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Assessment of tobacco heating product THP1.0. Part 9: The placement of a range of next-generation products on an emissions continuum relative to cigarettes via pre-clinical assessment studies

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ABSTRACT

This series of nine papers described the operation and pre-clinical assessment of a tobacco heating product THP1.0. This last paper contextualises the pre-clinical assessment data on THP1.0 with data from other next generation products relative to cigarette smoke.

The tobacco and nicotine risk continuum is a concept that ranks products according to their potential harm, with cigarettes at the highest risk extreme and Nicotine Replacement Therapy at the least risky extreme. Data generated in pre-clinical studies on THP1.0 and a range of Next Generation Products (NGPs) may provide some initial indication of potential ranking of these products, although importantly, data from such studies are limited and cannot take into consideration several important aspects for risk such as long term product use patterns.

In each of the studies, the responses to the emissions from THP1.0 were substantially reduced relative to cigarette smoke. Additionally, responses from THP1.0 were very similar to those from the other NGP emissions. A comparison of the results clearly showed the emissions from all the NGPs were considerably lower than those from cigarettes and all in around the same emissions level.

These results show that THP1.0 could have the potential to be a reduced risk product compared to cigarettes, though further studies assessing the exposure, individual and population risk reduction profile would be required to substantiate this potential.

1. Introduction

Tobacco has been used by people globally for centuries, and current estimates suggest that over 1 billion people are using products from oral smokeless, through pipe, shisha, factory-made cigarettes and roll-your-own tobacco products. Factory-made cigarettes are overwhelmingly the main form of tobacco used and when smoked. The tobacco is combusted at temperatures in excess of 900 °C, creating smoke that comprises more than 6500 different identified chemicals (Rodgman and Perfetti, 2013), of which around 150 constituents are thought to be toxicants (Fowles and Dybing, 2003). Continued exposure to these chemicals over time can lead to smoking-related diseases, such as cardiovascular disease, chronic obstructive pulmonary disease and cancer (US DHHS, 2014). Detailing the specific toxicants that are the prime causes of disease has been the focus of research for decades, and biological causes linking to specific toxicants or classes of toxicants are far from being fully understood. Presently, different priority toxicant lists have been proposed by the World Health Organization (WHO) (Burns et al., 2008), Health Canada (1999), and the US Food and Drug Administration (FDA), with both their shortened list and a list of harmful and potentially harmful constituents (HPHC) (FDA, 2012a).

Tobacco harm reduction, which 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 being considered by some regulators. In many countries, including the USA and European countries, the ability to market next-generation products (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

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Abbreviations

BoE	Biomarker of exposure
E-cigarettes	Electronic cigarettes
HCI	Health Canada intense
HPHC	Harmful and potentially harmful constituent
IOM	Institute of medicine
MMD	Mass median diameter
NGP	Next-generation products
RTP	Reduced toxicant prototype
THP	Tobacco heating product
THS	Tobacco heating system
TPM	Total particulate matter
WA	Whole aerosol
WHO	World Health Organization

requirements to introduce tobacco products onto the market place, either via the Substantial Equivalence pathway where a predicate product exists or the Premarket Tobacco Application approach for novel tobacco products (FDA 2016). In Europe, assessment of product performance and impact on users and non-users 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 FDA has detailed the questions and the types of studies that should be considered by a manufacturer to investigate the reduced-risk nature of novel products, and these form part of a Modified Risk Tobacco Products application (FDA, 2012b). In response to these guidelines, product assessment frameworks have been published (Berman et al., 2015; Murphy, 2017; Murphy et al., 2017; Smith et al., 2016) proposing series of pre-clinical, clinical and population studies for the assessment of the relative risk of NGPs versus cigarettes.

A vast number of smokers across the globe are using NGPs to reduce or replace their consumption of cigarettes. Electronic cigarettes (e-cigarettes), tobacco heating products (THPs), such as THP1.0 (British American Tobacco) and iQOS (Philip Morris International) and hybrid THPs that combine both vapour and tobacco technologies are examples of such products (Poynton et al., 2017). Current NGPs are designed and operate differently from cigarettes and, thus, generate very different aerosols (Eaton et al., 2017). THPs contain tobacco but operate at temperatures of typically 250–350 °C, which are much lower than the combustion temperature in cigarettes of around 900 °C (Eaton et al., 2017; Schaller et al., 2016). Recently, a hybrid THP was described as heating a glycerol-based formulation containing nicotine and flavourings at around 250 °C and passing the resulting aerosol over a bed of tobacco at 30-40 °C, eluting volatile tobacco flavourings (Poynton et al., 2017). E-cigarettes, however do not contain tobacco and also operate at temperatures around 250 °C briefly to aerosolise propylene glycol and glycerol based e-liquids (Etter, 2013).

The advent of the array of NGPs has led to questions regarding the relative risk of each of the product categories to cigarettes. McNeill and Munafò (2012) introduced the concept of the product risk continuum, which placed different products that contained tobacco and nicotine, including pipes, oral smokeless, shisha products and e-cigarettes on a continuum of risk, with cigarettes being at the highest risk extreme and nicotine-replacement therapy at the least risky extreme.

Building on this, Nutt et al. (2014) used a Delphi panel approach comprising global public health experts to estimate the relative harms from products across the risk continuum, using a multi-criteria decision analysis model. In this study, cigarettes were estimated to be the most harmful product owing to their associated mortality and morbidity in users and others, whereas the harms from products like snus (5%), e-cigarettes (4%) and nicotine-replacement therapy (2%) were estimated to be substantially less harmful (Nutt et al., 2014).

Currently, in the UK, after reviewing the available evidence, several public health agencies have advocated a potential role for novel nicotine products in tobacco harm reduction. Public Health England (McNeill et al., 2015) has stated that “The wider body of evidence consistently finds that (e-cigarettes) are less harmful than smoking” and that “The current best estimate is that (e-cigarettes) are around 95% less harmful than smoking”. In support of this, the Royal College of Physicians (2016) has urged public health strategies to promote e-cigarettes widely as substitute for smoking. Recently, Cancer Research UK (2017) has also publicly supported the use of e-cigarettes as a less risky product for smokers.

This paper describes the first comparative pre-clinical assessment of a range of tobacco and nicotine products. The data from a series of chemical and *in vitro* studies will enable the ranking of the emission responses to aerosol from THP1.0 against those to other NGPs and cigarettes. Furthermore, this assessment will give insight into the potential risk profiles of the different NGP categories relative to cigarettes, although clinical and population studies would be required to fully assess this risk profile at both the individual and population levels.

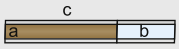
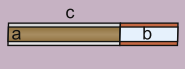
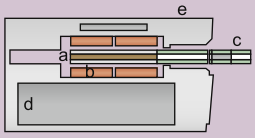
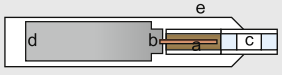
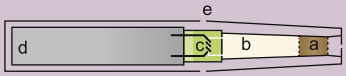
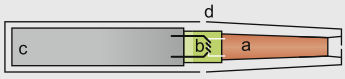
2. Product descriptions

This paper describes the results from testing seven products as described in Table 1. Three cigarettes were studied, including the research reference cigarette 3R4F (Center for Tobacco Reference Products, University of Kentucky, Lexington, KY, USA) and two commercial cigarettes, Lucky Strike Regular (LSR; non-mentholated) and DuMaurier Silver (DMS; British American Tobacco, London, UK). The THP1.0 device comprises the glo heating device with Bright Tobacco KENT Neosticks, and was tested with tobacco and menthol variant consumables, all sourced from Japan. The tobacco heating system (THS) was an iQOS heating device with Essence Marlboro HeatSticks (Philip Morris International), both sourced from Japan. The hybrid THP is a commercial product called KENT iFuse used with Neopod tobacco flavour consumables, which were sourced from Romania. The e-cigarette product tested was Vype ePen with blended tobacco flavour e-liquid cartridges (1.8% nicotine), which were sourced from the UK.

