




## REVIEW

# The product science of electrically heated tobacco products: a narrative review of the scientific literature [version 1; peer review: 1 approved, 1 approved with reservations]

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## Abstract

Heated tobacco products represent a novel category of tobacco products in which a tobacco consumable is heated to a temperature that releases nicotine from the tobacco leaf but not to a temperature sufficient to cause combustion. Heated tobacco products may therefore have the potential to be a less harmful alternative for adult smokers that would otherwise continue to smoke conventional cigarettes. Given the rapid development of this product category, the aim of this review was to examine the available peer-reviewed scientific evidence related to heated tobacco products and highlight any research gaps.

In recent years, manufacturers of heated tobacco products have published a number of studies on their respective heated tobacco products. Whilst there is limited research that is independent of commercial interests, the available scientific evidence indicates that heated tobacco products produce a much simpler aerosol than conventional cigarette smoke, with fewer and substantially lower levels of harmful toxicants. Toxicology assessments indicate these reductions in aerosol toxicants translate to reduced biological effects. Biomarker and clinical data from studies in which product use is controlled within a clinical setting, indicate changes in biomarker levels and clinical end-points similar to observations in cessation studies, indicating the potential for reduced harm. The scientific evidence also indicates that exposure of non-users to emissions from heated tobacco products in indoor environments is significantly reduced compared to exposure resulting from smoking conventional cigarettes.


Overall, the available scientific evidence indicates that heated tobacco products hold promise as a less harmful alternative to conventional cigarettes, but more independent data is required to validate industry

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findings. As a growing product category, epidemiological studies and independent population modelling studies are outstanding, and empirical data on how dual tobacco product category use by consumers affects their risk profile is lacking.

### Keywords

Heat-Not-Burn Tobacco, Heated Tobacco Products, Next Generation Products, Public Health, Risk Reduction, Smoking, Tobacco Harm Reduction, Tobacco Heating Products

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## Abbreviations

aHTPs: Aerosol Heated Tobacco Products  
 AIX: Augmentation Index  
 ARE: Antioxidant Response Element  
 BAT: British American Tobacco  
 cHTPs: Carbon Heated Tobacco Products  
 COPD: Chronic Obstructive Pulmonary Disease  
 CORESTA: Cooperation Centre for Scientific Research Relative to Tobacco  
 ECG: Electrocardiogram  
 EHCSS: Electrically Heated Cigarette Smoking System  
 eHTPs: Electrically Heated Tobacco Products  
 ETS: Environmental Tobacco Smoke  
 EVP: e-vapour product  
 FDA: Food and Drug Administration  
 FEF: Forced Expiratory Flow  
 FVC: Forced Vital Capacity  
 HCS: High Content Screening  
 HPHCs: Harmful and Potentially Harmful Constituents  
 IAQ: Indoor Air Quality  
 JTI: Japan Tobacco International  
 KT&G: Korea Tobacco and Ginseng  
 MOE: Margin of Exposure  
 NGPs: Next Generation Products  
 PMI: Philip Morris International  
 PWV: Pulse Wave Velocity  
 QSU: Questionnaire of Smoking Urges  
 THP: Tobacco Heating Product  
 THS: Tobacco Heating System  
 TPM: Total Particulate Matter  
 TSNAs: Tobacco-Specific Nitrosamines

## Introduction

The health effects associated with conventional cigarette smoking have been extensively documented by the public health and scientific communities (United States Surgeon General, 2010; International Agency for Research on Cancer, 2012; American Cancer Society, 2018). Smoking is a cause of serious diseases in smokers, including lung cancer, heart disease and emphysema. Given recent technological advances and new innovations that allow nicotine to be decoupled from harmful tobacco smoke, tobacco product manufacturers are now developing novel product categories which may be less harmful when compared to conventional cigarettes and offer a satisfying alternative for adult smokers. For this tobacco harm reduction approach to succeed in efficiently reducing harm compared with continued conventional cigarette smoking, two criteria must be fulfilled by these non-combustible nicotine-containing next-generation products (NGPs):

1. The NGP must be acceptable and satisfying for current adult smokers, so that they transition away from conventional cigarettes to the new product completely;
2. The NGP must have been scientifically demonstrated to be significantly less harmful than conventional cigarettes.

However, the potential consequences of unintended use (i.e. portions of the population for whom NGPs are not targeted towards, like youth or never smokers) must also be considered. Therefore, it is important to acknowledge that population level tobacco harm reduction can only be achieved if a scientifically-substantiated reduced harm product is accepted and used by a large number of adult smokers, who would otherwise continue to smoke, while never smokers, vulnerable populations and youth do not also begin using it. One such innovative product category within NGPs are heated tobacco products, also known as 'heat-not-burn' tobacco products. These products heat tobacco to a temperature that releases the nicotine and aromas from the tobacco leaf but not to a temperature high enough to cause combustion. By eliminating tobacco combustion, the levels of harmful chemicals and toxicants, including harmful and potentially harmful constituents (HPHCs), in the aerosol are substantially reduced. This in turn is expected to lead to a reduction in exposure to them for adult smokers and a subsequent reduction in toxicity in those adult smokers who use them as an alternative to conventional cigarettes. Consumer interest in heated tobacco products continues to increase as demonstrated by a rise in internet search engine searches relating to them (Caputi et al., 2017; Stall et al., 2018; Tabuchi et al., 2018) and an increased discussion of them on social media platforms (Hejlová et al., 2019; Kreitzberg et al., 2019; Jun, 2020; Barker et al., 2021).

Several different scientific assessment programs for assessing the harm reduction potential of NGPs have been proposed (Smith et al., 2016; Levy et al., 2017; Murphy et al., 2017). Imperial Brands has developed a 5-step scientific framework as shown in Figure 1. The framework employs five distinct components and is based in part on the US National Academy of Science's innovative blueprint for Toxicity Testing in the 21<sup>st</sup> Century which advocates for the use of *in vitro* testing using human cells as a more relevant alternative to traditional *in vivo* animal testing (Krewski et al., 2010). This narrative review will discuss the first three components of the scientific framework (product characterisation science, biological science and clinical science).

Heated tobacco products developed to date have taken three distinct engineering approaches:

1. **Electrically-heated tobacco products (eHTPs):** Use of a battery-powered handheld device which heats small non-combusted cigarette-like tobacco consumables (also known as “sticks”) which the adult smoker inserts into the device. The tobacco within the consumable is precisely heated by a number of heating elements or blades within the device to a specific temperature (Patskan and Reininghaus, 2003; Smith et al., 2016; Eaton et al., 2018).
2. **Aerosol heated tobacco products (aHTPs):** Use of a battery-powered handheld device which heats a liquid consumable to generate a hot aerosol; this aerosol then passes through a tobacco consumable to form a tobacco-containing aerosol. These products may also be known as “hybrid” devices (Breheny et al., 2017).
3. **Carbon heated tobacco products (cHTPs):** Use of a carbon tip which is lit by the adult smoker with a lighter or match, which heats incoming air which in turn heats a tobacco-cigarette-like product forming a tobacco flavoured aerosol containing mainly water, glycerol, nicotine and volatile tobacco components (Phillips et al., 2019).

Commercially available eHTPs include ‘IQOS’, produced by Philip Morris International (PMI), ‘glo’ produced by British American Tobacco (BAT;), ‘Pulze’ produced by Imperial Brands, ‘Ploom S’ produced by Japan Tobacco International (JTI;) and ‘lil’ produced by South Korea’s KT&G (Cho and Thrasher, 2019; Lee and Lee, 2019). Further details are shown for these products in Table 1. Ploom Tech produced by JTI is an example of a commercially available aHTP, whilst there are currently no commercially available cHTPs.



**Figure 1.** The 5-step scientific framework for assessment of the harm reduction potential of NGPs as developed by Imperial Brands.

**Table 1. Commercially available eHTPs as of September 2021.** N/A, Not Applicable; N/S, Not Stated; THS, Tobacco Heating System; THP, Tobacco Heating Product.

| Manufacturer                      | Product designation  | Commercialised name (if different) | Name of consumable sticks used with product                 | Operational temperature as reported by manufacturer (°C) | Website <sup>2</sup> /references  |
|-----------------------------------|----------------------|------------------------------------|---|--|---|
| British American Tobacco (BAT)    | THP1.0               | glo                                | Neostiks  | 240  | <a href="https://www.bat.com/">https://www.bat.com/</a>                                     |
| Philip Morris International (PMI) | THS2.2 <sup>1</sup>  | IQOS                               | HEETS Tobacco Sticks (9 variants currently available in UK) | 350  | <a href="https://uk.iqos.com">https://uk.iqos.com</a>                                       |
| Imperial Brands                   | Pulze                | N/A                                | iD Heat Sticks (5 variants currently available)             | N/S  | <a href="https://www.imperialbrandsplc.com">https://www.imperialbrandsplc.com</a>           |
| Japan Tobacco International (JTI) | Ploom S <sup>3</sup> | N/A                                | EVO Tobacco Sticks (4 variants currently available in UK)   | N/S  | <a href="https://www.ploom.co.uk/what-is-ploom/">https://www.ploom.co.uk/what-is-ploom/</a> |
| Korean Tobacco and Ginseng (KT&G) | lil                  | N/A                                | Fit Sticks (2 variants available as of 2019)                | N/S  | Cho and Thrasher, 2019; Lee and Lee, 2019   |

<sup>1</sup>Other variants of this eHTP have been reported in the literature including THS2.1 and THS2.4. It is assumed that these represent various generations of the handheld electrical device. THS2.2 is the most predominant designation found in the journal articles referenced in this review.

<sup>2</sup>All websites accessed on Monday 6<sup>th</sup> September 2021.

<sup>3</sup>Other Ploom products produced by JTI are regarded as being aHTPs and therefore are outside the remit of this narrative review.

The aim of this narrative review is to assess the most up to date and current available peer-reviewed scientific evidence on eHTPs and to determine if that evidence base supports their potential as a reduced harm product compared to conventional cigarettes for adult smokers that would otherwise continue to smoke. Four extensive reviews relating to heated tobacco products have already been published (Jankowski et al., 2019; Mallock et al., 2019; Simonavicius et al., 2019; Ratajczak et al., 2020) as well as a series of smaller reviews (Başaran et al., 2019; Signes-Costa et al., 2019; Znyk et al., 2021) and three journal issues published by PMI and BAT relating to their Heatbar, IQOS and glo eHTPs (Schorp et al., 2012; Smith et al., 2016; Proctor, 2018). This narrative review represents an extension of, and update to, these previous reviews.

This review will not discuss cHTPs, hybrid products (aHTPs), as such products possess engineering components of both a heated tobacco product and an e-vapour product (Glantz, 2018a) or loose-leaf tobacco vaporisers, as the design of such products permit use of non-tobacco plant-based substrates.

It should be assumed by the reader that where the descriptor “*heated tobacco product(s)*” is used in this review, it refers to eHTPs. It should also be noted that the descriptor used in each journal article to describe the heated tobacco product under discussion will be used in respect to that article in this review. The IQOS heated tobacco product can also be described as the Tobacco Heating System (THS, THS 2.1 or THS 2.2) in the literature, the glo heated tobacco product can also be described as the Tobacco Heating Product (THP1.0) whilst the Heatbar heated tobacco product (no longer commercially available) can also be described as the electrically heated cigarette smoking system (EHCSS).

## Review methodology

A manual interrogation of the PubMed database was conducted on Monday 24<sup>th</sup> May 2021 to identify all potentially relevant peer-reviewed journal articles relating to eHTPs using the following six search terms: “*heat-not-burn*”, “*heated tobacco*”, “*heated cigarette\**”, “*tobacco heating*”, “*heat tobacco*” and “*IQOS*”. IQOS was used as one of the search terms as the IQOS heated tobacco product was the first modern heated tobacco product to be commercially available, is the current market leader and was expected to have a broad range of publications relating to it. For each search term used, the interrogation was limited to it being present within the title and/or abstract of the journal article with the sole additional criteria applied being that all journal articles must have been published in English. No restriction was placed on date of publication. Each of these interrogations generated a separate list of journal articles with these six lists being combined into a single journal article list to remove multiples of any journal articles identified more than once. This single combined journal article list represented the initial starting point for this narrative review.

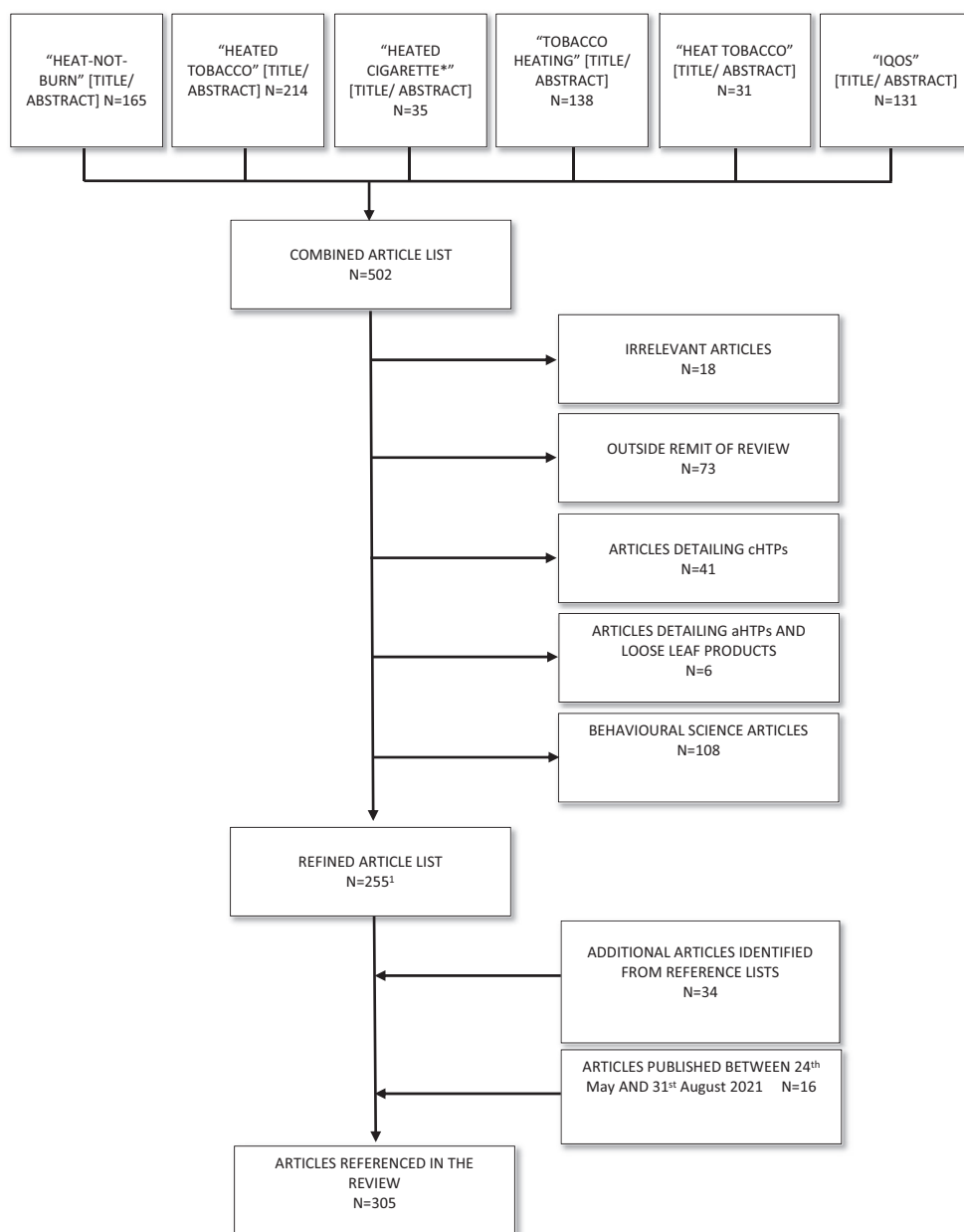
The abstract of each journal article in this combined article list was then read by one of the authors (KT) to identify those journal articles which were irrelevant to this narrative review (those which did not discuss heated tobacco products in any capacity), those deemed to be outside the remit of this review (any journal article published in any language other than English, any journal article which discussed regulation of, policy towards or advertising of heated tobacco products or journal articles which detailed tobacco industry activities or discussed non-peer reviewed internal tobacco industry documents), those which related to heated tobacco products which utilise a carbon heat source (cHTPs), those which related to hybrid products (also known as aHTPs and/or loose-leaf tobacco vaporisers and those which discussed the behavioural science of heated tobacco product use (which will be covered in a separate review article).

Additional relevant peer-reviewed journal articles were identified through manual review, analysis and cross-linking of reference lists contained in journal articles identified as described above. Conference posters, conference proceedings and journal abstracts (reported in the absence of an associated journal article) were excluded due to their lack of peer-review.

The identified relevant articles were reviewed in full by one of the authors (KT) to determine the key themes relevant to this review and extract the relevant data. Some articles contained data relevant to more than one end-point and so are discussed in more than one section.

Automatic e-mail alerts were created within the PubMed database for the search terms indicated above on Monday 24<sup>th</sup> May 2021 to identify relevant journal articles published between that date and Tuesday 31<sup>st</sup> August 2021 (with this latter date representing the cut-off date for inclusion in this narrative review). These additional journal articles were then included in the final list of journal articles referenced in the narrative review.

Citation details for all journal articles identified as discussed above are shown in the *Underlying data* (Supplementary File 1) (Mason, 2021). The overall search strategy and the numbers of journal articles identified at each stage of the search process are shown in Figure 2.



**Figure 2. Review methodology.** <sup>1</sup>The search strategy identified two separate citations for the Ioakeimidis et al. (2020) article, one of which was subsequently removed from the PubMed database. As such, the refined article list above is shown to contain 255 articles rather than 256 articles. See *Underlying data*: Supplementary File 1 for further details.

## Aerosol characterisation

This section of the review will discuss those articles that have reported on the characterisation of aerosols experimentally produced from heated tobacco products, both in terms of their physical properties and their chemical constituents.

### Aerosol physics

Characterisation of the aerosol produced by the THS 2.2 heated tobacco product and mainstream smoke produced by the 3R4F Kentucky Reference cigarette with respect to particle size indicated that the mean mass median aerodynamic diameters were 0.7µm and 0.8µm respectively based on ten replicate samples for each (Schaller et al., 2016a). In every instance, the upper boundary for particle size were below 2.5µm and as a result it was estimated that both the aerosol from the heated tobacco product and mainstream smoke from the 3R4F Kentucky Reference cigarette were respirable with a margin of error of 5%; more than 85% of the aerosol droplets could be reasonably expected to reach the alveoli of the lung.



A subsequent study characterised the particulates released by the IQOS heated tobacco device combined with four different stick variants (Pacitto et al., 2018). Median particle number concentration ranged from  $7.04 \times 10^7$  to  $9.64 \times 10^7$  particles/cm<sup>3</sup>, surface area concentrations ranged from  $2.04 \times 10^{12}$  to  $5.08 \times 10^{12}$  nm<sup>2</sup>/cm<sup>3</sup> whilst the mode of the particle number distribution ranged from 93 to 108nm. No statistically significant differences were observed between the different stick variants. Characterisation of the aerosol produced by the non-mentholated variant of the THP 1.0 heated tobacco product and mainstream smoke produced by the 3R4F Kentucky Reference cigarette with respect to particle size indicated mass median diameters of  $329 \pm 50$ nm and  $272 \pm 19$ nm respectively with geometric standard deviations of  $1.80 \pm 0.06$  and  $1.42 \pm 0.03$ , respectively (Forster et al., 2018a). Count median diameters for the non-mentholated variant of the heated tobacco product and mainstream smoke produced by the 3R4F Kentucky Reference cigarette were  $39 \pm 9$ nm and  $186 \pm 12$ nm respectively, with total particulate numbers per puff of  $5.26 \times 10^{10} \pm 1.77 \times 10^{10}$  and  $3.6 \times 10^{11} \pm 5.9 \times 10^{10}$ , respectively (Forster et al., 2018a).

Analysis of the aerosol produced by the THS 2.2 heated tobacco product and the mainstream smoke produced by the 3R4F Kentucky Reference cigarette indicated that whilst the conventional cigarette released significant quantities of solid carbon particles ( $10^{12}$  particles present in mainstream smoke over eleven puffs), no such particles were detected with the heated tobacco product, which was suggested by the authors to indicate the absence of both combustion and pyrolysis (Pratte et al., 2017). A subsequent study published by the same authors further corroborated this finding using a methodology involving a thermo-denuder operating at 300°C (Pratte et al., 2018). Results from this study indicated that whilst mainstream smoke from the 3R4F Kentucky Reference cigarettes contained solid particles or high boiling point droplets far above the lower limit of quantification of the analytical method employed, the aerosol produced from the THS 2.2 heated tobacco product did not. The absence of combustion in such products is corroborated by observations of no meaningful increase in levels of exhaled carbon monoxide in adult smoker volunteers after use of several different heated tobacco products under controlled laboratory conditions (Adriaens et al., 2018; Caponnetto et al., 2018; Maloney et al., 2021) and statistically significant decreases in exhaled carbon monoxide levels in adult smokers, not wishing to quit, who switched to a heated tobacco product for a period of six months (Beatrice and Massaro, 2019).

### Aerosol chemistry

This section of the review will detail those studies which have attempted to quantify the chemical constituents present in the aerosol of heated tobacco products when operated under experimentally controlled laboratory conditions. In the majority of studies discussed, the Health Canada Intense (ISO intense) analytical machine puffing regime has been widely used with some modifications given the absence of testing standards specific for heated tobacco products (Belushkin et al., 2018). It is apparent that detailed “real-world” puffing topography data is required in order to identify the most appropriate puffing parameters for heated tobacco products (McAdam et al., 2019).

This section of the review will not discuss those studies which have characterised flavour ingredients present in heated tobacco products (including menthol) (Reger et al., 2018; Jaccard et al., 2019a) or investigated their transfer rates to aerosol (Czégény et al., 2016; Blazsó et al., 2018). Toxicological studies have suggested that the presence of flavour ingredients does not modify the *in vitro* effects of heated tobacco product aerosols (Crooks et al., 2018; Le Godec et al., 2019). Nevertheless, separate authors have indicated a need for additional research on flavour ingredients in heated tobacco products (Kaur et al., 2018). This section of the review will, however, separately discuss nicotine and tobacco-specific nitrosamines (TSNAs) as articles specifically quantifying these chemical constituents have been published.

Using experimental designs where tobacco samples have been heated under precisely controlled conditions, it has been shown that yields of several chemical constituents are markedly reduced at lower temperatures (Torikai et al., 2004; Zhou et al., 2015; Forster et al., 2015). Using a sample of commercial blended tobacco, the yields of hydrogen cyanide, benzo [a] pyrene, formaldehyde, acrolein, isoprene, styrene, phenol and 1-aminonaphthalene were quantified as the sample was heated to either 300°C, 500°C, 800°C or 1000°C either in an air or nitrogen atmosphere (Torikai et al., 2004). In the case of each smoke constituent, yields increased significantly as the temperature increased in both atmospheres. When a range of samples of flue-cured tobacco were heated in a furnace with constant sample weight and air velocity and the temperature varied between 350°C and 750°C, it was observed that carbon monoxide yield was remarkably low when compared to yields seen with lit or smouldering tobacco (Zhou et al., 2015). A subsequent study employed a bench-top furnace to heat tobacco samples to between 100 and 200°C in 20°C increments and quantified the yields of numerous HPHCs released by the tobacco (Forster et al., 2015). Water, total and nicotine-free dry particulate matter and three TSNAs (NNN, NNK and NAT) were found at all temperatures tested. Several HPHCs were not detected at the lowest temperatures but were found as the temperature increased incrementally up to 200°C (nicotine, carbon monoxide, acetaldehyde, crotonaldehyde, formaldehyde, NAB, acetone, butyraldehyde, methyl ethyl ketone and propionaldehyde) whilst others were not detected at any temperature tested including ammonia, acrolein, hydrogen cyanide, phenol, benzene, 1,3-butadiene, isoprene and toluene.



