention of University Research Ethics Committee (UREC)?

No

CHECKLIST FOR ATTACHED DOCUMENTS

81. Please check that the following documents are attached to your application.

☒ Recruitment advertisement

☒ Participant information sheets (including easy-read where relevant)

☒ Consent forms (including easy-read where relevant)

☐ Assent form for children (where relevant)

☐ Evidence of any external approvals needed

☒ Questionnaire

☐ Interview Schedule or topic guide

☒ Risk assessment (where applicable)

☐ Overseas travel approval (where applicable)

81.1. Where it is not possible to attach the above materials, please provide an explanation below.

Recruitment will be via social media sites such as twitter/facebook. The researcher will seek support from all UEL available channels to reach out to smokers. For example advertising through InFocus and Universe newsletters, Plasma screens and Student Union webpages, etc. In addition, flyers will be distributed at various points throughout London in and outside the university.

The recruitment advertisement materials will be based on the following text:

Smokers wanted for a study on e-cigarettes at UEL

- *Researchers at UEL are exploring the potential of e-cigarettes as smoking cessation aids.*
- *The study involves 3 sessions in which you will be asked to use an e-cigarette whilst being video-recorded to measure your puffing patterns.*
- *You will be given the e-cigarette and ask to report your use for a period 4 weeks.*
- *To take part you must be:*
    - *Regular smoker (≥ 5 cigarettes/day for at least 1 year)*
    - *18 or over*
    - *Be willing to try and quit smoking and use an e-cigarette for a period of at least 2 weeks*
    - *Be willing to provide saliva samples and report on your cigarette and e-cigarette use daily for up to 4 weeks*

    - *If interested contact the researcher:*
- *Catherine Kimber at u0951767@uel.ac.uk or C.kimber@uel.ac.uk; 020 8223 4592 leaving your name and contact details. Find me on Twitter at @Cat_kimber*

APPROVAL LETTER

14 October 2015

Dear Catherine

Project Title: Cigalikes Versus Tank systems: Effects of Users' experience, Device Characteristics and Nicotine Concentration During a Quit Attempt

Principal Investigator: Dr Lynne Dawkins Researcher

Catherine Kimber Reference Number: UREC 1516 04

I am writing to confirm the outcome of your application to the University Research Ethics Committee (UREC), which was considered by UREC on Wednesday 16th September 2015.

The decision made by members of the Committee is Approved. The Committee's response is based on the protocol described in the application form and supporting documentation. Your study has received ethical approval from the date of this letter.

Should any significant adverse events or considerable changes occur in connection with this research project that may consequently alter relevant ethical considerations, this must be reported immediately to UREC. Subsequent to such changes an Ethical Amendment Form should be completed and submitted to UREC.

Approved Research Site

I am pleased to confirm that the approval of the proposed research applies to the following research site:

Research Site Principal Investigator / Local Collaborator

University of East London premises

Dr Lynne Dawkins

Approved Documents

The final list of documents reviewed and approved by the Committee is as follows:

Document Version Date

UREC application form 2.0

08 October 2015

Participant information sheet 2.0

08 October 2015

Consent form 1.0

27 August 2015

Baseline characteristic questionnaire 1.0

27 August 2015

Smoking history questionnaire 1.0

27 August 2015

Fagerström Test of Nicotine Dependence 1.0

27 August 2015

MPSS questionnaire 1.0

27 August 2015

Diary – E-cigarette use and Smoking behaviour 1.0

27 August 2015

Study 3 - Follow-up Interview questions 1.0

27 August 2015

Subjective effects questionnaire 1.0

27 August 2015

Approval is given on the understanding that the UEL Code of Good Practice in Research is adhered to.

The University will periodically audit a random sample of applications for ethical approval, to ensure that the research study is conducted in compliance with the consent given by the ethics Committee and to the highest standards of rigour and integrity.

Please note, it is your responsibility to retain this letter for your records.