3. Methods

The assessments described in this paper utilised a range of puffing regimes and chemical and *in vitro* toxicological methodologies for the assessment of the NGPs relative to cigarettes. Three cigarette controls were used for the studies namely 3R4F, LSR and DMS (Table 1). The scientific reference cigarette from the University of Kentucky, 3R4F, has been widely used in studies on tobacco products for over a decade (Roemer *et al.*, 2012), and was used as the control cigarette throughout all laboratory-based studies. The 3R4F cigarette was designed for research purposes only and not for consumer use. For the consumer-based studies (puffing behaviour and environmental emissions), therefore, only commercially available cigarettes were used. The two most popular styles of cigarettes sold globally are based on a blend of flue-cured Virginia tobacco or a blend of Virginia, Burley and Oriental tobaccos (“United States blended”), for which DSM and LSR were selected as representative examples, respectively.

Table 1
Summary of products tested in pre-clinical assessments of emissions.

Code	Product type and manufacturer	Temperature (°C) of consumable during operation	Aerosol formation mechanism	Reference
3R4F	**Cigarette:** 3R4F scientific reference cigarette, University of Kentucky a. Tobacco b. Filter c. Paper 	>900	Pyrolysis and combustion of tobacco	Roemer 2012
LSR	**Cigarette:** Lucky Strike Regular, British American Tobacco a. Tobacco b. Filter c. Paper 	>900	Pyrolysis and combustion of tobacco	N/A
DMS	**Cigarette:** Du Maurier Silver, British American Tobacco a. Tobacco b. Filter c. Paper 	>900	Pyrolysis and combustion of tobacco	N/A
THP	**Tobacco heating product (THP):** Glo and Kent Neosticks (Bright Tobacco) British American Tobacco a. Tobacco b. Heat source c. Mouthpieces d. Electronics and battery e. Device body 	245	Heating of a tobacco substrate	Forster 2017a (this series)
THS	**Tobacco heating product (THP):** iQOS and Marlboro Heatsticks (Essence), Philip Morris International a. Tobacco b. Heat source c. Mouthpieces d. Electronics and battery e. Device body 	340	Heating of a tobacco substrate	Forster 2017a (this series) Schaller 2016
hTHP	**Hybrid Tobacco Heating Product (hTHP):** Kent iFuse and Neopods (Blended Tobacco 1.8% Nicotine), British American Tobacco a. Tobacco b. E-liquid c. Wick and coil d. Electronics and battery e. Device body 	250/34	Vaporisation of a formulation and passage through a tobacco plug	Forster 2017b
EC	**Electronic cigarette (EC):** Vype ePen and eCaps (Blended Tobacco 1.8% Nicotine), British American Tobacco a. E-liquid b. Wick and coil c. Electronics and battery d. Device body 	250	Vaporisation of a formulation	Margham 2016

3.1. Puffing behaviour measurements

The majority of studies conducted in our assessment were laboratory-based chemical and *in vitro* biological tests, in which smoking machines were used to generate the aerosol emissions. The measurement of consumer in-use puffing behaviour, however, is key to ensure that the puffing regimes that are used in the laboratory tests are representative of actual consumer behaviour. Thus, studies have been done to compare the in-use consumer puffing behaviour when using NGPs from across the risk continuum, including Gee et al. (2017) using LSR, THP1.0 and THS. Cunningham et al. (2016) have also studied in-use consumer puffing behaviour using e-cigarettes and hybrid THPs.

3.2. Chemical and physical assessments

Four types of chemical studies were conducted to measure (i) the principal components in mainstream aerosol; (ii) mainstream aerosol toxicant levels; (iii) environmental emissions from product usage and (iv) physical characterisation of the aerosol. Details of each assessment are given below.

3.2.1. Measurement of principal components in mainstream aerosol

The focus of the first chemical study was to characterise the mainstream aerosol by measuring the principal aerosol components for all the products, using the puffing regimes described in Table 2 (the justification for using these regimes is summarised in section 4.1). They include the levels of total particulate matter

Table 2
Machine puffing regimens used in pre-clinical assessment.

Product	Regimen	Puff volume (ml)	Puff duration (s)	Puff interval (s)	Ventilation occlusion
3R4F	HCI[a]	55	2	30	100%
LSR	HCI[a]	55	2	30	100%
DMS	HCI[a]	55	2	30	100%
THP1.0	HCI[a]	55	2	30	NA
THS	HCI[a]	55	2	30	NA
hTHP	CRM81[b]	55	3	30	NA
EC	CRM81[b]	55	3	30	NA

[a] Health Canada, 1999.
[b] CORESTA, 2015. Abbreviations: HCI=Health Canada intense machine puffing regime; LSR = Lucky Strike Regular; DMS = DuMaurier Silver; THP = tobacco heating product; THS = tobacco heating system; hTHP = hybrid tobacco heating product; EC = electronic cigarette; NA = not applicable.

(TPM), water, nicotine, glycerol and propylene glycol. These five aerosol components are generally found in the emissions from cigarettes and NGPs to varying degrees.

The methodologies for measuring the components in the mainstream aerosol of all the products are summarised in Table 3.

3.2.2. Measurement of aerosol toxicant levels

The second chemical assessment focused on measuring a range of toxicants relevant to regulators and public health authorities. We focused on the WHO TobReg first nine toxicants mandated for lowering (Burns et al., 2008) and the chemicals from the FDA shortened list of HPHCs (FDA, 2012a). We used the toxicant levels reported for 3R4F and THP1.0 (Forster et al., 2017a) and previously reported data on THS (Schaller et al., 2016), hybrid THPs (Poynton et al., 2017) and e-cigarettes (Margham et al., 2016). Details on the experimental techniques adopted for those studies are available in each publication.

3.2.3. Environmental emissions from product usage

The third chemical assessment focused on measuring a range of toxicants in the environmental emissions. Forster et al. (2017b) outlined the study design and methodologies for quantifying 25 constituents and measuring the aerosol particle concentration in the environmental emissions for the control cigarettes (LSR and DMS) and THP1.0. Other NGPs, including a hybrid THP and an e-cigarette, were measured using similar methodologies.

3.3. Physical assessment: aerosol characterisation

The fourth assessment measured mass median diameter (MMD) and the average particle size of the aerosols produced from the NGPs. The aerosols generated from the products were characterised using a DMS500 Fast Particle Analyzer (Cambustion, Cambridge, UK) electrical mobility analyzer and a Spraytec laser diffraction system (Malvern Instruments, Malvern, UK) to measure particle size and particle number and to estimate particle mass. The methodology used was described by Forster et al. (2017b) for the assessment of LSR, THP1.0 and THS. The hybrid THP and e-cigarette were assessed using the same methodology, measuring the mass median aerosol diameter and the geometric standard deviation.

3.4. In vitro dosimetry

The quantification of exposure to aerosol components at the cellular level is of key importance to contextualise *in vitro* laboratory based exposures with consumer use. Programmed doses (e.g., air dilution factors) on any given smoking machine may not necessarily align with the delivered dose the cells experience throughout the exposure period. Thus, such measurements improve confidence in relating dose to biological effects. In vitro dosimetry from NGPs have previously been studied (Azzopardi et al., 2016; Schaller et al., 2016; Thorne et al., 2016) using *in vitro* assays targeting various toxicological end points. The assays that were described in this series (Thorne et al., 2017) used two machine-generated exposure techniques involving TPM trapped on a Cambridge filter pad and whole aerosol (WA) exposure. Dosimetry analyses were previously carried out for 3R4F, THP1.0 and THS (Jaunky et al., 2017), hybrid THPs (Breheny et al., 2017a) and e-cigarettes (Adamson et al., 2016) and similar methods have been used to assess THP1.0 and THS (Jaunky et al., 2017). Details on the experimental techniques used to measure the dosimetry are available in each publication.

3.5. In vitro assessment: regulatory toxicological endpoints

NGPs have previously been studied using standard regulatory *in vitro* assays to measure toxicological end points (Azzopardi et al., 2016; Schaller et al., 2016; Thorne et al., 2016). The assays are

Table 3
Methodologies used for the measurement of key components in mainstream aerosol.