The most comprehensive study currently available on the chemical characterisation of experimentally produced aerosols from heated tobacco products identified a total of 529 chemical constituents (excluding water, glycerol and nicotine) in the aerosol of the IQOS heated tobacco product at concentrations exceeding a concentration 100ng per stick (Bentley et al., 2020). The majority of the chemical constituents (n=402) were identified as being present in the particulate phase of the aerosol with thirty-nine of these being present in both the particulate and gas/vapour phases. The identification of 80% of the chemical constituents (representing more than 96% of the total determined mass) was confirmed using a range of analytical methodologies. Separate authors identified a total of 205 chemical constituents in the particulate phase of the aerosol derived from the same heated tobacco product (Savareear et al., 2017). Seventeen different chemical classes were identified including ketones (n=34), alcohols (n=31), aldehydes (n=22) and alicyclic hydrocarbons (n=20).

Using a similar approach on the vapour phase of the THP1.0/glo heated tobacco product, the same research group identified a total of 85 and 202 chemical constituents in the aerosol from the THP1.0/glo heated tobacco product and 3R4F Kentucky Reference cigarette respectively (Savareear et al., 2018). Thirty-five chemical constituents were found to be common to the vapour phase of both products. With respect to quantification of individual chemical constituents, a subsequent study analysed 126 chemical constituents in the aerosol from the THP1.0 heated tobacco product and directly compared these results with the 3R4F Kentucky Reference cigarette (Forster et al., 2018a). For the 102 chemical constituents which were detected in the aerosol of the THP1.0 heated tobacco product and in the mainstream smoke of the 3R4F Kentucky Reference cigarette, an overall average reduction of >95% was observed with the heated tobacco product. Data for the other 24 chemical constituents were excluded from the reduction calculations as their concentrations were below the limit of quantification in the aerosol from the heated tobacco product or mainstream smoke from the 3R4F Kentucky Reference cigarette or both. With respect to the particulate phase (and specifically the volatile organic compounds) of the aerosol produced from the THP1.0 heated tobacco product compared to mainstream smoke of the 3R4F Kentucky Reference cigarette, a total of 160 and 592 peaks were identified respectively using a methodology combining two-dimensional gas chromatography and time of flight mass spectrometry detection (Savareear et al., 2019). Ninety-three compounds were common to both sample preparations and in the vast majority of cases, observed levels were significantly lower with the heated tobacco product than with the reference cigarette. Aside from glycerine and its monoacetate, only nine compounds had marginally higher concentrations in the heated tobacco product aerosol when compared to mainstream smoke from the 3R4F Kentucky Reference cigarette with the increase being  $\leq 1\mu\text{g}$  between cigarette and stick (methylene chloride, hexane, 2-methylfuran, 1H-pyrrole, 2,5-furandione, 2-furanmethanol, 4-cyclopentene-1,3-dione, dihydro-2(3H)-furanone and alpha-monopropionin).

### Harmful and potentially harmful constituents (HPHCs)

In 2012, the United States Food and Drug Administration (FDA) published a list of 93 HPHCs present in tobacco products and tobacco smoke (US FDA, 2012). Table 2 shows data relating to these HPHCs with respect to their experimentally determined levels in the aerosol of heated tobacco products with direct comparison to their levels in the mainstream smoke of conventional cigarettes. Comparative data between heated tobacco products and conventional cigarettes is either based on direct quantitative results for both product groups reported within the same article, quantitative data reported elsewhere within the scientific literature for the conventional cigarette comparison, or a combination of both. Other approaches quantified HPHCs within aerosols experimentally produced from heated tobacco products without discussion of conventional cigarettes; in such cases, data can be extracted where the HPHC was determined to be below either the limit of detection or limit of quantification of the analytical method employed (Poget et al., 2017).

As indicated in Table 2, findings reported in the scientific literature with respect to HPHCs are categorised into five groups. Three of the groups are based on whether the individual HPHC has been experimentally quantified at levels lower than, comparable to or higher than those observed with conventional cigarette smoke. The remaining two groups are for those results where the experimentally determined value for the HPHC was reported as being below either the limit of detection and/or limit of quantification of the analytical method reported in the original article and those HPHCs for which no experimentally determined data could be identified. Table 2 shows that, for the 80 out of 86 (93%) of those HPHCs having data reported in the literature, levels observed with heated tobacco product aerosols are either below the limit of detection and/or limit of quantification of the analytical method employed, or substantially lower than those levels observed with conventional cigarette smoke. These observations have been reported to be maintained across a range of various high intensity puffing regimes for the THS 2.2 heated tobacco product based on a subset of 54 HPHCs with observed reductions being consistently more than 90% (Goujon et al., 2020). In addition, these quantitative results agree with separate data published within the scientific literature solely in a qualitative fashion where analysis of the IQOS heated tobacco product and 3R4F Kentucky reference cigarette GC-MS chromatographic fingerprints indicated a non-nicotine global component reduction of greater than 80% for the IQOS heated tobacco product in comparison to the 3R4F Kentucky reference cigarette (Ibañez et al., 2019). For three of the HPHCs (aflatoxin B1, coumarin and N-nitrososarcosine) no standardised methods have been developed to date for the quantification of these HPHCs in either conventional

**Table 2. Studies available in the scientific literature providing quantitative experimental data on levels of HPHCs produced by heated tobacco products in direct comparison with those produced by conventional cigarettes.** References on which this table is formulated are shown in Supplementary File 2 (*Underlying data* (Mason, 2021). AD, Addictive; CA, Carcinogen; CT, Cardiovascular Toxicant; Glu-P-1, 2-amino-6-methyldipyrido[1,2- $\alpha$ :3',2'- $d$ ]imidazole; Glu-P-2, 2-aminodipyrido[1,2- $\alpha$ :3',2'- $d$ ]imidazole; HPHC, Hazardous and Potentially Hazardous Constituent; IQ, 2-amino-3-methylimidazo[4,5- $f$ ]quinoline; LOD, Limit of Detection; LOQ, Limit of Quantification; MeA- $\alpha$ -C, 2-amino-3-methyl-9H-pyrido[2,3- $b$ ]indole; NNK, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone; PHIP, 2-amino-1-methyl-6-phenylimidazo[4,5- $b$ ]pyridine; RDT, Reproductive or Developmental Toxicant; RT, Respiratory Toxicant; Trp-P-1, 3-amino-1,4-dimethyl-5H-pyrido[4,3- $b$ ]indole; Trp-P-2, 1-methyl-3-amino-5H-pyrido[4,3- $b$ ]indole.

| HPHC  | Designation(s) of HPHC | Levels Quantified With Heated Tobacco Products Below LOD/LOQ of Analytical Method Employed <sup>1</sup> | Levels Quantified With Heated Tobacco Products Lower Than Those Quantified With Conventional Cigarettes <sup>1</sup> | Levels Quantified With Heated Tobacco Products Comparable To Those Quantified With Conventional Cigarettes | Levels Quantified With Heated Tobacco Products Higher Than Those Quantified With Conventional Cigarettes | No Quantitative Data Currently Reported In The Scientific Literature |
|---|------------------------|---|--|--|--|--|
| Acetaldehyde  | AD; CA; RT             |   |  |  |  |  |
| Acetamide   | CA                     |   |  |  |  |  |
| Acetone   | RT                     |   |  |  |  |  |
| Acrolein  | CT; RT                 |   |  |  |  |  |
| Acrylamide  | CA                     |   |  |  |  |  |
| Acrylonitrile   | CA; RT                 |   |  |  |  |  |
| Aflatoxin B1 <sup>2</sup>                             | CA                     |   |  |  |  |  |
| 4-Aminobiphenyl                                       | CA                     |   |  |  |  |  |
| 1-Aminonaphthalene                                    | CA                     |   |  |  |  |  |
| 2-Aminonaphthalene                                    | CA                     |   |  |  |  |  |
| Ammonia   | RT                     |   |  |  |  |  |
| Anabasine   | AD                     |   |  |  |  |  |
| <i>o</i> -Anisidine                                   | CA                     |   |  |  |  |  |
| Arsenic   | CA; CT; RDT            |   |  |  |  |  |
| 2-Amino-9H-pyrido[2,3- <i>b</i> ]indole               | CA                     |   |  |  |  |  |
| Benz[ <i>a</i> ]anthracene                            | CA; CT                 |   |  |  |  |  |
| Benz[ <i>j</i> ]aceanthrylene                         | CA                     |   |  |  |  |  |
| Benzene   | CA; CT; RDT            |   |  |  |  |  |
| Benzo[ <i>b</i> ]fluoranthene                         | CA; CT                 |   |  |  |  |  |
| Benzo[ <i>k</i> ]fluoranthene                         | CA; CT                 |   |  |  |  |  |
| Benzo[ <i>b</i> ]furan                                | CA                     |   |  |  |  |  |
| Benzo[ <i>a</i> ]pyrene                               | CA                     |   |  |  |  |  |
| Benzo[ <i>c</i> ]phenanthrene                         | CA                     |   |  |  |  |  |
| Beryllium   | CA                     |   |  |  |  |  |
| 1,3-Butadiene   | CA; RDT; RT            |   |  |  |  |  |
| Cadmium   | CA; RDT; RT            |   |  |  |  |  |
| Caffeic Acid  | CA                     |   |  |  |  |  |
| Carbon Monoxide                                       | RDT                    |   |  |  |  |  |
| Catechol  | CA                     |   |  |  |  |  |
| Chlorinated Dioxins/Furans                            | CA; RDT                |   |  |  |  |  |
| Chromium  | CA; RDT; RT            |   |  |  |  |  |
| Chrysene  | CA; CT                 |   |  |  |  |  |
| Cobalt  | CA; CT                 |   |  |  |  |  |
| Coumarin <sup>2</sup>                                 | <b>BANNED IN FOOD</b>  |   |  |  |  |  |
| Cresols ( <i>o</i> -, <i>m</i> - or <i>p</i> -cresol) | CA; RT                 |   |  |  |  |  |
| Crotonaldehyde  | CA                     |   |  |  |  |  |
| Cyclopenta[ <i>c,d</i> ]pyrene                        | CA                     |   |  |  |  |  |
| Dibenz[ <i>a,h</i> ]anthracene                        | CA                     |   |  |  |  |  |
| Dibenzo[ <i>a,e</i> ]pyrene                           | CA                     |   |  |  |  |  |
| Dibenzo[ <i>a,h</i> ]pyrene                           | CA                     |   |  |  |  |  |
| Dibenzo[ <i>a,i</i> ]pyrene                           | CA                     |   |  |  |  |  |
| Dibenzo[ <i>a,l</i> ]pyrene                           | CA                     |   |  |  |  |  |
| 2,6-Dimethylaniline                                   | CA                     |   |  |  |  |  |
| Ethyl Carbamate                                       | CA; RDT                |   |  |  |  |  |
| Ethylbenzene  | CA                     |   |  |  |  |  |
| Ethylene Oxide  | CA; RDT; RT            |   |  |  |  |  |
| Formaldehyde  | CA; RT                 |   |  |  |  |  |
| Furan   | CA                     |   |  |  |  |  |
| Glu-P-1   | CA                     |   |  |  |  |  |
| Glu-P-2   | CA                     |   |  |  |  |  |
| Hydrazine   | CA; RT                 |   |  |  |  |  |
| Hydrogen Cyanide                                      | CT; RT                 |   |  |  |  |  |
| Indeno[1,2,3- <i>cd</i> ]pyrene                       | CA                     |   |  |  |  |  |
| IQ  | CA                     |   |  |  |  |  |
| Isoprene  | CA                     |   |  |  |  |  |
| Lead  | CA; CT; RDT            |   |  |  |  |  |
| MeA- $\alpha$ -C                                      | CA                     |   |  |  |  |  |
| Mercury   | CA; RDT                |   |  |  |  |  |
| Methyl Ethyl Ketone                                   | RT                     |   |  |  |  |  |
| 5-Methylchrysene                                      | CA                     |   |  |  |  |  |
| NNK   | CA                     |   |  |  |  |  |
| Naphthalene   | CA; RT                 |   |  |  |  |  |
| Nickel  | CA; RT                 |   |  |  |  |  |

**Table 2.** *Continued*

| HPHC                            | Designation(s) of HPHC   | Levels Quantified With Heated Tobacco Products Below LOD/LOQ of Analytical Method Employed <sup>1</sup> | Levels Quantified With Heated Tobacco Products Lower Than Those Quantified With Conventional Cigarettes <sup>1</sup> | Levels Quantified With Heated Tobacco Products Comparable To Those Quantified With Conventional Cigarettes | Levels Quantified With Heated Tobacco Products Higher Than Those Quantified With Conventional Cigarettes | No Quantitative Data Currently Reported In The Scientific Literature |
|---------------------------------|--------------------------|---|--|--|--|--|
| Nicotine                        | AD; RDT                  | DISCUSSED SEPARATELY  | DISCUSSED SEPARATELY   | DISCUSSED SEPARATELY   | DISCUSSED SEPARATELY   | DISCUSSED SEPARATELY   |
| Nitrobenzene                    | CA; RDT; RT              |   |  |  |  |  |
| Nitromethane                    | CA                       |   |  |  |  |  |
| 2-Nitropropane                  | CA                       |   |  |  |  |  |
| N-Nitrosodiethanolamine         | CA                       |   |  |  |  |  |
| N-Nitrosodiethylamine           | CA                       |   |  |  |  |  |
| N-Nitrosodimethylamine          | CA                       |   |  |  |  |  |
| N-Nitrosomethylethylamine       | CA                       |   |  |  |  |  |
| N-Nitrosomorpholine             | CA                       |   |  |  |  |  |
| N-Nitrososarcosine <sup>2</sup> | CA                       |   |  |  |  |  |
| N-Nitrosopiperidine             | CA                       |   |  |  |  |  |
| N-Nitrosopyrrolidine            | CA                       |   |  |  |  |  |
| N-Nitrososarcosine <sup>2</sup> | CA                       |   |  |  |  |  |
| Nornicotine                     | AD                       |   |  |  |  |  |
| Phenol                          | CT; RT                   |   |  |  |  |  |
| PhIP                            | CA                       |   |  |  |  |  |
| Polonium-210                    | CA<br>(RADIONUCLIDE)     |   |  |  |  |  |
| Propionaldehyde                 | CT; RT                   |   |  |  |  |  |
| Propylene Oxide                 | CA; RT                   |   |  |  |  |  |
| Quinoline                       | CA                       |   |  |  |  |  |
| Selenium                        | RT                       |   |  |  |  |  |
| Styrene                         | CA                       |   |  |  |  |  |
| o-Toluidine                     | CA                       |   |  |  |  |  |
| Toluene                         | RDT; RT                  |   |  |  |  |  |
| Trp-P-1                         | CA                       |   |  |  |  |  |
| Trp-P-2                         | CA                       |   |  |  |  |  |
| Uranium-235                     | CA; RT<br>(RADIONUCLIDE) |   |  |  |  |  |
| Uranium-238                     | CA; RT<br>(RADIONUCLIDE) |   |  |  |  |  |
| Vinyl Acetate                   | CA; RT                   |   |  |  |  |  |
| Vinyl Chloride                  | CA                       |   |  |  |  |  |

<sup>1</sup>Where the result for a specific HPHC was reported for more than one heated tobacco product or different variants of the same heated tobacco product and was determined to be either below the limit of detection or limit of quantification for some, but not all, of the heated tobacco products or variants of the same heated tobacco product analysed, then the reference is shown in both columns. <sup>2</sup>Currently no standardised methods have been developed for the quantification of these HPHCs in either conventional cigarette smoke or aerosols from heated tobacco products (Forster et al., 2018).

cigarette smoke or aerosols from heated tobacco products, and as such, no data is reported in Table 2 (Forster et al., 2018a). The single experimental value for *N*-nitrosodiethanolamine suggesting higher levels with a heated tobacco product than with a conventional cigarette was reported by the original authors as being due to background contamination (Forster et al., 2018a). No data has been reported for the three radioactive HPHCs (polonium-210, uranium-235 and uranium-238) or for chlorinated dioxins/furans (although data has been reported for furan) (Crooks et al., 2018; Forster et al., 2018a). With respect to those results for individual HPHCs where specific studies have found higher levels with a heated tobacco product than with conventional cigarettes, and with this being in opposition to the other results available in the scientific literature, these results are associated with historical heated tobacco products which are no longer commercially available. Two specific exceptions are for chromium (Forster et al., 2018a) and benzo [c] phenanthrene (Dusautoir et al., 2021) where the reported results were obtained using the THP1.0 and IQOS heated tobacco products respectively.

The significant majority of studies reported in Table 2 used the 3R4F Kentucky reference cigarette as their comparator cigarette. The 3R4F Kentucky reference cigarette serves as an international standard for research purposes and was approved by representatives of commercial manufacturers. The 3R4F Kentucky reference is not commercially available and not intended for use by 'real-world' adult smokers. Furthermore, the 3R4F Kentucky reference cigarette was produced at a single time point as a single batch using a single set of tobacco blends. In light of these potential limitations, separate authors have compared the yields of HPHCs in the THS 2.2 heated tobacco product with the 3R4F Kentucky reference cigarette as well as an extensive range of commercial conventional cigarettes from numerous countries purchased in different years (Jaccard et al., 2017). The results from this study, where comparison was made with commercially available products, were found to be highly comparable to the reductions seen in HPHC levels (approximately 90%) when compared to the 3R4F Kentucky reference cigarette. Changes to the blend composition used in the tobacco of the THS 2.2 heated tobacco product has been demonstrated to have a minimal effect on the levels of HPHCs in the aerosol

subsequently produced by the product (Schaller et al., 2016b) whilst comparison between two tobacco blends (Burley and Virginia) have indicated comparable constituents being released after heating of the tobacco (Davies et al., 2018). It should also be noted that stocks of the 3R4F Kentucky reference cigarette are almost depleted. A new Kentucky reference cigarette, the 1R6F, has been produced and early initial data suggests comparable results for reductions in HPHCs for the THS 2.2 heated tobacco product compared to the 3R4F Kentucky reference cigarette (Jaccard et al., 2019b).

### Non-HPHC listed chemical constituents

With respect to chemical constituents present in the aerosol of heated tobacco products which are not listed on the 2012 US FDA HPHC list, the significant majority of studies have indicated that levels of numerous chemical constituents such as free radicals (Shein and Jeschke, 2019; Bitzer et al., 2020) are present at lower concentrations in the aerosol of heated tobacco products compared to concentrations found in conventional cigarette mainstream smoke. Nevertheless, several chemical constituents have been reported to be present in higher concentrations in the aerosol of heated tobacco products compared to mainstream smoke from conventional cigarettes under machine-generated experimental conditions (including valeraldehyde, glyoxal, methyl glyoxal and acenaphthene). Valeraldehyde, glyoxal, methyl glyoxal were reported to be absent from the mainstream smoke from Marlboro Red conventional cigarettes whilst present in quantifiable levels in the aerosol from the IQOS heated tobacco product (Salman et al., 2019) whilst a second study reported all three chemical constituents to be present in aerosols from two heated tobacco products (IQOS and glo) and three reference cigarettes [3R4F, 1R5F and CORESTA (Cooperation Centre for Scientific Research Relative to Tobacco) CM6 test-piece] with levels typically lower with heated tobacco products (Uchiyama et al., 2018). The observation of valeraldehyde, glyoxal, methyl glyoxal at higher levels in aerosol from the heated tobacco product than in the mainstream smoke from the Marlboro Red cigarette can likely be explained. Valeraldehyde is present due to the thermal degradation of flavour compounds present in the tobacco rod of the heated tobacco product which are not present in the tobacco rod of the conventional cigarette and glyoxal and methylglyoxal are present due to the thermal degradation of the propylene glycol present in the tobacco rod of the heated tobacco product. Acenaphthene was reported to be present in higher concentrations in the aerosol from a heated tobacco product compared to mainstream smoke from conventional cigarettes, albeit when assessed using a non-standardised testing methodology (Auer et al., 2017a). Separate researchers have indicated that the observed levels of acenaphthene, in both conventional cigarettes and heated tobacco products, do not pose a meaningful toxicological risk to smokers (Lachenmeier et al., 2018). Significant limitations of the experimental methodologies and analytical methods used to quantify the reported levels by Auer et al. have been subsequently highlighted by separate researchers (Caruso and Polosa, 2017; Maeder and Peitsch, 2017) but defended by the original researchers (Auer et al., 2017b).

### Nicotine

Nicotine levels present in heated tobacco products have been reported to be largely comparable to those observed with conventional cigarettes (Jaccard et al., 2017). Numerous studies have indicated that levels of nicotine in both tobacco filler (Bekki et al., 2017) and aerosol (Auer et al., 2017a; Bekki et al., 2017; Farsalinos et al., 2018a; Salman et al., 2019) are highly similar to those with conventional cigarettes confirming this aspect of the product design.

Nicotine-related alkaloids (nornicotine, anatabine, anabasine and myosmine) have been demonstrated to be present in both the tobacco filler and aerosol produced from three different heated tobacco products, none of which were identified by their commercial names (Jeong et al., 2018). Cotinine, whilst detected in the tobacco filler of the 3R4F reference cigarette and CORESTA Monitor (CM7) test piece, was not detected in either the tobacco filler or aerosol produced from the heated tobacco products (the presence or otherwise of cotinine in the mainstream smoke of the two reference cigarettes was not determined) (Jeong et al., 2018).

The presence of free-base nicotine within the aerosol produced by heated tobacco products is uncertain at this time. One study indicated that levels of free-base nicotine within the aerosol of the IQOS heated tobacco product were comparable to those found in conventional cigarette mainstream smoke when analysed under two different aerosol-generation regimes (Salman et al., 2019), whilst a second study failed to detect any meaningful levels of free-base nicotine in the aerosol from several variants of the same heated tobacco product (Meehan-Atrash et al., 2019). The pH of aerosol produced by the IQOS heated tobacco product and mainstream smoke produced from conventional cigarettes were found to be largely comparable (Salman et al., 2019) suggesting that a meaningful difference in the relative percentages of the various protonated forms of nicotine between the heated tobacco product and conventional cigarettes is unlikely. There is some evidence from a theoretical mechanistic study to suggest that nicotine degradation may be more rapidly initiated as temperature is reduced which was proposed by the authors to suggest that heated tobacco products may be more likely to experience nicotine degradation than conventional cigarettes (Chavarrio Cañas et al., 2021).

### Tobacco-specific nitrosamines (TSNAs)

Several articles have identified the presence of four TSNAs (NNN, NAT, NAB and NNK) both in the tobacco filler (Bekki et al., 2017; Jaccard et al., 2018; Jeong et al., 2018) and in the aerosols generated from heated tobacco products [only NNN and NNK are included in the 2012 US FDA HPHC list] (Bekki et al., 2017; Jaccard et al., 2018; Jeong et al., 2018; Leigh et al., 2018a; Ishizaki and Kataoka, 2019; Li et al., 2019). When compared on the basis of the sum of the four main TSNAs investigated (NNN, NAT, NAB and NNK), levels observed in the aerosols of the IQOS heated tobacco product were significantly lower than those observed in conventional cigarette smoke (Bekki et al., 2017; Li et al., 2019).