With the Committee's best wishes for the success of this project.

Yours sincerely,

Rosalind Eccles

University Research Ethics Committee (UREC)

UREC Servicing Officer

Email: researchethics@uel.ac.uk

RISK ASSESSMENT FOR STUDY 2

University of East London
School of Psychology

Risk assessment for testing participants

Puffing Patterns in Electronic Cigarettes: Self-titration of Nicotine

	Testing Location(s)	Possible Risk	Severity of hazard (H, M, L)	Likelihood of hazard (H, M, L)	Risk (H, M, L)	Mitigating Activity
1.	UEL clinical education building, Stratford campus	Participants experience adverse effect of nicotine	Low	Low	Low	Very unlikely as participants will be given their OWN STRENGTH and a LOWER strength nicotine e-liquid and using *ad libitum.* However, should a participant feel any adverse effects, s/he will be advised to stop using the e-cigarette immediately and the nurse and one of the researchers will stay with the participant until s/he feels better. Effects usually wear off within an hour. If needed, participant will be offered a taxi home.
2.	UEL clinical education building, Stratford campus	It is possible that blood samples could be spilt or dropped.	Low	Low	Low	All care will be taken to avoid this by ensuring that all the correct equipment is in place and is easily accessible. Only the qualified phlebotomy nurse and those with full Hepatitis B vaccination will

						handle blood samples.
4.	UEL clinical education building, Stratford campus	Participants might feel faint				All participants will be screened for previous history of feeling faint or fainting during blood samples collection. Participants will be asked to eat a substantial breakfast on the morning prior to each testing session. A telephone emergency services will be accessible and a first aider will be present throughout the duration of the experiment to intervene if need be.
5.	UEL clinical education building, Stratford campus	E-liquid spill				The device we have chosen is very easy to re-fill and the investigator and researcher have extensive experience in using e-liquid refills. We will only use 5ml of liquid at a time. In the case of accidental spillage or contact with the skin, the participant/researcher will use the sink in the laboratory to wash their hands immediately. No reports of adverse effects relating to exposure of this concentration and amount of e-liquid to

						the skin have been reported.

APPENDIX 17 - PARTICIPANT INVITATION LETTER

UNIVERSITY OF EAST LONDON

School of Psychology

Stratford Campus

Water Lane

London E15 4LZ

The Principal Investigator(s)

Dr Kirstie Soar; E-mail: k.soar@uel.ac.uk; tel: 020 8223 4082

Dr. Lynne Dawkins; E-mail: l.e.dawkins@uel.ac.uk ; tel: 020 8223 4421

Catherine Kimber; E-mail: C.Kimber@uel.ac.uk; tel: 020 8223 4592

Professor Olivia Corcoran; E-mail: o.corcoran@uel.ac.uk; tel: 020 8223 4034

Consent to Participate in a Research Study

The purpose of this letter is to provide you with the information that you need to consider in deciding whether to participate in a research study being conducted at the University of East London.

Project Title

Cigalikes Versus Tank systems: Effects of Users' experience, device characteristics and Nicotine Concentration during a Quit Attempt

Project Description

This study aims to compare e-cigarette puffing behaviour, nicotine delivery and quitting behaviour with the use of various e-cigarette brands and nicotine strengths. If you are eligible to take part, we would like to see you on three occasions for approximately 1 hour each time (day 1 and after 1 and 2 weeks). You will be asked to refrain from smoking and any nicotine use up to one hour prior to each session.

During the first testing session, you will be given an electronic cigarette to keep. This will be either 1) a 'cigarette-like' device plus 3 weeks supply of refill cartridges (18mg/ml); 2) a 'curve-mini' e-cigarette with 18mg/ml refill bottles; or 3) a 'curve-mini' e-cigarette with 6mg/ml refill bottles. Allocation to one of the three conditions will be randomly determined. We request that you use the device that we have provided for at least the first three weeks of the study, using your e-cigarette to replace as many tobacco cigarettes as you can with the aim of stopping smoking completely. After 3 weeks, you are free to use other electronic cigarettes or nicotine products or to change the flavour/strength of cartridges/e-liquid used but we will ask you to report this information to us.