Component	Methodology		
	3R4F	THP and THS	hTHP and EC
PM	Health Canada T-115	TMS-00115[a] Appendix D[b]	TMS-00115[a] Appendix E[c]
Water	Health Canada T-115	TMS-00115[a] Appendix D[b]	TMS-00115[a] Appendix E[c]
Nicotine	Health Canada T-115	TMS-00115[a] Appendix D[b]	TMS-00115[a] Appendix E[c]
Glycerol	TMS-00115[a] Appendix D[b]	TMS-00115[a] Appendix D[b]	TMS-00115[a] Appendix E[c]
Propylene glycol	TMS-00115[a] Appendix D[b]	TMS-00115[a] Appendix D[b]	TMS-00115[a] Appendix E[c]

[a] Official Method T-115, Determination of "Tar", Nicotine and Carbon Monoxide in Mainstream Tobacco Smoke, prepared by the Department of Health dated December 31, 1999.
[b] Determination of nicotine, water, propylene glycol, menthol, diethylene glycol, triacetin and glycerol in mainstream particulate phase emissions by gas-chromatographic method (modified T-115).
[c] Determination of nicotine, water, propylene glycol, menthol, ethylene glycol, diethylene, glycol, triacetin, glycidol and glycerol in e-cigarette liquids and aerosols. Abbreviations: LSR = Lucky Strike Regular; DMS = DuMaurier Silver; THP = tobacco heating product; THS = tobacco heating system; hTHP = hybrid tobacco heating product; EC = electronic cigarette.

summarised in Table 4 below, and the experimental techniques were described in this series (Thorne et al., 2017). Two machine-generated exposure techniques were used, involving TPM trapped on Cambridge filter pads and WA.

The full experimental techniques for toxicological evaluation of the range of products and both exposure types have been published for 3R4F, THP1.0 and THS (Jaunky et al., 2017; Thorne et al., 2017), the hybrid THP (Breheny et al., 2017a) and e-cigarettes (Azzopardi et al., 2016).

4. Results and discussion

This series of THP publications, has set out to describe the novel THP1.0 operation and pre-clinical assessment. Using the data from this series of publications and data that have been published in the literature on THS, hybrid THPs and e-cigarettes, they have been compared based on their pre-clinical responses relative to cigarettes.

4.1. Puffing behaviour measurements

The pre-clinical assessment consisted of chemical, physical and biological studies that were conducted in the laboratory using either machine or human puffing regimes to generate the aerosols. Standard machine smoking regimes have been published for cigarettes, with the Health Canada Intense (HCI) (Health Canada, 1999) method being the most commonly adopted by public health authorities, being 55 mL puff volume, 2 s duration and 30 s frequency, with ventilation holes completely (100%) blocked. The Cooperation Center for Scientific Research Relative to Tobacco (CORESTA) has published a recommended method No 81 for assessing e-cigarettes (CORESTA, 2015), 55 mL puff volume, 3 s duration and 30 s frequency, and no blocking of ventilation holes. To date, no standard machine puffing regimes have been published for THPs or other NGPs. Thus, human puffing behaviour studies were conducted to ensure that the laboratory-based puffing machine regimes were broadly reflective of consumer's puffing behaviour.

Puffing behaviour studies have previously been conducted on a range of NGPs, for example, on e-cigarettes (Cunningham et al., 2016; Dautzenberg and Bricard, 2015; Farsalinos et al., 2015), a hybrid THP (British American Tobacco, unpublished) and THPs (Gee et al., 2017; Haziza et al., 2016). Gee et al. (2017) measured the puffing behaviour of Japanese smokers who switched to either THP1.0 or THS for a 5-day home-use period before testing at a central location. In all studies, a device (SA7), was used to measure puff volume, duration, interval and number. Importantly for measurement of puff duration, the SA7 only starts measuring upon consumer puffing. For e-cigarettes, which is button actuated (THP1.0, THS and hybrid THP are not button actuated), the measured puff duration does not include the pre-puff button actuation time. The findings from these studies were used to guide the machine puffing regimes of the pre-clinical studies described here.

Using the published data, we can compare puffing behaviour for the different products across the risk continuum, inclusive of puff volume, puff duration and interval between puffs. Firstly, the average puff volumes measured in the studies (Fig. 1) showed that mean puff volume with LSR (48.8 ± 15.1 ml) was lower than the 55 ml puff volume stipulated in the HCI machine smoking regime (Health Canada, 1999). Average puff volumes were 66.7 ± 25.1 ml and 63.5 ± 20.8 ml when using THP1.0 and THS, respectively. Despite these volumes being higher than the average puff volume observed with LSR, the puff volume from the HCI regime is still relevant for the purpose of comparing emission levels for pre-clinical assessments. With cigarettes, larger puff volumes will add more oxygen into the combustion zone, resulting in more tobacco being burned per puff and, therefore, increasing the generation and subsequent delivery of smoke. Hypothetically, the effect of increasing puffing volume on aerosol delivery would be less for THP and THS products than for cigarettes, as it is independent of the heating of the tobacco and is principally used to condense the vapour into an aerosol. Further studies are required to verify this. The average volumes observed with the hybrid THP and e-cigarette, were 37.1 ± 15.0 ml and 35.7 ± 15.1 ml, respectively. Even though they were lower than the 55 ml machine puff volume recommended by CORESTA (2015), this volume is still relevant to consumers. Furthermore, Gee et al. (2017) have shown that marginal changes in puff volume did not increase the yield of the THPs. Thus, it was concluded that a 55 ml puff volume is suitable to set machine puffing engines for laboratory studies.

Secondly, the average puff duration measured in the studies (Fig. 2) was close to the 2 s stipulated in the HCI machine smoking regime (Health Canada, 1999) with LSR (1.8 ± 0.7 s). Similar average puff durations close to the puff duration of the HCI regime were seen with THP1.0 (1.8 ± 0.6 s) and THS (1.8 ± 0.6 s). The average puff durations observed with the hybrid THP and e-cigarette, were 1.8 ± 0.7 s and 1.7 ± 0.9 s, respectively. In each of the studies, the consumers depressed the activation button for approximately 1 s before puffing, meaning that the heating coil was activated for a 1 s longer than the measured puff duration. As a result, the hybrid THP and e-cigarette aerosolisation durations (or time when the heating coil was activated) were probably closer to 3 s (i.e., 2.8 s and 2.7 s, respectively), which is within the 3 s puff duration recommended by CORESTA (2015). In other words, the 1 s pre-heating duration is not covered by the puffing action itself, but rather is an active part of the aerosolisation mechanism.

In a separate study where 185 consumers used an open-tank e-cigarette, Dautzenberg and Bricard (2015) measured mean puff durations of 3.79 ± 1.89 s. They also observed that, as consumers became more familiar with the product, the puff duration increased from 3.4 ± 1.3 s (day 1) to 4.1 ± 1.6 s (Day 60). Furthermore, in a different study, Farsalinos et al. (2015) reported that with the same e-cigarette, the average puff duration of experienced users was 3.5 ± 0.2 s, whereas naïve users had average puff duration of 2.3 ± 0.2 s. These studies also suggest that a 3 s puff duration would be relevant for studying e-cigarettes.

Thus, it was concluded that for the study reported here, a 2 s period was a suitable puff duration for setting machine puffing engines for testing THP1.0 and THS, whereas the machine puff duration should be set to 3 s for testing hybrid THPs and e-

Table 4
Assessment of products using traditional toxicological endpoints.

Toxicological endpoint	Study	Matrix	Methodology reference
Mutagenicity	*Salmonella typhimurium* reverse-mutation assay (Ames test)	Total particulate matter	OECD, 1997a
Mutagenicity		Whole aerosol	
Cytotoxicity	Neutral red uptake assay	Whole aerosol	ICCVAM, 2006
Tumour promotion	Bhas 42 cell transformation assay	Total particulate matter	OECD, 2016

Abbreviations: OECD = Organization for Economic Co-operation and Development. ICCVAM=Interagency Coordinating Committee on the Validation of Alternative Methods.

