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Evaluation of behavioural, chemical, toxicological and clinical studies of a tobacco heated product glo™ and the potential for bridging from a foundational dataset to new product iterations

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ABSTRACT

Background: Tobacco Heating Products (THPs) are tobacco products that heat rather than burn tobacco with temperatures less than 350 °C. Because of this operating principle, they produce substantially fewer and lower levels of tobacco smoke toxicants than combustible cigarette smoke produced when tobacco is burnt, which occurs at much higher temperatures of around 900 °C. This paper analyses data on a THP, glo™, and assesses whether its use would result in reduced health risks compared to the health risks of smoking cigarettes. It also looks at the possibility of bridging datasets across the different variants of the glo™ product.

Methods: The approach is to consider whether datasets from behavioural, chemical, toxicological and clinical studies provide consistent findings of reductions in toxicant exposure with glo™ use by subjects who switch completely from smoking cigarettes to using glo™ and whether these reductions are similar to those who stop smoking cigarettes without switching to glo™ or any other tobacco or nicotine product. We also examine the similarities and differences of different versions of the glo™ product and benchmark it against a THP from another manufacturer.

Results: The studies indicate that the use of the glo™ results in substantial and prolonged reductions in toxicant exposure for smokers who switch to glo™ completely. A long-term clinical study shows substantial reductions in toxicant exposure over a period of time, similar to reduction of some biomarkers of exposure found following smoking cessation without switching to glo™ or any other tobacco product, and biomarkers of potential harm trending in a favourable manner for both groups that switch to glo™ and that quit all tobacco and nicotine use. Data suggests that all iterations of glo™ result in substantial reductions in toxicant exposure compared to smoking cigarettes and that bridging across datasets is feasible.

Conclusions: Given the accumulated scientific data summarised in this paper, and particularly the findings from a long-term clinical study, the data demonstrate that glo™ is a reduced exposure product compared to combustible cigarettes and is reasonably deemed to reduce the risk of smoking-related diseases and supports the conclusion that smokers who would have otherwise continued to smoke and instead switch entirely to THP glo™ use, will reduce their relative risk of developing smoking-related diseases as compared to continued smoking. The extent of reduction in risk compared to continuing to smoke is likely to vary by smoking-related disease and by an individuals' smoking history, other risk factors and an individual's susceptibility to disease. Use of the THP will present some level of increased health risk as compared to cessation of tobacco and nicotine products and will cause dependence. As long as the principles of heat-not-burn are maintained, THP use will result in substantially reduced exposure to smoke toxicants as compared to continued conventional cigarette smoking. It is possible to use bridging or read across to apply these conclusions to new iterations of the glo™ product, extending the utility and validity of the evidence generated through study of prior iterations.

Abbreviations: THP, tobacco heating product; BoE, biomarker of exposure; BoPH, biomarker of potential harm; WHO, World Health Organisation; TSNA, tobacco specific nitrosamines.

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1. Introduction

Cigarette smoking is a leading avoidable cause of premature morbidity and mortality globally and is a cause or a contributory factor to a wide range of diseases including lung cancer, chronic obstructive pulmonary disease and various cardiovascular diseases [1,2]. Most of the harm caused by cigarette smoking is the result of long-term persistent exposure to toxicants in tobacco smoke, many of which are formed during the combustion of tobacco [3]. Nicotine, the substance in tobacco and tobacco smoke identified as the main cause of dependence in smokers, is relatively benign toxicologically at the levels typically used in tobacco and nicotine products [2]. Just as epidemiology has authoritatively determined the health risks of smoking, it has also evaluated the impact of smoking cessation finding that following complete smoking cessation the relative risks of smoking-related diseases progressively reduce as a function of time since cessation and progression of certain smoking-related diseases slows [4,5].

A range of public health approaches have been used to reduce the number of cigarette smokers, and hence the public health impact of smoking, including on-pack health warnings, education, treatments to assist smoking-cessation, as well as marketing and public-place smoking restrictions, but the prevalence of cigarette smoking has not substantially declined in many countries [6]. Some governments have supported an additional approach of tobacco harm reduction where smokers who would not otherwise quit are encouraged to switch from cigarette smoking to the use of alternative tobacco and nicotine products (including tobacco heating products, vaping products, and nicotine pouches) as means to continue to enjoy nicotine and many of the gustatory, ritual and social pleasures provided by smoking, but reduce their exposure to toxicants [7]. Many of these products are relatively new, benefiting from recent advances in micro-electronics and batteries, and have not been in use by consumers for a sufficient period of time for epidemiological analysis to determine their longer-term health risks. Hence, a multi-disciplinary scientific approach is necessary to determine whether these products reduce relative health risks for smokers who switch to them in comparison to continued smoking. Several authors have suggested frameworks of studies needed to assess the reduced risk potential of new products [3,8–10]. Regulators in certain countries, such as the US, have formalised the process with mandatory authorizations with scientific data required to demonstrate reduced exposure or risk of new products as compared to conventional cigarettes.

The concept of tobacco heated products (THPs), also called heated tobacco products or heat-not-burn products, was developed in the US in the 1980 s [11,12], but has only become successful commercially over the past decade. Their core design principle is to use tobacco as the source of nicotine (unlike vaping products or nicotine pouches where pharmaceutical grade nicotine is used and do not contain tobacco) and to ensure that the tobacco is heated to a low enough temperature to release nicotine without causing ignition or combustion of the tobacco, resulting in much lower toxicant emissions than found in cigarette smoke. THPs typically comprise of an electronic device that provides the method of heating, and a consumable, usually in the form of a stick of tobacco wrapped in paper or foil, that is placed in the device and discarded after use.

A previous review [13] concluded that Type 1, THP 1.0 T [14], the original version of glo™, (Nicoventures Trading Limited) had the potential to be a reduced risk product as compared to conventional cigarettes. In this paper, we combine data from behavioural, chemical, toxicological and clinical studies on glo™ products, in a weight-of-evidence approach to assess whether it is a reduced risk product compared to cigarettes.

As was seen with vaping products [15], there is a rapid early-stage innovation cycle as the products are developed and evolve to become satisfactory alternatives to smoking cigarettes. There are now multiple iterations of the glo™ device that vary in size, weight and the technology used for heating that are used with a range of tobacco consumables that vary by size, the weight of tobacco used and flavours. Original, Type 1, THP 1.0 T uses a thin film resistive heater that heats to a maximum of 240 °C and a king-size superslim tobacco consumable. Newer glo™ device variants use induction heating, which comes to operating temperature more rapidly, can be adjusted by the consumer from a standard heating mode (typically at 250 °C) to a "boost" heating mode (of 260 °C or 270 °C) and can be paired with a tobacco consumable that has a larger diameter and contains more tobacco than the consumable used in the original, Type 1, THP 1.0 T.

Many of the scientific studies described below, including a key year-long ambulatory clinical study measuring biomarkers of exposure to toxicants and biomarkers of potential harm, have been conducted on the original, Type 1, THP1.0 product with various tobacco consumables. So, we also considered data necessary to bridge or read across from the foundational dataset from the original glo™ Type 1 THP1.0 to newer product iterations, to further support comparative risk analysis of subsequent device variants and various tobacco consumables.

It is important to assess the data regarding glo™ products and compare it with studies investigating other THPs and data on smoking cessation. Various public health authorities are reviewing the growing evidence on THPs generated from manufacturers, independent researchers and academic studies. Some public health authorities agree that the exclusive use of alternative tobacco and nicotine products, including THPs, is likely to be much less harmful than smoking cigarettes [16].

Because the operating principle of THPs leads to substantial reductions in toxicant formation in THP emissions in comparison to cigarette smoke, and consequently toxicant exposure, it is important to consider potential consequences of these reductions by examining dose-response relationships between tobacco-smoke toxicants and smoking-related disease, and the epidemiology of smoking cessation.

2. Dose-response relationships of tobacco smoke toxicants

The epidemiology of diseases caused by cigarette smoking shows a dose-response relationship with increased relative risks of disease with increased durations of exposure and amounts of daily exposure, illustrating that higher lifetime smoker toxicant exposure levels are associated with higher health risks [1,2]. Tobacco smoke is a complex mixture, containing thousands of individual chemicals and over one hundred chemicals that have been identified as carcinogenic, respiratory, or cardiovascular toxicants [12]. The levels of the emitted toxicants range widely from milligrams per cigarette smoked (e.g., carbon monoxide) to nanograms per cigarette smoked (e.g., tobacco-specific nitrosamines) [12].

Certain toxicants in cigarette smoke are likely more important to various disease risks than others. Fowles and Dybing [17] used cancer potency factors for 40 toxicants found in cigarette smoke, typically from studies of exposure to the individual toxicants, to calculate cancer risk index (CRI) values, finding for example that the CRI value for 1,3-butadiene was more than twice that of the next highest contributing compound, acrylonitrile. They also determined non-cancer risk index values for respiratory diseases (where acrolein and acetaldehyde were determined to be the most important cigarette smoke toxicants implicated in development of these diseases) and for cardiovascular diseases (where hydrogen cyanide and arsenic had the highest values). This study was central to the WHO Study Group on Tobacco Product Regulation's identification of toxicants that should be prioritised for reductions in cigarette smoke if possible [18], but it was noted that even if new types of conventional cigarette achieved the target smoke toxicant reductions, it would not be possible without additional data to determine that these reductions would result in reduced risks of smoking. However, the US Institute of Medicine (IoM) [3] considered that if scientific data demonstrated a substantial reduction in one or more tobacco smoke toxicant exposures from consumer use of a product serving as an alternative to combustible cigarettes, then if sufficient data was generated to

support the likelihood of reduced disease risk even in the absence of long-term epidemiology, the alternative product may be reasonably deemed reduced risk as compared to conventional cigarettes.

A study by Stephens [19] that modelled inhalation unit risks for cancer to compare emissions from a prototype THP with that of a conventional cigarette, suggested that the reduction in toxicant emissions would result in the THP emissions having lower cancer potencies than tobacco smoke by at least one order of magnitude. Furthermore, recent studies have estimated a large decrease in THP cancer and non-cancer risks in comparison with the predicted cancer and non-cancer risks induced by cigarettes [65,66].

So, while dose-response relationships for individual cigarette smoke toxicants are not precisely known, exposure to non-combusted aerosols with substantial reductions across a range of the most significant toxicants present in cigarette smoke would be expected to result in health risk reductions, but a weight-of-evidence approach using chemical, toxicological, and clinical assessments are needed to ensure that the reductions are meaningful [3].

3. Epidemiology of smoking cessation

Many studies have looked at whether cessation of smoking leads to a reduction in relative health risks. One of the most important looked at mortality in relation to smoking in a study that had 50 years of epidemiological observations of British doctors [20]. It found that prolonged smoking from early adulthood until late in life (in a cohort that was born in the 1920 s) tripled age-specific mortality rates, but that cessation at age 50 halved the rates experienced by continuing smokers and cessation at 30 avoided most of the excess risk of premature mortality associated with cigarette smoking. Reductions in relative risks after cessation varied by disease with a more rapid reduction for cardiovascular diseases than for lung cancer, and with the effect of smoking cessation on chronic obstructive pulmonary disease tending to slow onset and progression of the disease rather than fully reducing the risk to that of never smokers [21].

As mentioned, above, the US IoM, in its report assessing the scientific basis for tobacco harm reduction [3], took the view that a substantial reduction in one or more tobacco smoke toxicants in the emissions of an alternative product could reduce smoking-related disease risks and discussed what this might mean for individuals who switch from smoking conventional cigarettes to the alternative product. Utilizing smoking cessation data, IoM estimated that for cancer risks calculated with biomarkers as an indication of exposure, the shape of the dose-response curve was quadratic, suggesting greater risk reduction from switching to the model reduced-risk product for individuals who had smoked more but noting that potential reduction was likely to vary by individuals' age, race, ethnicity and gender. For cardiovascular risks, the report estimated that reductions in exposure would result in reductions in relative risk but that certain individuals and perhaps populations were likely to have varying susceptibility to cardiovascular disease, leading to less of a benefit from the modelled harm reduction approach. They noted that the data from studies of smoking cessation "indicate a considerable variance in the rate of offset of risk, which declines with time." [3].

