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Assessing modified risk tobacco and nicotine products: Description of the scientific framework and assessment of a closed modular electronic cigarette

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ABSTRACT

Cigarette smoking causes many human diseases including cardiovascular disease, lung disease and cancer. Novel tobacco products with reduced yields of toxicants compared to cigarettes, such as tobacco-heating products, snus and electronic cigarettes, hold great potential for reducing the harms associated with tobacco use. In the UK several public health agencies have advocated a potential role for novel products in tobacco harm reduction. Public Health England has stated that "The current best estimate is that e-cigarettes are around 95% less harmful than smoking" and the Royal College of Physicians has urged public health to "Promote e-cigarettes widely as substitute for smoking".

Health related claims on novel products such as 'reduced exposure' and 'reduced risk' should be substantiated using a weight of evidence approach based on a comprehensive scientific assessment. The US FDA, has provided draft guidance outlining a framework to assess novel products as Modified Risk Tobacco Products (MRTP). Based on this, we now propose a framework comprising pre-clinical, clinical, and population studies to assess the risk profile of novel tobacco products.

Additionally, the utility of this framework is assessed through the pre-clinical and part of the clinical comparison of a commercial e-cigarette (Vype ePen) with a scientific reference cigarette (3R4F) and the results of these studies suggest that ePen has the potential to be a reduced risk product.

1. Introduction

Many different tobacco products are currently used by consumers worldwide, from factory made cigarettes through pipe-tobacco, shisha to snus, and have been available worldwide for several hundred years. Numerous epidemiological studies have shown that cigarette smoking causes a variety of diseases such as cardiovascular disease (CVD), chronic obstructive pulmonary disease (COPD) and cancer (US Department of Health and Human Services, 2014), and current estimates put cigarette usage at 1.4 billion adults worldwide (MacKay et al., 2006). One potential route to reduce this harm is through tobacco harm reduction, was defined by the US Institute of Medicine (IOM) in 2001 as "decreasing total morbidity and mortality, without completely eliminating tobacco and nicotine use" (Stratton et al., 2001), is now being considered by some regulators.

The development of Next Generation Products (NGPs) is currently focussing on novel potentially reduced-risk products, including tobacco-heating products (THPs) and electronic cigarettes (e-cigarettes). In many countries, including the USA and European countries, the ability to market NGPs is subject to regulatory approval. Such approval needs to be obtained by submitting details of a new product's design, performance and impact on users and non-users. In the US, the FDA has outlined the requirements to introduce a novel tobacco onto the market place via the Substantial Equivalence or Premarket Tobacco Application approaches (FDA 2016). In Europe, they may become part of the requirements in the future revisions to the Tobacco Products Directive (European Parliament and the Council of the European Union 2014). Furthermore, the US FDA have detailed the questions and the types of studies that should be considered to investigate the reduced risk

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nature of novel products and these form part of a Modified Risk Tobacco Product (MRTP) application (FDA, 2012a).

E-cigarettes were conceived in 1927 by Joseph Robinson, but it wasn't until 1930 (Robinson 1930) that his first patent was accepted. In 1965 Herbert A. Gilbert received approval for his 'smokeless non-tobacco cigarette' (Gilbert 1965). Later, at the turn of the second millennium, e-cigarettes were first commercialized by the Chinese pharmacist Hon Lik. Today their use has become widespread in many countries around the world as an alternative to cigarette smoking. E-cigarette products have evolved rapidly, resulting in different generations of devices ranging from disposable single-piece cigarette-like products ('cigalikes'), to rechargeable closed modular and tank-style refillable devices with interchangeable parts. The majority of e-cigarettes work on the same principle, of heating a liquid formulation that may contain nicotine. The principle ingredients of these formulations are typically humectants (vegetable glycerine and/or propylene glycol), which, when heated, create a cloud of visible aerosol. These humectants give the aerosol it's body and stimulate sensorial attributes such as mouthfeel. The formulations are available both unflavoured and with myriad flavours and generally, but not always, contain nicotine. They typically do not contain tobacco (though some contain tobacco extracts) and heat the formulation at temperatures of around 250 °C during operation. Cigarettes, on the other hand contain tobacco which when burnt at temperatures up to 900 °C forms smoke containing more than 6500 compounds (Rodgman and Perfetti, 2013), including about 150 toxicants (Fowles and Dybing, 2003). The absence of tobacco, the use of high purity ingredients and an operating temperature substantially lower than that of combustion are the basis of the potential of e-cigarettes to generate aerosols with substantially lower levels of toxicants than cigarettes. E-cigarette product standards are evolving in various jurisdictions, with the aim at phasing out poorly-manufactured products and safeguarding consumers. France's association for standardisation, AFNOR, published the world's first national standards for e-cigarettes and e-liquids in 2015 (AFNOR 2015) with their British counterparts BSI, publishing their standards in the same year (BSI 2015).

As different products have different levels of toxicant emissions, they were placed on a risk continuum for tobacco and nicotine products was conceived in 2012 by Mufano et al. (McNeill and Munafò 2012). We have built on this concept and included tobacco-heating products (THPs) and e-cigarettes within the continuum (Lowe et al., 2015). However, as the different types of products are placed arbitrarily, there is a need to establish the supporting science that can help to place them accurately on the continuum. Evidence of this kind, preferably aligned to a standardised scientific framework devised and developed by regulatory, public health and industry scientists could lead to robust regulation for these new products and enable the substantiation of their harm reduction potential. Public Health England (McNeill et al., 2015) have recently stated that e-cigarettes could pose 95% less risk than combustible cigarettes and the UK Royal College of Physicians have supported the use of e-cigarettes as replacement products for smokers (Royal College of Physicians 2016). In addition to this, Cancer Research UK also endorsed (Cancer Research 2017) the improved safety profile of e-cigarettes relative to smoking following the completion of their long term clinical study (Shahab et al., 2017).

In this paper we initially describe a three-phase framework for the scientific evaluation of products across the risk continuum, exemplified by studies on e-cigarettes. The three key assessment phases in this framework include pre-clinical studies, clinical studies to assess toxicant exposure and individual risk relative to cigarettes and population studies to assess risk relative to cigarettes on a population level (Murphy 2017).

Following this, the utility of this framework is assessed in part, using published data from thirteen studies describing the comparison of Vype ePen and cigarettes. By collating these comparative studies, we provide the most comprehensive dataset gathered on a single e-cigarette to date. This data, along with other published datasets on e-cigarettes shows that these products have enormous promise for benefiting public health on a global scale.

2. Review of published scientific frameworks for the assessment of tobacco and nicotine products

The IOMs approach to tobacco harm reduction involved the proposal of switching smokers to PREPs (Potentially Reduced Exposure Products) as a means to reducing the harms caused by smoking (Stratton et al., 2001). In 2008 we presented a PREP assessment framework (Gregg et al., 2008) which comprised a series of tests that would profile emission, exposure and risk relative to conventional cigarettes. Importantly, the utility of this framework was demonstrated through the assessment of a Reduced Toxicant Prototype (RTP) cigarette (Dittrich et al., 2014), which showed reductions in toxicant emissions, *in vitro* toxicological endpoints (Combes et al., 2013 and Crooks et al., 2015) and biomarkers of exposures in comparison to a cigarette (Proctor et al., 2014). However, smokers who switched from a conventional cigarette to the RTP cigarette did not observe sufficient change in biomarkers of biological effect compared to those who continued smoking the conventional cigarette. These findings led us to conclude that there was insufficient evidence to support the RTP cigarette as a distinct category in a risk continuum and those products that emit lower levels of toxicants such as Tobacco Heating Products (Schaller et al., 2016) or e-cigarettes (Goniewicz et al., 2014, Margham et al., 2016) are more suitable candidates for assessment as potentially reduced risk products.

The assessment framework presented in this paper comprises a series of verifiable studies for comprehensive NGP evaluation and the substantiation of health-related claims (Fig. 1). It builds on the

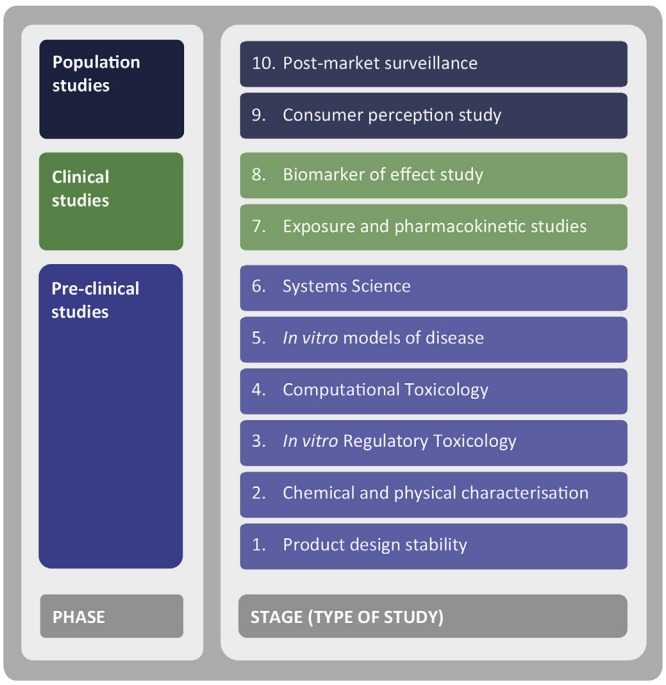

Fig. 1. Framework for the assessment of potentially reduced risk products.