### Conclusions

With respect to chemical constituents and specifically HPHCs, the data shown in Table 2 indicates that experimentally produced aerosols from heated tobacco products contain fewer and significantly lower levels of these chemical constituents compared to conventional cigarette mainstream smoke in almost all instances. Whilst some non-HPHC chemical constituents have been identified in aerosols from heated tobacco products at levels higher than those observed with conventional cigarette mainstream smoke, or have been quantified in aerosols from heated tobacco products but not in conventional cigarette mainstream smoke at all, the significance of these analytical observations to the toxicological profile of heated tobacco products remains unknown and requires further investigation (St Helen et al., 2018). These conclusions are in agreement with those of a separate review on the chemical content of aerosols from heated tobacco products which concluded that “the concentrations of chemical compounds in the aerosol were about 10 times lower than that of cigarette smoke” and that “replacing smoking in the traditional way by heating tobacco modified significantly the content of chemical substances found in aerosol” (Szparaga et al., 2021).

With respect to the physical characterisation of aerosols produced from heated tobacco products, it is evident that such aerosols differ significantly from conventional cigarette mainstream smoke. This conclusion is an agreement with those of a separate experimental study which investigated the suitability of existing analytical laboratory processes for use with heated tobacco products (Gasparyan et al., 2018). The authors observed significant differences for each characterising variable between the heated tobacco products under investigation (THS 2.2 and THP 1.0) and those values typically observed with both conventional cigarettes and e-vapour products (EVPs) suggesting that the aerosol from the heated tobacco products was fundamentally distinct in terms of its physical properties. The authors concluded that “taken collectively with other available aerosol chemistry and biological results on heated tobacco products in the literature, they show a fundamentally different aerosol in heated tobacco products and call for category-specific product standards and terminology” (Gasparyan et al., 2018).

### In vivo toxicology

This section of the review will discuss articles which have reported *in vivo* toxicological and histopathological effects of heated tobacco products. With the exception of a single mouse skin painting study, all of the available studies have exposed rodents to experimentally generated aerosols from heated tobacco products via inhalation using either a nose-only or whole-body experimental regime. It is important to note that the relevance of *in vivo* studies for assessing the effects of heated tobacco product aerosols for human exposure is unclear and results should be interpreted within this context.

### Mouse skin painting studies

The sole mouse skin painting study (conducted as part of a larger toxicological investigation) compared the carcinogenic potential of total particulate matter from the EHCSS Series K heated tobacco product (also known as Heatbar) with total particulate matter produced from the 2R4F Kentucky reference cigarette and two commercially available conventional cigarettes (Marlboro Lights and Marlboro Ultra Lights) over a 26-week exposure period using three dose levels (30mg, 60mg or 90mg total particulate matter per week) (Werley et al., 2008). No tumours were observed in the control group treated solely with dimethylbenzanthracene (the vehicle used for administration of the condensates). Condensate produced from the heated tobacco product demonstrated later dermal tumor onset, lower dermal tumor incidence, reduced dermal tumor multiplicity and a lower proportion of malignant dermal tumors than condensates produced from the conventional cigarettes. Treatment with a weekly dose of 30mg total particulate matter per week from the heated tobacco product showed a mean value for tumour multiplicity (number of tumours per mouse at the end of the application period), which was approximately 24% of the group given an equivalent dose from the 2R4F Kentucky reference cigarette. Non-statistically significant decreases were seen for the remaining two dose levels.

### Acute rodent exposure studies

To date, there have only been two *in vivo* studies reported that have utilised an acute exposure experimental methodology involving a heated tobacco product.

The first study investigated the effect of acute exposure to mainstream smoke from a commercial cigarette (Marlboro Red) or aerosol from a heated tobacco product (IQOS) on vascular endothelial function using flow-mediated dilation as



an experimental surrogate (Nabavizadeh et al., 2018). Anaesthetised male Sprague-Dawley rats (n=8 per group) were exposed via a nose cone to mainstream smoke from the Marlboro Red cigarette, aerosol from the IQOS heated tobacco product or fresh air (as a control group). The exposure regime consisted of a series of consecutive thirty-second cycles, each consisting of either five or fifteen seconds of exposure followed by removal of the nose cone for the remainder of the thirty second cycle. Each rat was exposed to either ten cycles over five minutes or three cycles over ninety seconds to approximate exposure to a single heated tobacco product use. Flow-mediated dilation (as an experimental estimate of vascular endothelial function) in the femoral artery was measured before and after exposure after temporary surgical occlusion of the common iliac artery. Flow-mediated dilation was reduced comparably by ten fifteen-second exposures to heated tobacco product aerosol and conventional cigarette smoke but not by exposure to fresh air. Flow-mediated dilation was also reduced comparably by ten five-second exposures to heated tobacco product aerosol and conventional cigarette smoke but not by exposure to fresh air. No statistical analyses were conducted to determine whether or not differences in the observed effects between the heated tobacco product and the conventional cigarette were statistically significant or not.

The second study investigated the effects of prenatal exposure to experimentally generated aerosol from the IQOS heated tobacco product, mainstream smoke from the 3R4F Kentucky reference cigarette or filtered air on testicular function in male offspring (Yoshida et al., 2020). Pregnant CD-1 mice (n=10 per group) were exposed using a whole-body exposure system for two 20-minute periods on days seven and fourteen of gestation. Adult male offspring were then divided into six groups based on the respective exposure history of the mothers and their age (five and fifteen-weeks old; age-points at which the male mice were sexually immature and sexually mature respectively). Spermatogenesis, sperm characteristics, serum testosterone and seminiferous morphology were evaluated. Prenatal exposure to experimentally produced aerosol from the IQOS heated tobacco product increased abnormal seminiferous tubule morphology and decreased sperm production at five week of age, but not at fifteen weeks of age, whilst exposure to mainstream smoke from the 3R4F Kentucky Reference cigarette did not. There were no statistically significant differences between the two exposed groups with regard to fertility, gestation period length, litter size or sex ratio at birth. There were no statistically significant differences in body weight, testicular weight, epididymis weight or serum sex hormones levels of the male offspring when stratified on the basis of the exposure group of their mothers.

### Chronic rodent exposure studies

Numerous chronic rodent exposure studies investigating the biological and histopathological effects of exposure to experimentally produced aerosols from the EHCSSS and THS 2.1/THS2.2 heated tobacco products (also known as Heatbar and IQOS respectively) have been published by PMI. In addition to those chronic rodent exposure studies reported by PMI, four studies undertaken by independent researchers have been published within the last year (Bhat et al., 2021; Scharf et al., 2021; Vivarelli et al., 2021; Daou et al., 2021) as well as the independent development of an aerosol exposure apparatus for *in vivo* experiments using heated tobacco products (Sawa et al., 2021).

The studies published by PMI have combined classical histopathological end-points with a Systems Toxicology Assessment to assess the transcriptomic and proteomic effects of exposure to experimentally generated aerosols from the various heated tobacco products on specific tissues, organs and the expression of specific proteins and/or nucleic acid species, primarily in the Apo E<sup>-/-</sup> mouse. In every instance, the reported effects have been directly compared to those observed using the 3R4F Kentucky reference cigarette as a comparison product. Exposure to aerosols has been on comparable nicotine concentrations (expressed either as mg/m<sup>3</sup> or µg/L) with several concentrations used for either or both of the aerosols. Exposure periods have ranged from thirty-five and/or ninety days (Terpstra et al., 2003; Moennikes et al., 2008; Werley et al., 2008; Oviedo et al., 2016; Wong et al., 2016) to six months or more [not including any additional recovery periods] (Phillips et al., 2016, 2019; Lo Sasso et al., 2016a; Titz et al., 2016, 2020a; Szostak et al., 2017; Choukrallah et al., 2019; Szostak et al., 2020; Wong et al., 2020; Battey et al., 2021). The suitability of the Apo E<sup>-/-</sup> mouse as an experimental model for studying the effects of both conventional cigarette smoke and heated tobacco product aerosol exposures on cardiovascular and respiratory disease end-points has been discussed elsewhere by separate authors (Lo Sasso et al., 2016b).

With respect to the findings of these studies, classical histopathological analyses have indicated that exposure to aerosol from heated tobacco products consistently yielded significantly reduced biological effects when compared to those observed with conventional cigarettes in a range of tissues including the respiratory tract (Phillips et al., 2016, 2019; Titz et al., 2016, 2020a; Oviedo et al., 2016; Wong et al., 2016), cardiovascular system (Phillips et al., 2016, 2019; Szostak et al., 2020), liver (Lo Sasso et al., 2016a; Wong et al., 2016) and gastrointestinal tract (Battey et al., 2021). Observations from a life-time exposure study using A/J mice with an 18-month exposure period indicated that chronic exposure to aerosol from the THS 2.2 heated tobacco product did not increase the incidence or multiplicity of bronchioalveolar adenomas or carcinomas relative to sham-exposed mice whilst exposure to mainstream smoke from the 3R4F Kentucky

reference cigarette did (Wong et al., 2020). The findings of one inhalation study (Wong et al., 2016) have been re-interpreted by separate authors to suggest that exposure to aerosol from the THS 2.2 heated tobacco product, but not to mainstream smoke from the 3R4F Kentucky reference cigarette, may have resulted in hepatotoxicity in the exposed rats based on a re-analysis of alanine aminotransferase enzymatic activity levels, liver weights and hepatocellular vacuolisation as reported in the original article (Chun et al., 2018). It should be noted, however, that a previous study (Lo Sasso et al., 2016a), which primarily focused on the potential effects of aerosol exposure from the same heated tobacco product on hepatic tissue, did not demonstrate any gross indicators for hepatotoxicity in its results.

Results from the Systems Toxicology Assessment of tissues obtained from the majority of the studies conducted by PMI have indicated a significantly lower response in terms of gene, microRNA and/or protein expression as a result of exposure to experimentally generated aerosols from heated tobacco products compared to exposure to mainstream smoke from the 3R4F Kentucky Reference cigarette (Kogel et al., 2016; Elamin et al., 2016; Lo Sasso et al., 2016a; Sewer et al., 2016; Szostak et al., 2017, 2020; Choukrallah et al., 2019, 2020; Lavrynenko et al., 2020; Titz et al., 2020b; van der Plas et al., 2020).

With respect to those studies published by independent researchers, three concluded that exposure to aerosols produced from the IQOS heated tobacco product produced detrimental effects on the respiratory system which were comparable, in some instances, to those effects seen with conventional cigarette mainstream smoke (Bhat et al., 2021; Vivarelli et al., 2021; Daou et al., 2021) whilst the fourth reported significantly higher levels of metallothionein I and II expression after exposure to conventional cigarette mainstream smoke than after exposure to aerosol from the IQOS heated tobacco product (Scharf et al., 2021). In the first study, male and female C57BL/6Ncr mice were exposed to experimentally-generated aerosol from the IQOS heated tobacco product or mainstream smoke from the 3R4F Kentucky Reference cigarette for a total of 5 hours per day for 2 weeks with 20 sticks being consumed each day (Bhat et al., 2021). Mice were exposed in a whole-body exposure system with aerosol being generated using the Health Canada Intense smoking regime. Exposure to aerosol from the IQOS heated tobacco product and mainstream smoke from the 3R4F Kentucky reference cigarette was reported to result in significantly increased levels of albumin in bronchoalveolar lavage fluid compared with air-exposed controls (reported to be a surrogate marker for lung epithelial cell damage). Albumin levels were lower after exposure to aerosol from the IQOS heated tobacco product than after exposure to mainstream smoke from the 3R4F Kentucky reference cigarette, although still significantly higher than those observed with controls, indicating lower lung damage with the IQOS heated tobacco product. Both exposure scenarios also produced increased infiltration of immune cells to the lungs of the mice compared with controls. Levels of neutrophils after exposure to IQOS aerosol showed no statistically significant differences when compared to controls whilst levels of macrophages were significantly decreased. In the second study, Sprague-Dawley rats were whole-body exposed to aerosol from the IQOS heated tobacco product for four weeks (Vivarelli et al., 2021). Rats were exposed to the aerosol produced from eight sticks per day, for five consecutive days per week for four weeks. Analysis of tracheal tissue using scanning electron microscopy showed marked changes from those observed in fresh air controls including the presence of erythrocytes and necrotic cells on the epithelial surface. Exposure was also indicated to increase expression of IL-13, IL-10, IL-12, TNF- $\alpha$  and INF- $\delta$ . Urinary mutagenicity was also significantly increased after exposure to IQOS aerosol compared to controls when assessed using the Ames test in the presence of S9 mixture. This study did not include a comparable treatment group of rats exposed to conventional cigarette mainstream smoke. In the third study, diabetic and non-diabetic mice were exposed to aerosol from the IQOS heated tobacco product or mainstream smoke from the 3R4F Kentucky reference cigarette using a nose-only regime (Daou et al., 2021). Mice were exposed for seven consecutive days with two three-hour exposure sessions per day. Exposure to aerosol from the heated tobacco product did not produce any significant oxidative stress or increase in apoptosis in either diabetic or non-diabetic mice whereas exposure to conventional cigarette mainstream smoke did. Exposure to heated tobacco product aerosol did produce an increase in albumin levels in bronchoalveolar lavage fluid in both diabetic and non-diabetic mice and an increase in TNF- $\alpha$  and IL-1 $\beta$  levels in diabetic mice only. In the fourth study, C57BL/6 mice were exposed to filtered air (as a control), to mainstream smoke from Marlboro Red conventional cigarettes or aerosol from the IQOS heated tobacco product (Scharf et al., 2021). Both products were smoked using the Health Canada Intense regime and mice were exposed for one hour twice per day for five days prior to sacrifice. The number of conventional cigarettes and sticks used was matched by the amount of nicotine for each hour of exposure. Analysis of liver and lung tissue collected sixteen hours after the final exposure indicated that metallothionein I and II expression was markedly enhanced in lung and liver tissue collected from mice exposed to conventional cigarette mainstream smoke than in tissues collected from mice exposed to filtered air or aerosol from the IQOS heated tobacco product with no statistically significant difference between these two groups.

## Conclusions

There appears to be a significant disparity in regard to the reported findings of *in vivo* studies investigating heated tobacco products. Those studies published by heated tobacco product manufacturers have indicated that exposure to aerosol from heated tobacco products consistently yielded significantly reduced biological effects when compared to those observed with conventional



cigarettes in a range of tissues including the respiratory tract based on both histopathological and toxicological analyses. In opposition, the small number of studies published by independent researchers have concluded that exposure to aerosol from heated tobacco products produced comparable effects to those seen with conventional cigarettes. This disparity may be due to the use of different species between studies (PMI typically used the A/J or Apo E<sup>-/-</sup> mouse in their studies compared to the Sprague-Dawley rat, CD-1 mouse or C57BL/6NCr mouse used in the independent studies) and/or use of different exposure methodologies. Specifically, it should be noted that whilst PMI investigated the effects of conventional cigarette mainstream smoke and heated tobacco product aerosol using comparable nicotine concentrations, the independent studies did not and instead compare the effects using exposure to mainstream smoke or aerosol produced from equivalent numbers of cigarettes/sticks per exposure session (Yoshida et al., 2020; Bhat et al., 2021). In addition, the study reported by Vivarelli et al. did not include a treatment arm involving exposure to conventional cigarette mainstream smoke (Vivarelli et al., 2021).

Further independent *in vivo* studies into the toxicological and histopathological effects of heated tobacco products may be warranted as well as the development of standardized methods for the *in vivo* toxicological assessment of heated tobacco products. A full assessment of biomarkers of exposure should be conducted in *in vivo* studies to ensure comparable exposure between treatment arms. In addition, it should be noted that only one currently commercially available heated tobacco product (THS 2.2/IQOS) has been investigated in *in vivo* studies and no data exists for other commercially available heated tobacco products at this time. It is important to note however that the relevance of *in vivo* studies for assessing the effects of heated tobacco product aerosols for human exposure is unclear. The strongest evidence for the assessment of heated tobacco products is likely to be derived from actual health outcomes in cohorts of heated tobacco product users compared to cohorts of smokers and non-smokers.

### ***In vitro* toxicology**

This section of the review will discuss those studies that have investigated the *in vitro* toxicological effects of heated tobacco products. These studies have investigated the toxicological properties of whole aerosol produced from heated tobacco products as well as that of total particulate matter (produced by passing the aerosol through a Cambridge filter pad), the gas-vapour phase and extracts produced by dissolving the total particulate matter in a range of solvents including water. Several of the methods used in these studies have been validated specifically for use with heated tobacco products (Buratto et al., 2018; Bozhilova et al., 2020).

### **CORESTA recommended assays**

CORESTA recommends three toxicology tests for the *in vitro* toxicological testing of tobacco smoke (CORESTA, 2004):

1. Ames *Salmonella typhimurium* test (bacterial mutagenicity assay),
2. Micronucleus assay and mouse lymphoma assay (mammalian genotoxicity assays),
3. Neutral Red Uptake assay (mammalian cytotoxicity assay)

With respect to the Ames *Salmonella typhimurium* test [also known as the Ames test], several heated tobacco products including the EHCSS, EHCSS Series K, THS 2.2 and THP 1.0 have been assessed using this assay (Tewes et al., 2003; Roemer et al., 2004, 2008; Werley et al., 2008; Zenzen et al., 2012; Schaller et al., 2016a; Breheny et al., 2017; Crooks et al., 2018; Thorne et al., 2018; Le Godec et al., 2019; Wang H et al., 2021). The results from these studies indicate that total particulate matter, the gas/vapour phase and/or whole aerosol produced from these heated tobacco products either demonstrated an absence of mutagenicity under test conditions or a significant reduction in mutagenicity when compared to conventional cigarettes. There are no studies available which suggest that heated tobacco products have either comparable or greater mutagenicity than conventional cigarettes in the Ames test. These observations are in line with an earlier experimental study in which tobacco tablets were heated to temperatures of between 250°C and 550°C, and which concluded that the temperature to which the tobacco was heated had a significant effect on the subsequent mutagenicity of the aerosol produced from the tobacco tablets (White et al., 2001). No bacterial mutagenicity was detected in this study for temperatures of between 250°C and 360°C for strain TA98 and for temperatures of between 250°C and 400°C for strain TA100.

With respect to those mammalian assays which are used to investigate cytogenetics/mutation [micronucleus assay and mouse lymphoma assay], several heated tobacco products including the EHCSS, EHCSS Series K, THS 2.2 and THP 1.0 have been assessed using these assays (Schramke et al., 2006; Roemer et al., 2008; Werley et al., 2008; Schaller et al., 2016a; Crooks et al., 2018; Thorne et al., 2018, 2019a, 2019b, 2020; Le Godec et al., 2019; Wang H et al., 2021). The results from these studies indicated that total particulate matter, the gas/vapour phase and/or whole aerosol produced from heated tobacco products either demonstrate an absence of genotoxicity under the conditions of test or a significant

reduction in genotoxicity when compared to conventional cigarettes in both assays. There are no studies available in the scientific literature which suggest that heated tobacco products have either comparable or greater genotoxicity than conventional cigarettes in either of the discussed assays.

With respect to those assays which are used to investigate cytotoxicity (Neutral Red uptake assay), several heated tobacco products including the EHCSS, EHCSS Series K, THS 2.2 and THP 1.0 have been assessed using this assay (Tewes et al., 2003; Roemer et al., 2004, 2008; Werley et al., 2008; Zenzen et al., 2012; Schaller et al., 2016a; Breheny et al., 2017; Adamson et al., 2018; Crooks et al., 2018; Jaunky et al., 2018; Leigh et al., 2018b; Murphy et al., 2018; Thorne et al., 2018; Davis et al., 2019a; Caruso et al., 2021). The results from these studies indicated that total particulate matter, the gas/vapour phase and/or whole aerosol produced from heated tobacco products typically demonstrate a significant reduction in cytotoxicity when compared to conventional cigarettes, with five studies reporting comparable cytotoxicity for some, but not all, of their experimental analyses. Only a single study has reported increased cytotoxicity for heated tobacco products compared to conventional cigarettes (Zenzen et al., 2012). In this study, three different variants of the heated tobacco product [which is no longer commercially available] were compared with a range of different commercially available conventional cigarettes ranging in stated tar yields including two 1 mg products.

Cytotoxicity, as assessed using *in vitro* assays other than the Neutral Red Assay, has also been shown to be absent for experimental reagents produced from heated tobacco products (Ito et al., 2019; Davis et al., 2019a; Bishop et al., 2020), present at lower levels than conventional cigarettes (Munakata et al., 2018; Bozhilova et al., 2020) or present at comparable levels to conventional cigarettes (Davis et al., 2019a).

#### Other *in vitro* toxicology assays

There are many studies, published both by heated tobacco product manufacturers and by independent researchers, which have discussed the *in vitro* toxicological effects of heated tobacco products using a range of assays other than the CORESTA recommended assays.

With respect to those published by heated tobacco product manufacturers, all have concluded that exposure to aerosols and/or extracts produced from heated tobacco products resulted in *in vitro* toxicological effects which were significantly reduced compared to those effects seen after treatment with conventional cigarette mainstream smoke and/or extracts.

Using human coronary arterial endothelial and THP-1 monocytic cells, chemotaxis and transendothelial migration has been assessed after exposure to aerosol extracts produced from the THS2.2 heated tobacco product as a surrogate for early pathological stages of atherosclerosis (Van der Toorn et al., 2015). Extracts produced from the THS2.2 heated tobacco products demonstrated a reduced effect, with extracts produced from the 3R4F Kentucky reference cigarette being approximately 18 times more potent than those produced from the THS2.2 heated tobacco product. A subsequent study quantified adhesion of monocytic cells to human coronary arterial endothelial cells after exposure to extracts produced from both 3R4F Kentucky reference cigarette and the THS2.2 heated tobacco product as an experimental surrogate for the initiation of atherogenesis (Poussin et al., 2016). The THS2.2 heated tobacco product displayed a reduced effect in both fresh direct and indirect exposure studies compared to the reference cigarette. Comparable results were observed with the THP1.0 heated tobacco product where no significant inhibition in wound healing was observed using aqueous extracts from the heated tobacco product in an endothelial migration assay (Bishop et al., 2020). Further studies investigated oxidative stress and inflammatory end-points in human bronchial epithelial cells after exposure to total particulate matter from both the THP1.0 and THS2.2 heated tobacco products and the 3R4F Kentucky reference cigarette (Taylor et al., 2018; van der Toorn et al., 2018). Using a luciferase-based reporter assay, transcriptional activation of the antioxidant response elements (ARE) was assessed 6 and 24 hours after exposure to the aerosols (Taylor et al., 2018). Using high-content screening (HCS), ten different toxicity and oxidative stress endpoints (ATP, cell count, glutathione content, mitochondrial mass, mitochondrial membrane potential, nuclear size, reactive oxygen species formation, DNA structure, DNA damage and c-Jun stress kinase) were assessed four and twenty-four hours after exposure. Exposure to total particulate matter from the 3R4F Kentucky reference cigarette after either 6- or 24-hours induced a significantly greater activation of the ARE than either of the heated tobacco products across all doses tested. Neither of the heated tobacco products elicited any responses in the ten HCS end-points at either time point, whilst the 3R4F Kentucky Reference cigarette caused statistically significant perturbations in four of the end-points (ATP, glutathione content, mitochondrial membrane potential and DNA damage) after 4 and/or 24 hours of exposure. The second study demonstrated that exposure of human bronchial epithelial cells (BEAS-2B) over a 12-week period to total particulate matter produced from the THS2.2 heated tobacco product did not produce an increase in inflammatory mediators at a five-fold higher concentration when compared to total particulate matter from the 3R4F Kentucky reference cigarette (van der Toorn et al., 2018).