On each of the three testing days, you will first be asked to use a smokerlyser which measures your carbon monoxide levels indicating how much you have smoked in the last 24 hours. This is a simple, non-invasive breath test which simply requires you to hold your breath and then breathe slowly into a tube. You will also be asked to provide a saliva sample which will be used to measure how much nicotine you are absorbing. You will then be asked to provide some background information about yourself and your smoking history and complete a brief questionnaire measuring craving, mood and physical symptoms. We will then ask you to use the e-cigarette for 20 minutes, using it as much or as little as you want during that time. We will video-record you doing this in order to capture your puffing patterns (i.e. puff frequency and duration), after which, you will complete 2 questionnaires about your mood state and experience of using the e-cigarette. Please note, you are free to take comfort breaks during this time.

At the end of the first testing session, you will be given a diary in which you will be asked to keep a record of your cigarette and e-cigarette use for the next 2 weeks. We will then arrange an appointment to see you again after 1 and 2 weeks at which point we will ask you more questions about your experience, smoking status and to video-record you using the e-cigarette again. Also, please keep all your empty cartridges or bottles and return them to us as we will use these to measure how much nicotine you consumed.

After the 2 week session, you can keep the e-cigarette and continue to use it to help you stop smoking or to help you not to relapse to smoking. We would like to contact you again at 4 week, 3 months and 6 months via telephone to ask you about your smoking status and e-cigarette use.

Study Eligibility and exclusion criteria

<u>To take part you must</u>:

- Be a regular smoker (i.e. smoke more than 5 cigarettes/day for at least 1 year)
- Fluent in written and spoken English
- aged 18 or over
- Willing to use an e-cigarette for up to 4 weeks to try and quit smoking.
- Willing to provide saliva sample and report on cigarette and e-cigarette use daily for 4 weeks
- available and willing to participate in three one-hour sessions at UEL, Stratford
- Willing to provide a contact telephone number and e-mail address and to agree to be contacted at 4 weeks, 3 and 12 months to report on your smoking status and e-cigarette use

<u>You cannot participate, if you</u>:

- are a non-smoker
- are under 18 years of age
- not fluent in written and spoken English
- are a current e-cigarette user, have used an e-cigarette on more than 5 occasions in the past or for more than a week continuously
- have any neurobiological or heart conditions
- are pregnant or a lactating female

Confidentiality of the Data

All data generated in the course of the research will be treated confidentially and will be retained in accordance with the University's Data Protection Policy and personal data will be kept in accordance with the Data Protection Act. All data will be numerically coded, consent forms will be detached and separated from the numerically-coded data and your questionnaire data and video recordings will be stored in a locked filing cabinet at UEL, analysed by two independent researchers at UEL, entered into a dataset then discarded securely. The anonymised data will be used for the current study only. It is aimed that the findings will be written up for publication in a peer reviewed journal and might be presented at conferences. Should any data be used in subsequent studies or for further analysis, the data will still be kept anonymous and confidential. Nevertheless, please be aware that the confidentiality of the information provided is subject to legal limitations in data confidentiality (i.e. the data may be subject to a subpoena, a freedom of information request or mandated reporting by some professions).

Providing Body fluids: Saliva Samples

The study involves salivary cotinine testing which is a non-invasive procedure whereby you will be instructed to gently place a swab in your mouth for a few minutes until

saturated. Saliva samples will be stored at the University of East London before being transported to the Advanced Bioanalysis Service Laboratories Ltd., Welwyn Garden City, UK, for analysis of the nicotine content. Samples will not be used for any other purposes or studies. Once saliva cotinine levels from all samples have been determined and recorded electronically, all samples will be disposed of in accordance MHRA, GLP (Good Laboratory Practise) and GCP (Good Clinical Practise) accreditation protocols.