The implication of the above is that reductions in relative health risks following switching from cigarette smoking to a reduced risk product may be generally found in populations of switchers but will vary by individual depending on age, history of smoking and varying susceptibilities to smoking-related diseases.

4. Methods

The general approach that we have taken is to compare three different conditions – continued cigarette smoke exposure, previous cigarette exposure switched to exposure to the THP emissions and previous cigarette exposure switched to no exposure (i.e., cessation without the use of any tobacco product). We evaluate data from chemical and toxicological laboratory studies, using behavioural studies in human volunteers to understand and ensure that product usage as well as dosing conditions for the laboratory studies are realistic, and compare these findings with data from several clinical studies.

4.1. Behavioural studies

Several approaches were taken to determine whether smokers who switched to the original, Type 1 THP changed their behaviour by either taking more puffs and/or larger puffs or consuming more sticks compared to their consumption when smoking cigarettes [22,23]. These approaches typically involve asking volunteer smokers to attend a central location after having been able to become accustomed to the way in which the original, Type 1, THP is used for around 5 days. Puffing topography is recorded using a smoking analyser (SA7) which is placed between the product and the volunteer and records puff volumes and duration. The mouth level exposure (MLE) to nicotine free dried particulate matter (NFDPM) and nicotine for THPs and cigarettes can be estimated using real-time measurement of optical obscuration for each puff of aerosol or smoke.

However, such observational studies are known to change subjects' behaviour, typically to more intensive puffing. Other types of studies, such as "Home Use Tests", where volunteers are asked to record numbers of study and non-study cigarettes or THP consumables used each day in the consumption diary provided, can be used to determine the number of cigarettes smoked or consumables used per day [23]. The 12-month ambulatory clinical study discussed below recorded daily consumption and biomarker levels to assess levels of exposure, though for this study the consumables were provided to the volunteers who may report higher consumption than under real-world conditions [24].

4.2. Analytical chemical studies

Four types of analytical chemical study have been used. The first type focuses on whether combustion is occurring in the THP and involves the measurement of markers of combustion including carbon monoxide and oxides of nitrogen.

The second type of study is a targeted analysis of the emissions from the THP. Analytical methods are similar to those developed for cigarette smoke toxicants, utilising linear smoke machines, where possible, and where a Cambridge Filter Pad is used to collect particulates with the neoprene washer removed from the holder. Due to very low levels of some toxicants under study in THP emissions, the use of air blanks ensures the identification of any artefactual levels of identified compounds.

The third type of study is untargeted analytical chemical scans that compare the relative complexity of cigarette smoke and THP aerosols and look for any substances that might be present in THP emissions but not in cigarette smoke (particularly metals given that a metallic device is involved in the heating of the THP). All three of the above analytical chemical studies used modified Health Canada Intense regime (55 ml puff volume, 2 s puff duration, 30 s puff interval with a bell-shaped puff profile), without blocking the perforations on the consumable (as studies described below show it is very difficult for a consumer to block these filter perforations in the THP format and that no such blocking takes place, whereas such blocking is possible in the case of a filtered cigarette) for the collection of the emissions.

The fourth type of study assesses the impact of THP emissions on Indoor Air Quality studies (IAQ), by simulating indoor exposure scenarios and measuring levels of known air quality toxicant markers. These studies are undertaken at a UK Accreditation Service-authorised testing laboratory (BRE Ltd, Watford, UK) using three ventilation conditions set out in the British Standard (BS EN 15251, BSI 2007) [25]. Known air quality chemical markers including fractions of particulate matter, gases and volatile organic compounds, were measured with

volunteers either smoking, using original, Type 1, THP1.0, or with no tobacco use [26].

4.3. In-vitro toxicology

Two broad categories of in vitro toxicological assessments have been conducted, comparing the biological impact of THP emissions versus cigarette smoke. The first are regulatory-accepted assessments based on international OECD guidelines or adapted OECD guidelines for the assessment of complex mixtures and aerosols. These include the neutral red uptake assay (NRU) for the assessment of acute toxicity testing, the bacterial reverse mutation assay (Ames) for mutagenicity assessment, the mouse lymphoma (MLA) or the in vitro micronucleus (IVMN) assays for genotoxicity and more recently the cell transformation assays such as the Bhas 42 assay which has a draft test guideline [27].

The second category of toxicological assessments have focused on contemporary 21st Century toxicological approaches [28] which include the use of human cell and tissue systems, disease modelling and mechanistic studies of endpoints associated with the development of smoking-related diseases. Such studies can support an adverse outcome pathway (AOP) approach, which are gaining regulatory traction as a framework to integrate and map in vitro and in vivo data against a known clinical outcome. For example, two cigarette smoking related AOPs have recently been developed for COPD and CVD related diseases. Respectively, these focus on a pathway for oxidative stress-mediated EGFR activation leading to decreased lung function [29,67] and the onset of hypertension by oxidative stress-mediated perturbation of endothelial nitric oxide bioavailability [30]. Examples of contemporary THP toxicological testing and mechanistic approaches include the application of high content screening methods to assess toxicity, DNA damage, cellular metabolism, mitochondrial health and oxidative stress in human lung cells exposed to THP or cigarette smoke. Cigarette smoke disease-related assays such as those that focus on lung function in a COPD model (including cell-tight junction assessments, cilia beating frequency and active area of cilia) coupled with measurement of the induction of mucin secreting cells as a model of goblet cell hyperplasia, have also been developed and used for the assessment of THP aerosols [31]. In addition, in vitro assays assessing vascular impairment in response to THP aerosols compared to cigarette smoke have also been employed [32].

More recently use of reporter gene analysis, such as ToxTracker®, developed by (Toxys, Netherlands) has been used to complement regulatory assays, high content multiplexing and disease mechanistic approaches [33]. ToxTracker® relies on the up regulation of gene expression for six receptor genes thought to be involved in intercellular processes such as oxidative stress, cell stress, protein damage and DNA damage to draw its mechanistic associated outcomes [33,63,64]. ToxTracker™ as an in vitro application is gaining momentum, due to its versatility in its application as it covers all three main aspects of in vitro testing and more importantly is showing good concordance with the traditional toxicological approaches (Ames, in vitro and in vivo IVMN). Such an assay could offer predictive mutagenicity and genotoxicological value and could be considered an instrumental tool in demonstrating concordance ("bridging") between product iterations linking back to a substantial historical dataset thus reducing the regulatory burden required to review extensive in vitro toxicological assessments for each subsequent product iteration. ToxTracker has been used to assess cytoxicity, oxidative and cellular stress biomarkers associated with disease AOPs for glo™ Type 1, original, to quantify the potential risk reduction associated with the use of the device, for which significant reductions were noted [68]. Local market regulations, such as the FDA regulations for the substantial equivalence pathway, will dictate the study and data requirements to demonstrate bridging between variants, however in countries with no established pathway, these assays may provide a valuable route to demonstrate comparability.

4.4. Clinical studies

Three types of clinical study have been undertaken, all registered before being conducted and performed under international standards for clinical studies (Good Clinical Practice (International Council for Harmonisation (ICH) E6 Consolidated Guidance, April 1996) and the ethical principles of the Declaration of Helsinki. The first are short-term studies where the volunteers are confined in clinic for around a week and randomised to one of three arms – a continue to smoke, a switch to the original, Type 1 THP and quit smoking without taking up the THP or other tobacco product (assisted where asked for with nicotine replacement therapy products) [34–36]. Biological samples are taken across the study period to track any changes in biomarkers of exposure from baseline to the end of the study. The second type is a long-term ambulatory clinical study where volunteers occasionally have visits to a clinic for the collection of biological samples, to be taken for both biomarkers of exposure and biomarkers of potential harm, as well as to have physiological measures taken [24,37]. Again, there are three key arms – a continue to smoke, a switch to the original, Type 1 THP and quitting smoking without taking up the THP or other tobacco product (assisted where asked for with nicotine replacement therapy products). These are not fully randomised for ethical reasons, with volunteers in the continue to smoke arm, the switch to the original, Type 1 THP arm consisted of randomised subjects who reported a low intent to quit smoking, while those in the cessation arm consisted of a separate group of subjects with a declared high intent to quit smoking. All subjects in these arms were cigarette smokers at baseline. Compliance with the protocol is important in such studies, and biomarkers that assess long-term compliance with designated smoking status were used in addition to short-term measures such as exhaled carbon monoxide levels and self-reporting.

Population surveys combined with Quality-of-Life measures have been used to assess the incidence of THP use in certain countries [38], and population health models have been used to estimate the consequences of smokers switching to THPs and the effects of making THPs available to the public including never and former smokers [39].

The third type of clinical study is the Abuse Liability study [40] where volunteers were confined overnight for 4 clinical visits in a randomised cross-over design. The study involved 4 products, which were 2 variants of, which differed in nicotine yield (0.46 and 0.68 mg nicotine/stick), an NRT which was the Nicorette inhalator (15 mg nicotine, Johnson & Johnson) and subjects' usual brand cigarette. Prior to each clinical visit subjects were supplied with the study product to be used in the next clinical visit for familiarisation, apart from their usual brand cigarette. For each clinical visit subjects were admitted to the clinic overnight for a 12-hour abstinence period from tobacco and nicotine use following which a baseline blood sample was taken for plasma nicotine analysis 5 min before product administration. Subjects used their assigned product for 5 min and multiple blood samples were taken for plasma nicotine analysis up to 240 min after the start of product use for a pharmacokinetic (PK) assessment. In addition, subjective effect questionnaires on product liking, overall intent to use again, urge to smoke, urge for product and product evaluation scale were administered at various points during the PK session.

5. Test products

The majority of studies discussed in this paper have used the original glo™ THP 1.0 T, Type 1. This comprises two functional parts: an electronic handheld device with a heating chamber, and a specially designed tobacco consumable that is inserted into the heating chamber (Fig. 1). Details of the formats of both the electronic heating device and tobacco consumable are summarised in Table 1. The electronic heating device contains a rechargeable battery that supplies the energy to the heating tube when switched on. The heating tube has two heater segments made of a thin-film resistive material, which are separately controlled by the inbuilt software. The tobacco rod is heated from the periphery.

The tobacco consumable has a diameter of ca. 5.0 mm and overall length of 82 mm, with a 42-mm long tobacco section. This is a smaller diameter than most cigarettes and enables quicker heat transfer from the peripheral surface into the inner core. The overall mass of the tobacco material is about 260 mg, which is less than the 700–800 mg of tobacco typically contained in a commercial king-size cigarette. Almost all the aerosol produced is emitted through the mouth end.

A user inserts the tobacco rod into the stainless-steel heating chamber; on pressing the activation button on the device, the heating chamber heats the tobacco rod to around 240 °C, significantly lower than the major pyrolysis and combustion temperature ranges seen in a lit cigarette (typically around 650 °C during smouldering and 900 °C during a puff) but sufficient to release nicotine, glycerol (added as the main aerosol agent) and volatile tobacco flavour compounds. The warming-up process takes some time and a haptic motor indication (vibrations) and an LED (visual indication) on the start button let the consumer know when the product is at the correct temperature and ready to use. The product stays at temperature for approximately 3–4 min before switching off. Several different external sizes of the device have been developed, all with similar internal dimensions to fit the same tobacco consumables.

The tobacco used in THP1.0, original, Type 1 consumable is tobacco processed by a paper-style reconstitution process to homogenise the chemical composition of finished material and hence provide a more consistent aerosol composition and delivery. Reconstitution is also necessary to incorporate a high level of glycerol as the main aerosol agent. Reconstitution incorporates glycerol into the inner structure of the material, which aids subsequent processing of the material. The tobacco consumable rod is typically one with a high inclusion (80–100 %) of reconstituted tobacco. A variety of consumables have been used in the studies, including some that have been mentholated. Nicotine levels in the consumable depend upon the blend of tobacco used.

In order to distinguish studies on the original format of resistive heating technology and a king size super slim (KSSS) tobacco consumable, we term this combination Type 1. This operates at 240 ºC and the user session ranges from 3 to 3.5 min depending upon the model of the device. Type 1 devices include the original glo™, nano and mini.