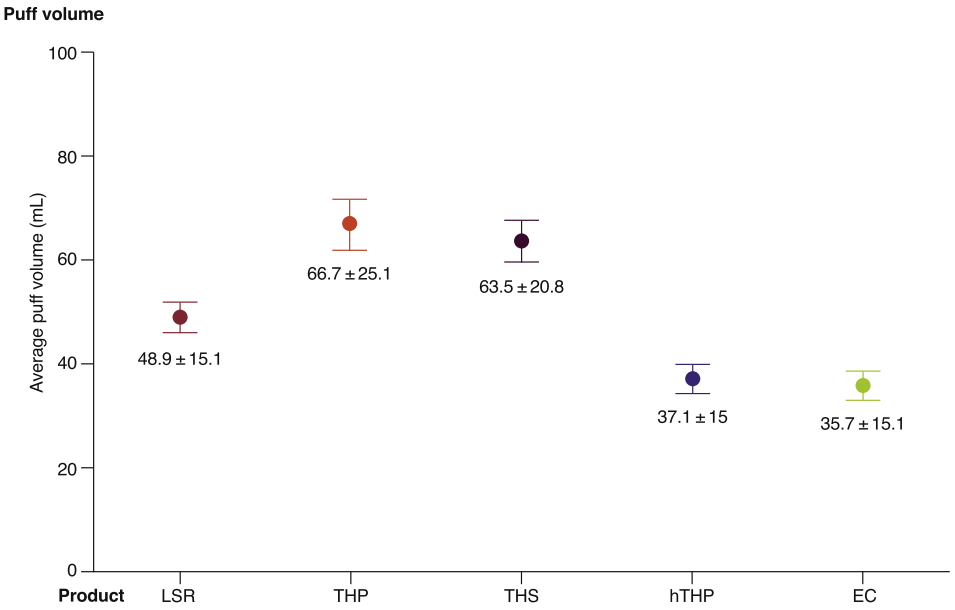


Fig. 1. Average human puff volume (±confidence interval) with a range of nicotine-containing products. Abbreviations: LSR = Lucky Strike Regular; THP = tobacco heating product; THS = tobacco heating system; hTHP = hybrid tobacco heating product; EC = electronic cigarette.

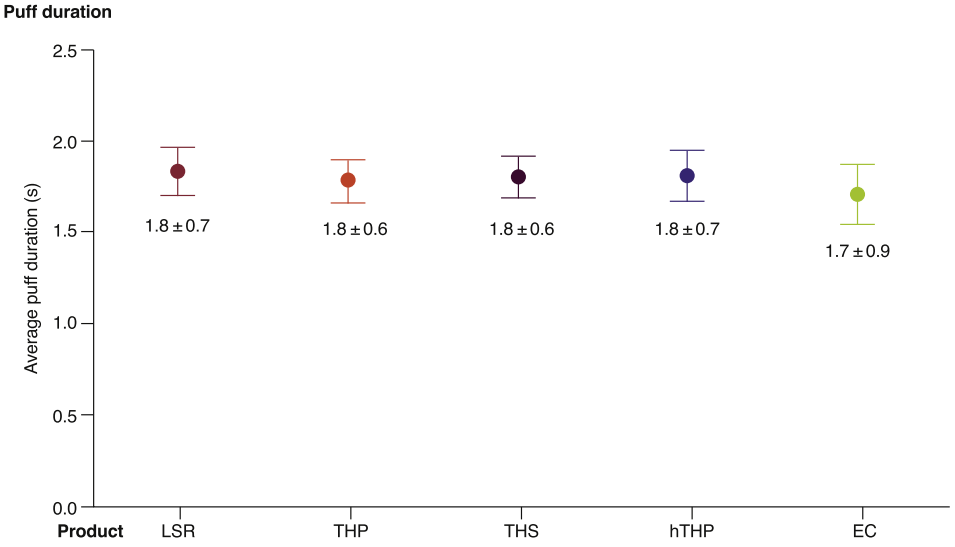


Fig. 2. Average puff duration (±confidence interval) with a range of nicotine containing products. Abbreviations: LSR = Lucky Strike Regular; THP = tobacco heating product; THS = tobacco heating system; hTHP = hybrid tobacco heating product; EC = electronic cigarette.

cigarettes.

The average puff interval was measured in the studies (Fig. 3), which showed that average puff intervals with LSR (11.84 ± 13.67 s), THP1.0 (9.42 ± 3.13 s) and THS (10.29 ± 3.38 s) were lower than the 30s puff interval stipulated in the HCI machine smoking regime (Health Canada, 1999). There is a defined operating duration for each of these products in which cigarettes burn down and electrically heated tobacco products have fixed durations of use or puff numbers when the heat of the device is applied to the tobacco (Eaton et al., 2017). As the consumers in the study were given a 5-day familiarisation period with each of the products, it was likely that they adjusted their puffing behaviour to achieve the number of puffs they desired from the product. Furthermore, despite the shorter observed *inter* puff interval, the mean number of puffs among users of THP1.0 was 10.9 ± 5.8–12.5 ± 7.4, which was similar to the puff numbers they stated when smoking cigarettes of 10.7 ± 5.1–10.9 ± 5.8 (Gee et al., 2017).

Hybrid THPs and e-cigarettes operate in a different manner to THPs, in that they can be puffed and then left for as long as the consumer wishes and then re-puffed. Experienced users of the products (≥6 months of use) that were recruited for the studies would have adjusted their behaviour to use the product in the way that suited them. As a result, the hybrid THP and e-cigarette had average puff intervals of 30.27 ± 24.58 s and 32.15 ± 31.51 s, which is closely aligned with the 30 s puff interval recommended by CORESTA (2015). Thus, it was concluded that for the study reported here, 30 s was a suitable puff duration for setting machine puffing engines for testing LSR, THP1.0, THS, hybrid THP and e-cigarette.

To determine whether where consumers' mouths touched the THP tobacco consumable, and hence whether the air inlet vent of

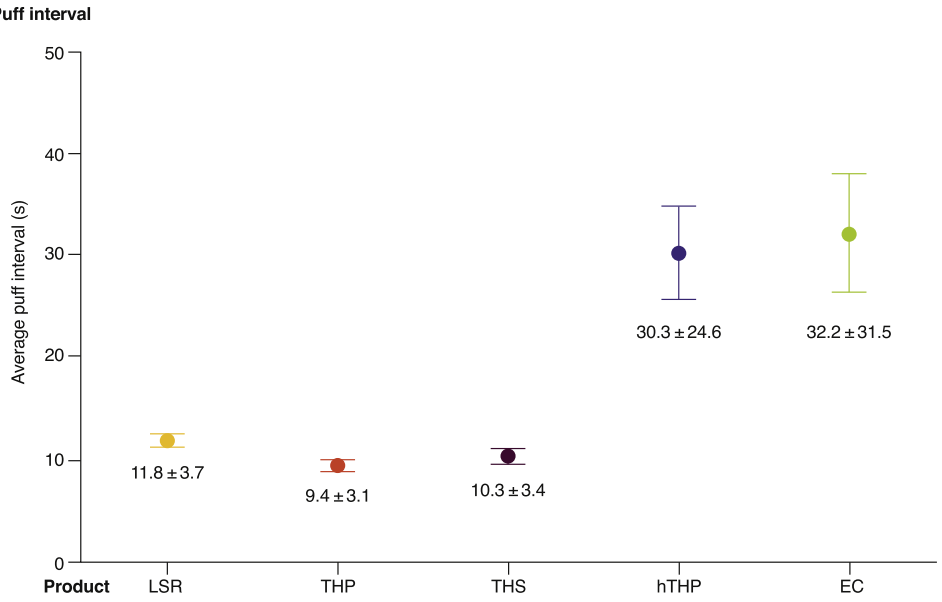


Fig. 3. Average puff interval (±confidence interval) with a range of nicotine containing products. Abbreviations: LSR = Lucky Strike Regular; THP = tobacco heating product; THS = tobacco heating system; hTHP = hybrid tobacco heating product; EC = electronic cigarette.

the THP1.0 would be blocked, preventing air cooling and expansion, ninhydrin, was applied to the THP tobacco consumables to detect saliva after use (Gee et al., 2017). None of the analysed consumables showed any evidence of air inlet blocking, and, therefore, it was deemed appropriate that the air inlet zone is not blocked during laboratory evaluation of THP1.0(T) and THP1.0(M).

4.2. Chemical and physical assessment

4.2.1. Measurement of principal components in mainstream aerosol

The products were assessed using a number of chemical studies, including targeted analysis measuring toxicant emission in aerosol and also environmental emissions. As a starting point, the TPM for each product (on a per-puff basis) was measured to quantify its major aerosol constituents (Fig. 4).