Location

The study will be conducted at the University East London, Stratford Campus in our research lab on the ground floor of the Arthur Edward building or at a mutually agreed location that is suitable.

Remuneration

There is no remuneration for taking part in the study, although, you will be given an e-cigarette device plus sufficient nicotine e-liquid refill bottle/cartridges to use for the first 1-2 weeks of the study.

Note, if you are a student, your participation, non-participation or later withdrawal will have no impact on your assessments (i.e.: no module credits involved) and learning experience at UEL. Equally, if you are a patient, service-user and/or receiving any form of treatment or medication, be advised that although e-cigarette use may increase the likelihood of a positive outcome in terms of cigarette consumption reduction, do not hold the belief that participation in the research guarantees such outcome.

Disclaimer

You are not obliged to take part in this study and should not feel coerced. You are free to withdraw at any time. Should you choose to withdraw from the study you may do so without disadvantage to yourself and without any obligation to give a reason. Your right to withdraw from the study includes the right to request the destruction of any of your data obtained from the study though you must memorise your personal participant number in order for the experimenter to delete your data. You can email us at any point prior stating your participation number, and we will remove your data for you.

Following completion of the study, none of your contact information will be retained without your specific permission.

Please feel free to ask any questions. If you are happy to continue you will be asked to sign a consent form prior to your participation. Please retain this invitation letter for reference.

This study has received full approval from the University of East London's Ethical Committee. If you have any questions or concerns about how the study has been conducted, please contact the study's Principle Investigator:

Dr. Kirstie Soar, School of Psychology, University of East London, Water Lane, London E15 4LZ. +44 (0)20 8223 4082. E-mail: k.soar@uel.ac.uk

Or

Catherine Fieulleteau, Ethics Integrity Manager, Graduate School, EB 1.43

University of East London, Docklands Campus, London E16 2RD

(Telephone: 020 8223 6683, Email: researchethics@uel.ac.uk).

APPENDIX 18 - CONSENT FORM

UNIVERSITY OF EAST LONDON

Consent to participate in a research study

Title

Cigalikes Versus Tank systems: Effects of Users' experience, device characteristics and Nicotine Concentration during a Quit Attempt

I have read the information sheet relating to the above research study and have been given a copy to retain. The nature and purpose of the research has been explained to me, and I have had the opportunity to discuss the details and ask questions about this information. I understand what is being proposed and the procedures in which I will be involved have been explained to me. I understand that I am able to take breaks whenever I want and that I am free to leave at any time.

I understand that my involvement in this study, and particular data from this research, will remain strictly confidential. Only the researcher(s) involved in the study will have access to identifying data. I understand that the findings from this research project may be written up, submitted for publication and presented at conferences. I understand that all collected data will remain confidential during this process. It has been explained to me what will happen once the research study has been completed.

I hereby freely and fully consent to participate in the study which has been fully explained to me. Having given this consent, I understand that I have the right to withdraw from the study at any time without disadvantage to myself and without being obliged to give any reason.

Participant's Name (BLOCK CAPITALS)

…….

Participant's Signature

……..

Researcher's Name (BLOCK CAPITALS)

……..

Researcher's Signature

……

Date: ……..…….

APPENDIX 19 - SMOKING HISTORY (STUDY 2)

Participant number: _____
Session number: _____
Date: _____
Condition allocated: _____

1. How long have you been smoking? …….

2. How many cigarettes do you smoke per day? …….

3. Have you tried to quit smoking before? ☐ Yes ☐ No

   i. If yes, how many times have you tried? …..
   ii. If yes, did you use a smoking cessation aid? ☐ Yes ☐ No
      a. Please state which type ……..