A newer version of the device replaces the thin-film resistive heating with induction heating which allows a more rapid heat-up of the chamber and hence consumable. We term the combination of induction heating and a KSSS tobacco consumable as Type 2. With Type 2 the device can operate in base mode (250 °C for a 4-minute use session) or boost mode (260 °C for a 3-minute session), the mode being chosen by the consumer. Induction heating also allows the possibility of using a demi-slim (DS) tobacco consumable with a larger circumference (21 mm) and containing more tobacco. This type of consumable is used in another device iteration termed Type 3 that can operate at either base mode (250 °C for a 4-minute session) or boost mode (260 °C or 270 °C depending upon the product for a 3-minute session) (see Fig. 1). Type 3 devices include hyper and hyper+ , with the hyper+ device operating at the increased boost temperature.

For laboratory studies, two reference cigarettes were used, both manufactured by the University of Kentucky for research purposes – 3R4F and the more recently introduced 1R6F. They are both well characterised in the scientific literature and are widely used in various laboratory studies pertaining to attributes of conventional cigarettes. These are not designed to be smoked by humans. For human studies, commercial cigarettes relevant to the country in which the study is

Table 1
Formats of devices and consumables and typical operating conditions for glo™ THP1.0 Type 1 (original, mini and nano), Type 2 (pro) and Type 3 (hyper and hyper+).

	THP Type 1 (original)	THP Type 1 (mini)	THP Type 1 (nano)	THP Type 2 (pro)	THP Type 3 (hyper)
Device format	Box	Smaller box	Pen	Box	Box
Tobacco rod format	KSSS	KSSS	KSSS	KSSS	DS
Heater	Resistive	Resistive	Resistive	Induction	Induction
Operating temperature (°C)	240	240	240	250–280	250–260
Ramp up time (time to first puff) (s)	40	40	40	20–10	20–15
Session length (s)	180–210	210	210	180–240	180–240
Number of puffs (n)	8–9	9	9	8–10	8–10

Type 1 (original, mini and nano), Type 2 (pro) and Type 3 (hyper). King size super slim (KSSS) – 82 mm length of rod (42 mm tobacco section length) Diameter. Demi slim (DS) – 75 mm length of rod (34 mm tobacco section length)

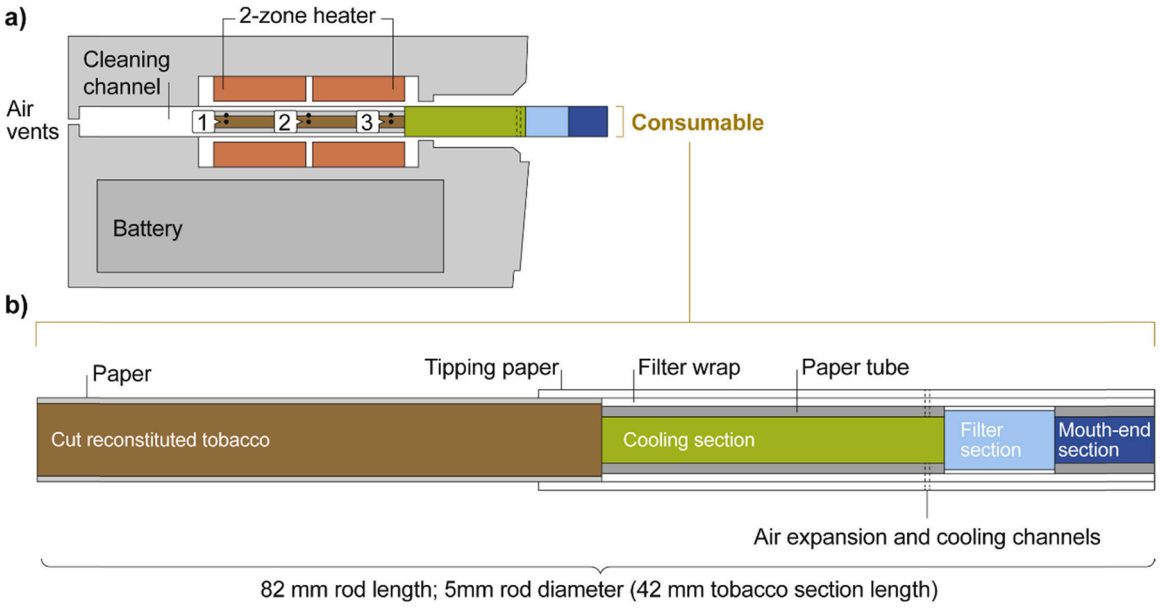

Fig. 1. Type 1 (original) schematic [14].

occurring are used.

6. Bridging or read across

Bridging of partial data sets is an established practice within various industrial sectors including food and pharmaceuticals, that seeks to improve the efficiency of regulatory product review and approvals while protecting consumers and supporting product innovation [40,42,43]. Bridging relevant data from an original product to a new version requires a substantial foundational data set for the original version (in this case THP 1.0 T, Type 1, original) [44]. Next, select studies are performed on the new variants of the original version to verify that any changes are unlikely to affect adversely the performance of the product relevant to compliance and product safety. This verification is enabled by fulfilling several requirements in the product stewardship process that is undertaken before a new product version comes to market. Three core stewardship aspects guide glo™ product development: toxicological assessment of all ingredients and materials used: safety of the device; and legal compliance. The process includes oversight of all new materials and ingredients used in new versions of either the device or the consumable to ensure that they do not add to any inherent risks presented by the original product. For example, in the case of THPs, chemical studies can ensure that the operating principle of heating but not burning the tobacco (and hence producing emissions substantially lower in toxicants than cigarette smoke) is maintained in the newer version. Eaton et al. [14] sets out a scheme for product stewardship assessment for THPs, which describes various pathways for evaluating any materials and ingredients that might be changed during the heating process. This risk assessment can be supported by studies on consumer use and in vitro toxicology data where necessary. The amount and type of additional study will depend on whether the new variant has minor or major changes from the original, and whether the change could impact toxicant formation.

The advantage of relying on data bridging arises both from the perspective of the regulator in terms of the time and effort it takes to approve or reject products in any pre-market assessment process, and from the manufacturer in terms of time and costs. This is most striking in clinical studies. A 360-day clinical study takes several years to complete in its planning, recruitment, conduct and analysis and it would be impractical to undertake one for every change made for a new product version (for example, running a new clinical study for each blend of tobacco used in the consumables). Bridging also provides a benefit to consumers in shortening the innovation cycle, and in the case of THPs makes it quicker to adapt product versions that provide more satisfactory, complete alternatives for cigarette smokers.

Below, we explore what changes might necessitate additional data collection and whether the general conclusion that we come to on the foundational dataset from THP, Type 1, original can reliably be extended to newer product versions through bridging.

7. Results

7.1. Behavioural studies

Table 2 compares puffing topography of volunteers using either cigarettes or a THP in two different populations, one in Japan [22] and one in Italy [23]. This information is used to determine whether the smoking regime used in emissions studies is appropriate. Standard methods have been developed for two regimes – an International Organisation for Standardisation (ISO) of 35 ml puffs of 2 s every 60 s, and what is known as the Health Canada Intense regime of 55 ml puffs of 2 s every 30 s, with filter perforation holes blocked. We typically use a modified HCI regime which is the same as the Health Canada method but with no filter perforation blocking [22].

The data show, apart from puff intervals, considerable variation both between subjects (each group shows the mean and standard deviation across around 50 volunteers) with in a study and between the studies in two countries. Total puff volumes were higher for the THP group than the cigarette group driven mainly by larger individual puffs, a shorter period between puffs and a shorter session time. This may be attributed to the limited time period of THP heater operation, compared with conventional cigarette where the burn down time depends on intensity and number of puffs taken.

Table 3 shows data on mouth level exposure to nicotine free dry particulate matter (NFDPM - tar) and nicotine for the same studies. In both populations, the per stick and daily mouth level exposure were much lower for both NFDPM and nicotine for THP use compared to cigarette use. This may be due in part to the lack of familiarity of the volunteers with the THP, despite a period of familiarisation, but also the difference in the chemical content of the emissions from a THP compared to a cigarette.

A 12-month clinical study was conducted in the UK and the data from the 180-day timepoint found that in this ambulatory study, the group that continued to smoke had an average daily consumption of 17.4 cigarettes per day and the group that switched to the THP averaged 21.9 sticks per day, though there was no significant difference in the level of total nicotine equivalents (TNeq) between the group that continued to

Table 3
Mouth level exposure (MLE) comparison of data from Japan and Italy on THP Type 1 (original).

Product/ country	Mean MLE to NFDPM (mg per stick) (± SD)	Mean MLE to NFDPM (mg per day) (± SD)	Mean MLE to nicotine (mg per stick) (± SD)	Mean MLE to nicotine (mg per day) (± SD)
Cigarette/ Japan	13.5 (± 6.2)	224.1 (± 154.9)	1.3 (± 0.5)	22.2 (± 14.1)
THP/ Japan	5.2 (± 3.4)	58.0 (± 56.4)	0.3 (± 0.2)	3.3 (± 2.8)
Cigarette/ Italy	16.7 (± 7.6)	218.2 (± 141.9)	1.36 (± 0.62)	17.8 (± 11.6)
THP/Italy	4.7 (± 2.9)	36.7 (± 40.4)	0.34 (± 0.21)	2.7 (± 2.9)

Table 2
Puffing topography comparison of data from Japan and Italy on THP Type 1 (original).

Product/ country	Total puff volume (ml) ± SD)	Mean puff number (n) (± SD)	Mean puff volume (ml) (± SD)	Mean puff duration (s) (± SD)	Mean puff interval (s) (± SD)	Mean session length (s) (± SD)
Cigarette/Japan [22]	489.0 (± 177.7)	10.7 (± 5.0)	48.9 (± 14.8)	1.8 (± 0.6)	9.7 (± 3.4)	NR
THP/Japan[22]	736.4 (± 415.8)	10.9 (± 5.6)	66.7 (± 23.7)	1.8 (± 0.6)	7.4 (± 2.7)	NR
Cigarette/Italy [23]	596.8 (± 197.1)	16.0 (± 37.6)	41.6 (± 16)	1.6 (± 0.5)	18.8 (± 10.6)	269.3 (± 88.0)
THP/Italy[23]	731.3 (± 437.6)	15.4 (± 7.4)	46.6 (± 16.8)	1.6 (± 0.5)	11.1 (± 5.8)	150.5 (± 40.5)

smoke and the group that switched to the THP [45].

The study conducted in Japan also examined used THP consumable filters from the volunteers with a ninhydrin staining technique that can detect saliva to see if filter perforation holes may have been partially or fully blocked during use and found that not one of the volunteers obscured the perforation holes when using the product [22].

The topography data suggests that the HCI regime is more likely to represent average THP behaviour than the ISO regime and given the lack of perforation blocking evidence in human studies and confirms the use of modified HCI puffing regime for laboratory chemical and toxicological studies.

7.2. Chemistry

7.2.1. Combustion chemistry

To ensure that the design principle of avoiding self-sustained combustion was achieved across the various THP types, a five-step test matrix was used which included temperature monitoring, mass loss measurement and observation after use, thermogravimetric analysis (TGA) and measurement of key markers of combustion [14]. In the TGA, which was conducted both in air and in nitrogen to help distinguish any losses due to pyrolysis or combustion, there was similar mass loss under both conditions up to 240 °C (the operating temperature of the THP type 1 device) due to evaporation of water, glycerol, nicotine and potentially other volatiles present in the tobacco, but little indication of pyrolysis or combustion, though some mass loses could be due to the thermal decomposition of hemi-cellulose which begins to thermally decompose above 220 °C. This was maintained up to around 350 °C (cigarettes' lower smouldering temperature and much higher than operating temperatures of the glo™ products) with little indication of oxidation occurring. Above 400 °C the samples in air showed a more rapid mass loss than those in nitrogen indicating oxidation of the key biomass substances of cellulose, hemicellulose and lignin.

The measurement of carbon monoxide, carbon dioxide and oxides of nitrogen emissions were measured for the THP consumable used in the THP device and a reference cigarette. As shown in Table 4, CO levels were below the level of quantification in the THP configuration and were much higher for the cigarette emissions. For CO_2, NO and NO_x there were detectible levels in the THP emissions, but these were much lower than in the cigarette emissions, indicating a small amount of thermal decomposition of the tobacco in the THP but no combustion.