Aerosol from the THS2.2 heated tobacco product has been demonstrated to have no quantifiable inhibitory effect on monoamine oxidase activity (whilst mainstream smoke from the 3R4F Kentucky reference displayed a significant inhibitory

effect) (van der Toorn et al., 2019) whilst separate studies have indicated that total particulate matter from the THS2.2 heated tobacco product produced a markedly lower effect on mitochondrial dynamics and biogenesis than total particulate matter from the 3R4F Kentucky reference cigarette (Malińska et al., 2018; Walczak et al., 2020). A dose of 150µg/ml TPM from the THS2.2 heated tobacco product was required to achieve the same effects on mitochondrial end-points as a dose of 7.5µg/ml TPM from the 3R4F Kentucky reference cigarette (twenty times higher dose) (Walczak et al., 2020).

Analysis of the transcriptomic changes (based on RNA sequencing) in 3D human airway cells due to exposure to experimentally-generated aerosols from the THP1.0 and THS2.2 heated tobacco products suggested that the THP1.0 heated tobacco product had a markedly lower transcriptomic effect compared to the THS2.2 heated tobacco product (Haswell et al., 2018). A further study employing a 3D model of goblet cell hyperplasia found that a six-week exposure period using whole smoke from the 3R4F Kentucky reference cigarette demonstrated a significant decrease in transepithelial electrical resistance and an increase in MUC5AC positive cells (cells expressing mucin) whilst exposure to aerosol from the THP1.0 did not (Haswell et al., 2021).

Systems Toxicology Assessments conducted by PMI have indicated a significantly lower and more transient response in terms of gene, microRNA and/or protein expression in cell lines as a result of exposure to experimentally generated aerosols or aqueous extracts from the THS2.2/IQOS heated tobacco product compared to comparable treatments produced from the 3R4F Kentucky Reference cigarette. Exposure to aerosols and/or extracts was conducted using comparable nicotine concentrations with several concentrations used for either or both of the exposure treatments. These studies have used the adhesion of monocytic cells to human coronary endothelial cells as a surrogate pathophysiologically relevant event in atherogenesis (Poussin et al., 2016, 2020), 3D nasal epithelial culture models (Iskandar et al., 2017a), organotypic nasal epithelial tissue cultures (Iskandar et al., 2017c), organotypic oral epithelial tissue cultures (Zanetti et al., 2016), organotypic buccal epithelial tissue cultures (Iskandar et al., 2017c), organotypic bronchial epithelial tissue cultures (Iskandar et al., 2017b, 2017c), organotypic gingival epithelial tissue cultures (Zanetti et al., 2017), small airway epithelium models (Iskandar et al., 2017d) and aortic smooth muscle cells (Poussin et al., 2021). A meta-analysis on the relative expression of microRNA using data collected from twelve *in vitro* studies investigating conventional cigarettes and potentially reduced risk products (including heated tobacco products) determined a 94% reduction in microRNA expression relative to conventional cigarette exposure (Sewer et al., 2020). No specific microRNA expression response pattern could be identified after heated tobacco product exposure.

With respect to those studies published by independent researchers, most, but not all, have concluded that exposure to aerosols and/or extracts produced from heated tobacco products produced *in vitro* toxicological effects which were comparable to those effects seen after treatment with conventional cigarette mainstream smoke and/or extracts produced from it.

With respect to those studies that have found comparable effects, one of the first studies published by independent researchers reported that the IQOS heated tobacco product demonstrated comparable effects to a conventional cigarette (Marlboro Red) on cell cytotoxicity as assessed using the MTT and lactate dehydrogenase assays (Sohal et al., 2019; McAlinden et al., 2019, 2020). A subsequent study also using the IQOS heated tobacco product reported a comparable oxidative stress response in primary rat alveolar epithelial cells after exposure to extracts produced from both the IQOS heated tobacco product and the Marlboro Red conventional cigarette (Ito et al., 2020). Assessment of oxidative stress by quantification of intracellular oxidised and reduced glutathione in bronchial epithelial cells (BEAS-2B) by separate authors reached comparable conclusions albeit after more intensive exposure conditions for the heated tobacco product under investigation (IQOS) (Dusautoir et al., 2021) whilst a second research group reported that changes in intracellular glutathione were significantly less pronounced for the IQOS heated tobacco product than for either a commercially available conventional cigarette or the 3R4F Kentucky Reference cigarette (Wang L et al., 2020). Exposure of Jurkat T cells to either filtered air, conventional cigarette mainstream smoke or aerosol from the IQOS heated tobacco product for a thirty-minute period demonstrated a statistically significant decrease in cell viability, an increase in necrotic cells and a greater percentage of early (but not late) apoptotic cells but no statically significant increases in oxygen and nitrogen reactive species and oxidative DNA damage (Scharf et al., 2021).

In opposition to these studies, several studies published by independent researchers have found no significant *in vitro* toxicological effects. A study investigating effects on adipocytes demonstrated no statistically significant effect on pre-adipocyte survival and differentiation to beige adipocyte after treatment with extracts produced from the IQOS heated tobacco products whilst extracts produced from mainstream smoke from the 1R6F Kentucky reference displayed a significant decrease in cell viability in a dose-dependent fashion (Zagoriti et al., 2020). Investigations into the effects of the IQOS heated tobacco product on osteoprogenitor cell viability and function found that aqueous extracts produced from the IQOS heated product were significantly less toxic to bone than aqueous extracts produced from Marlboro conventional cigarettes when analysed by mitochondrial and esterase activity (Aspera-Werz et al., 2020). Significant effects from treatment with the IQOS heated tobacco product extracts were only observed at very high, non-physiologically relevant

concentrations whilst extracts from the Marlboro conventional cigarette significantly reduced alkaline phosphatase activity and matrix mineralisation at low concentrations. A study investigating cell viability in human gingival fibroblasts and human keratinocytes using the MTT assay found that extracts produced from the IQOS heated tobacco products increased both cell viability (23% to 41% with fibroblasts and 30% to 79% with keratinocytes) and cell migration (Pagano *et al.*, 2021). IQOS extracts were not reported to induce an increase in cell death or produce morphological alterations although an increase in p53 expression in fibroblasts and a decrease in p53 expression in keratinocytes was observed. Exposure to total particulate matter produced from the MOK heated tobacco product (produced by the China Tobacco Hubei Industrial Company) and the 3R4F Kentucky Reference cigarette in human lung cancer cells (NCI-H292) showed that whilst the 3R4F Kentucky Reference cigarette demonstrated dose-dependent responses in the majority of the fifteen-toxicity end-points investigated, exposure to comparable doses produced from the MOK heated tobacco product did not (Wang H *et al.*, 2021).

### Tooth staining: experimental assessment of exposure

A small number of *in vitro* studies have investigated the effect of heated tobacco products on the discolouration of dental resin composites (Zhao *et al.*, 2017), extracted bovine enamel preparations (Dalrymple *et al.*, 2018; Haiduc *et al.*, 2020; Dalrymple *et al.*, 2021), extracted human teeth prepared with composite resin restorations (Zanetti *et al.*, 2019) and artificial denture teeth (Wang Y *et al.*, 2021) as experimental surrogates to assess whether or not heated tobacco product use has the potential to discolour the teeth of users. Experimentally produced samples were exposed to the particulate matter and/or aerosol produced from the THS2.2/IQOS or THP1.0 heated tobacco products and compared with samples exposed to conventional cigarette smoke. Sample colour was assessed quantitatively using spectrophotometric assessment. In each instance, the heated tobacco product under investigation was found to result in significantly less discolouration to the dental resin composites, extracted bovine enamel preparations, human teeth or artificial denture teeth than the smoke from conventional cigarettes.

### Toxicological risk assessment modelling

Given the data available in the scientific literature on the fewer and substantially lower levels of HPHCs present in the aerosols experimentally produced from heated tobacco products, it is possible to perform risk assessment modelling of the overall harm from their use and compare this with the harm estimated to be associated with conventional cigarette use (Stephens, 2018; Lachenmeier *et al.*, 2018; Slob *et al.*, 2020; Rodrigo *et al.*, 2021). This approach takes into consideration the different toxicological effects of each of the chemical constituents analysed and attempts to provide an overall estimate of harm.

Using data from a single study (Schaller *et al.*, 2016b), the first study attempted to estimate the cancer potencies of various product types using levels of chemical constituents found in the aerosols of each product type and their associated inhalation unit risks (Stephens, 2018). Using this approach, the author estimated that the heated tobacco product under investigation had a lower cancer potency (compared to that of conventional cigarettes) by at least one order of magnitude but a higher cancer potency than non-tobacco containing vapour products and a nicotine inhaler.

Using a margin of exposure (MOE) approach, subsequent authors attempted to conduct a quantitative risk assessment (Lachenmeier *et al.*, 2018). The MOE was defined as the ratio between the toxicological threshold of the chemical constituent and the estimated human intake of the same chemical constituent (with a higher MOE indicating a lower risk). This study noted the observations of the prior study of Auer *et al.* (2017a), which suggested that levels of acenaphthene were found to be higher in heated tobacco products than in conventional cigarettes. Lachenmeier *et al.* found that, with the exception of acenaphthene, the MOE of several chemical constituents (acetaldehyde, ammonia, arsenic, chromium, catechol, formaldehyde and pyridine) were increased by factors of between three and nine for heated tobacco products, compared to conventional cigarettes and for the rest for the chemical constituents exceeded a factor of ten up to a factor of 415 for isoprene. For several chemical constituents (acenaphthene, benzene, chromium, *m/p*-cresol, ethylene oxide, isoprene, NNN, NNK, quinoline and styrene), the MOE in heated tobacco products exceeded the threshold of 10,000 reported by the authors as being the threshold for genotoxic carcinogens. For conventional cigarettes, only acenaphthene exceeded this threshold. Overall, the results of this study indicated that the combined MOEs for all chemical constituents analysed (including acenaphthene) was twenty-three-fold higher for heated tobacco products compared to conventional cigarette use in the absence of nicotine and ten-fold (one order of magnitude) higher compared to conventional cigarette use with nicotine present. Furthermore, the study indicated that acenaphthene, present either in conventional cigarette smoke or in aerosol produced from heated tobacco products, is considered unlikely to pose any significant health risk to consumers from either product type use due to its MOE exceeding 10,000 for both conventional cigarettes and heated tobacco products.

Using an approach which focuses on the changes in cumulative exposure for a defined number of specific chemical constituents present in both the aerosol from heated tobacco products and mainstream smoke from conventional cigarettes, separate authors have attempted to compare the carcinogenicity of both product types (Slob et al., 2020). Using data from a single experimental study (Schaller et al., 2016b) which reported levels of eight known or suspected carcinogens (acrylonitrile, acetaldehyde, 1,3-butadiene, ethylene oxide, formaldehyde, benzo [a] pyrene, nitrobenzene and propylene oxide) in both the aerosol from heated tobacco products and mainstream smoke from conventional cigarettes, the changes in cumulative exposure was estimated to be between ten and twenty-five times lower when using heated tobacco products compared to conventional cigarettes. The authors concluded that “*such a change indicates a substantially smaller reduction in expected life span based on available dose-response information in smokers*”. Despite this conclusion, it was noted that “*an unfavourable health impact related to heated tobacco products remains as compared to complete abstinence*”. It should be noted that the findings of this study are limited by the fact that only eight chemical constituents were investigated.

The most recently published study estimated both cancer potency and mean lifetime cancer risk for HPHCs present in heated tobacco product aerosol and used an MOE approach for non-carcinogenic HPHCs (Rodrigo et al., 2021). The authors used experimentally-determined HPHC data obtained from commercially available HTPs (n=8) and conventional cigarettes (n=273) in the estimations. A total of forty-two and thirty-three HPHCs were quantified in heated tobacco product aerosol and conventional cigarette mainstream smoke respectively. Cancer potency was determined for each selected HPHC and translated to mean lifetime cancer risk for each evaluated product. Mean lifetime cancer risk values were estimated for conventional cigarettes and heated tobacco products with a median value of  $2.73 \times 10^{-2}$  (range of  $1.40 \times 10^{-2}$  to  $3.97 \times 10^{-2}$ ) for conventional cigarettes and a median value of  $1.106 \times 10^{-3}$  (range of  $4.53 \times 10^{-5}$  to  $3.95 \times 10^{-3}$ ) for heated tobacco products. Based on their median values, the authors reported that the relative cancer risk for a lifetime exposure to heated tobacco products was 0.039 when compared to conventional cigarettes. Total MOE values (including that for nicotine) for conventional cigarettes had a median value of  $1.36 \times 10^{-4}$  (range of  $1.03 \times 10^{-4}$  to  $2.16 \times 10^{-4}$ ) whilst the median value for heated tobacco products was  $4.49 \times 10^{-3}$  (range of  $1.40 \times 10^{-3}$  to  $1.42 \times 10^{-2}$ ). An increase in the median value for heated tobacco products was reported to indicate an estimated reduced non-cancer risk compared to conventional cigarette use.

## Conclusions

There appears to be a significant disparity in regard to the reported findings of *in vitro* studies investigating heated tobacco products. Those studies published by heated tobacco product manufacturers have indicated that exposure to aerosol, particulate matter, gaseous phase components or extracts experimentally produced from heated tobacco products consistently yielded significantly reduced toxicological effects when compared to those observed with conventional cigarettes in a range of cell lines including those derived from the respiratory tract and aerodigestive tracts across a range of different assays. In many instances, a several-fold/several order of magnitude reduction in biological effect was observed between conventional cigarettes and heated tobacco products. In opposition, a significant proportion of those number of studies published by independent researchers have concluded that exposure to aerosol, particulate matter, gaseous phase components or extracts experimentally produced from heated tobacco products produced comparable effects to those seen with conventional cigarettes. This disparity may be due to the use of different cell line exposure methodologies, and/or the use of different methods to generate heated tobacco product aerosol and conventional cigarette mainstream smoke and differences in data collection and data treatment. Further research is required in regard to the comparison between relative and absolute risk associated with heated tobacco product use (i.e. comparing differences between changes in single constituents and changes in cumulative levels). It should be noted, however, that heated tobacco products have been consistently demonstrated to have decreased mutagenic, genotoxic and cytotoxic effects when compared to conventional cigarettes in those *in vitro* assays recommended by CORESTA for use with tobacco smoke.

These conclusions are in agreement with those of a separate review published by PMI researchers, which concluded that “*the experimental results [from systems biology and systems toxicology studies] demonstrate a reduced impact on apical and molecular endpoints, no novel effect not seen with cigarette smoke exposure, and an effect of switching from cigarettes to either MRTP [two heated tobacco products; one electrically heated and one with a carbon-heating design] that is comparable to that of complete smoking cessation*” (Schlage et al., 2020). PMI researchers have also attempted to further validate their scientific approach to assessment of the THS2.2 heated tobacco product by providing their biological and clinical samples or data packages to external independent researchers (in a blinded fashion) to see whether or not they would reach the same conclusions as the original researchers (Poussin et al., 2017; Boué et al., 2019; Belcastro et al., 2020). In each instance reported, the external researchers corroborated with the conclusions reached by the original researchers based on their own independent analyses of the samples.



## Nicotine pharmacokinetics and pharmacodynamics

This section of the review will detail those studies that have investigated the pharmacokinetic and pharmacodynamic profile of nicotine delivery associated with the use of heated tobacco products under controlled clinical conditions.

### Pharmacokinetic and pharmacodynamic studies

Four controlled clinical studies involving adult smokers have been published which have attempted to estimate the pharmacokinetic profile of nicotine delivery from heated tobacco products and compare this delivery with that observed with conventional cigarettes. Two studies have been published by PMI (Picavet et al., 2016; Brossard et al., 2017) whilst two studies have been published by independent researchers (Maloney et al., 2021; Phillips-Waller et al., 2021).

With respect to those studies published by PMI, the first study was conducted using 28 adult smokers and investigated nicotine pharmacokinetics after single and *ad libitum* use of the THS 2.1 heated tobacco product and the subjects' own brand of conventional cigarettes during a 7-day clinical confinement period (Picavet et al., 2016). The median time to maximum nicotine concentration ( $T_{max}$ ) was eight minutes after single use of both products. The median time to the peak nicotine concentration following *ad libitum* use was similar for both product types. The average maximum plasma nicotine concentration ( $C_{max}$ ) after single use of the heated tobacco product was 8.4ng/ml, equivalent to approximately 70% of that obtained with the subjects' own brand of conventional cigarettes. A transient reduction from baseline in the urge to smoke of 40% was observed 15 minutes after single use of both product types whilst the mean Questionnaire of Smoking Urges (QSU-brief) total scores following both single and *ad libitum* use were similar for both product types. The second study was conducted with Japanese adult smokers using both mentholated and non-mentholated variants of the THS 2.2 heated tobacco product, subjects' own brand of conventional cigarettes and nicotine gum indicated that maximal nicotine concentration, overall nicotine exposure and urge-to-smoke scores were similar between the heated tobacco product and the conventional cigarette (Brossard et al., 2017). Maximal nicotine concentration ( $C_{max}$ ) and area under the curve from the start of product use to time of last quantifiable concentration ( $AUC_{0-last}$ ) were comparable between the mentholated and non-mentholated variants of the heated tobacco product and conventional cigarettes with ratios varying between 88 and 104% for  $C_{max}$  and from 96 to 98% for  $AUC_{0-last}$ . Pharmacokinetic profiles were largely comparable between the mentholated and non-mentholated variants of the heated tobacco product, although the mentholated variant did show a slightly lower  $C_{max}$  (10.70ng/ml) than the non-mentholated variant (14.30ng/ml).

With respect to those studies published by independent researchers, the first study assessed nicotine delivery and subjective effects in 18 adult smokers after controlled (10 puffs with a 30-second inter-puff interval) or *ad libitum* (90-minute session) use of the IQOS heated tobacco product, JUUL e-vapour product or the subjects' own brand of conventional cigarettes (Maloney et al., 2021). The subjects had no prior experience of the IQOS or JUUL products. Use of the IQOS heated tobacco product increased mean plasma nicotine levels from  $2.1 \pm 0.2$ ng/ml to  $12.7 \pm 6.2$ ng/ml after 10 puffs and to  $11.3 \pm 8.0$ ng/ml after *ad libitum* use. Use of the JUUL e-vapour product increased mean plasma nicotine levels from  $2.2 \pm 0.7$ ng/ml to  $9.8 \pm 4.9$ ng/ml after 10 puffs and to  $11.5 \pm 9.3$ ng/ml after *ad libitum* use. Use of the subjects' own brand of conventional cigarettes increased mean plasma nicotine levels from  $2.1 \pm 0.2$ ng/ml to  $20.4 \pm 1.4$ ng/ml after 10 puffs and to  $21.0 \pm 10.2$ ng/ml after *ad libitum* use. Mean plasma nicotine levels were significantly higher after use of the subjects' own brand of conventional cigarettes when compared to use of either IQOS or JUUL ( $p < 0.05$ ) although no information was provided as to whether or not there was a statistically significant difference between IQOS and JUUL use. Subjective measures of product use, determined using the QSU-brief and Visual Analogue Scales, indicated that nicotine cravings and urges to smoked were reduced significantly for all products investigated after controlled product use and for subjects' own brand of conventional cigarettes and the IQOS heated tobacco product after *ad libitum* use. The second study used a comparable study design to assess the pharmacokinetic profile of nicotine delivery after 5 minutes of *ad libitum* use of subjects' own brand of conventional cigarettes, the IQOS heated tobacco product, the JUUL e-vapour product and a separate refillable vaping product (Phillips-Waller et al., 2021). The study involved 22 daily e-vapour users who smoked less than one conventional cigarette a day and who had undergone overnight abstinence from smoking and e-vapour use prior to the experimental sessions. A baseline blood sample was obtained prior to product use and additional samples taken 2, 4, 6, 10 and 30 minutes after starting product use. Results indicated that use of the IQOS heated tobacco product delivered about half as much nicotine over the 30-minute period with a similar number of puffs than use of the subjects' own brand of conventional cigarette (median  $C_{max}$ , median  $T_{max}$  and median  $AUC_{0-30}$  values for IQOS and subjects' own brand of conventional cigarette were 8.3ng/ml, 4.0 minutes and 152.0 and 12.9ng/ml, 6.0 minutes and 314.7 respectively). Use of the IQOS heated tobacco product also had a lower median  $C_{max}$  and lower median  $AUC_{0-30}$  than use of the JUUL e-vapour product ( $C_{max}$  of 19.6ng/ml and  $AUC_{0-30}$  of 343.2) whilst the median  $T_{max}$  was the same (4.0 minutes).

### Conclusions

A key requisite for any heated tobacco product is that it provides a satisfying alternative to adult smokers who would otherwise continue to smoke. A heated tobacco product with a comparable nicotine uptake profile to conventional

cigarettes is likely to be more satisfying to more adult smokers considering heated tobacco products as an alternative to continued cigarette smoking. The available clinical evidence indicates that use of heated tobacco products provides a nicotine pharmacokinetic profile comparable to, or marginally less than, that observed with conventional cigarette use dependent on the study design employed and pharmacokinetic parameters quantified.

These findings are in agreement with the conclusions of a separately published review on nicotine pharmacokinetics associated with use of heated tobacco products (Marchand et al., 2017). This review, written by PMI researchers, compared the nicotine pharmacokinetics of the THS heated tobacco product with that observed with conventional cigarettes and two nicotine replacement therapy products (nicotine nasal spray and oral nicotine gum) based on data obtained from eight PMI-sponsored clinical trials (Marchand et al., 2017). The review concluded that background-adjusted exposure to nicotine was consistently lower with the THS heated tobacco product compared to that observed with conventional cigarettes by an average of 24% or 26% depending on whether the results were based on  $C_{max}$  or area under the curve (AUC).

## Biomarkers

This section of the review will detail those studies that have quantified levels of biomarkers of exposure and/or biomarkers of potential harm associated with the use of heated tobacco products in clinical assessments.

### Biomarkers of exposure

#### *Acute exposure studies*

Nine acute exposure studies are available that describe the effects of controlled use of heated tobacco products on exhaled carbon monoxide levels (Buchhalter and Eissenberg, 2000; Buchhalter et al., 2001; Breland et al., 2002; Adriaens et al., 2018; Caponnetto et al., 2018; Nga et al., 2020; Pataka et al., 2020; Ikonomidis et al., 2021; Maloney et al., 2021). Five studies reported no statistically significant increase in observed levels of exhaled carbon monoxide after controlled use of a heated tobacco product whilst reporting a statistically significant increase in observed levels after controlled use of conventional cigarettes (Buchhalter and Eissenberg, 2000; Buchhalter et al., 2001; Caponnetto et al., 2018; Ikonomidis et al., 2021; Maloney et al., 2021). The other four studies reported minimal, but statistically significant, increases in exhaled carbon monoxide levels when compared to baseline levels with heated tobacco product use (Breland et al., 2002; Adriaens et al., 2018; Nga et al., 2020; Pataka et al., 2020). In eight of the nine studies, at no time-point analysed did the exhaled carbon monoxide levels exceed 10ppm during and/or after heated tobacco product use, with this value being commonly accepted as the cut-off value for the reference range of non-smokers (Caponnetto et al., 2018). In the remaining study, inclusion criteria for the conventional cigarette smokers required an exhaled carbon monoxide levels exceeding 10ppm prior to conducting the study (Ikonomidis et al., 2021).