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previously published framework for assessing Potentially Reduced Exposure Products (PREPs) (Gregg et al., 2008, Proctor et al., 2014), augmenting that framework with studies that included cutting-edge chemical, biological and human clinical techniques. These include advances in tissue culture, systems biology and molecular biology that are paving the way as tools available to assess products for their risk reduction potential.

The framework comprises three assessment phases (i) pre-clinical studies; (ii) clinical studies and (iii) population studies and these phases are further broken down into ten stages (Fig. 1). Across each stage and where possible, regulatory-approved methods and key international standards are applied including International Organisation for Standardisation (ISO), International Council for Harmonisation (ICH) and Organisation for Economic Cooperation and Development (OECD) approved methods. These are supplemented by methods that have a long history of use in evaluating tobacco and nicotine products and novel high throughput methods outlined in the NRC's Toxicity Testing in the 21st Century strategy document (NRC 2007).

Smith et al. published a framework (Smith et al., 2016) describing similar approaches to assessing the risk profile of novel tobacco products. In this case, a pre-clinical, clinical and population assessment approach was also proposed, with one major differentiation, in that animal studies were anticipated as being necessary for the assessment of modified risk tobacco products. Underlying mechanisms for smoking related diseases such as cancer, COPD and CVD from long term exposure to smoke toxicants have been proposed as DNA damage, inflammation and oxidative stress (USDHSS 2010). In contrast, our framework aims to adhere to the 3R principles (Replacement, Reduction, and Refinement) by implementing alternative methods to animal testing where possible (National Centre for 3Rs 2017). Using *in vitro* toxicological and disease modelling techniques, we previously concluded that the vapour from the test e-cigarette induced no DNA damage (Thorne et al., 2016) and substantially reduced levels of oxidative stress (Taylor et al., 2016). Furthermore, the focus has been on harnessing *in vitro* tools and applications following the principles outlined in the National Research Council's Toxicity Testing in the 21st Century (National Research Council 2007) and the OECD's AOP, incorporating learnings from approaches taken by other industries for product assessment. These new disciplines and approaches have been incorporated into the assessment framework. For instance, we conducted an *in vitro* systems biology-based assessment of e-cigarette vapour in 3D primary tissues with added granularity regarding the mode of action of the tested aerosols compared to regulatory approved *in vitro* tests (Haswell et al., 2017). The combination of unbiased omics techniques mapping perturbations against Adverse Outcome Pathway key events could help build predictive models of adverse outcomes potentially reducing or eliminating the need for animal studies. A similar approach has been presented by Iskandar et al. for the *in vitro* assessment of e-liquids (Iskandar et al., 2017). In combination with chemical profiling, toxicant measurement of the e-liquid or aerosol and classical genotoxicity testing (e.g. Ames, micronucleus, mouse lymphoma, neutral red assays), high content screening of 2D tissues, and further systems toxicological assessment using 3D primary cultures is employed to help understand toxicological mechanism.

The Tobacco Product Assessment Consortium (TobPRAC) also proposed a tobacco product assessment framework which consisted of a four-stage model of assessment that included pre-market evaluations, pre-claim evaluations, post-market activities, and monitoring and re-evaluation of products (Berman et al., 2015). That framework highlighted key tests (chemical, toxicological and human studies) and reference products that would be required to demonstrate reductions in risk at the individual and population levels. Importantly, this framework emphasised a post market surveillance approach that would include claims testing with consumers, monitoring usage and measuring health related outcomes that could be linked back to 'pre-market' clinical outcomes. TobPRAC also recommend a monitoring and re-evaluation approach based on post market activities.

Farsalinos and Palosa used a similar approach in reviewing 114 published scientific reports that summarised the assessment of e-cigarettes grouping them into chemical, toxicological and clinical studies (Farsalinos and Polosa, 2014). They proposed that through the absence of tobacco and combustion in e-cigarettes users were exposed to less harmful toxic chemical relative to smoking. The authors did acknowledge that the data from the numerous studies demonstrated that there was likely some residual risk but that this would be trivial relative to the devastating consequences of smoking. Finally, they concluded that improvements in product design, safety and quality testing of ingredients and flavourings would ensure that e-cigarettes could fulfil their promise as revolutionary products in tobacco harm reduction.

3. Assessment framework studies and methodologies

The risk reduction potential and subsequent benefit to population health through the introduction of novel tobacco and nicotine products will depend on two main factors; (a) the reduction of toxicity relative to cigarettes (both to the individual user and bystanders) and (b) the number of smokers who switch (Bates 2013), which will depend on the acceptability of the new product in terms of sensory, ritual and use-behaviour. This framework incorporates studies that will enable the measurement of both (a) and (b) and will draw these together in a population effects model to assess the risk reduction potential of e-cigarettes.

The completion of this assessment will generate a large amount of data that will need to be consolidated and ordered logically into a narrative, which explains the assessment and demonstrates its relevance for morbidity and mortality risk reduction. A weight of evidence approach will be adopted primarily, assessing the relative risk of e-cigarettes versus cigarettes at each stage. To supplement this, the Adverse Outcome Pathway, (AOP) concept, as defined by Ankley et al. (Ankley et al., 2010) will also be adopted as it provides a knowledge-driven sequence of events from exposure to adverse outcome, encompassing all organizational levels of a given biological system. These data frameworks provide a way to assess disease-relevant risk reduction. The principles and potential applications of AOPs are well described by Villeneuve et al. (Villeneuve et al., 2014). Briefly, a molecular initiating event (the initial effect(s)) following exposure to a toxicant(s) is linked to an adverse outcome by a series of key events, which are placed in series according to causality and temporality, and organised by the lowest to the highest level of biological hierarchy (*e.g.* molecular level to population level). Each key event must be measurable in an experimental system or clinical studies, and in some cases, the same key event can be measured in both (which increases confidence in data from *in vitro* models should the data align with human clinical data). By establishing the key event data signature in studies of cigarette smoke, one can then conduct comparisons with novel products using the same studies. If the deleterious effects of the NGP(s) are significantly less than those induced by cigarette smoke, then there is a reasonable expectation that the NGP would lower the incidence of the adverse outcome in a population of smokers who switch to the NGP.

To date, we have proposed two AOPs (Lowe et al., 2017 and Luettich et al., 2016) to the OECD based on the available data from the literature. They are "oxidative stress to hypertension" (of

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relevance to cardiovascular disease) and "oxidative stress to mucus hypersecretion" (of relevance to COPD). The outputs from comparative pre-clinical clinical and population studies as summarised above could be placed into AOP frameworks to demonstrate that these NGPs reduce disease pathologies and adverse outcomes.

4. Assessment phase 1: pre-clinical studies

Pre-clinical studies form assessment phase 1 (stages 1–6) of the framework and include: (1) product design stability, (2) chemical and physical characterisation; (3) *in vitro* regulatory toxicology; (4) computational toxicology; (5) *in vitro* models of disease and (6) systems science. In addition to these we and others have published our approach to the stewardship of flavours, describing the requirement for full disclosure of ingredients in the e-liquid formulation and subsequent methods for hazard identification and risk assessment (Costigan and Meredith, 2015, Iskandar et al., 2017).

For the purposes of this paper, the framework has been tailored specifically for e-cigarettes. In general, it is applicable to all NGPs, however for other types of NGPs such as Tobacco Heating Products (THPs), some modifications to the framework may be necessary. For example, a thermo-physical analysis of the tobacco consumable may be required to evaluate whether it is heated and not combusted.

4.1. Stage 1: product design stability

Product development of e-cigarettes focusses on three key goals, namely ensuring optimum consumer acceptability of the product from a sensory, ritual and nicotine pharmacology perspective; characterisation of the product to ensure that minimum toxicant yields of are achieved with maximum sensory acceptability and finally building quality into the final product from the concept phase through to commercialisation. The aim of this stage is to confirm a product specification and establish it's stability over time via useful-life and shelf life studies. In the fast paced world of NGPs, as product innovation seeks to meet evolving consumer needs, it is likely that improvements to of the product will be designed. Thus, any new versions must meet the specification of the original product before entering the assessment framework if the datasets are to be bridged from the original product to new variants.

Two types of studies are used to assess e-cigarette stability (i) a 'useful life' assessment of the device life cycle and (ii) 'shelf life' studies on the consumable (eg. cartomisers stored in pack), under ambient conditions at 25°C/60% RH (relative humidity) and at elevated temperature and humidity conditions of 40°C/75% RH for the period of required shelf-life, typically 12 months. During shelf life testing, specifically for e-cigarettes, the formulation would be measured for compliance with the formulation specification; nicotine related substances including anatabine, anabasine, β-nicotyrine, cotinine, myosamine, nornicotine and nicotine N-oxide (European Pharmacopeia 2016), and microbiological and non-sterile compounds. Microbial and non-sterile compounds would include microbial enumeration (Current European Pharmacopeia - Section 2.6.12) and tests for specified micro-organisms (Current European Pharmacopeia – Section 2.6.13).

4.2. Stage 2: chemical and physical characterisation

Chemical and physical characterisation of the NGP aerosol focusses on assessing the reduction in Harmful and Potentially Harmful Constituents (HPHCs) in comparison to 3R4F and evaluate if the e-cigarette aerosol contains any new compounds. To ensure that machine studies are relevant to consumer usage, we conduct puffing behaviour studies with consumers measuring puff volume, puff duration and the interval between puffs (See stage 7). Four analyses are used characterise the product aerosols.