7.2.2. Targeted analytical chemistry

There are well developed analytical methods for identifying a number of toxicants found in tobacco smoke. One study compared the levels of 126 measurands in the emissions from THP Type 1, original, using two commercially available versions of the tobacco consumable (THP 1.0 T Bright tobacco and THP 1.0 M Intensely fresh (menthol)) compared with the University of Kentucky reference cigarette 1R6F or reference cigarette 3R4F [46]. The measurands were a combination of lists of toxicants developed by the US Food and Drug Administration (FDA), World Health Organisation (WHO) and Health Canada. Table 5 provides data on the WHO Tobacco Product Regulation Study Group (TobReg) 9 toxicants, showing substantial reductions in toxicant levels in the THP emissions compared to the reference cigarette, with levels of carbon monoxide, 1,3-butadiene and benzene either Below Detection Limits (BDL) or Not Quantifiable (NQ) in the THP emissions. Of the 126 measurands, 75 were greater than 90 per cent reduced in the THP emissions with a further 12 being between 70 % and 90 % reduced compared to the emissions from the reference cigarette 3R4F [46]. 21 were below levels of quantification or detection in both the THP and cigarette emissions. 7 were higher in the THP emissions (chromium (4 ng/consumable), propylene glycol (0.4 mg/consumable), glycidol (0.04 mg/consumable), glycerol (3 mg/consumable), N-nitrosodiethylene (0.6 ng/consumable), acetoin (6 μg/consumable) and methylglyoxal (28 μg/consumable)), several of these relating to the higher inclusion levels of propylene glycol and glycerol in the THP consumable compared to those in 3R4F. Compared to the reference cigarette 1R6F, 76 were greater than 90 % reduced, a further 13 between 70 % and 90 % reduced and another 23 were below limits of detection in both the THP emissions and the reference cigarette smoke. Only 4 measurands were higher in the THP emissions (chromium, glycidol, glycerol and N-nitrosodiethylene) [4].

7.2.3. Untargeted analytical chemistry

Studies have compared both the particulate phase and vapour phase of the emissions of THP, Type 1, original, with that of a reference cigarette [47,48]. In the particulate phase study, material was collected on glass wool before being introduced to analysis by thermal desorption and separated by two-dimensional gas chromatography with dual time of flight mass spectrometry and flame ionisation detection. Cigarette smoke created a much more complex particulate phase with around 590 peaks compared with the particulate phase from THP, Type 1, original, which had 160 identifiable peaks. 93 compounds were common between the two aerosols with the majority at lower concentrations in the THP aerosol. A few highly volatile compounds, mainly furans, glycerol and its acetate were found in higher concentrations in the THP sample. The study of the vapour phase used tenax as an adsorbent for its collection. Around 90 % of the compounds detected were identified through matching to libraries and data mining, and in terms of abundance, the study found the THP, Type 1, original, aerosol much simpler and around one-tenth the abundance of the reference cigarette sample.

Table 4
Mean (± SD) levels of four combustion markers from THP Type 1 (original) aerosol and in the emissions from a reference cigarette [14].

Combustion marker	Type 1 (original) (8 puffs per stick)	Reference cigarette (3R4F) (10.3 puffs per stick)
CO (mg)	NQ (<0.233)	32.0 (± 0.9)
CO_2 (mg)	2.35 (± 0.14)	85.1 (± 4.0)
NO (μg)	10.1 (± 0.4)	496 (± 16)
NO_x (μg)	12.0 (± 0.4)	533 (± 16)

Table 5
A comparison of mean (± SD) chemical emissions from a THP Type 1 (original) and two reference cigarettes [46].

Analyte	Type 1 (original, THP 1.0 T) (bright tobacco consumable)	Type 1 (original, THP 1.0 M) (menthol consumable)	Reference cigarette (3R4F)	Reference cigarette (1R6F)
Nicotine (mg/item)	0.446	0.365	2.02	2.00
Carbon monoxide (mg per stick)	NQ (0.233)	NQ (0.233)	32 ± 0.9	29.4 ± 0.6
Formaldehyde (μg per stick)	3.29 ± 0.30	3.51 ± 0.54	54.1 ± 6.0	68.4 ± 3.9
Acetaldehyde (μg per stick)	111 ± 8	115 ± 11	2200 ± 103	1859 ± 169
Acrolein (μg per stick)	2.22 ± 0.52	2.50 ± 0.11	157 ± 9	148 ± 22
1,3-butadiene (μg per stick)	BDL (0.029)	BDL (0.029)	108 ± 4	114 ± 4
Benzene (μg per stick)	NQ (0.056)	NQ (0.056)	78.6 ± 4.6	76.0 ± 5.8
Benzo(a)pyrene (ng per stick)	NQ (0.354)	0.356 ± 0.079	12.9 ± 1.3	11.4 ± 1.7
NNK (ng per stick)	6.61 ± 0.86	5.32 ± 0.89	281 ± 16	208 ± 7
NNN (ng per stick)	24.7 ± 2.5	19.1 ± 2.2	263 ± 12	191 ± 8

7.2.4. Indoor air quality (IAQ) studies

The indoor air quality (IAQ) studies involve volunteers spending several sessions of four hours in an environmentally controlled room set with air changes representing either residential, office or hospitality ventilation settings, and either smoking conventional cigarettes, using THP Type 1, original, or just sitting (to provide background readings with human activity). Measurements of CO, CO_2, NO_x, ozone, various fractions of particulate matter and volatile organic compounds were taken from the indoor air. Unlike a cigarette, where significant emissions are emitted into the air from the lit end, particularly between puffs, THP Type 1, original, does not generate sidestream emissions between puffs, though there will be a minor contribution to room air from exhaled THP emissions.

Significant reductions in ambient air levels of product emissions relative to conventional cigarettes were found for THP Type 1, original [26]. Most test analytes were below detectable levels or did not exceed baseline levels for the condition where the THP was used. For those that were measurable, namely nicotine, acetaldehyde and formaldehyde, the levels were typically $>$ 90 % lower than those from cigarette smoke. Particulate matter emitted from the THP was also $>$ 90 % reduced relative to cigarette smoke emissions within the laboratory conditions defined [26].

7.3. In vitro toxicology

7.3.1. Regulatory pre-clinical in vitro toxicology

Table 6 provides a summary of the key findings for all in vitro toxicological studies.

7.3.1.1. Regulatory in vitro toxicology. A number of toxicological studies have been conducted on THP Type 1 using various aerosol fractions, depending on the assay. Most of the studies have focused on the total particulate material captured on a Cambridge filter pad and eluted in DMSO up to 1 %. A smaller number of studies have employed an aqueous captured fraction termed 'aqueous extract' where the aerosol is bubbled through an aqueous trap which captures the volatile, semi-volatile and particulate compounds at various extraction efficiencies. Whole aerosol approaches do not fractionate the aerosol as per other approaches and capture the interactions between both the vapour and particulate phase in a manner more representative of consumer use.

As discussed, THP Type 1, original, has been investigated using a battery of in vitro mutagenicity and carcinogenicity toxicological assays conducted in accordance with GLP, following established international test guidelines. Across all assays, including the bacterial reverse mutation assay (Ames), in vitro micronucleus (IVMN), mouse lymphoma (MLA), Bhas 42 cell transformation assay and the neutral red uptake assay (NRU), results have all shown either limited activity or significant reductions in activity in tests of THP Type 1, original, emissions as compared to cigarette smoke [31,32,49–52].

Assessments of the THP particulate and whole aerosol using the bacterial reverse (Ames) assays found the THP emissions were non-mutagenic in all five-tester strains under conditions assessed. In contrast, cigarette smoke showed clear mutagenic activity. Both studies support the observation that THP emissions were non-mutagenic using the Ames assay [50,51]. Mixed responses were observed for MLA and IVMN assays. In the mouse lymphoma assay (MLA) mutagenic responses were found for both cigarette smoke and THP gas vapour phase (GVP) emissions, with the THP emissions giving a response 5–12 times lower than for cigarette smoke [51]. In contrast, another study found no mutagenic responses to THP particulate fraction using a cigarette smoke comparative study design [50]. Using the IVMN, THP particulate fraction was found to positively induce micronuclei formation at significantly higher doses than cigarette smoke [52]. However, another study found no activity to THP particulate fraction in either classical IVMN approaches or using contemporary screening methodologies, using a variety of cell lines including TK6 cells [49]. The Bhas 42 cell transformation assay was used to identify activity related to tumour promotion. Cigarette smoke was seen to cause changes related to tumour promotion while the THP emission samples showed no tumour promoting activity [50,52].

Studies utilising whole THP aerosols have been conducted to assess the toxicological impact of THP emissions [53]. Cytotoxicity was tested using the Neutral Red Uptake (NRU) assay. Because the toxic potential of the THP Type 1, original, and cigarette smoke are so different, dilution of cigarette smoke before exposure was essential to make any comparisons. Nicotine levels in cellular media were used to ascertain reasonably comparative exposures. At a dilution of 1:40 the cigarette smoke aerosol gave complete loss of cell viability while the THP aerosol still showed around 87 % viability. Even at the highest concentration (1 in 2 dilution) after a one-hour exposure complete cytotoxicity was not observed in the THP Type 1, original, aerosol [53].

7.3.1.2. Contemporary in vitro toxicology. A range of contemporary in vitro toxicology tests have been encouraged following the US National Research Council's report "Toxicity Testing in the 21st Century: A vision and a strategy" [28] (NRC 2018), and since the US Environmental Protection Agency's (EPA) introduction of ToxCast [54]. Contemporary approaches include those assays with a focus on toxicological disease (COPD, CVD, Cancer) relevant endpoints or mechanistic based approaches such as those used to assess oxidative stress. In addition, these approaches can be used to support an AOP framework and can also be used to extrapolate between in vitro and the in vivo setting. Discussed below are some examples of where THP have been assessed using these new contemporary or disease mechanistic toxicological approaches.

To complement the above toxicological assays advocated by regulatory authorities, a number of contemporary toxicity testing approaches have been included in the pre-clinical assessment of the THP Type 1, original, including screening assays such as high content screening approaches (HCS) and pathway-based assessments to obtain a multitude of readouts on pathways that may be involved in toxicity and disease. In addition, endpoints associated with CVD and COPD have been explored, while in vitro disease models have been used to gain further insight into disease mechanisms and the activation of key disease processes. A global untargeted systems biology approach has been conducted on 3D primary human lung cells to investigate the genomic response following exposures. In all cases, the assays and endpoints consistently show that exposure to reference cigarette TPM, AqE or smoke resulted in significant cellular and genomic responses that were measurable and consistent. The measurements show that many key pathways such as inflammation were activated and are likely to contribute to injury and disease. At doses equivalent to reference cigarette smoke, the THP Type 1, original, aerosol, TPM or AqE did not induce a significant response in any of the assays or endpoints tested [31,32,55–57].

For example, cell count, nuclear size, DNA structure, mitochondrial mass, mitochondrial membrane potential, formation of reactive oxygen species, glutathione content, cellular ATP, DNA damage and c-Jun stress kinase activation were all assessed in (H292) human lung epithelial cells using high-content screening. Results reported distinct toxicological activity of cigarette smoke and reduced or no activity of the emissions from the THPs [55,56]. Results also showed that THP responses were consistent between laboratories in an interlaboratory comparison using multiple oxidative stress and cytotoxicity assessments. The study demonstrated that THP Type 1, original, induced a very low level of oxidative stress and limited cytotoxicity, whereas cigarette smoke induced significant toxicity and oxidative stress up to 40-fold greater than baseline using particulate test matrices. In contrast, THP Type 1, original, produced only a 2-fold change in oxidative stress above baseline.

To assess vascular injury and impairment in response to cigarette

Table 6
Summary of in vitro toxicology studies emissions from a THP Type 1 and two reference cigarettes.