These findings are in line with those of a separate study which assessed the effect of increasing use of the Accord heated tobacco product on exhaled carbon monoxide levels (and number of cigarettes smoked per day) in eleven conventional cigarette smokers who were required to use increasing amounts of Accord (5, 10 and 15 sticks per day) whilst being allowed to continue to use their existing cigarette (Hughes and Keely, 2004). Use of the heated tobacco product decreased the number of conventional cigarettes smoked per day and exhaled carbon monoxide levels in dose-dependent manner. Using Accord fifteen times per day decreased the number of conventional cigarettes smoked by 32% (-8.6 cigarettes per day) and exhaled carbon monoxide levels by 27% (-5.9ppm).

#### *Forced switching studies*

Twenty-one forced switching studies are available which describe a study protocol involving volunteers within closed clinical settings and/or in “real-world” [also known as “ambulatory”] scenarios. Table 3 details the heated tobacco product(s) investigated, the number of study participants and switching period used and the biomarker(s) of exposure quantified in each study. A total of twenty-four separate biomarkers of exposure have been quantified across the reported studies. The volunteers involved in these studies were adult conventional cigarette smokers typically with a significant self-reported smoking history. In the majority of studies, the volunteers are brought into a closed clinical setting and allowed to settle for several days before being randomised into one of several specific groups for a period of five to eight days. One group continue to smoke conventional cigarettes [either brought into the clinic by the volunteers themselves or provided to them by the study staff], another group are switched to the heated tobacco product under investigation, whilst a final group (if present) do not use any tobacco- or nicotine-containing products (and act as the smoking abstinence/control group). Product consumption is controlled by the study staff to ensure approximately comparable consumption (in terms of number of sticks smoked per day) between the two product groups and typically takes into account the prior self-reported smoking history of the volunteers. Two of the twenty-one studies included an additional period of “real-



**Table 3. Studies available which detail forced switching to heated tobacco products from conventional cigarettes under controlled and defined conditions on quantified levels of twenty-four different biomarkers of exposure in experimental volunteers.** If the quantified biomarker of exposure was determined at the end of the clinical confinement period to be at a significantly lower level following heated tobacco product use when compared to conventional cigarette use, then this is indicated by a downwards arrow and green shading; if the quantified levels were comparable, with no statistically significant difference, after use of both product types, then this is indicated by an equivalency arrow and orange shading. 1-NA, 1-aminonaphthalene; 2-NA, 2-aminonaphthalene; 1-OHP, 1-hydroxypyrene; 3-HMPMA, 3-hydroxy-1-methylpropylmercapturic acid; 3-HPMA, 3-hydroxypropylmercapturic acid; 3-OH-B [a] P, 3-hydroxy-benzo [a] pyrene; 4-ABP, 4-aminobiphenyl; AAMA, N-acetyl-S-(2-carbamoyl-ethyl) cysteine; B [a] P, benzo [a] pyrene; CEMA, 2-cyanoethylmercapturic acid; COHb, carboxyhaemoglobin; EHCSS, Electrically Heated Cigarette Smoking System; eCO, exhaled carbon monoxide; GAMA, N-acetyl-S-(2-hydroxy-2-carbamoyl-ethyl)cysteine; HEMA, 2-hydroxyethylmercapturic acid; MHBMA, monohydroxybutenyl mercapturic acid; NNAL, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol; NNN, N-nitrosornicotine; S-PMA, S-phenylmercapturic acid; TMA, *trans*, *trans*-muconic acid.

| Reference(s)               | Heated Tobacco Product(s) Used <sup>1</sup>   | Switching Period <sup>2</sup> ; Number of Subjects | Quantified Biomarker of Exposure |      |                      |                      |                 |                 |            |           |        |       |             |             |       |         |       |      |      |      |      |     |      |      |                      |  |
|----------------------------|---|--|----------------------------------|------|----------------------|----------------------|-----------------|-----------------|------------|-----------|--------|-------|-------------|-------------|-------|---------|-------|------|------|------|------|-----|------|------|----------------------|--|
|                            |   |  | eCO                              | COHb | Urinary Mutagenicity | Nicotine Equivalents | Plasma nicotine | Plasma cotinine | Total NNAL | Total NNN | 3-HPMA | 5-PMA | Total 1-OHP | 0-toluidine | 4-ABP | 3-HMPMA | MHBMA | 1-NA | 2-NA | AAMA | GAMA | TMA | CEMA | HEMA | Total 3-OH-B[a]P/BBP |  |
| Roethig et al., 2005       | <b>Accord</b> (First Generation EHCSS; Series E4); <b>Oasis</b> (First Generation EHCSS, Series E4) | 8 days; 110  | ↓                                | ↓    | ↓                    | ↓                    |                 |                 |            |           |        |       |             |             |       |         |       |      |      |      |      |     |      |      |                      |  |
| Feng et al., 2006          |   | 8 days; 110  |                                  |      |                      |                      |                 |                 |            |           |        | ↓     | ↓           |             |       |         |       |      |      |      |      | ↓   |      |      |                      |  |
| Roethig et al., 2007       | <b>Accord</b> (Second Generation EHCSS; version JLI)  | 8 days; 100  |                                  | ↓    | ↓                    | ↓                    |                 | ↓               | ↓          |           | ↓      | ↓     | ↓           |             |       |         |       |      |      |      |      |     |      |      |                      |  |
| Frost-Pineda et al., 2008a | <b>EHCSS</b> (Third Generation; Series K)   | 8 days; 100  |                                  | ↓    | ↓                    | ↓                    |                 |                 | ↓          |           | ↓      | ↓     | ↓           | ↓           | ↓     | ↓       | ↓     |      | ↓    |      |      |     |      |      |                      |  |
| Frost-Pineda et al., 2008b | <b>EHCSS</b> (Third Generation; Series K)   | 12 weeks (“real-world setting”); 90                |                                  | ↓    |                      | ↓                    |                 |                 | ↓          |           | ↓      | ↓     | ↓           |             |       |         |       |      |      |      |      |     |      |      |                      |  |
| Roethig et al., 2008       | <b>EHCSS</b> (Second Generation)  | 12 months (“real-world setting”); 97               |                                  | ↓    | ↓                    | ↓                    |                 | ↓               | ↓          |           | ↓      |       | ↓           |             | ↓     |         |       |      |      |      |      |     |      |      |                      |  |
| Scherer et al., 2010       | <b>EHCSS</b> (Third Generation; Series K)   | 8 days; 100  |                                  |      |                      |                      |                 |                 |            |           |        |       |             |             |       |         |       |      |      |      |      | ↓   | ↓    |      |                      |  |
| Tricker et al., 2012a      | <b>EHCSS</b> (Series K; used with either K3 or K6 cigarettes)                                       | 8 days; 160  |                                  | ↓    | ↓                    | ↓                    |                 | ↓               | ↓          |           | ↓      | ↓     | ↓           |             | ↓     | ↓       |       |      |      |      |      |     |      |      |                      |  |
| Tricker et al., 2012b      | <b>EHCSS</b> (Series K; used with K3 cigarettes)  | 8 days; 72   |                                  | ↓    | ↓                    | ↓                    | ↓               | ↓               | ↓          |           | ↔      | ↓     | ↓           | ↓           | ↓     | ↔       | ↓     |      | ↓    | ↓    | ↓    |     |      |      |                      |  |
| Tricker et al., 2012c      | <b>EHCSS</b> (Series K; used with either K3 or K6 cigarettes)                                       | 8 days; 128  |                                  | ↓    | ↓                    | ↓                    | ↓               | ↓               | ↓          |           | ↓      | ↓     | ↓           | ↓           | ↓     | ↓       |       | ↓    | ↓    |      |      |     |      |      |                      |  |
| Tricker et al., 2012d      | <b>EHCSS</b> (Series K; used with K6 menthol cigarettes)  | 8 days; 100  |                                  | ↓    | ↓                    | ↓                    |                 | ↓               | ↓          |           | ↓      | ↓     | ↓           | ↓           | ↓     | ↓       |       | ↔    | ↔    |      |      |     |      |      |                      |  |

**Table 3.** *Continued*

| Referenc<br>e(s)                             | Heated<br>Tobacco<br>Product(s)<br>Used <sup>1</sup> | Switchin<br>g<br>Period <sup>2</sup> ;<br>Number<br>of<br>Subjects | Quantified Biomarker of Exposure |      |                      |                      |                 |                 |            |           |        |       |             |             |       |         |       |      |      |      |      |     |      |      |                                    |
|--|--|--|----------------------------------|------|----------------------|----------------------|-----------------|-----------------|------------|-----------|--------|-------|-------------|-------------|-------|---------|-------|------|------|------|------|-----|------|------|------------------------------------|
|  |  |  | eCO                              | COHb | Urinary Metagenicity | Nicotine Equivalents | Plasma nicotine | Plasma cotinine | Total NNAL | Total NNN | 3-HPMA | 5-PMA | Total 1-OHP | O-toluidine | 4-ABP | 3-HMPMA | MHBMA | 1-NA | 2-NA | AAMA | GAMA | TMA | CEMA | HEMA | Total 3-OH-B[a]P/[B]P <sup>3</sup> |
| Martin Leroy et al., 2012                    | EHCSS (Series K; used with K6 cigarettes)            | 1 month ("real-world setting"); 316                                | ↓                                | ↓    |                      | ↓                    |                 |                 | ↓          |           |        | ↓     | ↓           | ↓           | ↓     | ↓       | ↓     |      |      |      |      |     |      |      |                                    |
| Haziza et al., 2016a                         | THS2.2   | 5 days; 160  |                                  | ↓    |                      | ↔                    | ↔               | ↔               | ↓          | ↓         | ↓      | ↓     | ↓           | ↓           | ↓     | ↓       | ↓     | ↓    |      |      |      |     | ↓    | ↓    | ↓                                  |
| Haziza et al., 2016 <sup>4</sup>             | THS2.2   | 5 days; 160  |                                  | ↓    |                      | ↔                    | ↔               | ↔               | ↓          | ↓         | ↓      | ↓     | ↓           | ↓           | ↓     | ↓       | ↓     | ↓    |      |      |      |     | ↓    | ↓    | ↓                                  |
| Lüdicke et al., 2017                         | THS2.1   | 5 days; 40   |                                  | ↓    |                      | ↓                    | ↔               | ↔               | ↓          | ↓         | ↓      | ↓     | ↓           | ↓           |       |         | ↓     | ↓    |      |      |      |     | ↓    |      |                                    |
| Lüdicke et al., 2018a; Lüdicke et al., 2018b | THS2.2 [mentholated variant]                         | 5 days confined; 85 days "real world setting"; 160                 |                                  | ↓    |                      | ↔                    |                 |                 | ↓          | ↓         | ↓      | ↓     | ↓           | ↓           | ↓     | ↓       | ↓     | ↓    |      |      |      |     | ↓    | ↓    | ↓                                  |
| Gale et al., 2019 <sup>5</sup>               | Glo [THP1.0]; IQOS [THS]                             | 5 days; 180  | ↓                                |      |                      | ↓                    |                 |                 | ↓          | ↓         | ↓      | ↓     | ↓           | ↓           | ↓     | ↓       | ↓     | ↓    | ↓    | ↓    | ↓    | ↓   | ↓    | ↓    |                                    |
| Lüdicke et al., 2019 <sup>5</sup>            | THS2.2   | 6 months ("real world setting"); 984                               | ↓                                | ↓    |                      | ↔                    |                 |                 | ↓          | ↓         | ↓      |       | ↓           |             |       | ↓       | ↓     |      |      |      |      | ↓   |      | ↓    |                                    |
| Haziza et al., 2020a; Haziza et al. 2020b    | THS2.2 [mentholated variant]                         | 5 days confined + 86 days "real world setting"; 160                |                                  | ↓    |                      | ↔                    |                 |                 | ↓          | ↓         | ↓      | ↓     | ↓           | ↓           | ↓     | ↓       | ↓     | ↓    |      |      |      |     | ↓    | ↓    | ↓                                  |
| Gale et al., 2021a <sup>5</sup>              | Glo  | 90 days "real world setting"; 377                                  | ↓                                |      |                      | ↓                    |                 |                 | ↓          | ↓         | ↓      | ↓     |             | ↓           | ↓     | ↓       | ↓     | ↓    | ↓    |      |      |     | ↓    | ↓    |                                    |
| McEwan et al., 2021                          | Glo [non-mentholated variant]                        | 5 days; 148  | ↓                                |      |                      | ↓                    |                 |                 | ↓          | ↓         | ↓      | ↓     | ↓           | ↓           | ↓     | ↓       | ↓     | ↓    | ↓    | ↓    | ↓    | ↓   | ↓    | ↓    |                                    |
| Gale et al., 2021b <sup>5,6</sup>            | Glo  | 180 days "real world setting"; 285                                 | ↓                                |      |                      | ↔                    |                 |                 | ↓          |           |        |       |             |             |       |         |       |      |      |      |      |     |      |      |                                    |

<sup>1</sup>Where a study was reported to have used more than one heated tobacco product this refers either to use of different variants of the same heated tobacco product system produced by the same manufacturer [the same handheld electronic device used with different cigarette variants] or different heated tobacco products produced by different manufacturers.

<sup>2</sup>Switching Period refers to the period of time spent within a confined clinical setting in which volunteers were randomised to controlled use of the heated tobacco product(s) controlled continued use of conventional cigarettes or abstinence from use of any tobacco or nicotine-containing products. Majority of reported studies solely used a single period of clinical confinement. Minority of studies also included a "real-world" period after the controlled clinical confinement period in which the volunteers could use the randomised product as they wished in their normal lives or solely investigated effects of "real-world" exposure where smoking was un-restricted.

<sup>3</sup>In four studies, levels of 3-OH-B[a]P were quantified as a biomarker of exposure (Haziza et al., 2016a; Haziza et al., 2016b; Lüdicke et al., 2018a; Lüdicke et al., 2019) whilst single study (Haziza et al., 2020a) quantified B[a]P as a biomarker of exposure rather than its metabolite, 3-OH-B[a]P.

<sup>4</sup>The quantitative data reported in this study was subsequently re-published in a separate journal article (Haziza et al., 2017).

<sup>5</sup>Protocols for these studies have been published in separate journal articles (Gale et al., 2017; Ansari et al., 2018; Newland et al., 2019; Camacho et al., 2020).

<sup>6</sup>This article provides data for both 90- and 180-day time-points for the quantified biomarkers of exposure and represents an extension of the previous article by the same authors. This table only details biomarker of exposure data quantified beyond the 90 day time-point. eCO data was measured at the 120- and 150-day time-point whilst NNN and total nicotine equivalents were measured at the 180-day time-point.

world" use following the initial period of clinical confinement whilst five of the twenty-one studies did not include any period of clinical confinement and solely investigated "real-world" use with this approach reported to offer a more realistic method of assessing product use by consumers. The results shown in Table 3 indicate whether the quantified biomarker of exposure was determined to be at a significantly lower level following heated tobacco product use at the end of the clinical confinement period when compared to conventional cigarette use (as indicated by a downwards arrow and green shading) or whether observed levels were comparable with use of both product types (as indicated by an equivalency arrow and orange shading). The aim of this approach is to demonstrate that switching to the heated tobacco product(s) under investigation from conventional cigarettes resulted in a quantifiable, and statistically significant,

decrease in levels of biomarkers of exposure to select toxicants associated with cigarette smoking whilst maintaining comparable cigarette consumption between the two product use groups.

The results from these studies, as shown in [Table 3](#), indicate that use of those heated tobacco products which are currently commercially available (glo and IQOS) under highly defined and controlled conditions results in statistically significant reductions in quantified levels of all biomarkers of exposure when compared to those observed with continued use of conventional cigarettes under the same experimental and clinical conditions. In six of the twenty studies, several biomarkers of exposure associated with nicotine were found to be comparable between the two product types indicating a comparable nicotine delivery with the heated tobacco product (IQOS) and conventional cigarette under investigation.

These findings are in agreement with the conclusions of two published systematic reviews on the levels of biomarkers of exposure associated with use of heated tobacco products ([Drovandi et al., 2020](#); [Akiyama and Sherwood, 2021](#)). The first systematic review identified ten randomised controlled trials involving 1,766 participants published between January 2010 and August 2019 which quantified twelve biomarkers of exposure after controlled use of both heated tobacco products and conventional cigarettes ([Drovandi et al., 2020](#)). Seven of these ten studies are detailed in [Table 3](#) whilst the other three articles related to either use of a hybrid product (n=2) or a carbon-heated tobacco product (n=1) both of which are outside the remit of this review. The authors of this systematic review concluded that *“in comparison to conventional cigarettes, all twelve biomarkers of exposure assessed were significantly lower for participants assigned to a heat-not-burn device. In comparison to smoking abstinence, heat-not-burn devices were statistically equivalent for eight biomarkers of exposure and significantly elevated for four biomarkers of exposure”* ([Drovandi et al., 2020](#)). The second systematic review identified twenty-five randomised controlled trials published up to April 2020 which quantified twelve biomarkers of exposure after controlled use of both heated tobacco products and conventional cigarettes ([Akiyama and Sherwood, 2021](#)). Nineteen of these twenty-five studies are detailed in [Table 3](#) whilst the other six articles related to either use of a hybrid product (n=2) or a carbon-heated tobacco product (n=4). The authors of this systematic review concluded that *“taken together, all findings suggest that biomarker of exposure levels in users of e-cigarettes and heated tobacco products show a significant reduction compared to a cigarette condition (or cigarette baseline)”* ([Akiyama and Sherwood, 2021](#)).

#### *Survey cohort/smoking cessation studies*

A smoking cessation study demonstrated statistically significant decreases in both exhaled carbon monoxide and blood carboxyhaemoglobin levels in forty male adult conventional cigarette smokers who switched to either a heated tobacco product (THS 2.2) or an e-vapour product from their own brand of conventional cigarettes for a period of six months (n=20 in each group) ([Beatrice and Massaro, 2019](#)). Levels of both exhaled carbon monoxide and blood carboxyhaemoglobin with heated tobacco product use were reported as being within the range associated with non-smokers.

A study of 182 Japanese men aged between eighteen and sixty-four provided urinary biomarker data in relation to heated tobacco product use ([Kawasaki et al., 2021](#)). A lifestyle questionnaire was given to the participants as well as collection of a urine sample. Twenty-two men self-reported heated tobacco product use whilst forty-eight men self-reported conventional cigarette use. No information was provided with respect to duration or intensity of product use or incidence of dual product use. Urinary nicotine and cotinine levels did not show a statistically significant difference between heated tobacco product users and conventional cigarette smokers whilst levels of 3'-hydroxycotinine, total nicotine equivalents and NNAL were higher in conventional cigarette smokers when compared to heated tobacco product users (p<0.05 for 3'-hydroxycotinine and total nicotine equivalents and p<0.01 for NNAL).

A subsequent study investigated levels of six biomarkers of exposure (nicotine, cotinine, 3'-hydroxycotinine [metabolites of nicotine], NNAL [a metabolite of NNK], CEMA [a metabolite of acrolein] and CYMA [a metabolite of acrylonitrile]) in Korean adult smokers recruited into a cross-sectional study ([Rudasingwa et al., 2021](#)). Comparison of quantified levels for the six biomarkers of exposure between exclusive conventional cigarette smokers (n=403) and exclusive heated tobacco product users (n=76) showed that levels of nicotine, cotinine, 3'-hydroxycotinine and CYMA did not differ to a statistically significant extent between the user groups whilst levels of NNAL and CEMA were significantly lower in exclusive heated tobacco product users when compared to exclusive conventional cigarette smokers. Whilst the study did not provide quantitative data for dual product users, the authors did report that biomarker levels for dual users of heated tobacco products and conventional cigarette smokers were similar to those of exclusive conventional cigarette smokers.

### Biomarkers of potential harm: forced switching studies

Seven of the forced switching studies previously discussed and detailed in Table 3 quantified levels of biomarkers of potential harm in addition to levels of biomarkers of exposure (Roethig et al., 2008; Martin Leroy et al., 2012; Lüdicke et al., 2018b, 2019; Haziza et al., 2020b; McEwan et al., 2021; Gale et al., 2021b). Table 4 details the heated tobacco product(s) investigated, the number of study participants and switching period used and the biomarkers of potential harm quantified in each study. The study protocol for a seventh forced switching study discussed and detailed in Table 3 indicated the intention of the authors to quantify levels of 8-epi-prostaglandin F<sub>2α</sub> and white blood count in the urine and whole blood of volunteers respectively (Gale et al., 2017). However, the published results from this study do not include any such data (Gale et al., 2019).

The results shown in Table 4 indicate whether the quantified biomarker of potential harm demonstrated a statistically significant and physiologically favourable change with heated tobacco product use when compared to conventional cigarette use (as indicated by green shading) or whether no statistically significant physiological change was observed (as indicated by orange shading). It should be noted that unlike biomarkers of exposure, where a decrease in observed levels is invariably correlated to a decrease in exposure, physiologically favourable changes in biomarkers of potential

**Table 4. Studies available which detail forced switching to heated tobacco products from conventional cigarettes under controlled and defined conditions on quantified levels of twenty-six different biomarkers of potential harm in experimental volunteers.** If the quantified biomarker of potential harm was determined at the end of the clinical confinement period to be at more clinically favourable levels following heated tobacco product use when compared to conventional cigarette use, then this is indicated by green shading; if the quantified levels were comparable, with no statistically significant difference, after use of both product types, then this is indicated by orange shading. 8-EPF, 8-epi-prostaglandin F<sub>2α</sub>; 11-DTX, 11-dehydrothromboxane B<sub>2</sub>; EHCSS, Electrically Heated Cigarette Smoking System; Hb, Haemoglobin; HDL-C, High Density Lipoprotein Cholesterol; hs-CRP, High Sensitivity C-Reactive Protein; LDL-C, Low Density Lipoprotein Cholesterol; RBC, Red Blood Cell; THS, Tobacco Heating System; WBC, White Blood Cell; BP, Blood Pressure; FENO, Fractional Exhaled Nitric Oxide; FEV1, Forced Expiratory Volume in one second; HB A1c, Haemoglobin A1c; sICAM-1, soluble Intracellular Adhesion Molecule-1.

| Reference                              | Heated Tobacco Product(s) Used            | Switching Period <sup>1</sup> ; Number of Subjects  | Quantified Biomarker of Potential Harm |              |           |           |            |       |       |                |        |                 |                                |        |       |               |
|--|---|---|--|--------------|-----------|-----------|------------|-------|-------|----------------|--------|-----------------|--------------------------------|--------|-------|---------------|
|  |   |   | Hb                                     | Haema-tocrit | RBC Count | WBC Count | Fibrinogen | HDL-C | LDL-C | Tri-glycerides | hs-CRP | Total Bilirubin | von Wille-brand factor antigen | 11-DTX | 8-EPF | Micro-albumin |
| Roethig et al., 2008                   | EHCSS (Second Generation)                 | 12 months ("real-world setting"); 97                |  |              |           |           |            |       |       |                |        |                 |                                |        |       |               |
| Martin Leroy et al., 2012 <sup>2</sup> | EHCSS (Series K; used with K6 cigarettes) | 1 month ("real-world setting"); 316                 |  |              |           |           |            |       |       |                |        |                 |                                |        |       |               |
| Lüdicke et al., 2018b                  | THS2.2 [mentholated variant]              | 5 days confined; 85 days "real world setting"; 160  |  |              |           |           |            |       |       |                |        |                 |                                |        |       |               |
| Lüdicke et al., 2019                   | IQOS                                      | 6 months ("real world setting"); 984                |  |              |           |           |            |       |       |                |        |                 |                                |        |       |               |
| Haziza et al. 2020b                    | THS2.2 [mentholated variant]              | 5 days confined + 86 days "real world setting"; 160 |  |              |           |           |            |       |       |                |        |                 |                                |        |       |               |
| McEwan et al., 2021                    | Glo [non-mentholated variant]             | 5 days; 148   |  |              |           |           |            |       |       |                |        |                 |                                |        |       |               |
| Gale et al., 2021b                     | Glo                                       | 180 days "real world setting"; 285                  |  |              |           |           |            |       |       |                |        |                 |                                |        |       |               |

<sup>1</sup>Switching Period refers to the period of time spent within a confined clinical setting in which volunteers were randomised to controlled use of the heated tobacco product(s), controlled continued use of conventional cigarettes or abstinence from use of any tobacco or nicotine-containing products. With respect to those forced switching studies which investigated biomarkers of potential harm, four used "real-world" exposure scenarios only (Roethig et al., 2008; Martin Leroy et al., 2012; Lüdicke et al., 2019; Gale et al., 2021b), one used a single five-day period of clinical confinement (McEwan et al., 2021) and two used a five-day clinical confinement period combined with either an 85 or 86 period of "real-world" use (Lüdicke et al., 2018b; Haziza et al. 2020b).