Firstly, the major aerosol components (eg. glycerol, propylene glycol, water, nicotine etc) are quantified to assess the transfer of the individual eliquid components into aerosol.

Untargeted aerosol analysis to evaluate the complexity of NGP aerosols versus cigarette smoke can be achieved via two-dimensional gas chromatography and time-of-flight mass spectrometry (GC-TOF-MS) (Rawlinson et al., 2017).

Targeted aerosol analysis measures the levels of compounds in cigarette smoke and NGP aerosol. A number of regulatory lists have been published by the WHO (9 toxicants) (Burns et al., 2008), Health Canada (44 toxicants) (Health Canada, 1999) and FDA (both the shortened list of 18 chemicals and the full list of Harmful and Potentially Harmful Constituents) (FDA 2012b), which are comprised of chemicals that are proposed as the principle causation of harms from smoking.

Analysis of environmental emissions is necessary to understand and measure if e-cigarette users emit a second hand aerosol during use. Environmental tobacco smoke from cigarettes occurs when sidestream smoke is emitted from smouldering between puffs and exhalation of toxicants by smokers. These studies measure the potential of e-cigarettes to expose by-standers to nicotine or toxicants. As e-cigarettes typically operate upon puffing or using a button and will remain in rest mode unless activated by a consumer; they do not have any smouldering properties like cigarettes and do not generate side-stream tobacco smoke. However, consumers inhale and exhale e-cigarettes aerosols and studies investigating second hand exposure have previously been reported (Farmen 2014a and 2014b) showing reduced environmental toxicant emissions from users.

4.3. Stage 3: in vitro regulatory toxicology

A range of regulatory approved *in vitro* assays can be used to assess a variety of toxicological endpoints such as the Ames test to assess mutagenicity (OECD 1997a) and the Neutral red uptake assay to measure cytotoxicity (ICCVAM 2006). Other researchers investigating NGPs have used such methods (Schaller et al., 2016). Importantly, to improve the biological relevance of these assays, where available, they are performed with two exposure matrices including the whole aerosol (WA) and the aerosol condensed material (ACM), which is the condensed aerosol trapped on a Cambridge filter pad during machine puffing (Adamson et al., 2016, Alderman et al., 2014 and Margham et al., 2016).

4.4. Stage 4: computational toxicology

The data from the physico-chemical characterisation phase were used in the fourth stage computational risk assessment that calculates margins of exposure. Margin of exposure (MOE) assessments were used to set concern levels for individual toxicants and combine an estimate of human exposure with a reference point taken from the available toxicological data. Lower MOE values indicate greater concern, and toxicants with a MOE of greater than 10,000 can be considered a low priority for risk management actions (EFSA 2006 and Cunningham et al., 2011). This approach was applied here to generate MOEs for comparison, based on an estimated exposure to emissions from both e-cigarettes and cigarettes (3R4F) utilising an estimate of average daily use and machine generated yields. MOEs were generated for tobacco smoke toxicants using chronic inhalation data where possible (Cunningham et al., 2011 and Cunningham et al., 2012). Daily human exposure

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was estimated based on a conservative 150 puffs per day for e-cigarettes or 20 cigarettes per day and using product-specific yield data. The MOE calculations were carried out for six carbonyl compounds and two nitrosamines identified by Margham et al. (Margham et al., 2016) as detectable in e-cigarettes emissions. Where possible, multiple dose–response data sets were used to generate a MOE range for a single toxicant. For the purposes of this assessment, the lowest suitable MOE generated for a given toxicant was used to compare between e-cigarettes and cigarette emissions.

In addition to MOEs, mode of action (MOA) and physiologically-based pharmacokinetic (PBPK) studies could strengthen the findings from an *in silico* computational risk assessment if required. PBPK models (European Medicines Agency 2016) can be used to contextualise the concentration of toxicants required to cause biological responses *in vitro* and provide insight into species specific and dose related differences. One of the most technically challenging tasks however, in assessing the biological effects of exposure is to predict the target-tissue concentrations of toxicants in humans. To address this, we have developed PBPK models and recently published an assessment of 1,3-butadiene (Campbell et al., 2015).

4.5. Stage 5: in vitro models of disease

Technology advances in the areas of systems biology and molecular biology are driving the National Research Council's Toxicity testing in the 21st Century (National Research council 2007) towards becoming a reality. Other research shows how we stand at the edge of an unprecedented transformation in the conduct of toxicological evaluations (Sturla et al., 2014). Building on the traditional *in vitro* toxicological approaches in Stage 3 of the assessment framework, a range of assays with improved biological and disease relevance have been adopted and designed for the testing of e-cigarettes. To elucidate the disease relevance of the chemical and toxicological differences between the e-cigarette aerosol and cigarette smoke we conducted range of *in vitro* studies in Stage 5, investigating a series of endpoints associated with key smoking-related disease processes. Importantly, in addition to the ACM and WA exposure matrices outlined in stage 3, an additional exposure matrix that captured that aerosol as an aqueous extract (AqE) was used for the *in vitro* models of disease (Taylor et al., 2016).

4.6. Oxidative stress relevant assays

Inflammation and oxidative stress are important events that often follow exposure to toxicants and are critical for driving processes in the development of COPD, cardiovascular diseases and lung cancer. Inflammation and oxidative stress were assessed using a range of commercially available *in vitro* assays, including (caspase 3/7 activity), Reactive Oxygen Species (ROS) generation, intracellular glutathione antioxidant ratios and activation of a gene promoter region via an antioxidant response element (Taylor et al., 2016). This suite of assays is used to assess the oxidative stress response of human lung epithelial cells (NCI-H292) in response to AqE generated from 3R4F cigarette smoke and the aerosol from the e-cigarette.

4.7. CVD relevant assays

Cigarette smoking is a well-described risk factor for cardiovascular disease (CVD). This is due at least partly to the tendency of smoke and smoke toxicants to promote atherosclerosis within the cardiovascular system (Unverdorben et al., 2009 and Winkelmann et al., 2009). Previously we have used an *in vitro* assay to model a number of the CVD endpoints to assess the risk reduction potential of products using endothelial cells (HUVECs) (Fearon et al., 2012 and McQuillan et al., 2015). Building on this, the ability of HUVECs to migrate into and repair a mechanically induced wound in the presence of AqE generated from cigarette smoke and e-cigarette aerosol can compare endothelial dysfunction between the different product types (Taylor et al., 2016).

4.8. COPD relevant assays

COPD is a major cause of morbidity and mortality worldwide (Rabe et al., 2007), and is the result of chronic exposure to inhaled agents, such as cigarette smoke, noxious gases and particles, although over 90% of patients are reported to have a history of smoking (Maunders et al., 2007). We have used air:liquid interface (ALI) *in vitro* models of human bronchial epithelial cells and 3D reconstituted human respiratory tissue models eg. MucilAir™ (Baxter et al., 2015) and EpiAirway™ (Neilson et al., 2015) to assess the whole aerosol. The endpoints assessed included cytotoxicity, (Azzopardi et al., 2016), goblet cell hyperplasia (Haswell et al., 2010), mucus hypersecretion, ciliary beat frequency, and protein expression. Impaired muco-cilliary clearance, fibrosis and lung tissue re-modelling. These models also provided a source of material for further omics studies that are described in detail in Stage 7 (systems science).

4.9. Cancer relevant assays

Cancer, a leading cause of death worldwide, accounted for 8.2 million (22%) of all deaths from non-communicable disease in 2012 (WHO, 2012). Lung cancer is by far the biggest cause of death, accounting for 22% of all cancer deaths in the UK in 2012 (Cancer Research UK 2014). Cancer development is defined over three stages:

1) Initiation – irreversible changes to a cancer-related gene
2) Promotion – reversible selective clonal expansion of the initiated cell via growth stimulation or inhibition of apoptosis (programmed cell death)
3) Progression – stable alteration of genes in an initiated cell

The application of *in vitro* models of cancer initiation *ie*. DNA damage/mutation assays (Thorne et al., 2016), indirect quantification of DNA double-strand breaks by assessment of H_2AX histone phosphorylation in cell nuclei (Thorne et al., 2017) and promotion using cell transformation assays (Han et al., 2004; OECD, 2016) have previously been utilised to test the effects of cigarette smoke and toxicants. We have further assessed promoter activity using the Bhas transformation assays using PM generated from cigarette smoke and NGP aerosol (Breheny et al., 2017).

4.10. Stage 6: systems science

Further studies examining biological perturbations following an exposure and informative of the mechanism of toxicity were investigated using a global transcriptomics approach (Banerjee et al., 2015). In these studies, a reconstituted 3D human respiratory tissue, Muclilair™, was exposed to smoke from the reference cigarette 3R4F or e-cigarette aerosol followed by gene expression profiling. The regulation of a number of genes associated with disease relevant and biological functional endpoints (*eg.* tissue damage, inflammation, respiratory damage) was then monitored using tools such as enrichment analyses and downstream causal reasoning (Kramer et al., 2014). Other researchers have shown how a systems toxicology approach, combining advanced analytical and computational skills, can lead to more predictive and accurate risk-

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assessment approaches (Sturla et al., 2014).

5. Assessment phase 2: clinical studies

Assessment Phase 2 (Stages 7–8) builds on the pre-clinical assessment phase of the framework with the objective establishing whether changes in the levels of aerosol toxicants of e-cigarettes relative to cigarettes and subsequent responses to the aerosol in the range of *in vitro* toxicity impacts exposure and individual risk.