Assay	End point	Guideline	Test matrix	THP vs. Cigarette smoke dose	Observation	Ref
Classical Toxicology						
Neutral red uptake assay (NRU)	Acute toxicity testing	OECD 432	TPM	THP exceeding cigarette smoke doses. 240 μg/ml THP TPM top dose vs. cigarette TPM 140 μg/ml	Cigarette smoke cytotoxic. THP TPM deemed non-toxic at doses exceeding cigarette smoke	[50]
Neutral red uptake assay (NRU)	Acute toxicity testing	OECD 432	WA	THP exceeding cigarette smoke doses. 15,050 ng/ml THP top dose vs. cigarette 7863 ng/ml	Cigarette smoke cytotoxic. Low levels of cytotoxicity observed for THPs only at nicotine doses exceeding cigarette smoke	[53]
Bacterial reverse mutation assay (Ames)	Mutagenicity	OECD 471	TPM	THP and cigarette smoke at comparable doses. 2400 μg/plate THP TPM vs. cigarette TPM 2400 μg/ml	Cigarette positive in strains TA98, TA100 and TA1537. THP deemed non-mutagenic in all OECD five strains +/- metabolic activation	[50]
Bacterial reverse mutation assay (Ames)	Mutagenicity	OECD 471	WA	THP and cigarette smoke at comparable doses. ~240 μg/cm^2 deposited mass THP aerosol vs. ~240 μg/cm^2 deposited mass cigarette aerosol	Cigarette positive in strains TA98, TA100 and TA1537. THP deemed non-mutagenic in all OECD five strains +/- metabolic activation	[50]
Mouse lymphoma assay (MLA)	Mammalian Genotoxicity	OECD 490	TPM	THP exceeding cigarette smoke. 240 μg/ml THP TPM vs. cigarette TPM < 180 μg/ml (depending on treatment conditions)	Cigarette smoke genotoxic under all treatment conditions (+/- short and long). THP non-genotoxic under all conditions	[50]
Mouse lymphoma assay (MLA)	Mammalian Genotoxicity	OECD 490	GVP	THP exceeding cigarette smoke doses. 200 μg/TPMeq/ml THP GVP vs. < 250 μg/TPMeq/ml cigarette GVP (depending on treatment conditions)	Cigarette smoke positive under all treatment conditions (+/- short and long). THP only genotoxic at doses far exceeding cigarette smoke	[51]
In vitro micronucleus (IVMN)	Genotoxicity	OECD 487	TPM	THP exceeding cigarette smoke doses. 500 μg/ml THP TPM vs. cigarette smoke TPM < 140 μg/ml (depending on treatment conditions and cell lines)	Cigarette smoke positive under all treatment conditions (+/- short and long). THP negative at doses exceeding cigarette smoke using classical and contemporary scoring approaches	[49]
In vitro micronucleus (IVMN)	Genotoxicity	OECD 487	GVP	THP exceeding cigarette smoke doses. 1500 μg/ml THP TPM vs. < 200 μg/ml cigarette smoke TPM	Cigarette smoke positive under all treatment conditions (+/- short and long). THP activity observed only at doses 20x higher than cigarette smoke	[52]
Bhas 42 Cell transformation assay (Bhas)	Tumour Promotion	OECD Draft TG[1]	TPM	THP exceeding cigarette smoke doses. 120 μg/ml THP TPM vs. cigarette TPM 50 μg/ml	Cigarette smoke positive, no response observed for THP	[49, 52]
21st Century Toxicology						
Antioxidant response element (ARE)	Oxidative stress	n/a	TPM	THP and cigarette smoke at comparable doses. 200 μg/ml THP TPM vs. 200 μg/ml cigarette smoke TPM	Cigarette smoke induced significant oxidative stress and upregulation of ARE (>30-fold induction). THP produced limited increases in oxidative stress (<2.5 -fold induction)	[56]
High content screening approaches	ATP, Cell count, Glutathione content, Mitochondrial membrane potential, DNA damage	n/a	TPM	THP and cigarette smoke at comparable doses. 120 μg/ml THP TPM vs. 120 μg/ml cigarette smoke TPM	Cigarette smoke induced significant cellular changes in metabolism (ATP), toxicity (cell count), oxidative stress (GSH), DNA damage (p-H2AX) and impaired mitochondrial membrane potential. No effects were observed for THP	[56]
High content screening approaches	Glutathione, cytotoxicity	n/a	TPM	THP and cigarette smoke at comparable doses. 120 μg/ml THP TPM vs. 120 μg/ml cigarette smoke TPM	Induced oxidative stress and cytotoxicity in response to cigarette smoke exposure. Limited oxidative stress or cytotoxicity observed for THPs	[55]
Flow cytometry	Genotoxicity	OECD 487	TPM	THP exceeding cigarette smoke doses. 500 μg/ml THP TPM vs. cigarette smoke TPM < 140 μg/ml (depending on treatment conditions and cell lines)	Cigarette smoke positively induced micronuclei whereas THPs failed to elicit a response	[49]
Scratch assay	Vascular endothelial impairment	n/a	AqE	THP and cigarette smoke at comparable nicotine doses. 2500 ng/ml THP AqE (100 %) vs. cigarette ~2500 ng/ml (40 %)	Cigarette smoke significantly impaired endothelial cell migration, whereas, THP extracts had no obvious effect at concentrations far exceeding cigarette smoke	[32]
Lung 3D functionality	GCH, AA, TEER, CBF	n/a	WA	THP and cigarette smoke at comparable nicotine doses. ~1000 ng/ml THP WA (1:100	Repeated exposure to cigarette smoke resulted in significant increases in mucin producing MUC5AC cell populations, representing a clinically relevant in vitro endpoint. AA, TEERs and CBF were all	[31]

(*continued on next page*)

Table 6 (*continued*)

Assay	End point	Guideline	Test matrix	THP vs. Cigarette smoke dose	Observation	Ref
				dilution) vs. cigarette ~1000 ng/ml (1:40 dilution)	adversely effected in response to cigarette smoke exposure, demonstrating reduce lung function. THP aerosol did not adversely affect the cells and MUC5AC over-production was not observed	

Abbreviations: THP = tobacco heating product, WA = whole aerosol; GVP = gas vapour phase; n/a = not applicable; IVMN = in vitro micronucleus; AqE = aqueous extracts; GCH = goblet cell hyperplasia; TEER = transepithelial resistance; CBF= cilia beating frequency; AA = active area; TPM = total particulate matter

[1] Bhas_42_CTA_TG_HRI_Draft_Rev_7.pdf (oecd.org). The Bhas 42 cell are established from BALB/c 3T3 cells by the transfection of v-Ha-ras gene.

smoke and THPs, an endothelial migration assay was employed. To mimic the process of maintaining healthy blood vessels, uniform scratches were made on human umbilical vein endothelial cells to represent vascular damage. The tissues were exposed to either aqueous extracts of cigarette smoke or THP Type 1, original, emissions [32] and the ability of the tissue to repair the "wound" was recorded. Cigarette smoke caused cytotoxicity and vascular impairment, while THP extracts did not. A further mechanistic study investigated transcriptomic perturbations within 3D primary human lung cells resulting from either exposure to cigarette smoke or the emissions from THP Type 1, original [57]. 2809 RNAs were differentially expressed when exposed to cigarette smoke compared to 115 with THP aerosols. Quantification of a cytokine panel post-exposure found a pro-inflammatory effect of cigarette smoke but not for THP Type 1, original, emissions. Finally, in a similar study design using whole aerosol and 3D primary human lung cells, goblet cell hyperplasia (GCH) was investigated as a clinical phenotype of COPD coupled with cilia activity. Here the authors were able to demonstrate that cigarette smoke exposure increased the production of mucus secreting cells via MUC5AC expression with proportional decreases in cilia function and activity. Conversely, THP exposure did not result in increased mucin production or destruction of cellular tight junctions or similarly observed decreases in cilia or cilia activity [31].

7.4. Clinical studies

7.4.1. Biomarkers of exposure (BoE) clinical studies

Two short term clinical studies of similar design have been conducted, one in Japan [35] and the other in the UK [36], and one long-term study conducted in the UK [37]. For the short-term studies all volunteers were current and regular cigarette smokers and were randomised into one of three groups; continue to smoke, switch to THP or quit smoking without using any tobacco product. The volunteers were confined to clinic throughout the study period and so compliance to the protocol was likely to be high. In the Japanese study, which studied 180 volunteers, because of the high prevalence of smokers of mentholated cigarettes, mentholated products were included in the study as well as non-mentholated products. Non-menthol smokers were randomised between continuing to smoke, switching to a non-menthol (THP 1.0 T) consumable or smoking cessation, while the menthol smokers were randomised between continue to smoke, switching to a mentholated (THP 1.0 M) consumable or cessation. The UK study was of a similar design but because mentholated cigarette smoking was far less prevalent in the UK, it only included non-mentholated cigarettes and THP consumable (THP 1.0 T).

Table 7 gives the percentage of change in levels of BoE for both studies. In the study conducted in Japan, there was little difference in the percentage changes from baseline between the non-mentholated and mentholated consumables, and all were statistically significantly reduced at Day 7 as compared to baseline. This was also the case for BoE in the cessation group. For many BoEs, including e-CO (exhaled carbon monoxide), 3-HPMA (BoE for acrolein), HMPMA (BoE for crotonaldehyde), S-PMA (BoE for benzene), MHBMA (BoE for 1,3-butadiene), CEMA (BoE for acrylonitrile), 4-ABP (BoE for 4-aminobiphenyl), o-Tol (BoE for o-toluidine), 2-AN (BoE for 2-aminonaphthalene) and HEMA (BoE for ethylene oxide), the magnitude of reduction was similar in the switching to THP group and the smoking cessation group, indicating that the reductions in toxicant levels found in analytical chemical studies result in substantial reductions in actual exposure in volunteers. The BoE 1-OHP (a metabolite of the polycyclic aromatic hydrocarbon

Table 7
Percentage change in biomarkers of exposure between baseline and Day 7 in two short -term clinical studies for groups that continued to smoke, switched to THP 1.0 T or THP 1.0 M (both Type 1, original devices) or quit use of nicotine products [35,36].

BoE	Smoking[a] NM[d] Japan	Smoking M[e] Japan	THP 1.0 T[b] NM Japan	THP 1.0 M M Japan	Quit[c] Japan	Smoking NM UK	THP 1.0 T NM UK	Quit UK
eCO	+ 17 %	+ 18 % *f	87 % *	90 % *	87 % *	+ 5 %	81 % *	85 % *
TNeq	+ 11 %	+ 18 % *	25 % *	38 % *	94 % *	0	29 % *	97 % *
Total NNAL	+ 5 %	11 %	35 % *	37 % *	59 % *	3 %	26 % *	69 % *
Total NNN	0 %	+ 20 %	49 % *	52 % *	91 % *	+ 3 %	31 % *	77 % *
3-HPMA	+ 23 % *	+ 25 % *	53 % *	49 % *	47 % *	+ 12 %	72 % *	86 % *
HMPMA	+ 8 %	+ 6 %	79 % *	81 % *	80 % *	+ 1 %	84 % *	90 % *
S-PMA	+ 8 %	+ 5 %	89 % *	92 % *	91 % *	+ 18 %	93 % *	96 % *
MHBMA	+ 3 %	+ 7 %	91 % *	89 % *	85 % *	+ 5 %	82 % *	89 % *
CEMA	+ 3 %	+ 4 %	89 % *	88 % *	89 % *	+ 3 %	84 % *	89 % *
4-ABP	8 %	4 %	81 % *	82 % *	80 % *	+ 10 %	80 % *	86 % *
o-Tol	+ 18 %	+ 4 %	49 % *	63 % *	60 % *	1 %	70 % *	59 % *
2-AN	0	0	91 % *	90 % *	90 % *	+ 13 %	86 % *	91 % *
1-OHP	8 %	18 % *	64 % *	73 % *	82 % *	+ 5 %	48 % *	73 % *
HEMA	19 %	17 %	56 % *	61 % *	63 % *	12 %	61 % *	70 % *

Percentage reductions are mean difference/baseline mean x 100, with + indicating an increase rather than a reduction. (a) Continue to smoke from baseline to Day 7; (b) Switch to Type 1 (original) from baseline to Day 7; (c) Stop using nicotine products from baseline to Day 7; (d) NM – non-mentholated consumable; (e) M -mentholated consumable * statistically significant change, p value < 0.001; eCO – exhaled carbon monoxide; TNeq – total nicotine equivalents (nicotine, cotinine, 3-hydroxycotinine and their glucuronide conjugates); Total NNAL - 4-(methylnitrosamino)−1-(3-pyridyl)−1-butanol; Total NNN - N-nitrosonornicotine; 3-HPMA - 3-hydroxypropylmercapturic acid; HMPMA - 3-hydroxy-1-methylpropylmercapturic acid; S-PMA - S-phenylmercapturic acid; MHBMA - monohydroxybutenyl-mercapturic acid; CEMA - cyanoethylmercapturic acid; 4-ABP - 4-aminobiphenyl; o-Tol - o-toluidine; 2-AN −2-aminonaphthalene; 1-OHP - 1-hydroxypyrene; HEMA - 2-hydroxyethylmercapturic acid.

pyrene was used as an alternative BoE for Benzo[*a*]pyrene (McEwan et al. [36], Yuki et al., [69])) was reduced by 48–73 % in the group switching to the THP which was less than the reduction in the cessation groups of 73–82 %. For the two TSNAs measured, NNK (as total NNAL) and NNN (total NNN) the percentage reductions were between 35 % and 52 % for the group switching to the THP, less than those in the smoking cessation group of 59–91 %.