<sup>2</sup>In addition to those biomarkers of potential harm detailed above, this study also reported quantitative data on other biomarkers of potential harm associated with cardiovascular disease and found statistically significant, and physiologically favourable, changes for high-density lipoprotein (HDL), low-density lipoprotein (LDL), oxidised LDL, basophil count and ADP-induced platelet aggregation whilst reporting no statistically significant differences for neutrophil count, lymphocyte count, monocyte count, eosinophil count, interleukin-6 (IL-6), myeloperoxidase and platelet count.

**Table 4.** *Continued*

| Reference                              | Heated Tobacco Product(s) Used            | Switching Period <sup>1</sup> ; Number of Subjects  | Quantified Biomarker of Potential Harm |         |               |                   |        |             |                     |             |              |                  |                   |                   |      |
|--|---|---|--|---------|---------------|-------------------|--------|-------------|---------------------|-------------|--------------|------------------|-------------------|-------------------|------|
|  |   |   | sICAM-1                                | Glucose | Homo-cysteine | Total Cholesterol | HB A1c | Body Weight | Waist Circumference | Systolic BP | Diastolic BP | FEV <sub>1</sub> | Apolipoprotein A1 | Apolipoprotein B1 | FeNO |
| Roethig et al., 2008                   | EHCSS (Second Generation)                 | 12 months ("real-world setting"); 97                |  |         |               |                   |        |             |                     |             |              |                  |                   |                   |      |
| Martin Leroy et al., 2012 <sup>2</sup> | EHCSS (Series K; used with K6 cigarettes) | 1 month ("real-world setting"); 316                 |  |         |               |                   |        |             |                     |             |              |                  |                   |                   |      |
| Lüdicke et al., 2018b                  | THS2.2 [mentholated variant]              | 5 days confined; 85 days "real world setting"; 160  |  |         |               |                   |        |             |                     |             |              |                  |                   |                   |      |
| Lüdicke et al., 2019                   | IQOS                                      | 6 months ("real world setting"); 984                |  |         |               |                   |        |             |                     |             |              |                  |                   |                   |      |
| Haziza et al. 2020b                    | THS2.2 [mentholated variant]              | 5 days confined + 86 days "real world setting"; 160 |  |         |               |                   |        |             |                     |             |              |                  |                   |                   |      |
| McEwan et al., 2021                    | Glo                                       | 5 days; 148   |  |         |               |                   |        |             |                     |             |              |                  |                   |                   |      |
|  | [non-mentholated variant]                 |   |  |         |               |                   |        |             |                     |             |              |                  |                   |                   |      |
| Gale et al., 2021b                     | Glo                                       | 180 days "real world setting"; 285                  |  |         |               |                   |        |             |                     |             |              |                  |                   |                   |      |

<sup>1</sup>Switching Period refers to the period of time spent within a confined clinical setting in which volunteers were randomised to controlled use of the heated tobacco product(s), controlled continued use of conventional cigarettes or abstinence from use of any tobacco or nicotine-containing products. With respect to those forced switching studies which investigated biomarkers of potential harm, four used "real-world" exposure scenarios only (Roethig et al., 2008; Martin Leroy et al., 2012; Lüdicke et al., 2019; Gale et al., 2021b), one used a single five-day period of clinical confinement (McEwan et al., 2021) and two used a five-day clinical confinement period combined with either an 85 or 86 period of "real-world" use (Lüdicke et al., 2018b; Haziza et al. 2020b).

<sup>2</sup>In addition to those biomarkers of potential harm detailed above, this study also reported quantitative data on other biomarkers of potential harm associated with cardiovascular disease and found statistically significant, and physiologically favourable, changes for high-density lipoprotein (HDL), low-density lipoprotein (LDL), oxidised LDL, basophil count and ADP-induced platelet aggregation whilst reporting no statistically significant differences for neutrophil count, lymphocyte count, monocyte count, eosinophil count, interleukin-6 (IL-6), myeloperoxidase and platelet count.

harm may be associated with either a decrease or increase in their levels (such as with white blood cell count and high-density lipoprotein cholesterol respectively). Therefore, no arrows are included in Table 4 to avoid confusion. The aim of this approach is to demonstrate that switching to the heated tobacco product under investigation from conventional cigarettes resulted in a quantifiable, and statistically significant, physiologically favourable improvement in levels of biomarkers of potential harm whilst maintaining comparable product consumption between the two product use groups.

The results from these studies, as shown in Table 4, indicate that use of heated tobacco products results in statistically significant, and physiologically favourable improvements, in a small number of biomarkers of potential harm when compared to use of conventional cigarettes under the same experimental and clinical conditions. For the remaining biomarkers of potential harm, no significant differences between heated tobacco product use, conventional cigarette use and/or smoking abstinence were observed. The only biomarkers of potential harm which demonstrated physiologically favourable improvements with heated tobacco product use compared with conventional cigarette use across more than one of the six studies were white blood cell count (five studies), high-density lipoprotein cholesterol (three studies), 8-epi-prostaglandin F<sub>2α</sub> (four studies), 11-dehydrothromboxane B<sub>2</sub> (two studies), haemoglobin (two studies), haematocrit (two studies) and soluble intracellular adhesion molecule-1 (two studies). For the two studies that also included an abstinence group who refrained use of any tobacco- and/or nicotine-containing products (Lüdicke et al., 2018b; Haziza et al., 2020b), only a very small number of biomarkers of potential harm demonstrated a statistically significant difference between this group and those who used the heated tobacco product: triglycerides, body weight and glucose (Lüdicke et al., 2018b) and white blood cell count (Haziza et al., 2020b). Researchers from PMI have also applied a systems pharmacology approach in an attempt to demonstrate a reduced exposure response in adult subjects who switched to the THS2.2 heated tobacco product from their regular conventional cigarette as part of a forced switching study (Haziza et al., 2016b) on a whole-blood based eleven gene response signature (Martin et al., 2016). A reduced exposure response was observed in subjects that either stopped smoking or switched to the THS2.2 heated tobacco product compared to subjects who continued to smoke their regular conventional cigarette. Comparable findings of a reduction in the same eleven gene response signature in subjects who either stopped smoking or switched to the THS2.2

heated tobacco product compared to those who continued to smoke conventional cigarettes were found when data was pooled from four separate forced switching studies separately published by PMI in six articles (Haziza et al., 2016a, 2016b, 2020a, 2020b; Lüdicke et al., 2018a, 2018b) using largely comparable designs (Martin et al., 2019).

These findings are in agreement with the conclusions of a separately published systematic review on the levels of biomarkers of potential harm associated with use of heated tobacco products (Akiyama and Sherwood, 2021), a pooled analysis of data from three clinical studies (Roethig et al., 2010) whilst in disagreement with the conclusions of a third author who published a review on the same topic (Glantz, 2018b). The systematic review identified seven randomised controlled trials published up to April 2020 which quantified biomarkers of potential harm after controlled use of both heated tobacco products and conventional cigarettes. Five of the seven studies are detailed in Table 4 whilst the other two studies related to use of carbon-heated tobacco products which are outside the remit of this review. The authors of this systematic review concluded that “*regarding biomarkers of biological effect* [another descriptor used in the literature for biomarkers of potential harm], *the results show that levels found during the use of both e-cigarettes and heated tobacco products were generally moved in a direction believed to be consistent with improved health outcomes*” and “*since studies on biomarkers of biological effect may require longer intervention periods, the number of reports was limited without the necessary follow up time to show changes in biological functions*” (Akiyama and Sherwood, 2021). The pooled analysis combined haematological data from three clinical studies in which measurements were taken three days after switching to use of a heated tobacco product or stopping smoking from conventional cigarette use (Roethig et al., 2010). Switching to use of a heated tobacco product or stopping smoking resulted in statistically significant decreases of up to 9% in haematological parameters including white blood cell count within three days. The second review detailed the quantitative results of a PMI study conducted in Japan and published in 2018 (Lüdicke et al., 2018b) as well as those of an unpublished study conducted in the USA and concluded that “*studies conducted in people using Philip Morris International’s IQOS heated tobacco product did not reveal detectably better measures of biomarkers of potential harm than conventional cigarettes in human test*” (Glantz, 2018b).

### Future studies on biomarkers

To date, two study protocols have been published that describe clinical trials currently underway, which include some component related to heated tobacco products and biomarkers of exposure and/or potential harm.

The first study protocol described a prospective study that aims to compare changes in cigarette consumption and adoption rates among smokers randomised to use of either heated tobacco products or vaping products (Caponnetto et al., 2020). As part of the study design, 220 healthy smokers, who are not motivated to quit, will be randomised into a 12-week single-centre, open-label, study where the primary outcome will be biochemically-verified self-reported continuous smoking abstinence at 12 weeks from the previous visit. Secondary outcomes will include levels of selected biomarkers of exposure in exhaled breath (exhaled carbon monoxide) and in spot urine samples (total NNAL, HEMA, MHBMA, HMPMA, 3-HPMA, S-PMA, AAMA, GAMA, CEMA, 2-HPMA, 1-OHP and cotinine). The second study protocol described a controlled, single-centre study involving 60 healthy subjects, divided in 6 groups (5 nicotine product user groups and 1 non-user group) based on their sole use of the products of choice (Sibul et al., 2021). The subjects were confined in a clinical setting for a period of 76 hours during which unrestricted use of their product of choice was provided (conventional cigarettes, heated tobacco products, EVPs, oral tobacco products and oral/dermal nicotine replacement therapy products). The aim of the study is to identify biomarkers and/or biomarker patterns in body fluids which may be used to distinguish between product use categories as a means to the determination of product use compliance in long-term clinical studies.

### Conclusions

The results discussed in this section of the review clearly demonstrate that use of heated tobacco products in both clinical and “*real-world*” settings is associated with a statistically significant reduction in levels of non-nicotine related biomarkers of exposure to select toxicants associated with cigarette smoking. With respect to biomarkers of potential harm, the results indicate no statistically significant differences for the majority of the markers. This observation may be due to the follow-up periods reported by the studies being insufficient in length to observe physiologically favourable changes. It should also be noted that whilst the biomarkers of potential harm investigated in these studies may reflect processes on the pathway to smoking-related disease, their predictive and discriminative power has yet to be established so further studies such as long-term epidemiological studies are needed to show their relevance to tobacco-related disease and the subsequent impact of transitioning to heated tobacco product use (Akiyama and Sherwood, 2021). Clinical studies investigating heated tobacco product use and associated biomarkers of exposure and potential harm are currently underway. The results from these studies will further add to the available scientific literature.

For those HPHCs for which suitable biomarkers of exposure are not currently available, a modelling approach described as “*nicotine bridging*” has been proposed (Urban et al., 2012). In this model, exposure to HPHCs is estimated by quantifying levels of the HPHC under investigation in mainstream smoke/aerosol of the product under investigation



followed by *in vitro* toxicity parameter-to-nicotine regressions after use of several machine-smoking protocols. Exposure to the HPHC under investigation is then modelled using nicotine pharmacokinetic data from clinical trials. Using this approach and data from a separate study relating to two conventional cigarettes and a heated tobacco product (Zenzen et al., 2012), exposure to several HPHCs for which biomarkers of exposure are not currently available was reduced for the heated tobacco product compared to either of the conventional cigarettes (Urban et al., 2012).

## Health effects

This section of the review will discuss those articles that have investigated the potential health effects associated with heated tobacco product use.

### Cardiorespiratory effects

#### *Acute exposure studies (≤1 month)*

Several separate reviews and editorials have discussed the potential pulmonary and cardiovascular effects associated with heated tobacco product use (Conklin et al., 2019; Biondi Zoccai et al., 2020a; Fried and Gardner, 2020; Münzel et al., 2020; Peruzzi et al., 2020a; St Claire et al., 2020; Bravo-Gutiérrez et al., 2021). However, most have provided only minimal discussion of quantitative data relating specifically to heated tobacco product use in experimental volunteers (Biondi Zoccai et al., 2020a; Münzel et al., 2020; Peruzzi et al., 2020a) whilst others have referenced no studies at all relating specifically to the cardiovascular and/or pulmonary effects of heated tobacco product use in humans (Conklin et al., 2019; St Claire et al., 2020; Bravo-Gutiérrez et al., 2021). Only a single review has provided a detailed discussion of the current literature on the potential cardiovascular effects of heated tobacco product use (Fried and Gardner, 2020). The authors of this review concluded that “current evidence suggests that heat-not-burn tobacco products (and electronic cigarettes) are less dangerous than combustible cigarettes, but not without health risk” and that “further clinical, animal, and *in vitro* studies must be developed to explore the cardiovascular effects of heat-not-burn tobacco products” (Fried and Gardner, 2020).

In the first reported study, eighteen adult conventional cigarette smokers were randomised to continued use of conventional cigarettes, use of a second-generation heated tobacco product (Accord) or abstinence from any form of product use for a period of three days prior to symptom-limited spirometry, as an estimation of exercise performance, in a three-period crossover study (Unverdorben et al., 2007). Use of the heated tobacco product resulted in less severe shortness of breath and higher working capacity, peak oxygen uptake, anaerobic threshold and maximum rate-pressure when compared to continued smoking of conventional cigarettes. Subsequent articles which reported on the same experimental design indicated a reduction in heart rate and rate-pressure product with both use of the same heated tobacco product and smoking abstinence when compared to continued use of the conventional cigarettes (Unverdorben et al., 2008), as well as an increase in heart rate variability as determined through use of 24-hour electrocardiogram (ECG) measurements (Munjal et al., 2009) and a physiologically favourable increase in pulmonary function parameters (Unverdorben et al., 2010). It should be noted, however, that the observed effects in these studies were more pronounced with smoking abstinence than with use of the heated tobacco product when compared to continued smoking of conventional cigarettes.

With respect to the potential cardiovascular and respiratory effects of those heated tobacco products which are currently commercially available, several experimental clinical studies have been reported.

The first study assessed the acute effects of smoking a single conventional cigarette (Marlboro Gold; nicotine content of 0.60mg per cigarette), taking nine puffs on an e-vapour product (blu Pro; 0.58mg nicotine content in 9 puffs based on a cartridge with a nicotine content of 16mg for 250 puffs), or consumption of a single heated tobacco product (IQOS; nicotine content of 0.50mg per stick) on several cardiovascular end-points including oxidative stress, antioxidant reserve, platelet activation and endothelial dysfunction (Biondi-Zoccai et al., 2019). Twenty healthy conventional cigarette smokers were assigned to use each product in a randomised order with a one-week washout period between each product use. Blood samples were taken just before and immediately after use of each allocated product and analysed for markers of oxidative stress (as determined by levels of sNox2-dp, a small peptide released after platelet activation, which is a measure of Nox2 activation, H<sub>2</sub>O<sub>2</sub> production and 8-iso-PGF<sub>2α</sub>), antioxidant reserve (as determined by levels of vitamin E and HBA [serum H<sub>2</sub>O<sub>2</sub> breakdown activity]), platelet function (as determined by levels of sCD40L and soluble P-selectin, two markers of platelet activation) and endothelial dysfunction (as determined by flow-mediated dilation, NO availability and blood pressure). Single use of any of the product was reported to have an adverse impact on the assessed cardiovascular parameters. However, heated tobacco product use was reported to have a less significant effect than e-vapour and conventional cigarette use on sNox2-dp levels (p=0.004 and p=0.001 respectively), 8-iso-PGF<sub>2α</sub> levels (p=0.004 and p<0.001 respectively) and vitamin E levels (p=0.018 and p=0.044 respectively). Heated tobacco product and e-vapour use were reported to have a comparably lesser effect than conventional cigarette use on flow-mediated



dilation ( $p=0.872$  for heated tobacco product versus e-vapour use),  $H_2O_2$  production ( $p=0.522$  for heated tobacco product versus e-vapour use), HBA ( $p=0.091$  for heated tobacco product versus e-vapour use), sCD40L ( $p=0.849$  for heated tobacco product versus e-vapour use) and soluble P-selectin sCD40L ( $p=0.821$  for heated tobacco product versus e-vapour use). A subsequent study from the same researchers suggested that individual responses to acute heated tobacco product use differed specifically with respect to oxidative stress and platelet aggregation based on cluster analysis of data derived from 60 individuals including those who participated in their first study (Frati et al., 2020).

Two studies have reported the effects of acute heated tobacco product on experimental parameters of arterial stiffness (Ioakeimidis et al., 2020; Franzen et al., 2020). In the first study, 22 current conventional cigarette smokers were randomly assigned to use of either a heated tobacco product (IQOS), a conventional cigarette or a sham cigarette for a five-minute period during three separate experimental sessions which were conducted at least two days apart from each other (Ioakeimidis et al., 2020). The mean nicotine content for both the heated tobacco product and conventional cigarette was reported to be 0.5mg. Each experimental session was conducted in the morning after a minimum four hour fasting period during which time the subjects did not smoke or consumer any caffeinated drinks. Heart rate, blood pressure (both brachial and aortic), augmentation index corrected for heart rate (AIx@75), carotid-femoral pulse wave velocity (cfPWV) and brachial-ankle pulse wave velocity (bcPWV) were assessed immediately before and after smoking and then 5, 10, 20 and 30 minutes after product use. Baseline measurements were comparable across all three sessions with no statistically significant differences. Heart rate increased in a similar fashion after both conventional cigarette and heated tobacco product use (with a maximum increase of 10 beats per minute). Both brachial and aortic systolic blood pressure increased immediately after the end of smoking of conventional cigarettes (by 11.5 and 10.5mmHg;  $p<0.001$  and  $p<0.01$  respectively) and the heated tobacco product (by 7.5 and 6mmHg;  $p<0.01$  in both cases). Blood pressure responses from baseline between the two product groups were not statistically significant at any time point through the experimental sessions ( $p>0.05$ ). Compared with sham smoking, cfPWV, bcPWV and AIx@75 increased immediately after the end of conventional cigarette use (by 0.29m/s, 93cm/s and 3.3% respectively) as well as after heated tobacco product use (by 0.30m/s, 86cm/s and 3.5% respectively). Whilst heated tobacco product use when compared with conventional cigarette use resulted in less potent numerical increases in arterial stiffness indices after the end of smoking, the changes between the two product types were not different. The mean differences of cfPWV, bcPWV and AIx@75 area-under-the-curves between heated tobacco product use and conventional cigarette use (by 0.06m/s, 4.50cm/s and 1.97% respectively) were all statistically insignificant (all  $p>0.05$ ). The authors concluded that *“in the present cross-over, randomized trial comparing the acute effects of heat-not-burn cigarettes and tobacco cigarettes based on equivalent nicotine consumption in young smokers we found that use of any of these two products was associated with comparable acute detrimental effects on arterial stiffness”* and that *“it is likely that the acute effect of heat-not-burn cigarettes on arterial stiffness is mediated, at least in part, by nicotine and its effect on blood pressure”*. The second study used a highly comparable experimental design with 20 healthy smokers and a two-hour follow-up after either smoking a conventional cigarette or use of the IQOS heated tobacco product (Franzen et al., 2020). Peripheral systolic blood pressure and heart rate increased significantly by more than 3% and 9% respectively compared to baseline after conventional cigarette and heated tobacco product use and returned to baseline levels after 60 and 45 minutes respectively. The augmentation index was significantly increased after 5, 10 and 15 minutes after conventional cigarette use and after 5 minutes for heated tobacco product use. The pulse wave velocity showed a trend towards being altered but this did not meet statistical significance ( $p=0.066$ ).

With respect to pulmonary function parameters, the acute effect of heated tobacco product use in both smokers and non-smokers has been investigated (Pataka et al., 2020; Polosa et al., 2021a). In the first study, a total of 50 healthy male volunteers were recruited to the study (25 non-smokers and 25 current conventional cigarette smokers) (Pataka et al., 2020). Subjects underwent exhaled CO measurement, pulse oximetry (to determine blood oxygen saturation levels), pulmonary function tests (forced expiratory flow at 25% and 50% of vital capacity; FEF25% and FEF50%), peak expiratory flow (PEF) and airway resistances before and immediately after use of an IQOS heated tobacco product over a total of five to six minutes. Overall, oxygen saturation levels ( $98.4\pm1.2\%$  before and  $97.9\pm1.06\%$  after;  $p=0.002$ ), FEF25% ( $7.38\pm1.9L$  before and  $6.98\pm1.92L$  after;  $p=0.002$ ), FEF50% ( $5.00\pm1.42L$  before and  $4.84\pm1.45L$  after;  $p=0.03$ ) and PEF ( $7.9\pm2.16L$  before and  $7.3\pm2.08L$  after;  $p<0.001$ ) decreased significantly after use of the IQOS heated tobacco product whilst exhaled CO ( $1.75\pm1.02ppm$  before and  $4.89\pm1.4ppm$  after respectively;  $p<0.001$ ) and airway resistances at all tested frequencies (5, 10, 20, 25 and 35Hz) increased significantly. The authors concluded that *“IQOS had an impact on exhaled CO, blood oxygen saturation and airway function immediately after use. Even those these changes were rather small to be considered of major clinical importance, they should raise concerns regarding the long-term safety of this product”*. In the second study, the effect of heated tobacco product use on mucociliary clearance was investigated using saccharin test transit time (Polosa et al., 2021a). The saccharin test transit time was assessed in 39 current conventional cigarette smokers, 40 former conventional cigarette smokers, 40 never smokers and in 20 exclusive e-vapour users and 20 exclusive heated tobacco product users. The exclusive heated tobacco product users had

not smoked conventional cigarettes for at least three to six months after switching to their heated tobacco product, had an exhaled carbon monoxide level of less than 7ppm and had been using heated tobacco products for a median of seven months. Conventional cigarette smokers had a median saccharin test transit time of 13.15 minutes which was significantly longer compared with that of all other groups. Exclusive heated tobacco product users had a median saccharin test transit time of 8.00 minutes which was not statistically different from that of exclusive e-vapour users (7.00 minutes), former conventional cigarette smokers (7.26 minutes) and never smokers (7.24 minutes).