4. Which of the following describes you?

☐ I don't want to stop smoking ☐ I think I should stop smoking but don't really want to☐ I want to stop smoking but haven't thought about when ☐ I REALLY want to stop smoking but I don't know when I will ☐ I want to stop smoking and hope to soon ☐ I REALLY want to stop smoking and intend to in the next 3 months ☐ I REALLY want to stop smoking and intend to in the next month ☐ I don't know

5. How confident are you in succeeding in your quit attempt?

☐ Not at all confident ☐ A little confident ☐ Moderately confident ☐ Fairly confident ☐ Extremely confident

6. Have you tried an electronic cigarette in the past?

☐ Yes ☐ No

   i. If yes, how many times? …..
   ii. What e-cigarette model did you try? ☐ Cigalike ☐ Later generation ☐ Both
   iii. If applicable, add a quick statement on why you did not sustain the use of e-cigarette (eg. Did not like it, I wanted to try out of curiosity eg. I tried somebody else's)
   ……

APPENDIX 20 - FAGERSTRÖM TEST FOR CIGARETTE DEPENDENCE

Participant number: _____
Session number: _____
Date: _____
Condition allocated: _____
CO levels: _____

Please answer each question by ticking the response with which you agree most

1. How soon after you wake up do you smoke your first cigarette?

Within 5 minutes	□
6 - 30 minutes	□
31 - 60 minutes	□
After 60 minutes	□

2. Do you find it difficult to refrain from smoking in places where it is forbidden, e.g. in church, at the library, in cinema etc.?

Yes □ No □

3. Which cigarette would you hate most to give up?

□ The first one in the morning □ Any other one

4. How many cigarettes a day do you smoke?

10 or less	□
11 - 20	□
21 - 30	□
31 or more	□

5. Do you smoke more frequently during the first hours after waking than during the rest of the day?

Yes □ No □

6. Do you smoke if you are so ill that you are in bed most of the day?

□ Yes □ No

APPENDIX 21 - E-CIGARETTE USE AND SMOKING BEHAVIOUR DIARY

Participant number: _____
Condition allocated: _____
Week beginning: _____
Date of completion: _____

	Monday	Tuesday	Wednesday	Thursday	Friday	Saturday	Sunday
How many tobacco cigarettes have you smoked today?*							
Please try to estimate how many puffs you have taken on your e-cig today: **							
How many cartridges/ml of nicotine e-liquid have you used today?***							
How satisfying did you find your e-cigarette today (0-100%)?							
How pleasant was your e-cigarette today (0-100%)?							
How much did your e-cigarette reduce your craving for smoking							

today (0-100%)?							
How much of a 'hit' did you feel from the e-cigarette today (0-100%)?							

*Place a small vertical line inside the box every time you smoke a tobacco cigarette as shown below:

would mean you have smoked 7 cigarettes today

**Place a small vertical line inside the box every time you use your e-cigarette and add the numbers of puffs underneath the line as shown below:

would mean you have used your e-cigarette 4 times today and had 40 puffs overall.

***The tank has a 2 mL capacity so if you topped up your tank today and used half of it following the top up, you used approximately 3 mL.

APPENDIX 22 - FOLLOW-UP INTERVIEW QUESTIONS AT 1, 3 AND 6-MONTHS (STUDY 3)

Participant number: _____
Session number: _____
Date: _____
Condition allocated: _____

Cigarettes consumption

1. Have you smoked at all in the last 7 days?
   If YES. How many? ……

   i. How many cigarettes do you smoke per day?
   ……..

   b. If NO. When did you last smoke
   ……

iii. How did you quit smoking?
……
……
……

Have you used any smoking cessation aids (NRTs, Counselling) in the last 12 months?

1. If yes please state what type, when, how often you have used it

……

If you answered NO to Question I.1, answer I.1.b then go to Question II (e-cigarette use).

2. How soon after you wake up do you smoke your first cigarette?

Within 5 minutes ☐
6 - 30 minutes ☐
31 - 60 minutes ☐
After 60 minutes ☐

3. Do you find it difficult to refrain from smoking in places where it is forbidden, e.g. in church, at the library, in cinema etc.?