The UK short-term study gave similar findings to the one conducted in Japan. The data for the continuing to smoke arm were similar in both countries, with most volunteers maintaining or slightly increasing measured BoEs over the study period. For the arm that switched to the THP, the BoE reductions were similar to those reported in the study conducted in Japan, between baseline and end of the study for many of the BoEs (such as S-PMA, 4-ABP, 2-AN, HEMA) and a little different in some BoEs (for example 3-HPMA was reduced between 49 % and 53 % in Japan, and by 72 % in the UK). It is not possible to make calculations of statistical significance across the two studies. For the group of volunteers who quit smoking, the percentage reductions in BoEs were similar in the two studies except for 3-HPMA which was 47 % reduced in Japan and 86 % reduced in the UK.

Table 8 shows percentage changes in BoEs between baseline and Day 180 in the long-term ambulatory clinical study conducted in the UK [45]. Such a study is more susceptible to a lack of compliance with protocol as subjects only visit the clinic on occasion. Any non-compliant dual use of cigarettes and THP in the switch to THP arm, or use of cigarettes in the cessation arm, would likely result in higher levels of BoEs than in groups that stayed compliant. Because of this, a biological marker of compliance, a haemoglobin adduct of acrylonitrile (CEVal) with a long half-life, was used as an additional way to assess volunteer compliance. The data showed similar results to the short-term studies, with percentage reductions in BoEs from baseline to Day 180 being similar for the groups switching to the THP (Type 1, original) and the group quitting (in this case many with assistance of nicotine replacement therapy) for most of the BoEs (e-CO, 3-HPMA, HMPMA, S-PMA, MHBMA, CEMA, 4-ABP, o-Tol, 2-AN and HEMA.). The TSNA biomarker reductions were 45–49 % in the switch to THP group and 73–75 % in the cessation group. The reductions in total nicotine equivalents were 33 % in the switch to THP group and 83 % in the cessation group, the latter less than found in the short-term clinical studies perhaps because of the use of NRT by subjects in the smoking cessation group in the long-term study.

The data suggest that exposure to many important toxicants in cigarette smoke are substantially reduced over the course of just a few days when either switching completely to the THP or when quitting smoking without THP use, and these reductions can be sustained for many months.

Table 8
Percentage reductions in biomarkers of exposure between Day 1 and Day 180 in groups that continued to smoke, switch to THP 1.0 T (Type 1, original) or quit smoking [45].

BoE	Continue to smoke	Switch to Type 1 (original)	Cessation
eCO	+ 5 %	81 %	88 %
TNeq	26 %	33 %	83 %
Total NNAL	+ 2 %	49 %	75 %
Total NNN	8 %	45 %	73 %
3-HPMA	1 %	68 %	71 %
HMPMA	15 %	75 %	75 %
S-PMA	10 %	91 %	87 %
MHBMA	10 %	91 %	88 %
CEMA	+ 3 %	93 %	87 %
4-ABP	11 %	78 %	75 %
o-Tol	27 %	67 %	58 %
2-AN	5 %	85 %	85 %
1-OHP	+ 25 %	33 %	64 %
HEMA	52 %	84 %	84 %

eCO – exhaled carbon monoxide; TNeq – total nicotine equivalents (nicotine, cotinine, 3-hydroxycotinine and their glucuronide conjugates); Total NNAL - 4-(methylnitrosamino)−1-(3-pyridyl)−1-butanol; Total NNN - N-nitrosonornicotine; 3-HPMA - 3-hydroxypropylmercapturic acid; HMPMA - 3-hydroxy-1-methylpropylmercapturic acid; S-PMA - S-phenylmercapturic acid; MHBMA - monohydroxybutenyl-mercapturic acid; CEMA -cyanoethylmercapturic acid; 4-ABP - 4-aminobiphenyl; o-Tol - o-toluidine; 2-AN −2-aminonaphthalene; 1-OHP - 1-hydroxypyrene; HEMA - 2-hydroxyethylmercapturic acid

7.4.2. *Biomarkers of potential harm (BoPH)*

BoPH are biomarkers that are related to biological processes thought to be involved in smoking-related diseases and that have been found to be reversible following smoking cessation. The long term clinical study described above [45] collected data on BoPH in urine, 11-dehydrothromboxane B2 [11-dTx B2] (an indicator of platelet activation/coagulation and related to cardiovascular disease (CVD)), 8-epi-prostaglandin F2a type III [8-Epi-PGF2α type III] (an indicator of oxidative stress that may be involved in CVD, COPD and carcinogenesis); in whole blood, white blood cell [WBC] count (an indicator of general inflammation and associated with CVD, COPD, and carcinogenesis); in plasma, soluble intercellular adhesion molecule-1 [sICAM-1] (an indicator of endothelial dysfunction and associated with CVD); in serum, high-density lipoprotein [HDL]; (an indicator of lipid metabolism and associated with CVD) and exhaled breath (FeNO) (an indicator of bronchodilation and vascular tone associated with respiratory disease and CVD). Additionally, forced expiratory volume in 1 s (FEV_1) (an indicator of lung health and associated with respiratory disease) was assessed using spirometry. NNAL, often measured as a BoE, is also seen as a BoPH given its specificity to tobacco and its association with lung cancer risk.

These biomarkers will typically take much longer to change than most BoEs and will be more variable among a population as a result of varying genetics and exposures other than tobacco, with the exception being NNAL as a BoE to a TSNA. Hence the need for longer-term study and expectations that directional trends rather than statistically significant changes are likely to be the outcome. The study looked to see if biomarker level changes were different between the groups that continued to smoke, switched to the THP Type 1, original, or quit.

Table 9 presents the mean absolute change in BoPHs from baseline to Day 180, for subjects with a valid result at both baseline and Day 180 (the biomarker of compliance, CEVal, was used to remove data from subjects who were clearly not compliant with protocol in the group that switched to the THP). For 11-dTx B2, a favourable change would be a reduction in absolute levels. This BoPH was reduced in all three groups, but the reductions were much greater in the switch to THP (−274 ng/24 h) and the quit group (- 302 ng/24 h) compared to the continue to smoke group (−100 ng/24 h). For sICAM-1, where a lower value would also be favourable, there was a slight increase in the continue to smoke arm (+27 ng/ml) and a decrease in the switch to THP arm (−33 ng/ml) that was greater than in the quit arm (−10 ng/ml). For FEV_1, where an increase in value would be an indicator of a favourable change, the continue to smoke arm decreased (−3 %) while both the switch to THP and the quit arms improved slightly by 0.3 %.

There was little indication that levels of any of the BoPH markers were getting directionally worse during the six months of exposure in either the switch to THP or the cessation group, while some of the BoPH levels in the group that continued to smoke did continue to move in an unfavourable direction with regards to possible health risks.

7.5. *Abuse Liability studies*

An abuse liability study (Hardie et al., [41]) involving nicotine PK analysis along with subjective effects questionnaires was conducted. This showed that nicotine uptake in terms of Cmax was highest for the cigarette (22.7 ng/ml) compared to the THPs (8.6 for THP1.0(RT) and 10.5 ng/ml for THP1.1(RT)) with the NRT giving the lowest value (2.3 ng/ml). In addition, the subjective questionnaires for product liking

Table 9
Absolute value changes in biomarkers of potential harm between Day 1 and Day 180 [45].

BOPH	Continue to smoke	Switch to THP Type 1 (original)	Cessation	Direction of a beneficial change
11-dTx B2 (ng/24 h)	-100.45	-273.65	-302.06	Lower
8-Epi-PGF2α (ng/24 h)	-40.71	-116.43	-73.74	Lower
WBC (x 109/L)	-0.05	-1.24	-0.63	Lower
sICAM-1 (ng/ml)	+ 27.49	-32.98	-10.38	Lower
HDL (mmol/L)	-0.015	+ 0.081	-0.003	Higher
FeNO (ppb)	+ 0.30	+ 5.65	+ 9.78	Higher
FEV_1 %pred (%)	-2.69	+ 0.22	-0.41	Higher
NNAL (ng/ 24 h)	+ 4.70	-96.28	-163.67	Lower

11-dTx B2 - 11-dehydrothromboxane B2; 8-Epi-PGF2α - 8-epi-Prostaglandin F2a type III; WBC - white blood cell count; sICAM-1 - soluble intercellular adhesion molecule-1; HDL -high-density lipoprotein; FeNO – fractional concentration of exhaled nitric oxide; FEV1 %pred - forced expiratory volume in 1 s as % of predicted FEV1; NNAL - 4-(methylnitrosamino)−1-(3-pyridyl)−1-butanol

and overall intent to use again were highest in the cigarette, compared to the THPs and lowest values being reported for the NRT. Urge to smoke was reduced more by the cigarette than the other three products. Thus, these results suggest that the abuse liability of the cigarette was highest with the THPs lying between the usual brand cigarette and the NRT.

7.6. Population studies

Gathering data on THP consumption on a population level is important to assess both the impact and likelihood of a complete switch to THPs from smoking. One cross-sectional study was conducted during 2018 in three urban regions of Japan (a country where THP prevalence was higher than most), just a few years after the introduction of these products. Of around 4000 participants in the study, cigarette smoking was still the most prevalent form of tobacco use, but 27 % of tobacco users reported use of THPs [38]. The study was a follow up of a similar study undertaken in 2017 and found that while 67.4 % of tobacco users were still smoking cigarettes, 14.6 % had switched to THP use only and 10.5 % were dual users of THP and cigarettes. There was little evidence of a gateway effect of never users starting to use THPs and then switching to cigarettes. The quality of life (QOL) survey suggested some improvements in those switching to a THP including a reduction in self-reported frequency of cough. The most frequent reason reported for switching to THPs was reducing harm to others and to self [58].

In a population health modelling study of THPs in Japan, assuming equal risk for dual use and smoking, it was estimated that THP risk would need to be at least 10 % lower than smoking to achieve a population health benefit by 2100. Potential reduction in life-years lost due to smoking with the introduction of THPs was 13 million by 2100 compared with a scenario where THPs did not exist [39].

7.7. Bridging studies

Studies on data bridging for THP product iterations have considered three possible scenarios: comparing different consumables (i.e. changes in the tobacco composition or the ingredients used) in the same device; comparing the same method of heating but in devices of different dimensions; or comparing devices with different forms of heating and maximum temperatures.

Table 1 provides details of the formats of devices, consumables, and typical operating conditions for glo™ used in the bridging studies. Table 5 provides emissions analysis of the TobReg 9 mean toxicant levels using the same device (THP Type 1, original) but two different consumables of a similar format, one a blend of Virginia tobacco reconstituted and including 15 % glycerol dry weight basis, and the other of a similar blend but with menthol added. The toxicant levels found in the emissions of the two consumables were similar and were always substantially lower than the emissions of either of the reference cigarettes.