4.2.2. Measurement of aerosol toxicant levels

Previous studies have been conducted to assess the chemical constituents present in NGPs specifically the toxicant levels. Recently Schaller et al. (2016) measured the emissions from THS, concluding that relative to 3R4F, a reduction of approximately 90% was evident for the majority of the analysed HPHCs. Studies have also been conducted on the chemical emissions of e-cigarettes (Burstyn, 2014; Goniewicz et al., 2017; Margham et al., 2016). For example, Burstyn (2014) concluded that although there was no evidence that vaping produced inhalable exposure to aerosol contaminants that would warrant health concerns, surveillance is recommended due to the high levels of propylene glycol and or glycerol inhaled. Furthermore, Goniewicz et al. (2017) analysed the aerosol emissions of 12 different e-cigarette products and found traces of toxic substances, although they were reported at 9 to 450 times lower than the levels in cigarette smoke.

To summarise the differences in levels of aerosol toxicant for the range of NGPs and 3R4F, we grouped together the percentage differences on a per-puff basis for two regulatory lists: the WHO TobReg first nine toxicants that have been mandated for lowering (Burns et al., 2008) and the FDA shortened list of HPHCs (FDA, 2012a). All emissions data is summarised in Supplementary Table 1. For the WHO list, all of the NGPs had substantially reduced levels of toxicants, with THS (95% reduction) and THP1.0(T)

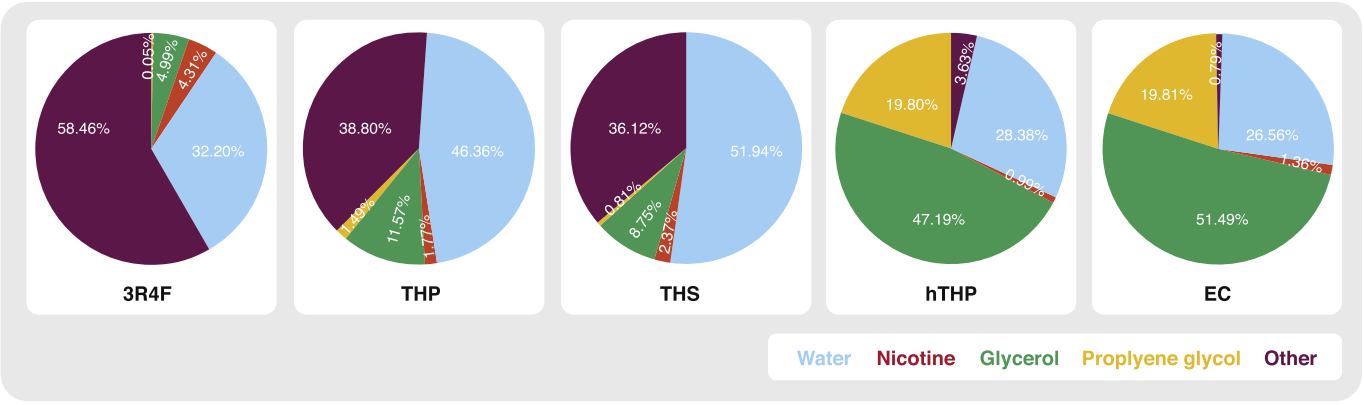


Fig. 4. Measurement of key components in the mainstream aerosol. Abbreviations: THP = tobacco heating product; THS = tobacco heating system; hTHP = hybrid tobacco heating product; EC = electronic cigarette.

(96% reduction) (Forster et al., 2017a), having similar levels of reduction to hybrid THP (>99% reduction) (Poynton et al., 2017) and e-cigarettes (>99%) (Margham et al., 2016). For the FDA abbreviated list of 18 toxicants (except for nicotine), all of the NGPs also had substantially reduced levels of toxicants, with THS (92% reduction) and THP1.0(T) (97% reduction) (Forster et al., 2017a), having similar levels of reduction to hybrid THP (>99% reduction) (Poynton et al., 2017) and e-cigarettes (>99%) (Margham et al., 2016).

The blocking of cigarette filter ventilation in HCI machine smoking regimen (55 ml puff volume/2 s puff duration/30 s puff interval, better to provide a reference here), is known to be a more intensive puffing condition for cigarettes to increase 'tar', nicotine and carbon monoxide yields. The ISO machine smoking regimen (35 ml puff volume/2 s puff duration/30 s puff interval, ISO4387:2009), which does not stipulate the blocking of ventilation holes, has been in regulatory and product development use for several decades, for example, to derive cigarette pack printed yields of 'tar', nicotine and carbon monoxide yields in some jurisdictions (ISO, 2000). Therefore, to give insight into the levels of toxicants in the emissions from the range of the test NGPs relative to cigarette smoke generated without ventilation blocking, we compared the NGP yields versus those of the cigarette generated under the ISO regime.

Focusing on the WHO list, the toxicant emission levels were compared to 3R4F yields measured at both Health Canada Intense (HCI_{3R4F}) and ISO (ISO_{3R4F}). THS was 95% reduced versus HCI_{3R4F} and 85% versus ISO_{3R4F} whereas THP1.0(T) was reduced by 96% compared to HCI_{3R4F} and 87% relative to ISO_{3R4F}. Hybrid THP was >99% reduced relative to HCI_{3R4F} and 98% versus ISO_{3R4F} and e-cigarettes was 99% reduced versus HCI_{3R4F} and 92% compared to ISO_{3R4F}.

For the FDA abbreviated list of 18 toxicants (excluding nicotine), toxicant emission levels were also compared to 3R4F yields measured at both HCI and ISO. THS was 95% reduced versus HCI_{3R4F} and 79% versus ISO_{3R4F} whereas THP1.0(T) was reduced by 97% compared to HCI_{3R4F} and 83% reduced relative ISO_{3R4F}. Hybrid THP was >99% reduced relative to HCI_{3R4F} and 98% compared to ISO_{3R4F} and e-cigarettes was >99% reduced versus HCI_{3R4F} and 92% versus ISO_{3R4F}.

4.2.3. Environmental emissions from product usage

Smoking can introduce emissions into the environment through the exhalation of smoke by the smoker and from the smouldering of the cigarette between puffs. For some regulators, the period of where a tobacco heating product is switched on but not puffed may also require assessment to determine the level of possible aerosol escape through the device. Several studies have been conducted (Baker and Proctor, 1990) on cigarettes to assess the levels of emissions from environmental emissions. NGPs operate differently from cigarettes, in that they do not facilitate smouldering. However, when consumers use these products they typically produce an exhalate into the environment. In this work, the levels of environmental emissions were quantified from the range of NGPs.

A study was conducted with smokers using each of the products in a room with a controlled environment (fixed air flows and air changes), where the air flow could be changed to reflect conditions relevant to home, office and hospitality settings (Forster et al., 2017b). A range of markers relevant to environmental emissions were measured including volatile organic compounds, carbonyl-containing compounds and other compounds, including tobacco-specific nitrosamines and oxides of nitrogen and carbon. Table 5 presents the results under the home condition (1.2 air changes per h), giving an example of the least ventilated environment investigated in the study.

Targeted environmental emissions analysis revealed that toxicants in the room from using THP1.0 relative to the WHO TobReg list (Burns et al., 2008) were reduced by approximately 90% compared with LSR smoke (Forster et al., 2017a). In comparison to cigarette smoke, the levels of measurable environmental emissions from THP1.0 were substantially reduced for acetaldehyde, benzene, formaldehyde and CO, to the extent that they were very similar to those of the baseline measurements (i.e., occupants in the room with no product usage). The techniques were not sufficiently sensitive to measure environmental levels of 1,3-butadiene, benzo[a] pyrene, acrolein, 4-*N*-nitrosonornicotine or 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone from either the cigarette or THP 1.0. In this series, Eaton et al. (2017) found that THP1.0 did not combust tobacco with its maximum heater operating temperature of 240 °C, which is thought to be the main reason behind the substantial reduction on these environmental toxicant levels. This is also supported by the fact that levels of oxides of carbon and nitrogen in the environmental emissions, which are markers of tobacco combustion, were substantially reduced when using THP1.0 versus LSR, to the extent that they were similar to baseline levels in all the cases. Additionally, from the $PM_{1.0}$ mobility measurements (Table 5), the particle mass in THP1.0 aerosol was 99% lower than in LSR smoke. Importantly, the reductions in environmental emissions for THP1.0 are relevant, whether they are compared to the US blended LSR cigarette or the flue-cured blended DMS cigarettes (Table 5). These findings are similar to those published on THS by Mitova et al. (2016).