Yes □
No □

4. Which cigarette would you hate most to give up?

The first one in the morning □
Any other one □

5. Do you smoke more frequently during the first hours after waking than during the rest of the day?

Yes □
No □

6. Do you smoke if you are so ill that you are in bed most of the day? □ Yes □ No

E-cigarette use questionnaire

1. Are you still using the e-cigarette we provided?
   i. If no, when did you stop using the device?
   ……
   ii. Did you stop using the e-cigarette because? (Choose the option (only one) which feels the most relevant)
      a. Malfunction issues The e-cigarette required to be charged too often □
      b. Was not convenient – felt a burden □
      c. The e-cigarette was not satisfying □
      d. Urge to smoke was too great □
      e. I have upgraded to a better model □
      f. Other □
      ……
      ……
      ……
      ……

2. Which type of device do you currently use most (please circle one):

   a. A disposable e-cigarette (non-rechargeable)

b. A rechargeable e-cigarette (sometimes called ‘stick-like’, ‘cigarette-like’ or ‘cartomizers’ without fluid)
c. A rechargeable e-cigarette (‘stick-like/cigarette-like/cartomizer’ without fluid) starter kit
d. A rechargeable e-cigarette (non cigarette-like with fluid)
e. A rechargeable e-cigarette (non cigarette-like with fluid) starter kit
f. A modular system (your own combination of separate parts: battery, atomizer, fluid etc).
g. E-cigar

3. Which brand and model of e-cigarette are you currently using the most?

Brand (please state) _____
Model (please state)_____

4. Which strength(s) of nicotine fluid/cartridge are you currently using? (circle all that apply).
   a. 0mg
   b. 4 mg
   c. 6mg
   d. 8mg
   e. 10 mg
   f. 11mg
   g. 15 mg
   h. 16mg
   i. 18mg
   j. 24mg
   k. 36mg
   l. Higher than 36mg
   m. I don’t know

5. If you have circled more than one strength, please state which strength you use most: _____

6. Please try to estimate the amount you use per day:
   a. In puffs _____
   b. In cartridges _____
   c. In ml _____

7. Which is your preferred flavour? (please circle one)
   a. Tobacco
   b. Mint/menthol
   c. Fruit (various)
   d. Coffee

e. Vanilla
f. RY4
g. Chocolate
h. Cinnamon
i. Tea
j. Alcohol-related
k. Other (please state)

8. How soon after you wake up do you use your electronic cigarette?

    a. Within 5 minutes
    b. 6 – 30 minutes
    c. 31 – 60 minutes
    d. After 60 minutes

9. Do you find it difficult to refrain from using your electronic cigarette in places where it is forbidden (e.g. on the train, in a business meeting)

    a. Yes
    b. No

10. Which puffs on your e-cigarette would you hate most to give up?

    a. The first ones in the morning
    b. Any other one

11. Do you puff more frequently on your e-cigarette during the first hours after waking than during the rest of the day?

    a. Yes
    b. No

12. Do you use your e-cigarette if you are so ill that you are in bed most of the day?

    a. Yes
    b. No

13. Please rate your addiction to e-cigarettes on a scale of 0 to 100:_____

    I am NOT addicted at all = 0
    I am extremely addicted = 100

14. For you, stopping using the e-cigarette for good would be:

    a. Impossible
    b. Very difficult
    c. Fairly difficult
    d. Fairly easy
    e. Very easy

15. How much of the time have you felt the urge to ***vape (***i.e. use your e-cigarette) in the past week? *(Circle one number)*

All the time	Almost all the time	A lot of the time	Some of the time	A little of the time	Not at all
5	4	3	2	1	0