Table 10 considers the percentage reductions compared to the emissions of 1R6F reference cigarette for three types of THP Type 1 devices (in three different sizes, original, mini and nano). The same tobacco consumable was used for all tests and was a commercially available Rich Tobacco blend in a KSSS format. For analytes that were not expected to be formed in a THP, similar reductions were found across the series (for example, 99.5 % for CO, 99.9 % for 1,3-butadiene and 99.9 % for benzene). For toxicants that are expected to be present in THP emissions, similar reductions were found (for example, 97.3–97.5 % for formaldehyde, 95.0–95.8 % for acetaldehyde, 96.5–97.2 % for NNK and 89.8–93.9 % for NNN.). All versions gave greater than 90 % reductions apart for one case (NNK from THP1.0 nano which was 89.8 % reduced) suggesting that the principle of heat not burn was maintained across these variants as in the original version of the product.

Table 11 compares mean toxicant levels and percentage reductions compared to reference cigarette 1R4F for THP, Type 1, original, device which heats to around 240 °C through thin-film resistive heating and a king-size super slim combustible, and the Type 3, hyper, device that uses both induction heating and a larger demi-slim tobacco consumable. The same tobacco blend was used for all tests, though there was a greater weight of tobacco in THP Type 3, hyper. For THP Type 3, hyper, the test was conducted in both its Standard mode (maximum temperature 250 °C) and its Boost mode (maximum temperature 260 °C). Comparing the original THP Type 1 with the newer THP Type 3, hyper, in Standard mode (i.e., increase in tobacco weight and operating temperature) levels of carbon monoxide, 1,3-butadiene and benzo(a)pyrene were identical within any analytical variability, levels of NNK, NNN and benzene were similar, and levels of formaldehyde, acetaldehyde and acrolein were slightly higher, though still substantially reduced compared to the reference cigarette. Comparing the original THP Type 1 with THP Type 3, hyper, at Boost temperature (i.e., maximum temperature of 260 °C and greater tobacco weight, levels of NNK and NNN were reduced slightly, levels of 1,3-butadiene and benzo(a)pyrene were identical, levels of benzene and carbon monoxide were similar, and levels of

Table 10
Percentage reductions in toxicants for several sizes of the device THP Type 1 as compared to the reference cigarette 1R6F.

Analyte	THP 1.0 T, original[a] KSSS	THP 1.0 T mini[b] KSSS	THP 1.0 T nano[c] KSSS
Carbon monoxide	99.5	99.5	99.5
Formaldehyde	97.3	97.5	97.5
Acetaldehyde	95.8	95.6	95.0
Acrolein	99.1	99.1	99.0
1,3-butadiene	> 99.9	> 99.9	> 99.9
Benzene	> 99.9	99.9	99.9
Benzo(a) pyrene	98.6	98.4	98.4
NNK[d]	97.2	96.6	96.5
NNN[e]	93.9	90.6	89.8

All analytical chemistry was performed at Labstat, Kitchener, ON, Canada using Health Canada Intense smoking regime with vents unblocked for THP, vents blocked for 1R6F

[a] Original size, thin-film resistive heating, 40 s to first puff, 3.5 min heating at 240 °C. King-size super slim (KSSS) consumable. Nicotine 0.607 mg/item

[b] Smaller size, thin-film resistive heating, 40 s to first puff, 3.5 min heating at 240 °C, KSSS. Nicotine 0.635 mg/item.

[c] Smallest size, thin-film resistive heating, 40 s to first puff, 3.5 min heating at 240 °C, KSSS. Nicotine 0.506 mg/item.

[d] Nicotine-derived nitrosamine ketone.

[e] *N*-Nitrosonornicotine.

Table 11
Comparisons of the levels and percentage reductions compared to 1R6F emissions in emissions of TobReg 9 analytes between the original Type 1 (original) and THP Type 3 (hyper, DS consumable) (at two different maximum operating temperatures).

Analyte	THP Type 1 (original) KSSS	THP Type 3 (hyper, Standard mode) DS	THP Type 3 (hyper, Boost mode) DS
Maximum operating temperature (°C)	240	250	260
Nicotine (mg/item)	0.462	0.613	0.596
Carbon monoxide (mg per stick)	NQ < 0.22 (>99.5)	NQ < 0.22 (>99.3)	0.241 (99.1)
Formaldehyde (μg per stick)	3.29 (95.2)	2.21 (95.2)	1.93 (95.8)
Acetaldehyde (μg per stick)	111 (94.0)	131 (91.4)	133 (91.3)
Acrolein (μg per stick)	2.22 (98.5)	3.02 (98.1)	2.58 (98.4)
1,3-butadiene (μg per stick)	BDL < 0.029 (> 99.9)	BDL > 0.029 (>99.9)	BDL < 0.029 (>99.9)
Benzene (μg per stick)	NQ < 0.056 (> 99.9)	0.066 (99.9)	0.060 (99.9)
Benzo(a)pyrene (ng per stick)	NQ < 0.35 (> 97.4)	NQ < 0.35 (>97.8)	NQ < 0.35 (>98.4)
NNKd (ng per stick)	6.61 (96.8)	7.49 (95.9)	4.46 (97.8)
NNNe (ng per stick)	24.7 (87)	26.5 (83.5)	18.1 (88.7)

formaldehyde, acetaldehyde and acrolein were slightly increased, though again all substantially reduced compared to the reference cigarette emissions).

In a study by Jaunky et al. [59], six variants of the glo™ THP Type 1, original, (including the original THP 1.0 T) and THS2.2 were evaluated against a scientific reference cigarette using analytical chemical measurement of toxicants and in vitro toxicology. The variants included changes in flavour ingredients, nicotine content and emissions, aesthetics and the addition of a foil wrap for the tobacco consumable. The chemical analysis found that the five new variants had similar toxicant profiles, based on the original TobReg9 list of priority toxicants [18] and were all (when toxicant reductions are combined) between 94 % and 97 % significantly reduced compared to the reference cigarette. All variants' emissions were significantly less cytotoxic, using an aerosol-based air-liquid interface approach, than the reference cigarette smoke. Combining emissions chemistry with toxicological data, the THP variants showed a 94 % reduction compared to the reference cigarette, and lower toxicity compared to the other THP comparator THS2.2.

The ToxTracker assay suite was used to compare the genotoxic potential of aqueous extracts from original THP Type 1 and 3, hyper. The extracts were tested at multiple concentrations to build dose-response curves at relevant concentration ranges (Fig. 2). The ToxTracker assay is considered positive for a particular endpoint if the fold-change in reporter gene expression is at least two-fold compared to the 0 % concentration baseline [33,63,64]. A 1.5-fold-change is also considered an early indication of product deviation from the baseline. For the purposes of bridging, the point of departure chosen to perform comparisons between products is the tested concentration at which original THP Type 1 passes the 1.5-fold-change; this is to allow comparisons between products even when original THP Type 1 does not cross the 2-fold threshold. When the THP samples do not cross the 1.5-fold-change threshold, comparisons are performed at the highest concentration tested.

Table 12 shows the numerical estimates of the fold-change in reporter gene expression between original THP Type 1 and Type 3, hyper, and the corresponding values of fold-change in reporter gene expression for each product compared to baseline, upon which the comparison between products is calculated. In all cases, fold-changes between the two products calculated at the point of departure are below the 2-fold-change threshold.

Consistent with the similarity in toxicant emissions between original THP Type 1, original, and 3, hyper, in this assay, there were similar toxicological results despite the change in format, weight of tobacco and maximum temperature in THP Type 3, hyper, compared to Type 1, original, suggesting that bridging between the two products is possible.

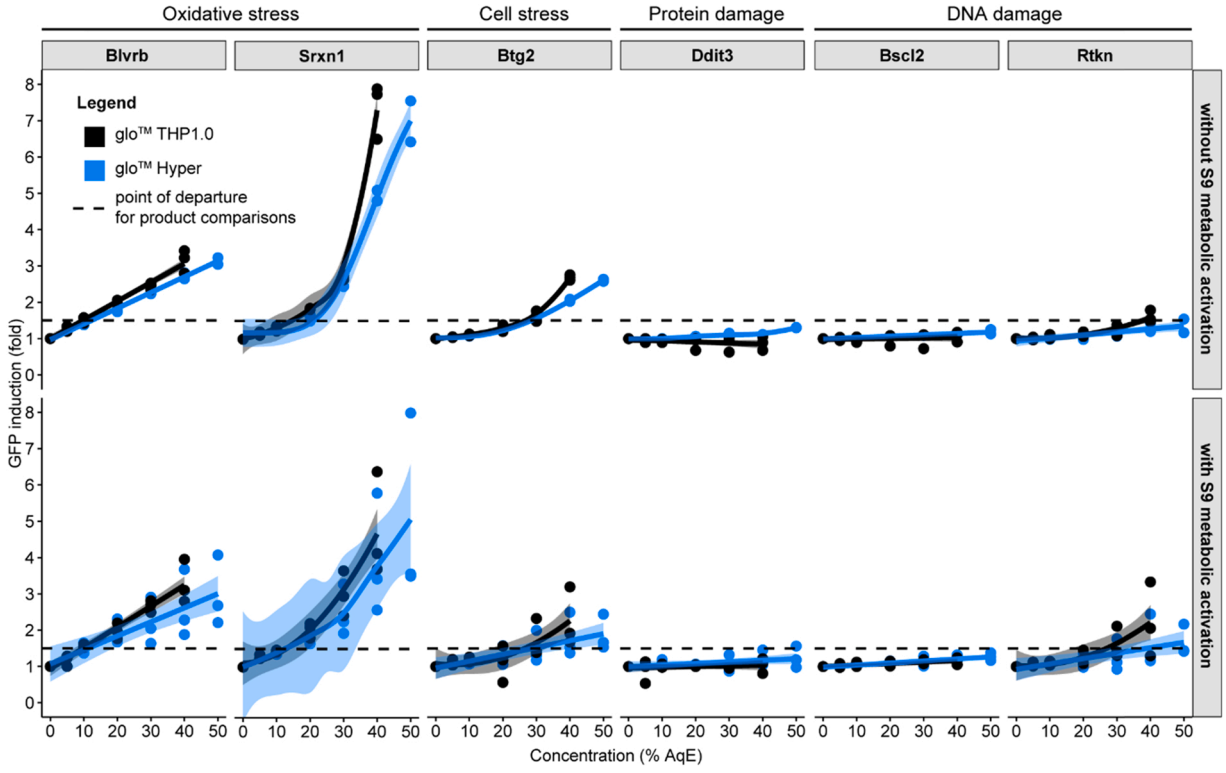

Fig. 2. ToxTracker assay suite for THP Type 1 (original, in black) compared to THP Type 3 (hyper+, in blue). Curves show bests fit and 95 % confidence interval of the fit.

Table 12
Fold change in GFP induction values compared to baseline (mean ± standard deviation) and fold-changes in gene expression between THP Type 3 (hyper+) and THP Type 1 (original) for each endpoint tested in the ToxTracker suite. Values calculated at the first concentration tested above the selected point of departure [59].