5.1. Stage 7: exposure and pharmacokinetic studies

A range of different studies are useful for understanding and quantifying exposure beginning with puffing behaviour and mouth level exposure studies giving insight into how consumers use new products and quantifying the maximum yield possible. Furthermore, puffing behaviour studies are important for assessing if the machine smoking regimes used in laboratory-based pre-clinical measurements are reflective of consumer's actual behaviour. Short term clinical based studies are useful for quantifying both nicotine uptake through pharmacokinetic studies (Fearon et al., 2016) and measuring the levels of toxicants in man via biomarker of exposure studies (Gregg et al., 2013).

5.2. Consumer behaviour

A two-part consumer study is conducted to measure consumption and puffing behaviour. The consumption study includes respondents who are requested to complete a questionnaire detailing their daily usage of e-cigarettes (*eg.* volume of liquid, number of cartomisers). The second part is a puffing behaviour study which measures the puff volume, puff duration and interval between puffs from consumers using a portable Smoking Analyser instrument (Slayford and Frost, 2014).

5.3. Mouth level exposure study

In addition to the puffing behaviour study it is also possible to provide estimates of mouth level exposure to the e-cigarette aerosol. The basic approach involves determining the loss of mass from the cartridge (or device). Further determinations of consumers' exposure to the constituents of aerosol can be achieved by replaying a consumer's puffing topography record on a laboratory puffing machine, a process known as duplication. The resultant aerosol from duplication can be captured and analysed for the constituents of interest.

5.4. Clinical pharmacokinetic studies

A key part to be examined in an assessment of the reduced risk potential of a novel product is the measurement of the effect on that product on the behaviour of the existing tobacco users (FDA 2012a). One such necessary assessment is of the abuse liability (abuse potential) of the new product in comparison to other tobacco products currently marketed. This has the potential to inform an assessment of the population level impact of the introduction of the new product and inform on the wider implications of a new product on the potential for uptake by non-users, relapse in ex-users or dual use by current smokers. Inclusion of non-users and ex-users in such studies, however, is difficult to rationalise in interventional studies for ethical reasons and as such, post-market data of real-world use patterns will be key to obtaining data in such groups.

Based on the guidance given by Carter et al., (Carter et al., 2009) amongst others, we define abuse liability as "The potential for a nicotine-containing product to create dependence behaviours and promote compulsive self-administration with negative consequences of use". Physical dependence is characterised by the development of tolerance to tobacco product and/or the onset of withdrawal symptoms upon stopping use. Psychological dependence is characterised by persistent tobacco-seeking and tobacco-use behaviours, impairment in behavioural control and craving, and inability to abstain consistently. Currently, while no specific guidance exists for assessment of abuse liability for products across the risk continuum we believe that such assessments in clinical studies should examine:

i) Nicotine uptake in pharmacokinetic studies of healthy smokers, under defined and *ad libitum* puffing conditions and following overnight abstention; as well as measuring subjective assessments of constructs such as craving relief, satisfaction and intent to use again
ii) How the novel product affects symptoms of withdrawal, using standard scales (eg., the Minnesota Nicotine Withdrawal Scale) in abstinent smokers (Hughes and Hatsukami, 1986)
iii) The degree to which a subject is willing to pay or work to "earn" use of the novel product. Such behavioural econometric assessment could allow cross-sectional examination of withdrawal symptoms and "value" of allowing product use in accustomed, abstaining users of different types of products.

5.5. Clinical biomarker of exposure study

Clinical studies using biomarkers are useful for assessing whether any pre-clinical reductions in toxicant that are observed with e-cigarettes are measurable in humans. The hypothesis to be proven would be that biomarkers of exposure and potential harm would significantly change in the correct biological direction for smokers who switch to an e-cigarette in comparison to those who stop smoking (with or without the aid of nicotine replacement therapy). Additionally, as the Institute of Medicine (IOM, 2012) has stated that complete cessation is the gold standard for tobacco harm reduction, and because risks of chronic disease have been shown through epidemiology to reduce or at least slow in their progression following cessation, biomarker changes for e-cigarette would be expected to follow similar trends, direction and levels of change to the cessation arm. In practise, not all smokers who are asked to quit in the study may be able to abstain for the duration of the study and relapse into smoking. As the relapsers would be kept in the studies, this design may remove the need for a specific smoker arm, thus reducing the burden on subjects. It is important to monitor subjects using e-cigarettes in short- and long-term studies to assess their initial BoE levels relative to smoking, and to assess if any reductions observed in BoEs are sustained over significant time periods.

5.6. Stage 8: biomarker of biological effect study

Stage 8 builds on the assessment of toxicant exposure with the objective of both understanding and measuring if a change in exposure impacts individual risk using clinical biomarkers of biological effect (BoBE) (Haswell et al., 2014). We previously conducted a clinical study using BoBEs that were deemed appropriate for assessing the individual risk of a novel RTP cigarette (Shepperd et al., 2015). These BoBEs were recently presented at an FDA public workshop where they have asked experts from public health, regulatory, academia and tobacco manufacturers to present their views on biomarkers of potential harm (an equivalent terminology

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for biomarkers of biological effect), that would have utility in assessing the relative individual risk of novel tobacco products in comparison to cigarettes. We presented our approach to both single BoBE, physiological measures and novel 'omic' based biomarkers (Proctor 2016) (a full list of BoBEs can be found in Supplementary Table 2).

A series of biomarker development studies were conducted through the comparison of smoker, never-smoker and ex-smoker cohorts, and combined with literature reviews, we have elucidated a number of proteomic (Haswell et al., 2016) and transcriptomic (Garcia-Perez et al., 2014, Kaluarachchi et al., 2016 and Shepperd et al., 2013b) markers relevant to a variety of smoking-related disease processes.

Looking at each BoBE in isolation does not give a comprehensive picture of the physiological response of subjects exposed to a product. Furthermore the use of an array of biomarkers developed in the context of cigarette product testing can miss adverse events potentially indicative of toxicity caused by nicotine products. Toxic insults lead to tissue damage with secretion and leakage of cellular material in biofluids that can be quantified in many matrices such as serum, sputum, saliva, and urine. These possess the added advantage of minimizing the need for invasive sample collection procedures.

Multiplatform metabolomics, transcriptomics, epigenetics and proteomics analyses allow the quantification of thousands of metabolites, transcripts, proteins, and microRNAs (miRNA) in these biofluids, which not only could be BoBEs in their own right, but also advise on entire biological network perturbations and aid the biological interpretation of product risk reduction potential. For example, in recent metabolomic studies, we and others have clearly highlighted that smoking alters metabolites that are part of the glutathione pathway, a well-known antioxidant (Garcia-Perez et al., 2014). In a screen of plasma miRNAs we identified changes in the level of two tumour suppressors (Banerjee et al., 2015) in healthy smokers associated with human lung tumour prognosis (Berghmans et al., 2013) of which one was also correlated with a BoE to a smoke toxicant.

6. Phase 3: population studies

The focus of tobacco harm reduction is ultimately to have a beneficial impact on health (reduction in tobacco based morbidity and mortality) at a population level. Therefore, a series of population studies are required pre- and post-marketing of the modified risk product inclusive of testing any regulatory approved modified product claims. Some regulators, including the FDA, are proposing to make the assessment of population level risks part of the regulatory process (FDA, 2012a,b).

6.1. Stage 9. consumer perception studies

Although the required population level tests have not yet been fully defined, we anticipate that they will include studies examining risk perception, uptake, impact of marketing and other information and level of risk imparted to individuals by use of the product alone or in combination with other tobacco or nicotine products. Data will need to be collected from current and non-users in qualitative and quantitative studies, and before and after a new product is marketed and computational models will be needed to estimate the effects.

6.2. Stage 10. post market surveillance (PMS)

This framework proposes a series of pre-clinical, clinical and population studies on products across the risk continuum before launch to ensure that risks are minimised. However, once products are in general use, post market surveillance would be carried out to identify adverse events, unintended consequences and/or unexpected disease outcomes. Data collected during post-market surveillance would inform any likely intervention by either the manufacturer or the regulator in the interest of public health. Approaches would include passive surveillance which relies on data reported spontaneously by consumers and healthcare professionals, and active surveillance data collected through intervention and epidemiological studies and population wide surveillance.

A PMS programme would include information about product usage patterns, consumer perception; provide data with respect to the health risks (inclusive of the measurement of BOEs and BoBEs), and the effect on morbidity and mortality as compared to using other products or quitting use of tobacco products. Specific information could also be collected such as health care visits, physiological measurements to assess if the continued use of ends lead to (i) Adverse events; (ii) Reduction and/or reversal of smoking related disease symptoms; (iii) Improvement in quality of life (Kulasekaran et al., 2015) and (iv) Reduction in health visits.

Historically, epidemiological studies have been used to substantiate the effects of smoking on population health and it can take up to a generation (*ie*. 25 years or longer) to gather the required datasets. In the absence of epidemiology, the immediate impact of e-cigarettes on population health could be assessed using mathematical modelling. We and others have proposed a population model (Hill and Camacho, 2017; Weitkunat et al., 2017) where usage status (*eg*. non-smoker, smoker, former smoker, dual e-cigarettes and e-cigarette user) are represented as stocks and the probabilities of changing user status as transitions or flows (Fig. 2).