16. How strong have the urges been? *(Circle one number)*

Extremely strong	Very strong	Strong	Moderate	Slight	No urges
5	4	3	2	1	0

17. Since you started using the electronic cigarette, have you attempted to cut down the amount that you use it?

    a. Yes
    b. No

If YES: In your attempt to cut down your use of the electronic cigarette, how successful have you been?

    g. Extremely successful: I have stopped using it completely now
    h. Very successful: I only use it occasionally now
    i. Quite successful: I use it much less that I did initially
    j. Not very successful: I have only cut down slightly
    k. Unsuccessful: My use has not changed
    l. Very unsuccessful: I use it more than I did initially

18. Is it likely that in one month from now, you will have stopped using the electronic cigarette?

    a. Very likely
    b. Rather likely
    c. Rather unlikely
    d. Very unlikely

APPENDIX 23 - TABLES 3.6 AND 3.7 MEAN (SD) SUBJECTIVE EFFECTS STUDY 2

Table 3.6 *Mean (SD) per condition in positive effects associated with e-cigarette use.*

	Cigalikes		Tank 18		Tank 6	
	Mean (%)	SE	Mean (%)	SE	Mean (%)	SE
'Hit'	47.78	5.74	59.93	4.26	50.10	4.37
'Satisfaction'	42.77	4.12	61.75	3.06	59.29	3.14
'Pleasant'	46.55	6.86	55.46	5.08	64.53	5.22
'Tastes good'	45.02	6.62	46.59	4.91	50.84	5.07
'Reduced my craving'	51.07	5.43	62.36	4.03	58.71	4.14
'helped concentration'	36.83	6.44	41.13	4.77	41.18	4.90
'helped me feel calmer'	48.21	11.27	49.49	8.36	56.73	8.58
'feel more awake'	32.36	5.97	32.33	4.43	32.11	4.54
'reduced my hunger'	23.55	6.14	34.01	4.55	29.56	4.67
'tastes like usual brand/model'	22.52	6.08	22.68	4.51	22.05	4.62
'feels like using usual brand/model'	22.83	5.65	24.49	4.19	27.46	4.30
Overall mean	38.13	4.12	44.56	3.06	44.78	3.14

Table 3.7 *Mean (SD) per condition in adverse effects associated with e-cigarette use*

	Cigalikes		Tank 18		Tank 6	
	Mean (%)	SE	Mean (%)	SE	Mean (%)	SE
'Confused'	7.74	2.81	10.58	2.08	8.69	2.14
'Dizzy'	10.49	3.36	8.27	2.49	13.23	2.55
'Headache'	7.13	3.02	7.12	2.59	13.68	2.30
'Pounding heart'	7.80	2.45	8.13	2.10	9.57	1.86
'Light headed'	15.59	3.62	11.59	3.10	15.45	2.76
Nausea, feeling sick	5.55	2.34	7.93	2.01	11.14	1.78
'Nervous'	5.21	2.08	7.17	1.78	8.23	1.58
'Salivation'	10.21	3.06	9.56	2.62	12.65	2.33
'Sweaty'	5.64	2.41	6.28	2.07	9.73	1.84
'Weak'	10.89	3.39	9.88	2.91	10.35	2.58
'Mouth irritation'	8.98	2.21	9.07	1.90	9.43	1.68
'Throat irritation'	14.68	4.68	18.86	4.00	16.50	3.56
'Aching jaws'	6.58	2.89	7.24	2.48	9.86	2.20
'Vomiting'	5.56	1.65	6.53	1.41	7.29	1.25
'Flatulence/Bloating'	6.47	2.37	10.91	2.03	7.06	1.81
'Stomach ache'	4.84	1.72	6.15	1.47	6.83	1.31
'Heartburn'	7.17	2.11	6.21	1.81	7.50	1.61
'Diarrhoea'	4.66	2.24	8.91	1.92	6.09	1.70
'Hiccups'	4.30	1.59	6.26	1.36	5.99	1.21
'Cold hands feet'	5.88	3.19	6.32	2.73	12	2.43
'Palpitations'	5.49	2.01	7.35	1.72	7.13	1.53
Overall mean	7.67	2.16	11.60	1.60	9.92	1.64