Furthermore, the hybrid THP and e-cigarette were assessed using this methodology (Forster et al., 2017b) and the levels of environmental emissions were substantially reduced in

Table 5
Levels of toxicants in the environmental emissions from cigarettes and a range of next-generation products.

	Baseline	LSR	DMS	THP1.0	hTHP	EC
VOCs ($\mu g \cdot m^{-3}$)						
1,3-butadiene	ND	ND	ND	ND	ND	ND
Isoprene	17	191	255	16	17	19
Acrylonitrile	ND	ND	ND	ND	ND	ND
Benzene	1	16	21	1	1	1
Toluene	2	29	32	3	2	2
Propylene glycol	ND	ND	ND	ND	ND	ND
Acrylamide	ND	ND	ND	ND	ND	ND
Carbonyls ($\mu g \cdot m^{-3}$)						
Formaldehyde	16	33	43	18	18	17
Acetaldehyde	8	100	118	10	9	9
Acrolein	ND	ND	ND	ND	ND	ND
Crotonaldehyde	ND	ND	ND	ND	ND	ND
Other ($\mu g \cdot m^{-3}$)						
Nicotine	1.3	47	33	0.32	0.79	1
3-ethenyl pyridine	0.2	9	8	ND	0.24	0.35
TSNAs[a] (μg on filter)	ND	ND	ND	ND	ND	ND
PAHs[b]	ND	ND	ND	ND	ND	ND
Glycerol	ND	ND	ND	ND	ND	ND
CO (ppm)	ND	ND	1.4	ND	ND	ND
NO (ppb)	12	30	22	4	4	7
NO_2 (ppb)	9	12	11	8	8	10
NO_X (ppb)	20	42	33	12	12	16
[c]$PM_{1.0}$ – mobility	23	510	572	5	3	3

[a] 4-*N*-nitrosonornicotine (NNN), 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK), *N*-nitrosoanatabine (NAT), *N*-nitrosoanabasine (NAB).

[b] Acenaphthene, acenaphthylene, anthracene, benzo(a)anthracene, benzo(a) pyrene, benzo(b/k)fluoranthene, benzo(ghi)perylene, chrysene, dibenzo(ah) anthracene, fluoranthene, fluorene, indeno(123-cd)pyrene, naphthalene, phenanthrene, pyrene.

[c] PM1.0 = respirable fraction of particulate matter ≤ 1 μm in diameter; Abbreviations: LSR = Lucky Strike Regular; DMS = DuMaurier Silver; THP = tobacco heating product; THS = tobacco heating system; hTHP = hybrid tobacco heating product; EC = electronic cigarette; VOCs = volatile organic compounds; TSNAs = tobacco-specific nitrosamines; PAHs = polycyclic aromatic hydrocarbons; ND = not detected.

comparison to the cigarette, with levels close to that measured in the baseline. Importantly, the environmental emissions for all the NGPs tested were substantially reduced, irrelevant of the cigarette comparator used (Table 5).

There has been much discussion regarding air pollution and the WHO recently recommended indoor air quality (WHO, 2010) and outside air quality standards (WHO, 2016) for $PM_{1.0}$ of 10 μg/m^3. The particle masses, measured from all the products across the risk continuum, were compared and a magnitude of difference was found for the environmental particle emissions between the cigarettes (LSR and DMS) and the NGPs. Furthermore, it was concluded that all NGP particle emissions would be compliant with the proposed WHO outdoor air quality standard.

4.3. Physical assessment: aerosol characterisation

A physical assessment was made on the aerosols from each of the products, inclusive of particle concentration and mass median diameter. Five measurements were made on each of the products and the results are summarised in Table 6.

To ensure accuracy in the different particle size ranges covered, measurements were obtained using both a Spraytec and DMS technique (Table 6) (Forster et al., 2017a). In general, the particle size distribution and number density were similar for 3R4F mainstream smoke and the aerosol emitted by THP1.0. The similarities noted above also indicate that the THP1.0 aerosol can be sampled effectively using the same techniques as for mainstream smoke. Furthermore, comparing MMDs and (Table 6), the aerosols from the cigarettes, THP, hybrid THP and e-cigarettes were within the respirable range (ICRP, 1994).

4.4. In vitro dosimetry

Deposited aerosol particle mass was determined from all the products assessed at the exposure interface within *in vitro* aerosol exposure systems by use of quartz crystal microbalance technology (Table 7).

The results demonstrate that at the ranges selected, aerosol dose (deposited mass) was clearly being delivered to and detected at the cellular exposure interface irrespective of the product and puffing regimes used, and there is a clear overlap in the range between the two exposure systems (Borgwaldt RM20S and Vitrocell VC10).

4.5. In vitro assessment: regulatory toxicological endpoints

For each of the NGPs described above, the product was evaluated in isolation in comparison to cigarettes. In this pre-clinical assessment, responses are summarised for a range of NGPs relative to a cigarette in Table 8, allowing for a more systematic comparison across the risk continuum.

4.5.1. Mutagenicity assessment

Salmonella typhimurium strains TA98 and TA100, in the presence of metabolic activation (Aroclor 1254-induced rat liver S9), were used to test the TPM and WA from the various products. No positive responses were seen with the TPM from THP1.0, THS, hybrid THP or e-cigarettes for either strain, whereas mutagenic activity was observed after exposure to 3R4F smoke TPM. Similarly, both TA98 + S9 and TA100 + S9 strains showed a positive response to WA from the 3R4F reference cigarette under the test conditions, but not to WA from the other test products (Breheny et al., 2017a; Thorne et al., 2016, Thorne et al., 2017).

4.5.2. Cytotoxicity assessment

For whole aerosol analysis, Jaunky et al. (2017); Azzopardi et al. (2016) and Breheny et al. (2017a), described the cytotoxicity for all the NGPs discussed over a range of doses (Seven biologically relevant aerosol dilutions were generated using the RM20S, ranging 1:20–1:10000 for the reference cigarette and 1:2–1:200 for the NGPs (aerosol:air, v:v)). In this series, assessments were made on all products and the comparison of cytotoxicity was made at a dose of 1:40 dilution of aerosol to air. At this dose, 3R4F cigarette smoke was 100% cytotoxic and used as a comparator for the NGPs. Relative to cigarette smoke, THS was 13% cytotoxic, THP1.0 was 3% cytotoxic (Jaunky et al., 2017), hybrid THP (Breheny et al., 2017a) and e-cigarettes (Azzopardi et al., 2016) were non-cytotoxic (i.e. 0% cytotoxic).

4.5.3. Assessment of tumour-promoting potential

In the Bhas 42 cell transformation assay, using the promotion protocol, responses were compared across all the products up to 48 μg/ml TPM concentrations. 3R4F reference cigarette TPM was classified as positive in the assay (i.e., the number of transformed foci increased significantly in at least two consecutive concentrations). 3R4F TPM induced significantly higher numbers of foci than the control treatment at concentrations of 6 μg/mL and above (Dunnett's test, $p < 0.001$).

By contrast, the overall activity observed after exposure to TPM from the other products did not differ significantly from that seen after exposure to a DMSO control at any concentration in the above range ($p > 0.05$) (Breheny et al., 2017a, 2017b; Thorne et al., 2017).

5. Results and discussion: summary

The emissions from a range of NGPs were assessed in chemical and *in vitro* biological studies to compare their responses relative to cigarette smoke (Fig. 5). In chemical studies, all of the NGPs had measured average reductions of toxicants levels relative to 3R4F for the WHO TobReg list (Burns et al., 2008) and the shortened FDA HPHC list (FDA 2012a) of 96–97% for THP1.0; 92–95% for THS; >99% for hybrid THP and >99% for e-cigarettes.

Table 6
Physical assessment of the aerosols from 3R4F, THP1.0, hTHP and EC.