A Risk Factor, RF, would then be assigned for each of these stocks. For example, cigarettes would have a RF = 1.00, whereas an e-cigarettes could have a RF = 0.05 (if they were proven to be 95% less risky than cigarettes as stated by Public Health England (McNeill et al., 2015) and the Royal College of Physicians (Royal College of Physicians, 2016)). In order to calculate the impact on population health, subjects would be surveyed once the product had been placed on market for 3–6 months and placed into the stocks as defined in the model. Then, by applying the risk factors for each stock, conclusions surrounding different scenarios of potential population effects could be drawn.

PMS will play an important role in monitoring and re-evaluation in the case of a regulatory claim being approved, for example an MRTP and will probably need to be designed differently for reduced exposure and reduced risk claims. These activities are needed to monitor post launch product changes and consequent effect on the population to ensure consumer safety and regulatory compliance.

7. Results from the assessment of a closed modular EC

This section of the publication presents an example of the application of the proposed framework for the assessment of the Vype ePen e-cigarette (now referred to as EC), specifically; its operation, pre-clinical assessment and initial assessment of consumer usage, behaviour and pharmacokinetics. The objective is to demonstrate in-part, how the assessment framework could be put into practice, by summarising data from studies assessing EC compared to both scientific reference cigarettes and conventional cigarettes. Further studies are required to complete the dataset, and will be the subject of future publications.

8. Products

The EC used in the assessments described in this paper was the Vype ePen (Nicoventures, Blackburn, UK), which has been

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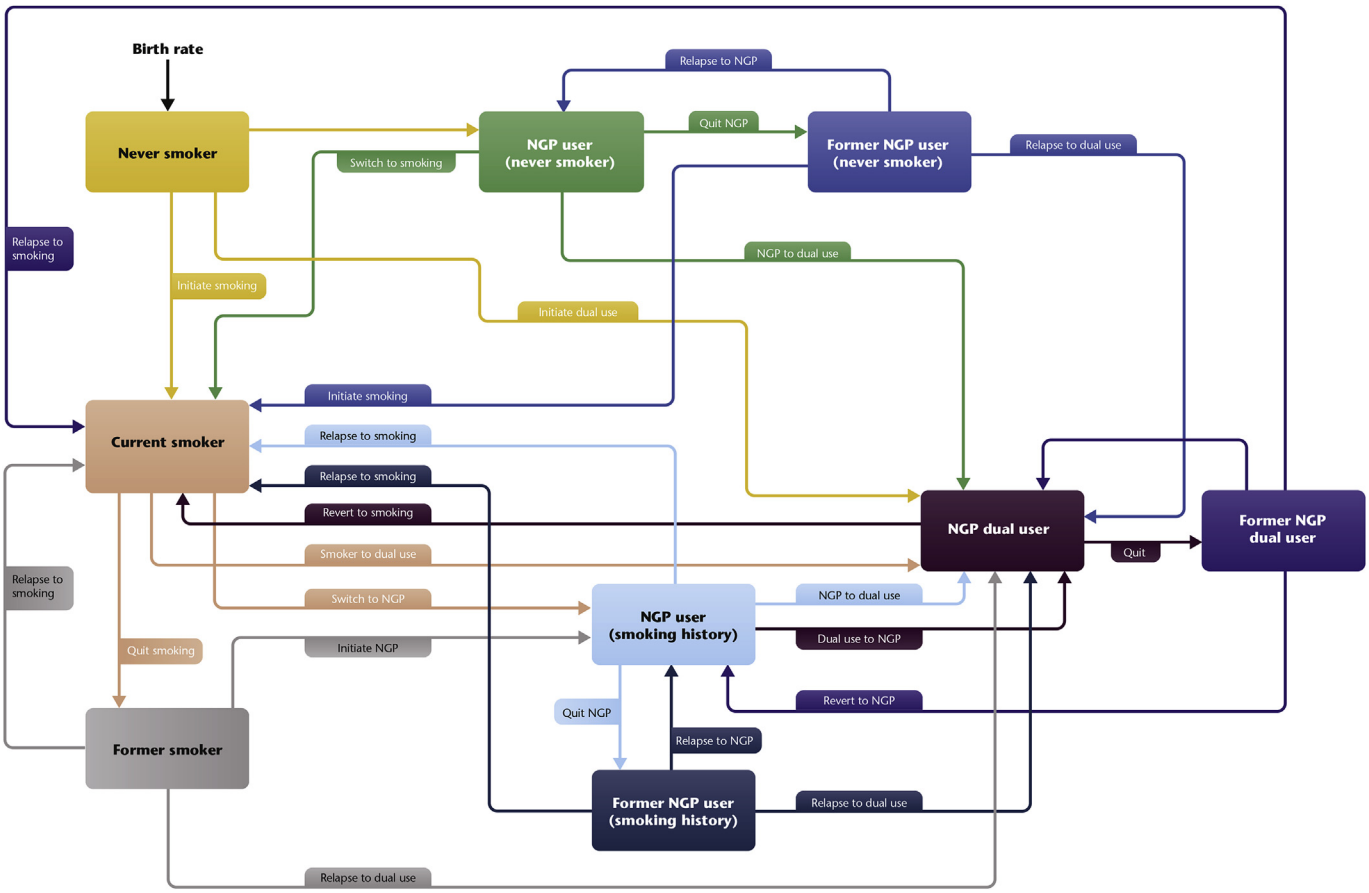

Fig. 2. Population effects model schematic.

described in detail previously (Margham et al., 2016). In summary, it is a non-refillable, closed modular system comprising a 2 ml cartomiser. The formulation used in these studies was the commercially available Blended Tobacco formulation containing 1.86% v/v nicotine (further information is available in the Supplementary Information, Supplementary Fig. 1).

For laboratory studies, the 3R4F scientific reference cigarette from the University of Kentucky (Roemer et al., 2012) was adopted. 3R4F is a Kingsize 9mg/cig ISO 'tar' product containing a 'United States' blend of tobacco (a combination of burley, oriental and Virginia tobaccos) and a single filter piece comprised of cellulose acetate. It was selected as the comparator for the pre-clinical studies as this product has a history of use in tobacco studies and is used by regulatory and public health scientists globally.

In clinical/human/consumer tests, a commercial cigarette was used. The cigarette, on commercial sale in the United States of America, was a Kingsize 6mg/cig ISO 'tar', 0.7mg/cig ISO nicotine product also containing a 'United States' blend' tobacco and a single filter piece comprised of cellulose acetate.

9. Phase 1. pre-clinical studies

9.1. Stage 1. product design stability

Studies investigating both the shelf life and the useful life of EC are underway and will be the subject of a future publication.

9.2. Stage 2. chemical and physical characterisation

A smoking regime in active regulatory use was used for the cigarette, namely the Health Canada Intense (HCI) machine puffing regime. This comprised of a 55 mL puff with a 2 s duration and a 30 s interval between puffs, with a bell-shaped puff profile and the cigarette filter ventilation 100% occluded (Health Canada 1999). In contrast, for the EC, the CORESTA recommended test method, CRM81, which is a 55 mL puff with a 3s duration and a 30s interval between puffs, a square wave puff profile and no ventilation occlusion, was used for the EC (CORESTA 2015).

A chemical analysis was conducted to quantify the amounts of the major components in the EC aerosol and cigarette smoke (Margham et al., 2016). The majority of the aerosol from EC was comprised of humectants, glycerol and propylene glycol (71.3%), water (26.6%), nicotine (1.4%) and other constituents *ie.* flavours etc. (0.8%). In contrast, cigarette smoke was significantly different comprising humectants, glycerol and propylene glycol (5.0%); water (32.2%); nicotine (4.3%) and other constituents ie. combustion by-products of carbohydrates, proteins, lignins, peptides etc. (63.3%). In addition to this, the pads used to trap the condensed aerosol from both products were inspected and brown residue ('tar') was collected on the pad from the 3R4F, whereas the condensed aerosol from EC was collected as colourless droplets very similar in appearance to the e-liquid (Fig. 3).

An untargeted chemical screen was performed (Rawlinson et al., 2017) using a GC-MS analysis of 3R4F reference cigarette smoke and EC aerosol and on comparison of the 2D, GC-MS chromatograms (Fig. 4), it was evident that the aerosol from EC was chemically much simpler than smoke, comprising significantly fewer chromatographic peaks.

Vapour from a range of twelve e-cigarette products was previously measured by Goniewicz et al. (Goniewicz et al., 2014), who

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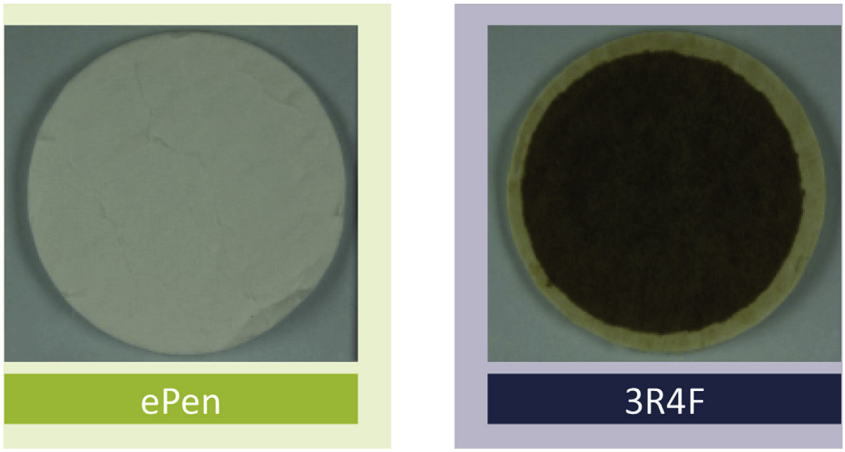

Fig. 3. Filter pads with condensed EC aerosol (left) and smoke from 3R4F cigarettes (right).