Products	Parameter	BLVRB	BLVRB	SRXN1	SRXN1	BTG2	BTG2
		-S9	+S9	-S9	+S9	-S9	+S9
THP Type 1	Fold change to baseline	1.52 ± 0.09	1.56 ± 0.05	1.83 ± 0.02	2.03 ± 0.21	1.63 ± 0.14	1.72 ± 0.53
THP Type 3	Fold change to baseline	1.41 ± 0.04	1.49 ± 0.14	1.58 ± 0.12	1.86 ± 0.26	1.55 ± 0.04	1.49 ± 0.44
THP Type 3 cf THP Type 1	Fold change to Series 1	0.93	0.96	0.86	0.92	0.95	0.87
Products	Parameter	Ddit3	Ddit3	BSCL2	BSCL2	RTKN	RTKN
		-S9	+S9	-S9	+S9	-S9	+S9
THP Type 1	Fold change to baseline	0.87 ± 0.19	1.04 ± 0.21	1.07 ± 0.14	1.16 ± 0.10	1.59 ± 0.17	1.61 ± 0.44
THP Type 3	Fold change to baseline	1.11 ± 0.01	1.20 ± 0.23	1.14 ± 0.04	1.22 ± 0.09	1.32 ± 0.17	1.27 ± 0.44
THP Type 3 cf THP Type 1	Fold change to Series 1	1.28	1.15	1.07	1.05	0.83	0.79

GFP – green fluorescent protein; BLVRB (oxidative stress), SRX1 (oxidative stress), BTG2 (cell stress), Ddit3 (protein damage), BSCL2 (DNA damage), RTKN (DNA damage) – reporter cell lines; -S9 without metabolic activation; +S9 with metabolic activation

Consideration of consumer use and behaviour is important when determining the potential health impact of new product iterations. The average daily consumption (ADC) of the original, Type 1 device was reported in the long term 180-day clinical study, investigating changes in BoEs and BoPHs, to be 22 ± 10 sticks per day at the 180-day point [35,37]. In a Japanese consumer behaviour study using the original, Type 1 device, an ADC of 10 ± 6 sticks per day was reported [22]. The ADC of Type 3 products is expected to be substantially lower than that reported for the Type 1 device in the above clinical study, as whilst there have been changes to the heater technology, the user behaviour should remain unchanged.

8. Discussion

Public health authorities are reviewing the growing evidence on THPs, generated from manufacturers, independent researchers and academic studies. Many agree that exclusive use of alternative tobacco and nicotine products, including THPs, is likely to be much less harmful than smoking cigarettes [7,16]. Public Health England [7] and the UK Committee on Toxicology (COT) [16] concluded that THPs are likely to result in reduced exposure to tobacco smoke toxicants and COT took the view that they were likely to present reduced health risks compared to continued smoking but not as greatly reduced as occurs after cessation of all tobacco product use. The WHO Study Group on Tobacco Product Regulation (TobReg) reported that more independent science was needed to determine the risks associated with THPs [12], the WHO takes a similar view with respect to ENDS [60]. The US FDA, to date, has granted marketing authorisation for a range of THPs from one manufacturer for reduced exposure but not reduced risk marketing claims.

The studies on original THP Type 1 discussed here show a strong consistency in the large differences between this THP and that of conventional cigarettes for chemical emissions, biological activity in toxicological tests and long-term toxicant exposure in clinical studies.

Chemical studies show that where the principle of heat-not-burn is maintained many of the toxicants found in tobacco smoke are not present in the emissions from the studied THPs (eg 1,3-butadiene, acrylonitrile, benzene) and other toxicants are substantially reduced. Studies also find few additional substances being formed by the THP process or with interactions of the tobacco with the device. Indoor air quality studies show a substantially lower impact of THP use on IAQ as compared to cigarette smoking.

Toxicological studies of the THPs consistently have shown reduced biological activity, compared to cigarette smoke, across a wide range of toxicological end-points. The emissions from original THP 1.0 T, Type 1 are, in comparison to cigarette smoke, less toxicologically active in regulatory tests for cytotoxicity, mutagenicity and genotoxicity, and contemporary toxicological tests report striking differences between cigarette smoke exposure and the THP aerosol exposure for a variety of end-points associated with smoking-related disease development. We take a weight of evidence approach to data across different types of studies, not in a formalised manner where different weights are given to different types of evidence, but rather in an analysis of whether the data across studies is consistently in a similar direction or whether there are inconsistencies across the data sets. When reviewed in this way, the data show that for THPs where a response is observed, such responses are significantly lower in activity when compared to traditional combustible cigarettes.

Short- and long-term clinical studies show substantial reductions in a wide range of BoE, more similar to volunteers that quit smoking than volunteers who continue to smoke. The long-term clinical study of original THP 1.0 T, Type 1 found favourable changes in BoPH, again similar in the group that switched to the THP and the group that quit smoking, and no unfavourable changes. In contrast, the group that continued to smoke showed some unfavourable changes in BoPH six months into the study.

Several of the THP 1.0 T studies have included a THP from another manufacturer THS2.2 (IQOS from Philip Morris International (PMI)) and there are substantial published datasets on this product looking at similar studies to those described above as well as several in vivo toxicology studies not undertaken by BAT. THS2.2 heats to a higher temperature and in a different manner than THP 1.0 T., THS2.2 studies have been conducted to ensure that the increased temperature does not adversely alter relevant toxicological parameters established through studies of THP 1.0 T.

From the BAT studies, at equivalent doses to cigarette smoke, the commercially available THP 1.0 T and THS2.2 emissions produced substantially lower toxicological activity when compared to cigarette smoke across a range of in vitro toxicological endpoints [50]. In a study comparing transcriptomic perturbations after acute exposure to 3D primary human lung cells, both THP 1.0 T and THS2.2 gave much lower numbers of genesets being differentially expressed compared to cigarette smoke [56].

The 8th Report of the WHO Study Group on Tobacco Product Regulation [12] reviewed behavioural, chemical, toxicological and clinical data published by BAT, PMI and academic researchers. The report noted that the levels of many harmful constituents that originate from the combustion process in cigarettes were consistently significantly lower in the THP aerosols than in cigarette smoke.

In comparing BAT, PMI and academic researchers' data on THP 1.0 T and THS2.2, the report noted that for many of the toxicants found in THP emissions (eg TSNAs, B(a)P, carbonyls, and some volatile organic compounds) both industry and academic researchers found much lower emission levels in THP emissions than in cigarette smoke, though absolute reductions varied with methods used.

The Report commented on several in vivo toxicological studies using experimental animals conducted by PMI. For example, in one in vivo study cardiovascular effects were examined and it was reported that continuous exposure to the THP aerosol did not affect atherosclerotic progression, heart function, left ventricular structure or the cardiovascular transcriptome [61] though the Report suggested that there were

non-statistically significant increases in many of the treatment outcomes in the animals treated with the THP emissions [12].

A 180-day long-term clinical study conducted by PMI [62] was evaluated in the Report, but the results of the BAT long-term study was not published at the time for analysis in the WHO Report (though have subsequently been published and are discussed here). The PMI long term study (at 180 days) reported reductions in BoEs in the group switched to the THP of between 16 % and 49 % in volunteers who reported mainly THP use during the study. The BAT long term study (at 180 days) reported reductions in BoE of between 33 % and 93 % in volunteers that confirmed protocol compliance with a long-term biomarker CeVal. Both studies reported directional favourable changes in some BoPH.

This comparison of data from these two different THPs suggests that the principle of operation, heat-not-burn, is likely to be the key factor in reducing users' long-term exposure to smoke toxicants, not the specific design of the individual products, however, this does influence other factors such as consumer preference.

The similarity in toxicant reduction levels within the range of variants from the original THP 1.0 T, including device size changes, consumable blend and flavour changes, increased operating temperature, increased weight of tobacco used and change method of heating, and the similarity between the findings of research on THP 1.0 T and THS2.2 suggests that as long as the principle of heat-not-burn is maintained in any variant from the original, the products should generally have substantially lower toxicant emissions resulting in lower biological activity in toxicological tests than cigarette smoke.

However, this general observation should be qualified. It assumes that any new features of subsequent versions of the device or the consumable are unlikely to affect consumption behaviour and that any new flavours do not change the toxicant profile by thermally degrading in the THP system. In our studies, doses were matched or exceed those doses for THP compared to cigarette smoke including nicotine and TPM measurements. Dosing beyond that of a cigarette does provide increased confidence in the results and the ability to translate to the variable THP consumer behaviour observed (see Table 3 for example). Ingredient selection should maintain the principle of avoiding ingredients of toxicological concern in unheated and heated forms.

The amount of data required to bridge between product versions will depend on the change made to the new products compared to the original, THP Type 1, original, device. A minimal change that does not impact on the pathway or process of aerosol formation, such as a change in material to the external surfaces of the device, should not require additional data. Substantial modifications that may require additional data collection include changes in heating profile that cause temperatures greater than those set for non-combustion (the limit criteria for carbon monoxide and nitrogen oxide); the use of a novel tobacco substrate with properties that could change the toxicant profile; and the use of technologies that might increase nicotine delivery to levels above those delivered by a cigarette. The data required in these cases to see if bridging to the original foundational data set is possible would include consumer behavioural studies and analytical chemical studies at first instance, followed by toxicological testing. If the data values produced on the new variant was outside of the range of data collected for the THP variants discussed in this paper, then further studies, including additional toxicological testing and clinical studies may be required.

While the bridging framework outline provides a foundation from which to demonstrate potential comparability between product iterations, there are some limitations. A consideration of the impact of product modifications on nicotine delivery should be noted to ensure that consumer consumption behaviour does not alter significantly compared to the original product and therefore impact chemical emissions and toxicology.

More work is required to evolve the statistical analysis of data generated; future studies may need to incorporate the use of a non-superiority analysis to demonstrate that newer THP iterations are comparable to the original dataset. Further investigation is also required to understand the nuances of how product changes, such as heater technology or consumable format, can impact consumer use and consumption, as well as on chemical emissions and toxicology. A bridging framework should be under constant scrutiny as products and technologies evolve and due to growing complexities.

The significant reductions in toxicant emissions and exposure, and little or no biological activity in toxicological tests between cigarette smoke and THP emissions, demonstrate that the glo™ original, Type 1, THP 1.0 T device and its current range of iterations are reduced exposure products compared to combustible cigarettes and can be reasonably deemed to reduce the risk of smoking-related diseases. Regulators in certain countries have formalised the process for this determination with mandatory authorizations with scientific data required to demonstrate reduced risk or exposure of new products, such as the Modified Risk Tobacco Product (MRTP) route set out by the FDA. THP use results in some toxicant exposure, albeit much lower than from cigarette smoking, as well as nicotine exposures similar to those provided by cigarettes and so, are likely to present some health risks and cause dependency. The health consequences for any individual switching from smoking to THP use, given what is known about the epidemiology of smoking cessation, is likely to vary depending on a variety of factors including age, history of smoking and susceptibility to diseases.

9. Conclusions

Cigarette smoking causes high levels of health risks for a large range of smoking-related diseases, many caused by persistent exposure over years to carcinogens and respiratory and cardiovascular toxicants in cigarette smoke. A substantial reduction in these toxicants in the emissions of THPs as compared to cigarette smoke has been observed in many studies and is a consequence of the lower heating conditions and absence of ignition and combustion. It has been demonstrated that smokers switching entirely to THPs reduce their toxicant exposure which is also reflected in their reduction of BoPH.

The data presented in this study, on original THP 1.0 T, Type 1 demonstrate that across a multi-disciplinary testing framework, behavioural, chemical, toxicological and clinical studies show good consistency and establishes large differences between cigarette smoking and THP. There is sufficient data to show that subsequent iterations of the original THP 1.0 T, Type 1 (albeit using different forms of heating, different amounts of tobacco used in the consumables and slightly different maximum operating temperatures) have maintained the principle of heat-not-burn, resulting in maintaining substantially reduced toxicant emissions and exposure to smoke toxicants, compared to smoking cigarettes. It is, therefore, possible to use bridging or read across approaches to future variants of the product as long as product stewardship standards and product quality standards are maintained and there is not a major change that could impact toxicant formation.

BAT has conducted a wide-ranging scientific evaluation of the individual and population-level health impact of the glo™ (THP 1.0 T, Type1, original) that encompasses many aspects of emissions chemistry, toxicological and biological properties, effects on users and non-users including changes in exposure and disease risk, and population dynamics. A multitude of studies conducted on the glo™, based on a weight-of-evidence approach, supports the conclusion that the glo™ is a reduced exposure tobacco product that provides a satisfying alternative to combustible cigarettes and is reasonably deemed to reduce the risk of developing smoking-related diseases associated with cigarette smoking for both smokers and non-smokers when smokers switch completely to using the glo™. The extent of reductions in risk compared to continuing to smoke are likely to vary by smoking-related disease and by an individual's smoking history, other risk factors and an individual's susceptibility to disease.

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Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: SG, NG, DT, SH, KP, IG, and FM were employees of a tobacco and nicotine product manufacturer, British American Tobacco (Investments) Limited at the time the studies were conducted. CP is a former employee of British American Tobacco (Investments) Limited and contributed as a paid consultant to British American Tobacco (Investments) Limited.

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