Using transthoracic echocardiography, the acute effects of heated tobacco product use on myocardial systolic and diastolic function has been investigated (Yaman et al., 2021), involving thirty-eight current IQOS users. Volunteers used their own IQOS product which were all adjusted to achieve a two-second puff duration. Transthoracic echocardiography was performed three times for each volunteer: prior to use of any tobacco product, ten minutes after use of the IQOS heated tobacco product and ten minutes after smoking a single conventional cigarette. Each volunteer was randomised with respect to product use order with measurements being conducted on separate days. A total of ten puffs were taken for each product over a five-minute period. Heart rate increased significantly after heated tobacco product use ( $74.4 \pm 9.4$  bpm before and  $81.8 \pm 8.7$  bpm after;  $p < 0.01$ ) whilst systolic blood pressure ( $111.3 \pm 13.5$  mmHg before and  $114.1 \pm 16.8$  mmHg after;  $p = 0.229$ ) and diastolic blood pressure ( $71 \pm 10$  mmHg before and  $71.9 \pm 10.1$  mmHg after;  $p = 0.515$ ) did not vary. All three parameters increased to a statistically significant extent after smoking a single conventional cigarette. With respect to transthoracic echocardiography parameters, heated tobacco product and conventional cigarette use resulted in a decrease in left ventricle global longitudinal strain, left ventricle global circumference strain and right ventricle global longitudinal strain compared to baseline values. No indication was provided as to whether or not these parameters returned to baseline values and, if so, after what period of time.

The most recent study investigated the effect of heated tobacco product use (IQOS) and conventional cigarette use (Marlboro Red) on myocardial, coronary and arterial function in addition to oxidative stress and arterial function in 75 current conventional cigarette smokers using both acute (60-minute) and chronic (1-month) exposure scenarios (Ikonomidis et al., 2021). In the acute exposure scenario, 50 current conventional cigarette smokers were randomised to use of a single conventional cigarette or use of a single heated tobacco product followed with being crossed over to the alternative product after one hour. In the chronic exposure scenario, 50 current conventional cigarette smokers were switched to use of the heated tobacco product and compared to a separate group of 25 conventional cigarette smokers before and after one month of product use. Exhaled carbon monoxide, pulse wave velocity, malondialdehyde and 11-dehydrothromboxane  $B_2$  were assessed in both exposure scenarios whilst global longitudinal strain, myocardial work index, wasted myocardial work, coronary flow reserve, total arterial compliance and flow-mediated dilation were assessed in the chronic exposure scenario only. Acute use of the heated tobacco product resulted in a smaller increase in pulse wave velocity when compared to use of a conventional cigarette (change of 1.1 m/s and 0.54 m/s respectively;  $p < 0.05$ ). No statistically significant changes in exhaled carbon monoxide levels were reported after acute heated tobacco product compared to baseline values ( $14.2 \pm 7.3$  ppm and  $14.9 \pm 7.4$  ppm respectively;  $p = 0.1$ ) whilst levels were significantly elevated following conventional cigarette use compared to baseline values ( $17.5 \pm 7.8$  ppm;  $p < 0.001$ ). Levels of malondialdehyde and 11-dehydrothromboxane  $B_2$  followed a comparable pattern with levels increased after conventional cigarette use, but not after heated tobacco product use, when compared to baseline levels. Malondialdehyde levels at baseline, after heated tobacco product use and after conventional cigarette use were  $1.34 \pm 0.72$  nmol/L,  $1.28 \pm 0.95$  nmol/L ( $p = 0.55$ ) and  $2.56 \pm 0.85$  nmol/L ( $p = 0.03$ ) respectively. 11-dehydrothromboxane  $B_2$  levels at baseline, after heated tobacco product use and after conventional cigarette use were  $378 \pm 103$  pg/ml,  $362 \pm 113$  pg/ml ( $p = 0.16$ ) and  $398 \pm 103$  pg/ml ( $p = 0.02$ ) respectively. With respect to the chronic exposure scenario, switching to the heated tobacco product resulted in statistically significant improvements in exhaled carbon monoxide levels, flow-mediated dilation, coronary flow reserve, total arterial compliance, global longitudinal strain, wasted myocardial work, malondialdehyde and 11-dehydrothromboxane  $B_2$  with differences of 10.42 ppm, 4.3%, 0.98, 1.8 ml/mmHg, 2.35%, 19.72 mmHg%, 0.38 nmol/L and 45 pg/ml respectively ( $p < 0.05$  in each instance).

#### *Chronic use studies (>1 month)*

Two studies have reported on the potential cardiorespiratory effects of heated tobacco product use over a time-period exceeding one month.

In the first study, markers for oxidative stress, endothelial dysfunction and platelet activation were quantified in twenty chronic heated tobacco product users, twenty chronic conventional cigarette smokers and twenty non-smokers (Loffredo et al., 2021). Chronic use was defined as use exceeding a one-month period. All heated tobacco product users were reported as being former conventional cigarette smokers and had used their heated tobacco product for a mean of eighteen months. Oxidative stress was assessed by quantification of sNox2-dp levels and  $H_2O_2$  production, platelet activation was

assessed by quantification of platelet aggregation, sCD40L and soluble P-selectin levels and endothelial dysfunction was assessed by quantification of flow-mediated dilation and NO availability. Both measures of oxidative stress were increased in conventional cigarette smokers and heated tobacco product users compared to non-smokers with sNox2-dp showing statistically elevated levels in conventional cigarette smokers compared to heated tobacco product users whilst  $H_2O_2$  production showed no statistically significant difference between use of the two product types. A comparable relationship was observed for endothelial dysfunction with conventional cigarette use having a more profound effect on flow-mediated dilation than the heated tobacco product whilst both product types produced comparable decreases in NO availability. With respect to platelet activation, heated tobacco product use produced less profound increases in sCD40L and soluble P-selectin compared with conventional cigarette use although differences in sCD40L levels were not statistically significant whilst differences in soluble P-selection levels were.

In the second study, health parameters were monitored for a period of three years in Chronic Obstructive Pulmonary Disease (COPD) smoking patients who substantially attenuated or ceased conventional cigarette consumption after transitioning to heated tobacco products (Polosa et al., 2021b). Changes in daily cigarette consumption, annualised disease exacerbations, lung function indices, self-reported outcomes and 6-minute walk distance from baseline were measured in the COPD patients using heated tobacco products after 12, 24 and 36 months. These were compared to a group of age and gender-matched COPD patients who continued to smoke conventional cigarettes throughout the 3-year period. Data was obtained for a total of 38 patients (with 19 in each group). COPD patients who transitioned to use of a heated tobacco product reported a substantial decrease in annualised COPD exacerbations from  $2.1 \pm 1.9$  (mean  $\pm$  SD) at baseline to  $1.4 \pm 0.8$ ,  $1.2 \pm 0.8$  and  $1.3 \pm 0.8$  at 12, 24 and 36-month follow up ( $p < 0.05$  for all time-points). No statistically significant changes were observed in COPD patients who continued to smoke conventional cigarettes over the same time period.

#### Poison control centre reporting data

A single study has reported on the incidence of acute exposure to heated tobacco products as documented by a national poison control centre.

The study reported a statistical analysis of calls received by the Czech Toxicological Information Centre over a seven-year period from 2012 to 2018 (Obertova et al., 2020). A total of 148 calls were received over the seven-year period in relation to exposures to vaping products, e-liquids or heated tobacco products (with three of these calls relating to animal exposures rather than human exposures). This cohort of 148 calls was equivalent to 0.12% of all calls received during the seven-year period ( $n = 119,229$ ). Heated tobacco products were reported as being the source of the acute exposure in 9 of the 148 calls (6%). The authors described exposure as being exposed to the “*heat-not-burn cigarette refill*” and these were reported by the authors as containing 5mg of nicotine. No further information was provided in the article with respect to heated tobacco products, as the authors did not stratify the results of their statistical analyses with respect to EVPs and heated tobacco products, but rather classed them together as a single product category.

#### Medical case reports

To date, five medical case reports have been published in the scientific literature pertaining to heated tobacco products (Kamada et al., 2016; Aokage et al., 2019; Hitosugi et al., 2019; Tajiri et al., 2020; Yumoto et al., 2020). All of the medical case reports originated in Japan; three involved the development of acute eosinophilic pneumonia in users of heated tobacco products after normal use of their products (Kamada et al., 2016; Aokage et al., 2019; Tajiri et al., 2020) whilst two involved the intentional misuse of heated tobacco products or their constituents (Hitosugi et al., 2019; Yumoto et al., 2020). Of these two medical case reports, one detailed a case of attempted homicide in a user after tampering of his heated tobacco product with elemental mercury by a third party (Hitosugi et al., 2019), whilst the second involved the intentional ingestion of sticks associated with a heated tobacco product (Yumoto et al., 2020). Of the five medical case reports, two provided details as to the heated tobacco product involved (Hitosugi et al., 2019; Yumoto et al., 2020), two provided no details (Aokage et al., 2019; Tajiri et al., 2020) whilst for the final medical case report, the heated tobacco product involved could be deduced from one of the pictures provided in the original article (Kamada et al., 2016). An overview of the five medical case reports is provided in Table 5.

It should be noted that those medical case reports which described health effects resulting from normal use of heated tobacco products (Kamada et al., 2016; Aokage et al., 2019; Tajiri et al., 2020) can only provide anecdotal evidence as it is possible that the observed health effect (reported as acute eosinophilic pneumonia in all three cases) could have been caused by another factor or exposure not reported in the original articles or not reported by the patients to the authors of the original articles. Acute eosinophilic pneumonia is a rare disorder characterised by marked accumulation of eosinophils in lung tissues and/or bronchoalveolar fluid with most patients recovering completely following treatment with corticosteroids (Suzuki and Suda, 2019). It is of note that tobacco product use has been separately reported to be a significant factor in the development of acute eosinophilic pneumonia (Chaaban, 2020; Sakao, 2020).

**Table 5. Medical case reports documenting health effects associated with heated tobacco products.** F, female; M, male.

| Reference             | Health effect described                                     | Exposure scenario <sup>1</sup> | Heated tobacco product/ constituent involved  | Age/ gender of patient | Clinical treatment and outcome  |
|-----------------------|---|--------------------------------|---|------------------------|---|
| Kamada et al., 2016   | Acute eosinophilic pneumonia                                | Normal use                     | IQOS [presumed] <sup>2</sup>                  | 20/M <sup>3</sup>      | Patient treated with two-week course of prednisolone. By the fourth day, abdominal shadows on chest X-ray had improved and oxygen therapy was no longer required. No relapse was reported after steroid treatment WAS stopped.  |
| Aokage et al., 2019   | Acute eosinophilic pneumonia                                | Normal use                     | Not Reported                                  | 16/M <sup>3</sup>      | Patient treated with extracorporeal membrane oxygenation (ECMO) for severe respiratory failure and treated with methylprednisolone. The patient was weaned off ECMO four days after initiation and extubated the day after. He fully recovered without any sequelae.          |
| Hitosugi et al., 2019 | Mercury poisoning   | Intentional misuse             | IQOS  | 36/M                   | No treatment administered. His initial symptoms (headache, appetite loss and shivering flu-like symptoms) gradually improved after stopping use of his heated tobacco product.  |
| Tajiri et al., 2020   | Acute eosinophilic pneumonia                                | Normal use                     | Not Reported                                  | 47/F <sup>4</sup>      | Patient treated with daily course of 25mg prednisolone. Respiratory symptoms, respiratory function and chest x-ray abnormalities improved once steroid treatment was initiated. No relapse in her condition was observed after she stopped use of the heated tobacco product. |
| Yumoto et al., 2020   | Cigarette ingestion (with potential for nicotine poisoning) | Intentional misuse             | Heatstick Menthol cigarettes (used with IQOS) | 59/M                   | Patient underwent gastric lavage to remove stomach contents. Patient was asymptomatic after 24 hours and subsequently discharged from hospital.   |

<sup>1</sup>Normal use defined as use of the heated tobacco product as per the manufacturer's instructions. Intentional misuse defined as use of a heated tobacco product, or any of its constituent parts, as part of a deliberate attempt to cause harm to either yourself or others.

<sup>2</sup>Presumed to be IQOS based on the picture shown in [Figure 1](#) of the original article.

<sup>3</sup>Both of these patients were reported by a separate author as not being current conventional cigarette smokers ([Chaaban, 2020](#)). However, no such information was identified by this author in either of the two original medical case reports.

<sup>4</sup>The patient had a significant known history of conventional cigarette use (27 years) and had switched to heated tobacco products from conventional cigarettes. In the two other patients diagnosed with acute eosinophilic pneumonia detailed above ([Kamada et al., 2016](#); [Aokage et al., 2019](#)), it is not known whether or not they had previously smoked, or were current smokers, of conventional cigarettes.

### Epidemiological studies

To date, four epidemiological studies have been reported which have attempted to correlate heated tobacco product use with health-related endpoints.

Using cross-sectional data derived from a cohort of 58,336 Korean adolescents aged between twelve and eighteen years of age, one study attempted to assess the association between heated tobacco product use and the risk of allergic diseases ([Lee et al., 2019](#)). The data was derived from the 2018 Korea Youth Risk Behaviour Survey, and of all participants included in the survey, 2.4% (n=1,443), 20.9% (n=11,884) and 7.2% (n=4,198) reported a diagnosis of asthma, allergic

rhinitis and atopic dermatitis within the previous year respectively. Conventional cigarettes, vapour products and heated tobacco products were reported as being used by 13.9% (n=8,129), 7.1% (n=4,114) and 2.4% (n=1,414) of participants, respectively. 82.4% of heated tobacco products were reported as being triple users (concurrent use of all three product types). Sole use of heated tobacco products was associated with a statistically significant increased risk of asthma; dual use of heated tobacco products and conventional cigarettes was associated with a statistically significant increased risk of asthma and atopic dermatitis whilst triple use of heated tobacco products, conventional cigarettes and vapour products was associated with a statistically significant increased risk of asthma, allergic rhinitis and atopic dermatitis. Dual use of heated tobacco products and vapour products was not associated with a statistically significant risk for any of the three allergic diseases. However, limitations such as the cross-sectional nature of the study and survey design prevents a causal relationship from being established.

A subsequent article used data from the same survey with different inclusion criteria to assess the association between heated tobacco product use and the risk of allergic rhinitis and asthma (Chung et al., 2020). In this study, the researchers used a cohort of 60,040 Korean adolescents aged between thirteen and eighteen years of age. Within the cohort, 2.5% and 20.8% of participants reported having prevalent asthma and allergic rhinitis respectively. 2.9% of the participants (n=1,568) reported themselves as ever having used a heated tobacco product. With respect to allergic rhinitis, ever use of a heated tobacco product alone [combined with never use of both conventional cigarettes and vapour products] did not increase the risk of developing the condition (odds ratio of 1.9; 95% confidence intervals of 0.7 to 5.1). The sole product use scenarios which increased the risk for developing allergic rhinitis were ever use of heated tobacco products combined with both former use of vapour products and former use of conventional cigarettes (odds ratio of 1.9; 95% confidence intervals of 1.1 to 3.2) and ever use of heated tobacco products combined with current use of both vapour products and conventional cigarettes (odds ratio of 1.6; 95% confidence intervals of 1.1 to 2.2). With respect to asthma, ever use of a heated tobacco product alone [combined with never use of both conventional cigarettes and vapour products] increased the risk of developing the condition (odds ratio of 3.8; 95% confidence intervals of 1.5 to 9.6). The other product use scenarios which increased the risk for developing asthma were ever use of heated tobacco products combined with never use of vapour products and current use of conventional cigarettes (odds ratio of 6.0; 95% confidence intervals of 3.1 to 11.5) and ever use of heated tobacco products combined with current use of both vapour products and conventional cigarettes (odds ratio of 1.7; 95% confidence intervals of 1.1 to 2.7). It should be noted that the statistically significant observation that ever use of a heated tobacco product alone [combined with never use of vapour products and conventional cigarettes] resulted in an increase in risk of developing asthma was based on only fifty-nine individuals (out of 60,040 in the cohort) who reported ever use of a heated tobacco product and never use of both vapour products and conventional cigarettes.

A subsequent study aimed to investigate the potential association between conventional cigarette use, heated tobacco product use and dual product use and self-reported periodontal disease using data from the 2019 arm of the Japan “Society and New Tobacco” internet survey (JASTIS) (Yoshioka and Tabuchi, 2021). Of the 10,439 JASTIS survey respondents, the number of current exclusive conventional cigarette smokers, current exclusive heated tobacco product users, and current dual product users was 1,304, 437 and 1,049 respectively. Compared with never users, current exclusive heated tobacco product use was significantly associated with an increased prevalence of self-reported periodontal diseases (prevalence ratio of 1.43; 95% CI of 1.08 to 1.88). Compared with never users, current exclusive conventional cigarette smoking (prevalence ratio of 1.29; 95% CI of 1.03 to 1.62) and current dual product use (prevalence ratio of 1.55; 95% CI of 1.20 to 1.99) were also significantly associated with an increased prevalence of self-reported periodontal diseases.

The most recent epidemiological study investigated the association between heated tobacco product use and respiratory symptoms in Hong Kong adolescents (Wang L et al., 2021). Using a cross-sectional school-based survey which provided an anonymous questionnaire to 33,627 students, the association between persistent respiratory symptoms and use of heated tobacco products was investigated. The main outcome of the study was self-reported respiratory symptoms which lasted for three consecutive months in the previous twelve months. Compared to never users of heated tobacco products, both former users (prevalence ratio of 1.30; 95% confidence intervals of 1.06 to 1.59) and current users (prevalence ratio of 1.59; 95% confidence intervals of 1.23 to 2.06) were more likely to self-report respiratory symptoms. Compared to current exclusive conventional cigarette smokers, both current exclusive heated tobacco product users (prevalence ratio of 1.40; 95% confidence intervals of 0.93 to 2.11) and current dual users of both products (prevalence ratio of 1.19; 95% confidence intervals of 0.94 to 1.49) were not more likely to self-report respiratory symptoms.

### Future studies on health effects

In addition to those studies discussed in this review, several study protocols have been published for clinical trials currently underway which are investigating potential health effects associated with heated tobacco products and which will likely produce articles of interest in the future.



A study protocol for a five-year single centre observational study, partially funded by PMI, has been published which aims to evaluate frequency of exacerbations, respiratory symptoms, physical exercise intolerance and abnormal lung functions in men and women aged 40 to 59 years who live in Almaty, Kazakhstan who use the IQOS heated tobacco product compared to those who smoke conventional cigarettes (Sharman et al., 2018). Participant recruitment began in December 2018 and enrolment was expected to last until late summer 2018. The authors reported that the study results will be published in a peer-reviewed scientific journal once the study has been completed.

PMI have completed a randomised, controlled two-arm parallel-group multicentre Japanese study which investigated the effect of switching to a heated tobacco product on periodontal endpoints in patients with generalised chronic periodontitis (Pouly et al., 2021). A total of 172 subjects were randomised to continued conventional cigarette smoking (n=86) or switching to the heated tobacco product with all subjects completing the study. The conduct phase of the study has been completed and the data cleaning and statistical analyses currently underway.

The SUR-VAPES 3 study aims to compare the acute coronary effects of EVP and heated tobacco product use in chronic conventional cigarette smokers admitted to hospital for invasive coronary evaluation (Biondi Zoccai et al., 2020b). In patients with a confirmed angiographic intermediate coronary stenosis, who have never quit smoking before, the study will measure coronary flow reserve as the primary end-point. Twenty patients will be randomised to either single use of an EVP (n=10) or single use of a heated tobacco product (IQOS; n=10) whilst in the catheterisation laboratory, followed by repeat coronary flow reserve measurement (Lombardi et al., 2020).

The DIASMOKE study aims to investigate whether or not switching from conventional cigarettes to NGPs (including EVPs or heated tobacco products) in type 2 diabetic smokers will yield a quantifiable improvement in metabolic syndrome factors within a two-year time period (Krysinski et al., 2021). The study aims to recruit a total of 576 diabetic patients who will be randomised in a non-blinded fashion to receive either a referral to smoking cessation services or to receive an NGP of their choice. It is anticipated that results will be available between 2023 and 2024.

The 12-month randomised controlled trial involving “real-world” use of heated tobacco products which is currently being conducted by BAT includes several health-related secondary endpoints in its study design (Newland et al., 2019; Camacho et al., 2020). These include augmentation index, pulse wave velocity, reactive hyperaemia index, lung spirometry and brachial systolic and diastolic blood pressure measurements with measurements throughout the 12-month period. Interim analyses up to 90 and 180 days have been published (Gale et al., 2021; Gale et al., 2021b) and it is anticipated that future publications relating to the 12-month analysis will include data relating to these secondary endpoints.

## Conclusions

The results discussed in this section of the review demonstrate that whilst several studies have reported that the use of heated tobacco products under acute exposure settings may have both physiologically favourable and unfavourable effects on both the cardiovascular and pulmonary systems, studies into their long-term health effects are currently lacking. Of the two available studies into long-term health effects, one reported physiologically favourable outcomes (Polosa et al., 2021b) whilst the other did not (Loffredo et al., 2021). There are currently no studies on the potential risk of heated tobacco product use on development of any form of cancer and long-term epidemiological studies into this may be warranted.

There are suggestions from medical case reports that the use of heated tobacco products may be associated with the development of the rare disorder, acute eosinophilic pneumonia (Kamada et al., 2016; Aokage et al., 2019; Tajiri et al., 2020). However, the number of reported cases of acute eosinophilic pneumonia is extremely small compared to the number of heated tobacco product users. Furthermore, there is no data currently available to indicate whether or not the incidence of acute eosinophilic pneumonia secondary to use of heated tobacco products is higher than that associated with conventional cigarette use.

## Heated tobacco products, indoor air quality and bystander exposure

This section of the review will discuss those articles which have investigated the effects of heated tobacco product use on indoor air quality (IAQ) and the potential health effects of bystander exposure.

The International Agency for Research on Cancer has concluded that there is sufficient evidence in humans for the carcinogenicity of environmental tobacco smoke (ETS) produced through use of conventional cigarettes and other combustible tobacco products within indoor environments (International Agency for Research on Cancer, 2012). In addition, the United States Surgeon General has concluded that there is no risk-free level of exposure to ETS and that exposure to ETS in adults has immediate adverse effects on the cardiovascular system and causes coronary heart disease and lung cancer (United States Surgeon General, 2006).

Given these findings from the public health community, there is concern that use of heated tobacco products in indoor environments may negatively impact IAQ and may subsequently result in health effects in bystanders (Tabuchi et al., 2018; Imura and Tabuchi, 2021). A web-based survey conducted in Japan in early 2018 reported that 15.6% of current tobacco users used heated tobacco products within indoor public spaces whilst 72.0% of the same survey respondents reported using conventional cigarettes within indoor public spaces (Sutanto et al., 2019). For dual users, conventional cigarette use within public indoor spaces was reported as being more frequent (97.7%) than heated tobacco product use (76.0%).

ETS released into indoor environments from the smoking of conventional cigarettes is an aged and diluted mixture comprised of sidestream smoke released directly from the cigarette and aerosol exhaled by the smokers after their inhalation of mainstream smoke (Tricker et al., 2009). The emissions present in indoor environments as a result of heated tobacco product use are principally comprised of aged and diluted aerosol exhaled by users after inhalation on the heated tobacco product, since there is no sidestream smoke produced by heated tobacco products, although an exploratory analytical analysis indicated some emissions may be released both whilst heated tobacco products are turned on and whilst they are actively being used (O'Connell et al., 2015).

### Chemical characterisation studies

Table 6 details those experimental studies which have characterised the emissions present in indoor environments after controlled use of heated tobacco products. These studies investigated a range of historical and currently commercially available heated tobacco products including Accord, IQOS and glo and employed a range of analytical methodologies with some being specifically validated for use with heated tobacco products (Mottier et al., 2016; Gómez Lueso et al., 2018). Most of these studies have reported on IAQ levels before, during and after heated tobacco product use within an experimental indoor environment, with a single study reporting on IAQ levels during and after experimentally controlled heated tobacco product use within a non-operational nightclub which featured an external smoking area (Kaunelienė et al., 2019). Two additional studies reported on heated tobacco product use within different models of passenger car (Schober et al., 2019; Savdie et al., 2020) whilst a single study detailed the effects of heated tobacco product use on outdoor air quality (Cammalleri et al., 2020).