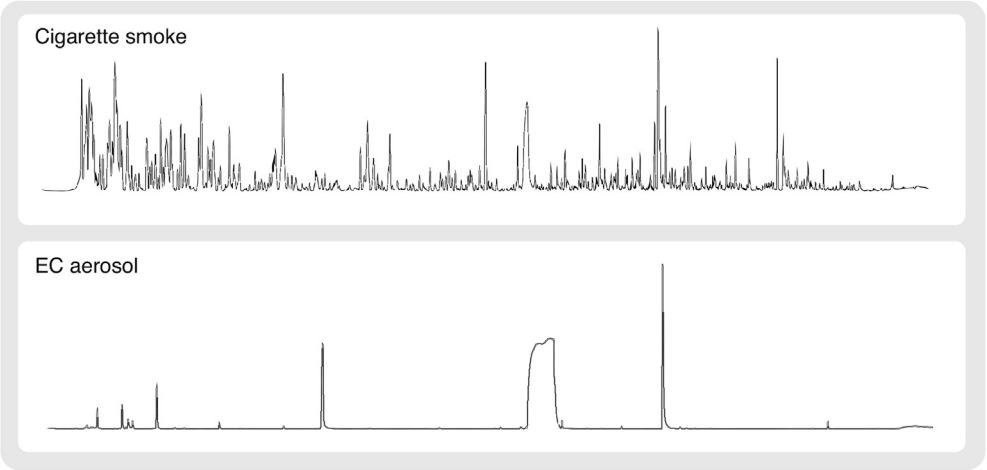

Fig. 4. Chromatographic analysis of 3R4F cigarette smoke and EC aerosol.

found that the levels of selected carcinogens and toxicants was greatly reduced in comparison to cigarette smoke. More recently, Margham et al., (Margham et al., 2016) performed a series of targeted chemical analyses on cigarette smoke and EC aerosol, measuring 150 harmful or potentially harmful compounds in both samples. Results were presented both individually and summarised by a series of regulatory toxicant lists. The levels of toxicants in EC aerosol were substantially reduced in comparison to cigarette smoke; against the WHO list of 9 toxicants and the FDA reporting list >99% reductions were found; against the Health Canada list of 44 toxicants 95% reduction were found, and against the full list of FDA HPHCs average reductions of 92% were found. These chemical studies also showed that EC aerosol contained much fewer numbers of smoke toxicants than the reference cigarette.

9.3. Stage 3. in vitro regulatory toxicology

To assess the toxicological impact of the chemical differences between the EC aerosol and cigarette smoke, a series of regulatory-approved *in vitro* toxicological tests were conducted (Fig. 5), including, an Ames mutagenicity test (Thorne et al., 2016) and cellular cytotoxicity endpoints (Azzopardi et al., 2016). In each of these studies the cigarette smoke caused both mutagenic and cytotoxic responses, in contrast to EC aerosol which was shown to be non-mutagenic and substantially less cytotoxic (around 91% reduced versus 3R4F). Similarly, when we assessed DNA damage in human lung cells (BEAS 2B) it was observed that cigarette smoke induced extensive DNA damage and the EC aerosol produced no DNA damage (Thorne et al., 2017). In addition to these assays described above, other studies which evaluate mammalian genotoxicity such as *in vitro* micronucleus (OECD, 2010) and mouse lymphoma assays (OECD, 1997b) could have utility for assessing the toxicity of EC.

9.4. Stage 4. computational toxicology

Despite the substantial reductions in targeted chemistry emissions and toxicological responses for EC relative to 3R4F, it is necessary to assess the potential residual risk. Thus, an *in silico* approach using MOEs was used to evaluate the selected carbonyls and nitrosamines detected in EC emissions (Margham et al., 2016). The MOEs generated for acetaldehyde, propionaldehyde, butyraldehyde and acetone were all above 10,000, indicating a low priority for risk management actions. The MOEs generated for acrolein and formaldehyde, based on EC use were below 10,000 indicating a higher priority category. However, when comparing to the MOEs generated for smoking exposure for the same toxicants, the MOEs for EC use were greater by 1–2 orders of magnitude. For the two

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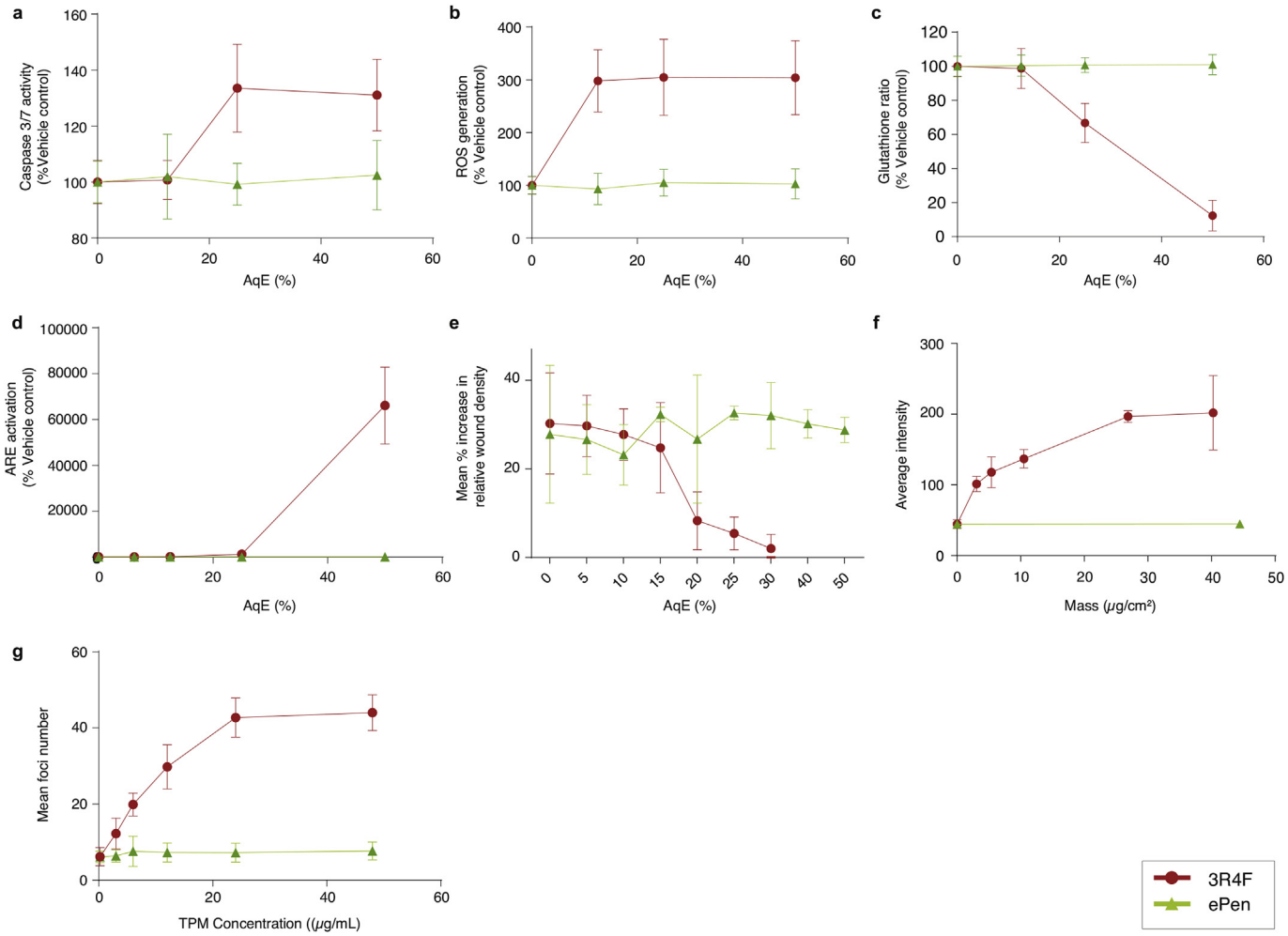

Fig. 5. Results from the comparison of 3R4F cigarette and EC using *in vitro* tests that assess oxidative stress (a–d); endothelial dysfunction (e); oxidative DNA damage (f) and Tumour promotion (g). (a) Apoptosis measuring Caspase 3/7 activity; (b) Reactive Oxygen Species (ROS) generation; (c) Intracellular glutathione antioxidant ratio; (d) Antioxidant Response Element (ARE) activation; (e) increase in wound density; (f) H2AX and (g) Bhas Cell 42 assay.

nitrosamines, N-Nitrosonornicotine (NNN) and N-Nitrosodimethylamine (NDMA), the MOEs based on EC emissions were greater than 10,000. The MOEs for NNN, acetone and butyraldehyde were based on limited data from either acute exposure or non-inhalation studies. From these studies it can be concluded that reduced levels of emissions from EC relative to cigarette smoking result in them having a reduced computational toxicological profile.

9.5. Stage5. in vitro models of disease

The products were assessed in a range of *in vitro* assays to compare biological effects of emissions from EC and 3R4F (Fig. 5). Commercially-available oxidative stress assays were performed using H292 human lung epithelial cells and AqE generated from EC aerosols and 3R4F smoke. In each of the assays (Fig. 5a–d), the cigarette smoke induced extensive oxidative stress whereas the EC aerosol did not induce any oxidative stress (Taylor et al., 2016).