DMS				
Consumable	3R4F	THP1.0	hTHP	EC
MMD (nm)	272 ± 19	329 ± 50	–	–
GSD	1.42 ± 0.03	1.80 ± 0.06	–	–
Number of particles per puff	3.6E+11 ± 5.9E+10	2.6E+10 ± 1.1E+10	–	–
Spraytec				
DV_{50} (nm)	–	575 ± 94	519 ± 94	532 ± 94
D4,3 (nm)	–	723 ± 212	585 ± 86	619 ± 86
GSD	–	1.84 ± 0.21	1.64 ± 0.14	1.73 ± 0.18

Abbreviations: THP = tobacco heating product; THS = tobacco heating system; hTHP = hybrid tobacco heating product; EC = electronic cigarette; MMD = mass median diameter; bGSD = geometric standard deviation; DV50 = particle size below which 50% of the aerosol lies; DV4,3 = volume mean diameter.

Table 7
Deposited aerosol mass across all products on two *in vitro* exposure systems.

System	3R4F	THP1.0	THS	hTHP	EC
RM20S[a] (1:5 aerosol:air) μg/cm^2 per puff (±SD)	0.51 (0.09)	0.10 (0.03)	0.18 (0.06)	0.43 (0.10)	0.85 (0.21)
VC10[b] (1 L/min) μg/cm^2 per puff (±SD)	0.36 (0.04)	0.06 (0.01)	0.06 (0.02)	0.44 (0.03)	0.34 (0.03)

[a] Borgwaldt-KC, Hamburg, Germany.
[b] Vitrocell® systems, Waldkirch, Germany. Abbreviations: THP = tobacco heating product; THS = tobacco heating system; hTHP = hybrid tobacco heating product; EC = electronic cigarette; SD = standard deviation.

Table 8
Summary of results from *in vitro* toxicological assessment.

Product	Ames (TPM)		Ames (WA)		NRU (WA)		Bhas 42 cell transformation (PM)	
	Result[a]	Reference	Result[a]	Reference	Result[b]	Reference	Result[a]	Reference
3R4F	Mutagenic	Thorne et al., 2017	Mutagenic	Thorne et al., 2017	100% cytotoxic	Azzopardi et al., 2016	Tumour promotion	Breheny et al., 2017a
THP1.0	Non-mutagenic	Thorne et al., 2017	Non-mutagenic	Thorne et al., 2017	3% cytotoxic[a]	Jaunky et al., 2017	No tumour promotion	Thorne et al., 2017
THS	Non-mutagenic	Thorne et al., 2017 and Schaller et al., 2016	Non-mutagenic	Thorne et al., 2017	13% cytotoxic[a]	Jaunky et al., 2017 and Schaller et al., 2016	No tumour promotion	Thorne et al., 2017
hTHP	Non-mutagenic	Breheny et al., 2017a	Non-mutagenic	Breheny et al., 2017a	0% cytotoxic[a]	Breheny et al., 2017a	No tumour promotion	Breheny et al., 2017a
EC	Non-mutagenic	Thorne et al., 2016	Non-mutagenic	Thorne et al., 2016	0% cytotoxic[a]	Azzopardi et al., 2016	No tumour promotion	Breheny et al., 2017b

[a] At all doses.
[b] Reductions relative to cigarette smoke at doses of [1:40]. Abbreviations: TPM = total particulate matter; WA = whole aerosol; NRU = neutral red uptake; THP = tobacco heating product; THS = tobacco heating system; hTHP = hybrid tobacco heating product; EC = electronic cigarette; SD = standard deviation.

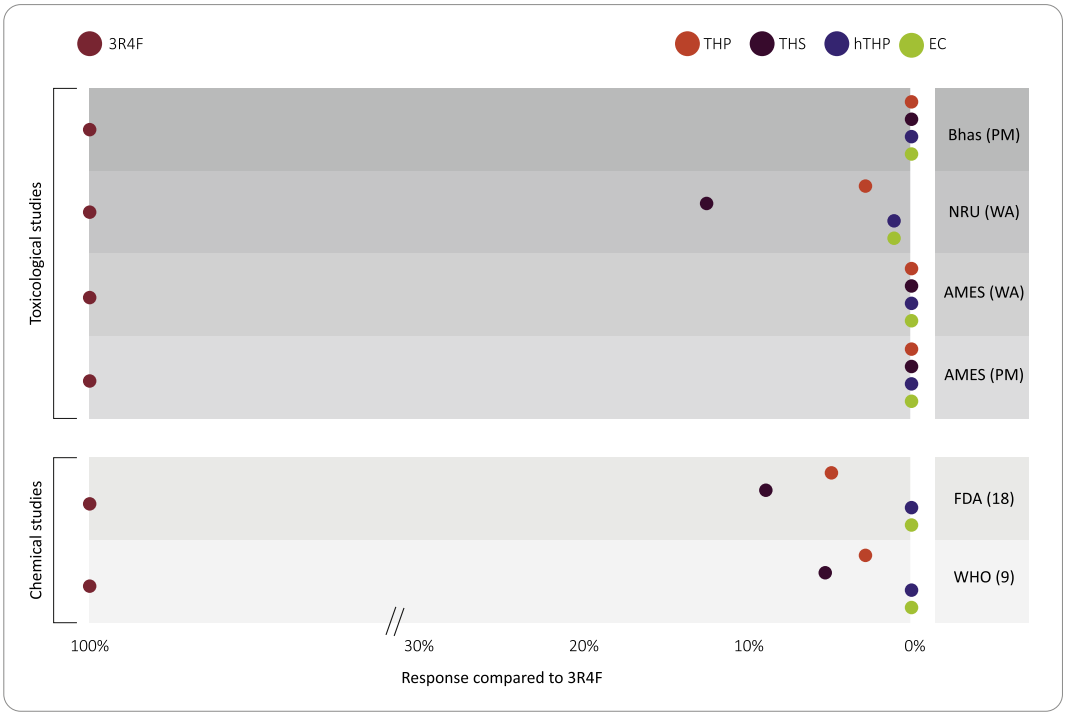


Fig. 5. Comparison of NGP responses in pre-clinical assessment studies compared to a scientific reference cigarette. Abbreviations: THP = tobacco heating product; THS = tobacco heating system; hTHP = hybrid tobacco heating product; EC = electronic cigarette.

For the past six decades, scientists have developed and used *in vitro* assays measuring relevant end points to enable the toxicological assessment characterisation of tobacco products. Historically, the end points included bacterial mutagenicity (Ames test), cytotoxicity (neutral red uptake assay) and mammalian genotoxicity (*in vitro* micronucleus test and mouse lymphoma assay). Traditionally, these assays have been used to study combustible cigarettes to understand, for example, the impact of different design features, ingredients and materials on smoke toxicity (Baker et al., 2004; Rustemeier et al., 2002). A general product stewardship paradigm with combustible cigarettes has emerged, based on the principle that a novel design, material and/or ingredient used in the product would not increase the toxicological burden in comparison to conventional cigarettes. Noteworthy was the development and

evaluation of a reduced-toxicant prototype (RTP) cigarette (Dittrich et al., 2014), which comprised a variety of blend and filter toxicant-reducing technologies. When tested in comparison to a commercially available cigarette of matched 'tar', the RTP was found to be less mutagenic and cytotoxic, with no new mutagenic hazards (Crooks et al., 2015).

Toxicological assays have also been adapted for evaluating next generation products, including cigarette-shaped THPs that used charcoal as the heat source (Foy et al., 2004), electrically heated THPs (Roemer et al., 2008; Schaller et al., 2016), hybrid THPs (Breheny et al., 2017a) and e-cigarettes (Thorne et al., 2016; Azzopardi et al., 2016; Breheny et al., 2017b). Toxicological endpoints, including mutagenicity and cytotoxicity, were measured using *in vitro* assays on the aerosol condensate of the cigarette-shaped charcoal-fuelled THPs (Foy et al., 2004). Collectively, the reductions in responses in each of the tests led the authors to conclude that the toxicity of the THPs was significantly reduced relative to cigarettes. The first-generation electrically heated cigarette smoking system was assessed for cytotoxicity in the neutral red assay and genotoxicity of TPM in the *S. typhimurium* reverse-mutation assay and the mouse lymphoma thymidine kinase assay, and showed lower toxicological responses compared to conventional reference cigarettes (Roemer et al., 2008). More recently, a number of toxicological assays were used to assess THS (Schaller et al., 2016), including cytotoxicity (neutral red uptake assay) and mutagenic potency (mouse lymphoma assay), which were both reduced by approximately 90%, relative to 3R4F. The *in vitro* studies with all NGPs showed no mutagenicity or tumour promotion activity, whereas 3R4F was both mutagenic and showed tumour promotion activity. In assessing cytotoxicity, all NGPs had responses less than 90% relative to 3R4F.