APPENDIX 24 - PRESENTATIONS & CONFERENCES

- 22nd February 2019 "Predictors of Smoking Cessation in an E-Cigarette Quit Attempt: Device Type, Craving And Cigarette Dependence" at SRNT 25th Annual Meeting, Hilton San Francisco Union Square, San Francisco, California
- 8-9th November 2018 "Effects of Device type and Nicotine Concentrations on E-Cigarette Naïve Smokers' Puffing Topography in a Two-Week Smoking Reduction Attempt" Poster presentation at the Society for the Study of Addiction (SSA) at the Crowne Plaza Hotel, Newcastle, UK.
- 7th September 2018 "Changes in puffing topography in e-cigarette naïve smokers in the initial two weeks of a cessation attempt" Oral presentation at the Society for Nicotine and Tobacco Research Europe (SRNTe), Ludwig-Maximilians-University psychiatric clinic, Munich, Germany
- 23rd February 2018 "The Early Stage of a Smoking Cessation Attempt Using a cigalike Versus a Tank System E-cigarette: Effects on Subjective Symptoms" Poster presentation at the Society for Nicotine and Tobacco Research, The Hilton hotel, Baltimore, US.
- 8th November 2017 "Cigalikes versus tank system E-cigarettes: Effects on smoking behaviours at the early stage of a quit attempt". Poster presentation at the Society for the Study of Addiction (SSA) at the Crowne Plaza Hotel, Newcastle, UK.
- 17th July 2017 Invited speaker at a round table discussion on the role and impact of e-cigarettes in the UK and as part of a harm reduction strategy to a delegation sent by the Brazilian authorities. This was organised by the Knowledge Exchange Action group (KAP) and Nicotine Science and Policy group at the Guildhall, city of London Guildhall, Gresham Street, London, EC2V 7HH.
- 15th – 17th June 2017 *"Cigalikes vs Tank Systems: Effects on Smoking Reduction, Self-reported Satisfaction, Craving and Withdrawal Relief"*. Invited speaker at the 4th Global Forum on Nicotine (GFN) 2017 at the Marriot Hotel, Warsaw, Poland.
- 8th – 11th March 2017 Annual Meeting of the Society for Research on Nicotine and Tobacco (SRNT) Lynne Dawkins (LSBU), Catherine Kimber (UEL), Mira Doig (ABS labs), Colin Feyerabend (ABS labs) and Olivia Corcoran (UEL), Leon Kosmider, Jolanta Kurek (IOM) *Compensatory Puffing Behaviour with Lower Nicotine Strength E-Liquid can Increase Carbonyl Exposure*
- 9th - 10th June 2016 Kimber CF, Kośmider L., Corcoran O., Dawkins L. (2016) *Liquid Chromatographic Analysis of Carbonyl Compounds in Aerosols from High and Low Nicotine E-Cigarette Liquids Mirroring Realistic Puffing Topography*. Poster presentation at the UK Nicotine & Smoking Cessation Conference (UKNSCC) at the Victoria Park Plaza Hotel, London, UK
- 17th - 18th June 2016 Kimber CF, Kośmider L., Corcoran O., Dawkins L. (2016) *Liquid Chromatographic Analysis of Carbonyl Compounds in Aerosols from High and Low Nicotine E-Cigarette Liquids Mirroring Realistic Puffing Topography*. Poster presentation at the Global Forum on Nicotine (GFN) in Warsaw.
- 26th - 29th July 2015 in Bristol Kimber CF, Kośmider L., Corcoran O., Dawkins L. (2015) Do e-cigarette users alter their puffing behaviours when given lower nicotine Concentrations?. Journal of Psychopharmacology. Abstract supplement 29(8) ISSN 0269-8811. Poster presentation at the British Association for Psychopharmacology (BAP).