The EC aerosol had no impact on the ability of a cellular system to repair a wound, whereas cigarette smoke completely impaired the wound repair (Fig. 5e) (Taylor et al., 2017). Thus we can conclude that EC aerosol AqE at these dose levels does not impair endothelial cells motility and ability to divide to form a confluent cell ability to migrate and close an artificially induced wound as opposed to cigarette smoke AqE. Further studies showed that whole mainstream smoke aerosol induced oxidative DNA damage in lung cells, H2AX whereas EC vapour induced no oxidative DNA damage (Fig. 5f) (Thorne et al., 2017). Furthermore, cigarette smoke particulate matter acted as a weak initiator and strong promoter *in vitro* using the Bhas 42 cell transformation assay whereas EC ACM did not (Fig. 5g) (Breheny et al., 2017).

In addition to their role in the totality of the assessment framework, *in vitro* disease models are extremely useful for early stage screening and assessing products rapidly, which is advantageous in such a fast-paced category.

9.6. Stage 6. systems science

In this stage, transcriptomics approaches were applied to a 3D lung tissue model to screen for biological perturbations potentially associated with adverse events leading to diseases following exposure to aerosols from EC and 3R4F (Banerjee et al., 2017 and Haswell et al., 2017). An exemplar heatmap showing the comparative differential gene response between EC aerosol and cigarette smoke is illustrated for key biological functions including inflammation (Fig. 6). Gene markers significant at pFDR<0.05 in one or more of the treatment contrasts are shown. Gene expression was assessed by RNA-seq at 24 and 48 h post-exposure and adjusted for post-exposure time. Post-exposure time adjustment combined the

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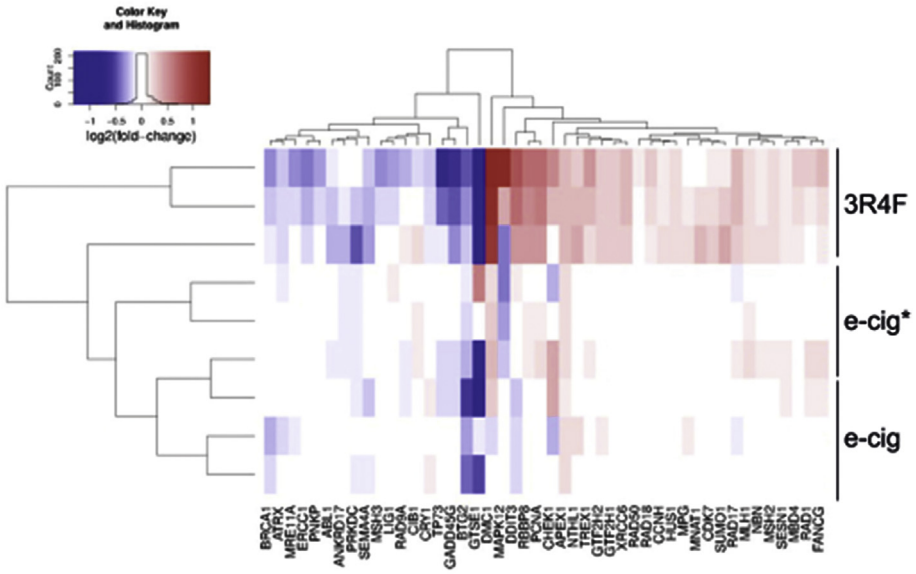

Fig. 6. Unsupervised hierarchical clustering for inflammation genes from cells treated with Vype EC aerosol and 3R4F cigarette smoke. DNA damage signalling pathway markers significant in one or more of the comparisons.

time points to increase statistical power and tease out potential responsive genes especially in the e-cigarette treatment group. EC aerosols dilutions were selected (1/3 and 1/7) to match and double nicotine delivery compared to 3R4F smoke (1/30). There was a distinct reduction in response observed following exposure to EC aerosol to 3R4F cigarette smoke.

Analysis of the data showed that numerous biological networks associated with smoking-related diseases were upregulated following exposure to cigarette smoke, whereas those same networks were either not upregulated, or the level of upregulation was substantially lower, following exposure to the EC aerosol. Other research using a systems toxicology approach has shown significantly reduced effects of an NGP aerosol on a surrogate for atherogenesis compared to cigarette smoke (Poussin et al., 2016). While these studies only examined a subset of possible disease processes and a limited part of what could potentially be achieved using other systems science models, the results are indicative of the potential reduced risk nature of EC.

10. Phase 2. clinical studies

10.1. Stage 7. exposure and PK studies

The chemical and *in vitro* studies were laboratory based, using machine puffing engines to replicate human puffing. Therefore, there is an important need for EC use data to ensure that machine-testing data reflects real-world use; therefore, we performed an initial study to measure consumer puffing behaviour with a variety of e-cigarette products including EC (Cunningham et al., 2016). This study was one of the largest consumption survey studies conducted on e-cigarettes to date, comprising over 1000 subjects who used 'cigalike' e-cigarette (products that have a similar format and appearance and conventional cigarettes), modular and refillable tank systems. From our study of over 1000 subjects using a web based questionnaire, we found that consumption can vary between both consumers and product types (Cunningham et al., 2016).

As well as gross usage patterns, a further consideration is how consumers use products during a single use period. Therefore, a smaller subset of 60 subjects who used EC took part in a puffing behaviour study and the results showed that their behaviour comprised an average puff volume of 52.2–83 ml, an average puff duration of 2.0–2.2s and average interval between puffs of 23.2–29.3s (Cunningham et al., 2016). This consumer behaviour study showed that the machine puffing methodology recommended by the CORESTA (Cooperation Centre for Scientific Research Relative to Tobacco) organisation, '*CORESTA recommended method CRM No.81'* which is a machine puffing regimen of a 55 ml puff, with a 3s duration and a puff interval of 30s between puffs (CORESTA 2015) was representative of what was measured with consumers for EC. However, as consumers use different products in different ways, even between different formats within one category (Hajek et al., 2014, Farsalinos et al., 2015 and Dautzenberg and Bricard, 2015), it may be necessary to conduct consumer studies to determine the machine puffing regime again in the future for novel e-cigarette technologies.

To further investigate consumer usage of EC, two nicotine pharmacokinetic studies were conducted in which we assessed nicotine uptake into the blood of users during a single product use session, comparing EC to both currently-marketed conventional cigarettes and a cig-a-like e-cigarette (Fearon et al., 2016 and Fig. 7). The first study conducted in the UK included smokers who were naïve users of e-cigarettes, whereas the second study was conducted using experience US vapers. As EC was the same in both studies, the user experience was a key determinant for nicotine uptake.

In the first study, C_{max} (geometric mean (CV)) during the 5-minute puffing period was 13.4 (51.4%) ng/ml for a regular cigarette. EC C_{max} was significantly lower at 2.5 (67.8%) ng/ml. In the second study, during the 5-minute *ad libitum* puffing period cigarette C_{max} was 7.2 (130.8%) ng/mL, and it was 7.8 (108.2%) ng/mL for EC. In this study, nicotine C_{max} for a cig-a-like EC was lower than that seen for the EC (Fig. 5), with a mean value of 4.7 (93.6%) ng/ml. These findings are similar to those of Farsalinos et al. (Farsalinos et al., 2015), where more experienced users of e-cigarettes adapted their behaviour to achieve their required nicotine uptake. Furthermore, Vansickel et al. (Vansickel et al., 2012) conducted an abuse liability assessment using PK measurements in conjunction with subjective effect questionnaires. The nicotine plasma levels measured from subjects using an e-cigarette were in-line with those found in our studies (ca. 7 ng/ml). Vansickel et al. concluded

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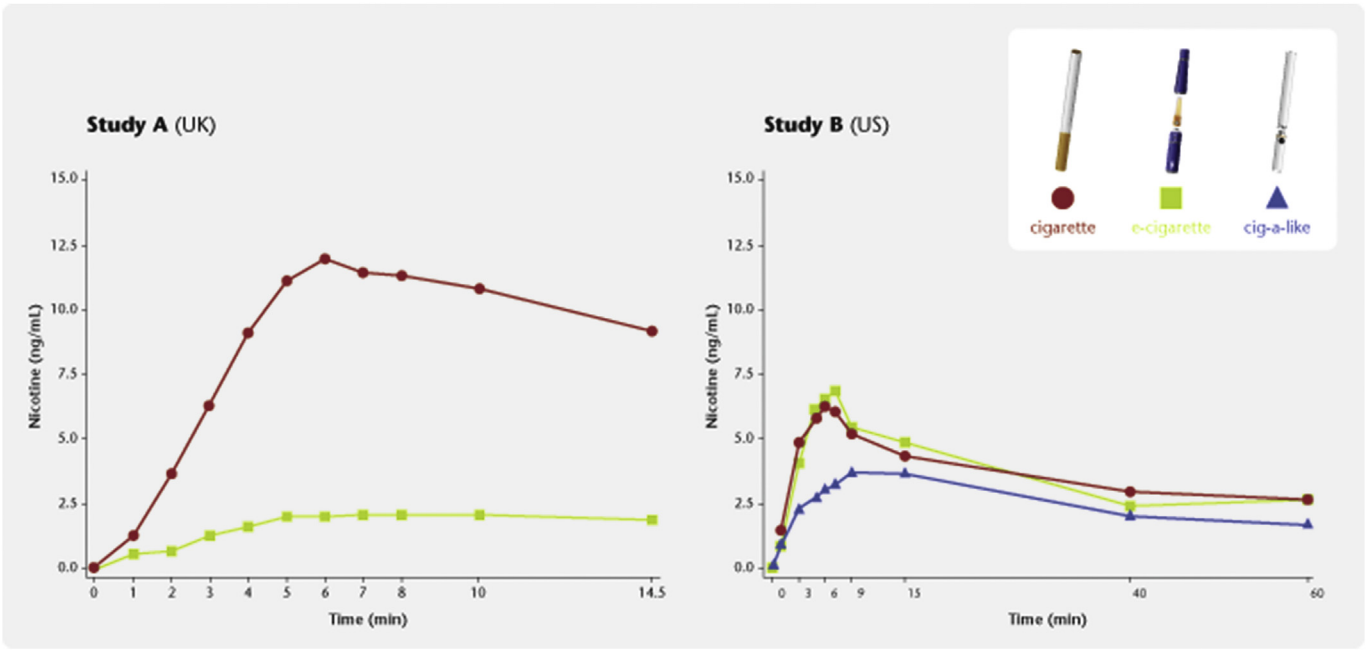