However, the usage of the product will play an equally important role in the determination of the level of toxicants to which consumers would be exposed. Puffing behaviour and consumption studies (Dautzenberg and Bricard, 2015; Gee et al., 2017; Farsalinos et al., 2015; Haziza et al., 2016) followed by clinical studies measuring biomarkers of exposure (BoE) (D'Ruiz et al., 2016; Goniewicz et al., 2017; Haziza et al., 2016; Shahab et al., 2017) are key to assessing whether NGPs reduce toxicant exposure in comparison to smoking. The measurement of daily consumption as part of the puffing behaviour study (Gee et al., 2017) showed that when smokers switched from cigarettes to THP1.0, there was a small decrease in use of consumables per day in comparison with daily cigarette use. As exposure to toxicants from THP1.0 will be dependent on both daily consumption and the toxicant levels in the emissions, this is encouraging for assessing the BoEs from smokers using cigarettes in confined clinical studies who would then be switched to THP1.0.

The IOM, has previously defined cessation as the gold standard for tobacco harm reduction (IOM, 2012). Two studies have so far demonstrated that switching smokers to solus use of THPs (Haziza et al., 2016) and solus use of e-cigarettes (D'Ruiz et al., 2016) reduces BoEs to similar levels as those from smokers who quit smoking for the duration of the studies. Furthermore, Shahab et al. (2017) evaluated BoE levels from solus e-cigarette users in comparison to smokers over a 6- month period and found that reductions were maintained as being substantially reduced throughout the duration of the study.

Importantly, the dose response of exposure to NGPs and how it relates to relative disease risk is yet to be determined. Previous studies with an RTP cigarette demonstrated that biomarkers of exposure could be reduced for both vapour phase constituents, such as crotonaldehyde, and particulate-phase constituents, such as 4-*N*-nitrosonornicotine (Shepperd et al., 2015; Proctor, 2015). This RTP cigarette was further evaluated to see whether reductions in exposure to toxicants would favourably change indicators of health outcomes, such as biomarkers of biological effect. When consumers smoked the RTP cigarette over a 6-month period, biomarkers related to inflammation and oxidative stress and cardiovascular endpoints did not show any biologically relevant change (Shepperd et al., 2015; Proctor, 2015). Therefore, it is expected that substantial reductions in exposure to both vapour and particulate phase toxicants will be required to manifest a reduction in both individual and population risk.

The findings from this series of papers suggest that in pre-clinical assessments, all the NGPs had substantially reduced responses in each study relative to cigarettes. Future work will assess whether these reduced responses in laboratory-based tests translate into reductions in exposure and individual risk in clinical studies, along with a range of population studies focusing on consumer perceptions of NGPs and studies to assess their risk on a population basis. Once these studies are complete, the quantitative placement of NGPs on a risk continuum relative to cigarettes would be possible.

6. Conclusions

NGPs, such as THPs, novel tobacco products and e-cigarettes, are being widely used by smokers who wish to reduce or replace their use of cigarettes. To date, the majority of research studies have been conducted on e-cigarettes and, as a result of the findings of those studies, public health bodies in the UK such as Public Health England (McNeill et al., 2015) have estimated that they may be 95% less risky relative to cigarettes. Furthermore, other UK public health authorities, such as the Royal College of Physicians (2016) and Cancer Research UK (2017), have publicly supported the potential role that e-cigarettes could play in the reduction of harms from smoking. Although epidemiological evidence is the gold standard to substantiate the full disease risk reduction potential of a modified tobacco product, providing early scientific evidences to the scientific community and regulators alike should allow the comprehensive assessment of any novel tobacco products at both individual and population levels, in parallel with the pace of consumer uptake of the product.

There has been less research conducted to date on THPs, and this compendium of nine papers summarises a series of studies that describe both the operation of the new THP1.0 and its' assessment in a series of pre-clinical chemical, *in vitro* biological studies and human studies. Behavioural studies showed that the machine puffing regime of a 55 ml puff, 2 s duration and a 30 s interval used in laboratory studies would be a reasonable regime to replicate human use with this type of THP. Furthermore, in-use evidence gathered on THP1.0 have shown that, provided the product is well designed to address potential lip coverage of air holes during use by consumers, with no blocking found during use (Gee et al., 2017). This supports the use of the 55 ml puff, 2 s duration and a 30 s interval without vent blocking for laboratory testing.

With no combustion and maximum heating of the tobacco to 240 °C ± 5 °C in THP1.0 (Eaton et al., 2017), the tobacco consumable did not form any ash as is found with cigarette smoking. The emissions of THP1.0 correspondingly showed approximately 90% less toxicants than those measured in cigarette smoke (Forster et al., 2017a). Furthermore, a range of analyses of physical properties concluded that the aerosol produced was respirable (Forster et al., 2017a). The environmental emissions were substantially reduced when consumers used THP1.0 compared to when they smoked cigarettes (Forster et al., 2017b), to the extent that for the majority of measured constituents, the environmental emissions were at similar levels as those from the baseline measurements, when the consumers were not using any products. Furthermore,

the PM_{10} measurement of the aerosol would conform with the recommended WHO outdoor air limit of 10 μg/m^3. This reduction in environmental emissions led to a reduction in the tobacco odour on hands, hair and fabric being perceived from using THP1.0 compared to smoking cigarettes under a set of laboratory tests (Forster et al., 2017b). Importantly, the reduction in environmental emissions and tobacco odour with THP1.0 were measured versus both the flue-cured blended DMS and the US blended LSR cigarettes.

A series of *in vitro* toxicological studies showed that THP1.0 was non-mutagenic, showed no tumour promotion activity and elicited a substantially reduced cytotoxic response which was 97% reduced relative to the response from cigarette smoke (Thorne et al., 2017; Jaunky et al., 2017). A separate study that used a high-content screening approach with eight end points showed similar substantially reduced responses for THP1.0 relative to the cigarette control (Taylor et al., 2017).

Finally, when smokers switched to using THP1.0, their daily consumption levels, in terms of the number of consumables used, did not increase in comparison to their daily cigarette consumption before the switch (Gee et al., 2017). Furthermore, the daily consumption of THP1.0 and THS was found to be similar. The exposure to toxicants, measured in clinical studies, is dependent on both the individual's daily consumption of the consumables and the toxicant levels from THP1.0. Therefore, it is promising that daily consumption did not increase during this 5-day placement study, for the exposure reduction potential. Further studies with a longer placement period may be needed to fully validate this trend.

Using the concept of the risk continuum, the data from the chemical and *in vitro* biological assays presented here were placed relative to cigarettes, on a preclinical assessment basis. To further contextualise the emissions data from THP1.0 with another commercial THP, THS (Fig. 5). It was clear that in all the studies, both THP and THS had elicited very similar responses, which were substantially reduced relative to 3R4F or LSR. Based on the evidence from the scientific assessment of THS to date, Smith et al. (2016) concluded that THS had the potential to be a reduced-risk product. Based on the similarity of the evidence from the pre-clinical assessment of THP1.0 in comparison to THS, it would appear that they have similar potentially reduced-risk profiles relative to cigarettes. However, in both cases, clinical and population studies would be required to substantiate this risk profile.

In these preliminary studies, THP1.0 and the other test NGPs (hybrid THP and e-cigarettes), elicited similar substantially reduced *in vitro* responses in the studies in comparison to cigarettes (Fig. 5). On the basis of product emissions, it appears that there is a wide gap between all the NGPs assessed in this study and cigarettes. However, as the dose response is a critical factor in assessing risk at both an individual and population basis, future studies will focus on clinical studies to measure whether the reduced emissions translate to reduced exposure and subsequent disease-relevant outcome markers. Data from studies assessing exposure and disease related risk are required to quantitatively place products on a risk continuum and these will be the subject of future publications on THP1.0.

Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.yrtph.2017.10.001.

Transparency document

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