The results shown in Table 6 indicate whether the quantified chemical constituent was determined to be at or below the limit of quantification or limit of detection of the analytical method employed (as indicated by the abbreviation ND and dark green shading) or at a significantly lower level within the indoor environment following heated tobacco product use when compared to conventional cigarette use (as indicated by a downwards arrow and light green shading). The aim of this approach is to demonstrate that heated tobacco product use under controlled conditions resulted in a quantifiable, and statistically significant, decrease in levels of chemical constituents when compared to conventional cigarette use under the same experimental conditions. The results from these studies, as shown in Table 6, indicate that a range of IAQ chemical constituent markers are either present in the experimental indoor environments at markedly lower concentrations when compared to levels observed with conventional cigarette use within the same indoor environments or determined to be below the level of detection or limit of quantification of the analytical methods employed.

Several of the studies have compared their analytical measurements of IAQ markers with heated tobacco product use with occupational exposure limits and/or air quality guidelines. The authors of a controlled study using the THP1.0 heated tobacco product observed that “environmental emissions from the THP1.0 would conform to published IAQ guidelines, such as those from the World Health Organisation and, for particles, would conform to target the World Health Organisation outdoor air annual mean limits of  $10\mu\text{g}/\text{m}^3$   $\text{PM}_{2.5}$ ” (Forster et al., 2018b). Comparable conclusions have been reported for the THS2.2 heated tobacco product including with respect to its nicotine emissions (Mitova et al., 2016; Prodanchuk et al., 2017).

### Toxicological risk assessment modelling

Using data from their own analytical assessment study (Hirano et al., 2020), separate authors attempted to estimate the excess cancer risk in bystanders exposed to either ETS or exhaled heated tobacco product aerosol under usual indoor conditions (Hirano and Takei, 2020). Based on the assumption that nicotine inhalation is proportional to cancer potency, the lifetime cancer risk for heated tobacco product exhaled aerosol exposure was estimated to be  $2.7 \times 10^{-6}$  (below  $1 \times 10^{-5}$ ; 1 in 100,000) with this being three orders of magnitude lower than that estimated for exposure to ETS ( $8.3 \times 10^{-3}$ ) using the same approach.

### Potential health effects in bystanders after exposure to emissions produced from heated tobacco product use in indoor environments

There are currently no clinical or epidemiological studies which have attempted to associate exposure to emissions produced from heated tobacco product use within indoor environments with any subsequent potential health effects in bystanders.



**Table 6. Studies detailing levels of chemical constituents present in experimental indoor environments after controlled heated tobacco product use.** If the quantified chemical constituent was determined to be at below the limit of quantification or limit of detection of the analytical method employed then this is indicated by the abbreviation ND and dark green shading. If the quantified chemical constituent was determined to be at a significantly lower level within the indoor environment following heated tobacco product use when compared to conventional cigarette use then this is indicated by a downwards arrow and light green shading). **3-EP**, 3-ethenylpyridine; **AA**, Acetaldehyde; **CO**, Carbon monoxide; **CO<sub>2</sub>**, Carbon dioxide; **FPM**, Fluorescing particulate matter; **ND**, Not detected [experimental results below the limit of detection or limit of quantification of the analytical method employed]; **NH<sub>3</sub>**, Ammonia; **NO**, Nitrogen oxide; **NO<sub>x</sub>**, Nitrogen oxides; **PM<sub>x</sub>**, Particulate matter with a mean aerodynamic diameter below x µm; **RSP**, Respirable suspended particulates; **SMP**, Submicronic particles; **Sol**, Solanesol; **Sol-PM**, Solanesol-related particulate matter; **TVOC**, Total volatile organic compounds; **UVPM**, Ultraviolet absorbing particulate matter.

| Reference                          | Heated Tobacco Product(s) Used <sup>1</sup> | Chemical Constituent |    |      |        |      |     |          |      |                 |                 |    |                 |     |    |     |                 |                   |                  |
|------------------------------------|---|----------------------|----|------|--------|------|-----|----------|------|-----------------|-----------------|----|-----------------|-----|----|-----|-----------------|-------------------|------------------|
|                                    |   | RSP                  | CO | TVOC | Sol-PM | UVPM | FPM | Nicotine | 3-EP | NH <sub>3</sub> | CO <sub>2</sub> | NO | NO <sub>x</sub> | Sol | AA | SMP | PM <sub>1</sub> | PM <sub>2.5</sub> | PM <sub>10</sub> |
| Roethig et al., 2005               | Accord [1 <sup>st</sup> generation]         | ↓                    | ND | ↓    |        |      |     |          |      |                 |                 |    |                 |     |    |     |                 |                   |                  |
| Oey et al., 2008                   | Accord [2 <sup>nd</sup> generation]         | ↓                    | ND | ↓    | ND/↓   | ↓    | ↓   | ↓        | ND   |                 |                 |    |                 |     |    |     |                 |                   |                  |
| Frost Pineda et al., 2008c         | Accord [3 <sup>rd</sup> generation]         | ND                   | ↓  | ↓    | ↓      | ↓    | ↓   | ND       | ND   | ↓               |                 |    |                 |     |    |     |                 |                   |                  |
| Tricker et al., 2009               | Accord                                      | ↓                    | ↓  |      |        | ↓    | ↓   | ↓        | ↓    |                 | ND              |    |                 | ↓   |    |     |                 |                   |                  |
| Mitova et al., 2016                | THS 2.2                                     | ND                   | ND | ND   |        | ND   | ND  | ↓        | ND   |                 |                 | ND | ND/↓            | ND  | ↓  |     |                 |                   |                  |
| Protano et al., 2016               | IQOS  |                      |    |      |        |      |     |          |      |                 |                 |    |                 |     | ↓  |     |                 |                   |                  |
| Protano et al., 2017               | IQOS  |                      |    |      |        |      |     |          |      |                 |                 |    |                 |     | ↓  |     |                 |                   |                  |
| Prodanchuk et al., 2017            | IQOS  |                      | ↓  |      |        |      |     | ND       |      | ND              | ↓               |    |                 |     |    |     |                 |                   |                  |
| Ruprecht et al., 2017              | IQOS  |                      |    |      |        |      |     |          |      |                 |                 |    |                 |     | ↓  |     | ↓               | ↓                 | ↓                |
| Forster et al., 2018b              | THP 1.0                                     |                      |    | ↓    |        |      |     | ↓        | ND   |                 |                 | ↓  | ↓               |     | ↓  |     | ↓               | ↓                 | ↓                |
| Cancelada et al., 2019             | IQOS  |                      |    | ↓    |        |      |     |          |      |                 |                 |    |                 |     |    |     |                 |                   |                  |
| Loupa et al., 2019                 | THS   |                      |    |      |        |      |     |          |      |                 |                 |    |                 |     |    |     |                 | ↓                 | ↓                |
| Meišutovič-Akhtarieva et al., 2019 | THS   |                      | ND |      |        |      |     | ↓        | ND   |                 | ND              |    |                 |     | ↓  |     |                 | ↓                 | ↓                |
| Mitova et al., 2019                | IQOS  |                      | ↓  |      |        | ND   | ND  | ↓        | ND   |                 |                 | ↓  |                 | ND  | ↓  |     | ND              | ND                |                  |
| Kaunelienė et al., 2019            | IQOS  |                      |    |      |        |      |     |          |      |                 |                 |    |                 |     |    |     |                 | ↓                 | ↓                |
| Protano et al., 2020               | IQOS and glo                                |                      |    |      |        |      |     |          |      |                 |                 |    |                 |     |    |     | ↓               |                   |                  |
| Savdie et al., 2020                | IQOS  |                      | ↓  |      |        |      |     |          |      |                 |                 |    |                 |     |    |     | ↓               | ↓                 | ↓                |
| Peruzzi et al., 2020b              | IQOS and glo                                |                      |    |      |        |      |     |          |      |                 |                 |    |                 |     |    |     | ↓               | ↓                 | ↓                |
| Hirano et al., 2020                | IQOS and glo                                |                      |    |      |        |      |     | ↓        |      |                 |                 |    |                 |     |    |     |                 | ↓                 |                  |
| Meišutovič-Akhtarieva et al., 2021 | Pulze                                       |                      |    |      |        |      |     |          |      |                 |                 |    |                 |     | ↓  |     |                 |                   |                  |

<sup>1</sup>In each study, the comparison product analysed was a conventional cigarette with some studies comparing the heated tobacco product(s) to multiple different conventional cigarettes. The conventional cigarette(s) used was/were either a commercially available product or a University of Kentucky Reference cigarette [specifically the 1R4F, 1R5F, 2R4F and 3R4F variants].

However, two surveys conducted in Japan in 2017 and 2019 respectively have investigated the frequency of self-reported symptoms occurring from such an exposure scenario (Tabuchi et al., 2018; Imura and Tabuchi, 2021). In the first survey, a total of 12% of the survey respondents reported as having been exposed to emissions from heated tobacco product use in their vicinity (977 respondents of a total survey size of 8,240 respondents) (Tabuchi et al., 2018). Of this group, 37% experienced at least one symptom as a result of the exposure with the most common complaint being feeling generally ill (25%) followed by eye discomfort/pain (22%), a sore throat (21%) and any other [unspecified] injury or symptom (13%). Only 26% of current heated tobacco product users reported having experienced at least one symptom compared to 41% of former users of heated tobacco products and 49% of never users of any tobacco product. In the second survey, a total of 33% of the survey respondents reported as having been exposed to emissions from heated tobacco use in their vicinity (2,923 respondents of a total survey size of 8,784 respondents) (Imura and Tabuchi, 2021). Of this group, 40% experienced at least one symptom as a result of the exposure with most common complaint being nausea (32%),

respiratory symptoms (29%), sore throat (23%), cough (23%), eye pain (19%), headache (18%), chest pain (12%), any other [unspecified] symptom (12%) and asthma attack (11%).

### Surface staining: experimental assessment of “third-hand” exposure

In addition to the potential for inhalation of emissions produced by heated tobacco product use present by bystanders, it has also been claimed there is a potential for exposure through residues present on indoor surfaces which deposit onto them through diffusion and sedimentation after heated tobacco product use. Such an exposure scenario is referred to as “third-hand” exposure.

Using a novel aerosol exposure chamber, surface staining of wallpaper and cotton samples after exposure to mainstream smoke from 3R4F Kentucky Reference cigarettes, aerosol from a heated tobacco product (THP 1.0) and e-vapour products has been reported (Dalrymple et al., 2020). The exposure chamber had an internal volume of 885cm<sup>3</sup> with the wallpaper and cotton samples being exposed to 200, 400, 600, 800 or 1,000 puffs of undiluted conventional cigarette mainstream smoke or heated tobacco product aerosol produced using the Health Canada Intense regime. After delivery of 50 puffs to the chamber, a settling time of five minutes was included to allow for aerosol deposition by diffusion and sedimentation within the chamber. Following exposure to 200 puffs, wallpaper and cotton samples were removed from the exposure chamber and staining levels determined quantitatively using a spectrophotometer. Exposure to between 200 and 1,000 puffs from the 3R4F Kentucky Reference cigarette yielded a visible dose-response effect with respect to the wallpaper and cotton samples whilst exposure to aerosols from both the heated tobacco product and EVPs after the same number of puffs showed relatively little colour changes. The authors concluded that *“the method developed demonstrated that cigarette smoke exposure significantly increased the level of wallpaper and cotton sample staining in a dose dependent manner, whereas glo, glo pro, glo sens [heated tobacco products] or iSwitch Maxx [an EVP] exposure resulted in significantly reduced levels of staining. This data suggests that PRRPs [potentially reduced risk products] may have additional social benefits for consumers and others”*.

### Conclusions

The results discussed in this section of the review demonstrate that a range of IAQ chemical constituent markers are present at markedly lower concentrations when compared to levels observed with conventional cigarette use within indoor environments; these are typically comparable to background levels of below IAQ standards. Furthermore, the intensity of indoor surface staining is markedly less with heated tobacco product use when compared to conventional cigarette use and the lifetime cancer risk for heated tobacco product exhaled aerosol exposure has been estimated to be three orders of magnitude lower than that estimated for exposure to ETS when using the same risk assessment modelling approach.

These conclusions are comparable to those reported by separate authors from both independent and heated tobacco product manufacturers. A review on the impacts of use of the THS heated tobacco product on IAQ concluded that *“generally, the usage of THS has been associated with lower or comparable indoor air pollutant concentrations compared against other conventional indoor sources or environments, in most cases distinguishable above background”* (Kaunelienė et al., 2018) whilst a systematic review of heated tobacco products concluded that *“evidence on heat-not-burn secondhand emissions suggested that heat-not-burn [tobacco product use] exposes users and bystanders to substantially lower but measurable levels of particulate matter and HPHC”* (Simonavicius et al., 2019) and an analytical study concluded that *“heated tobacco products are a weaker indoor pollution source than conventional cigarettes, but their impacts are neither negligible nor yet fully understood”* (Cancelada et al., 2019). A further commentary also noted that *“there is a reduction in risk to bystanders where conventional [cigarette] smokers switch to heat-not-burn products”* (Górski, 2019). These conclusions are comparable to those reached by Public Health England who concluded that *“compared with cigarettes, heated tobacco products are likely to expose users and bystanders to lower levels of particulate matter and harmful and potentially harmful compounds (HPHC). The extent of the reduction found varies between studies”* (Public Health England, 2018).

Finally, it should be noted that no clinical or epidemiological studies have been reported which have attempted to associate exposure to emissions produced from heated tobacco product use within indoor environments with subsequent health effects in bystanders. Such studies may be informative.

### Overall summary

The vast majority of the articles discussed in this review which provide quantitative data have concluded that heated tobacco products show a favourable tobacco harm reduction potential compared with conventional cigarettes. Although not risk free, this reduced risk profile may have been reported as a substantial decrease in levels of toxicants present in aerosol, coupled with lower levels of biomarkers of exposure, effect and/or function after controlled product use in

clinical settings, with significantly lower biological effects in *in vitro* or *in vivo* experimental studies or lower levels of indoor air quality markers in indoor environments after controlled product use.

With respect to those studies published to date which have suggested that, overall, heated tobacco products may be equally harmful or more harmful than conventional cigarettes (Auer et al., 2017a; Chun et al., 2018; Leigh et al., 2018a; Nabavizadeh et al., 2018; Salman et al., 2019; Sohal et al., 2019; Ioakeimidis et al., 2020), the experimental findings can be either explained by reference to the known effects of nicotine where heated tobacco products deliver comparable levels of nicotine to conventional cigarettes (Nabavizadeh et al., 2018; Ioakeimidis et al., 2020), to the findings of other relevant studies which contradict their findings (Chun et al., 2018; Leigh et al., 2018a; Sohal et al., 2019), by limitations with respect to the experimental methodologies employed (Auer et al., 2017a) or due to expected differences between the compositions of tobacco rods between heated tobacco products and conventional cigarettes (Salman et al., 2019).

To date, no novel health effects have been associated with heated tobacco product use which have not been reported with the use of tobacco and/or nicotine containing products. Long term research is warranted in this regard. Three medical case reports of acute eosinophilic pneumonia have been published which reported that heated tobacco product use was associated with development of the condition (Kamada et al., 2016; Aokage et al., 2019; Tajiri et al., 2020) and the use of conventional cigarettes is known to be a significant risk factor for development of the condition (Chaaban, 2020). However, the number of reported cases of acute eosinophilic pneumonia is extremely small compared to the number of heated tobacco product users. Furthermore, there is no data currently available to indicate whether or not the incidence of acute eosinophilic pneumonia secondary to use of heated tobacco products is higher than that associated with conventional cigarette use.

Given the enormous tobacco harm reduction potential that heated tobacco products offer to adult smokers who would otherwise continue to smoke due to the substantial reduction in numbers and levels of toxicants present in their aerosols compared to combustible tobacco smoke, there is a need for more research that is independent of commercial interests to be conducted with data from independent sources required to validate industry findings (Stepanov and Woodward, 2018). The totality of the currently available scientific evidence indicates heated tobacco products are likely to be significantly less harmful than conventional cigarettes, and have a risk profile much closer to that of non-tobacco containing products (Leigh et al., 2018a; Stephens, 2018; McEwan et al., 2021). These findings are consistent with the concept of a continuum of risk for non-combustible tobacco and tobacco-free nicotine products (McAdam et al., 2018), and are supported by the conclusions from a recent UK government review on vapour products and heated tobacco products (Public Health England, 2018).

Given the limited time during which heated tobacco products have been commercially available, there is limited data on long term use and more research is needed. Such studies will be of significant benefit and there are indications that such studies are underway (Sharman et al., 2018; Newland et al., 2019; Camacho et al., 2020).

Finally, the available scientific evidence suggests a significant reduction in smoking-related risk for conventional cigarette adult smokers who transition to heated tobacco products completely whilst quitting the use of all tobacco (and nicotine) products irrespective of their method of operation will lead to the greatest overall reduction in risk. This is consistent with public health messaging and the conclusions of separate authors (Slob et al., 2020).

### Future research

Based on the content of this review, there are several areas of research related to the potential health effects associated with heated tobacco products which may warrant further investigation:

1. **There is currently insufficient epidemiological data on the health effects of heated tobacco product use.** To date, only four epidemiological studies have been published (Lee et al., 2019; Chung et al., 2020; Yoshioka and Tabuchi, 2021; Wang L et al., 2021). Further studies are warranted into the long-term health effects of heated tobacco product use.
2. **There is currently no epidemiological data in relation to the potential carcinogenicity of heated tobacco product use.** Long-term epidemiological studies are warranted in this matter to determine if a transition from conventional cigarette use to heated tobacco product is associated with a decreased incidence of smoking-related cancers.
3. **There is currently a lack of clinical or epidemiological data on the effects of exposure to emissions produced from heated tobacco product use within indoor environments on potential health effects in bystanders.** Acute exposure and long-term epidemiological studies would be informative.

4. **There is a currently insufficient experimental data in relation to the potential health effects associated with use of heated tobacco products in pregnancy.** To date, no human data has been reported on the potential effects of heated tobacco product use in pregnancy on maternal and/or foetal health (Li et al., 2018; Kopa and Pawliczak, 2020). Currently, only a single *in vivo* animal study is available with investigates fetal effects of maternal exposure to heated tobacco products in mice (Yoshida et al., 2020). Although pregnant women should not use any tobacco or nicotine products, the potential risks to both mother and child associated with this exposure scenario should be investigated further.
5. **There is currently insufficient experimental data on the role of electrically heated tobacco product components on HPHC production.** Whilst the overwhelming evidence indicates that aerosols experimentally produced from heated tobacco products contain lower quantities of HPHCs than mainstream smoke from both reference conventional cigarettes and commercially available conventional cigarettes, there is limited evidence to suggest that non-tobacco components of the heated tobacco products such as the filter present in the tobacco stick and the heating elements of the electrical device can produce chemical constituents of toxicological concern such as acrolein, cyanohydrin and formaldehyde in their own right (Davis et al., 2019b; Kim et al., 2020; Kim and An, 2020). Further studies are required to investigate this phenomenon.

## Data availability

### Underlying data

Open Science Framework: The product science of electrically heated tobacco products, <https://doi.org/10.17605/OSF.IO/XQ3HB> (Mason, 2021).

This project contains the following underlying data:

- Supplementary\_File\_1.xlsx (Citation details for all journal articles identified in the review)
- Supplementary\_File\_2.docx (Table detailing the studies available in the scientific literature providing quantitative experimental data on levels of HPHCs produced by heated tobacco products in direct comparison with those produced by conventional cigarettes)

Data are available under the terms of the [Creative Commons Attribution 4.0 International license](#) (CC-BY 4.0).

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# Open Peer Review

Current Peer Review Status:



Version 1

Reviewer Report 21 December 2022

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**Chris Bullen** 

School of Population Health, Faculty of Medical and Health Science, University of Auckland, Auckland, New Zealand

This is a thorough, comprehensive account of the current scientific evidence on heated tobacco products and their potential health effects.

The authors have used a broad 'scoping review' approach to identifying key papers, studies and reviews pertinent to the topic. They used appropriate search terms and specifications. They provide a suitable flow chart to show the selection process they used to identify relevant articles. It is unlikely that their review has missed important reviews or studies.

They make a fair case in support of the overall evidence pointing to these products being less likely to cause harm than conventional combusted tobacco products because of the constituents in their emissions, their toxicological profiles from in vitro studies and in vivo animal studies, changes in biomarkers among users relative to smokers and non-users/non-smokers, and second-hand exposures. They identify several key issues that are 'live' and need resolution

1. there is a paucity of non-industry studies. Most research studies are tobacco industry-funded (including the present paper).
2. The conclusions tend to differ regarding assessment of harm, between industry and independent research.

The conclusions about where research in this field needs to go are correct: 1) first and foremost, far more independent (non-industry) research is needed; 2) there is diversity in assessment methods, especially between industry and independent researchers; and agreement is needed in the scientific community about the optimal standards for assessing harms; 3) a gap exists in evidence from long-term follow-up studies of the health of cohorts of heated product users compared with smokers and non-user/non-smokers; and another gap exists in research around population-level impacts of these products. Perhaps the best source of such data could come from Japan where heated tobacco products have emerged as a popular cigarette substitute. 4) Dual use (smoking conventional and heated products) needs further investigation. I would add to this list

the combination of e-cigarette use and heated tobacco product use as a potential 'dual use' phenomenon that is likely to grow in popularity as changes continue to occur in the nicotine product marketplace.

Overall, the paper is a comprehensive, balanced overview and makes a useful contribution to the literature on these novel products.

**Is the topic of the review discussed comprehensively in the context of the current literature?**

Yes

**Are all factual statements correct and adequately supported by citations?**

Yes

**Is the review written in accessible language?**

Yes

**Are the conclusions drawn appropriate in the context of the current research literature?**

Yes

**Competing Interests:** No competing interests were disclosed.

**Reviewer Expertise:** Tobacco control, smoking cessation, clinical trials

**I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.**

Reviewer Report 04 July 2022

<https://doi.org/10.5256/f1000research.78497.r135110>

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**Gagandeep Kaur** 

Dept of Environmental Medicine, School of Medicine and Dentistry, University of Rochester, Rochester, NY, USA

The current review is a very comprehensive review of the toxicological and risk assessment of electronically heated tobacco products. The authors have done a commendable job in putting together all the existing literature comparing the toxicology and health effects of aHTPs with conventional cigarettes. However this reviewer feels that the length of this review with so many different aspects being discussed makes it a tough read.

Here are a few suggestions to improve upon this manuscript:

1. The way the review is structured it might as well be structured as a systematic review. Authors must check the guidelines and restructure the manuscript accordingly.
2. The manuscript will benefit much with some serious editing. Repetitions and redundant statements and studies can be removed from discussion and key ideas should be focused on in terms of the public health risk.
3. The quality of Tables could be improved.

**Is the topic of the review discussed comprehensively in the context of the current literature?**

Yes

**Are all factual statements correct and adequately supported by citations?**

Yes

**Is the review written in accessible language?**

Yes

**Are the conclusions drawn appropriate in the context of the current research literature?**

Yes

**Competing Interests:** No competing interests were disclosed.

**Reviewer Expertise:** Inhalation toxicology, epigenetics, smoking, senescence

**I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.**

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