Fig. 7. Pharmacokinetic data illustrating blood nicotine profile for EC compared to commercial cigarettes and a cig-a-like EC. Study A – Plasma nicotine levels in smokers smoking a cigarette or using an e-cigarette. Nicotine levels in blood of smokers using an e-cigarette are much lower than those when they smoke a cigarette. Study B – Plasma nicotine levels in e-cigarette users smoking a cigarette or using one of two different e-cigarettes. Vapers blood nicotine levels reach those seen when they smoked a cigarette.

that electronic cigarettes have a lower potential for abuse relative to traditional cigarettes, under the measured laboratory conditions (Vansickel et al., 2012).

Other researchers have conducted comparative nicotine PK studies and have found that NGPs deliver nicotine with kinetics close to cigarettes (Teichert et al., 2017) and an MHRA-approved NRT (Walele et al., 2016a and 2016b).

We have previously reviewed biomarkers of exposure (BoEs) for use in tobacco product assessments (Puntmann, 2009, Schmidt 2006, Gregg et al., 2013 and Proctor 2015) and the recommended BoEs are outlined in Supplementary Table 1. BoE studies on subjects who switched from a conventional cigarette to a Reduced Toxicant Prototype (RTP) cigarette have also been conducted and reported (Shepperd et al., 2013a, 2013b and 2015).

Recently, clinical studies have been used to quantify consumer's exposure to toxicants from different products using biomarkers of exposure (BoE). Cravo et al. found lower levels of BoEs with e-cigarette use than with smoking; were a decrease of up to 54.5% in BoEs related to smoke toxicants was found (Cravo et al., 2016). Two other clinical studies (Goniewicz et al., 2017 and D'Ruiz et al., 2016) investigated switching smokers to e-cigarettes solus use in comparison to dual use and found that BoE reductions were greatest with solus use. Furthermore, one of the studies (D'Ruiz et al., 2016), showed that solus e-cigarettes use resulted in BoE reductions that were comparable to those from continued cessation. The two studies were conducted over a short period time of two weeks and one week respectively (Goniewicz et al., 2017 and D'Ruiz et al., 2016). However, it is noteworthy, that a recent study sponsored by Cancer Research UK (Shahab et al., 2017), which was conducted over a six month period, showed similar results that solus e-cigarettes (or NRT use) resulted in sustained lower levels of BoEs than both smoking and dual use.

10.2. Stage 8. biomarker of biological effect study

Modified Risk Tobacco Product (MRTP) applications for novel tobacco products are now required to show risk reduction to individuals' relative to the risk of continued smoking. The objective of the biomarker of effect study is to monitor health effect indicators (BoBEs) in smoking subjects who continue smoking; switch to using a Next Generation Product (NGP) or cease smoking over a time period of 6–12 months.

Other research has shown switching to e-cigarettes reduces disease-related effects. For example, harm from smoking in asthma patients can be reversed, with persisting long-term benefits, by dual use or sole use of e-cigarettes; dual users substantially reduced their daily tobacco consumption by 70–80% (Polosa et al., 2016a). And a retrospective study suggests e-cigarettes may help smokers with COPD substantially reduce cigarette consumption by at least 75% or remain abstinent, markedly improving COPD symptoms and ability to perform physical activities (Polosa et al., 2016b).

Current research is focussed on executing clinical studies measuring both biomarkers or exposure and effect to assess if similar reductions in BoEs and favourable changes in BoBEs from the published studies occur when smokers switch to EC. The findings from these studies will be the subject of future publications.

11. Phase 3. population studies

Using publically available data from the UK, Hill et al. (Hill and Camacho, 2017) modelled the potential population health outcomes of introducing e-cigarettes into the market place. Mortality over a 50-year period (2000–2050) was the health outcome of interest, and was compared between two scenarios, with and without e-cigarettes being introduced. The results suggest that by 2050, smoking prevalence in adults was 12.4% in the core model and 9.7% (including dual users) in the counterfactual base model. Smoking-related mortality was 8.4% and 8.1%, respectively. The results suggested an overall beneficial effect from launching e-cigarettes and that system dynamics could be a useful approach to assess the potential population health effects of nicotine products when epidemiological data are not available.

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Considered in their totality, these studies have laid the foundations for ePen being characterised as a potentially reduced risk product, in line with findings from both PHE (McNeill et al., 2015) and RCP (The Royal College of Physicians, 2016), though more studies would be required to confirm this at the individual and population level to both users and non-users.

12. Future outlook, challenges and limitations

Innovation is the cornerstone of the development of any new-to-world product category. For e-cigarettes to fully meet the harm reduction potential proposed by Nutt et al. (Nutt et al., 2014) currently supported and endorsed by PHE, RCP and Cancer Research UK (McNeill et al., 2015, RCP 2016 and Cancer Research 2017), on-going product innovation will be required to meet consumer's evolving expectations. The pace of innovation in this new product category is such that a new approach is required to ensure that the necessary datasets can be compiled on the products (eg. safety assessments, regulatory submissions etc.), in tandem with current product life cycles which are currently measured in months. A bridging framework is required to ensure that incremental improvements in these innovations can quickly have their harm reduction potential assessed and, more importantly, that products with a greater potential impact on harm reduction find their way into the marketplace in a manner commensurate with that potential while maintaining safety standards. Taking and modifying best practice from the 'biosimilars' approach (European Medicines Agency 2014), could facilitate this by allowing data from an original 'reference' product to be added to on a 'need' basis to the new variant 'similar' product.

The nature of tobacco products, their use and subsequent impact on health is complex, so a multi-disciplinary framework is required for the comprehensive evaluation of novel nicotine and tobacco products and the substantiation of health related claims. Some of the challenges of this approach will be the harmonisation of approaches, agreement of methodologies and standardisation across the various studies.

In the interest of public health, it is imperative that information on the potential risk, and potential reduction in risk compared to smoking cigarettes is provided. It is the responsibility of regulatory, public health and industry scientists to agree an approach for the independent verification of data. Transparency is key and would be facilitated through peer reviewed publication of data and making datasets publicly available.

The advent of the workshops moderated by groups such as the Institute for In Vitro Sciences (IIVS) (Institute for In Vitro Sciences, 2017), is an example of how regulatory, public health, academia and industry scientists could work together to agree and harmonise on a science framework, which could be used for evidence based regulation of products across the risk continuum. Furthermore, a need for consolidation around data standards and terminology remains, and methods need to be harmonised by regulatory, public health and industry scientists.

13. Conclusions

Millions of smokers worldwide are now using e-cigarettes and in the UK, these products are gaining support from public health authorities as a wide body of evidence consistently finds e-cigarettes are less harmful than smoking, with the current best estimate of around 95% less harmful. In support of this, the Royal College of Physicians has urged public health to "Promote e-cigarettes widely as substitute for smoking.

This paper described a three-phase framework for assessing the potential of novel tobacco and nicotine products as reduced risk products. Furthermore, the AOP approach is incorporated synergistically in this framework as it has the potential to map key events from toxicant exposure through to smoking related diseases using a variety of chemical, *in vitro*, 'omics' and biomarker studies. Additionally, the utility of the framework was piloted by comparing a closed modular e-cigarette (Vype ePen) against both reference and conventional cigarettes in a range of chemical, *in silico*, *in vitro* biological and human studies. The results form the most comprehensive dataset on a single e-cigarette to date and when considered in their totality are in line with the findings of Public Health England (McNeill et al., 2015), that ePen has the potential to be a reduced risk product in comparison to cigarettes. However, longer term clinical studies will be required to fully determine this potential and to demonstrate individual risk reduction. Furthermore, a range of pre- and post-market studies are required to substantiate them as products that can reduce risk on a population level.

The proposed framework would generate large and heterogeneous foundational datasets on an original 'reference' product. When complete, datasets could be compared between other NGPs, such as THPs, enabling the ranking of products across the risk continuum. Additionally, in the fast paced world of NGP innovation, datasets could be transferred from the 'reference' NGP to 'similar' variants of that product, with data sets added on a needs basis.

An approach that meets the requirements of regulators, public health and manufacturers is required to ensure that the best possible NGPs, like e-cigarettes are being made available on the market, and that consumers are informed of their reduced risk potential in comparison to smoking cigarettes.

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Appendix A. Supplementary data

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Transparency document

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