Contributions to Tobacco & Nicotine Research
formerly: Beiträge zur Tabakforschung International
Volume 31 · No. 1 · March 2022
DOI: 10.2478/cttr-2022-0001

Short Communication

An Experimental Analytical and *In Vitro* Approach to Bridge Between Different Heated Tobacco Product Variants *

by

Tomasz Jaunky, David Thorne, Andrew Baxter, Simone Hadley, Justin Frosina, Damien Breheny, James Murphy, and Marianna Gaça

British American Tobacco, R&D, Southampton, Hampshire SO15 8TL, UK

SUMMARY

Tobacco heating products (THPs) have reduced toxicant emissions relative to cigarettes. THPs are continually evolving, but safety and efficacy studies on each new variant involve considerable resources. As employed by the pharmaceutical industry, a "bridging" process could be used to demonstrate product equivalence.
Therefore, we investigated the feasibility of a bridging approach by evaluating aerosol emissions and *in vitro* cytotoxicity of five variant THPs in relation to a base product. All products were compared to a reference cigarette and a commercial benchmark. Relative to smoke, chemical reductions in THP aerosols were comparable among the THPs at 94–97%. The aerosols showed similar cytotoxicity in human lung tissues exposed at the air-liquid interface ($p = 0.8378$) but were significantly less toxic than smoke ($p = 0.04$). Relative to the THP benchmark, variant THPs showed lower cytotoxicity ($p = 0.0141$). Emissions and cytotoxicity data demonstrated that the variant THPs were comparable to the base THP, irrespective of consumable format or flavour. This dataset demonstrates the feasibility of a bridging approach and can inform an evidence-based strategy in developing sufficient data to predict similarity against an already established dataset. Therefore, avoiding repetition of vast data generation could ease authorisation requirements of newer products. Finally, we propose that more work is required to understand chemical, biological (*in vitro*), human consumption, and clinical data before the equivalence of these products (and others) can be definitively demonstrated. Future studies maybe needed to assess additional chemical and biological outputs and all data will need to be contextualised against human consumption data in terms of a bridging framework.
[Contrib. Tob. Nicotine Res. 31 (2022) 1–9]

KEYWORDS

In vitro; analytical; tobacco heating products; cytotoxicity;

ZUSAMMENFASSUNG

Bei Tabakerhitzern (THPs) entstehen im Verhältnis zu Zigaretten geringere Schadstoffemissionen. Die THPs werden kontinuierlich weiterentwickelt, jedoch ist für Studien zur Sicherheit und Wirksamkeit jeder neuen Variante der Einsatz beträchtlicher Ressourcen nötig. Zum Nachweis der Äquivalenz von Produkten könnte hierbei das in der pharmazeutischen Industrie verwendete Verfahren des „Bridging" genutzt werden.
In der vorliegenden Studie wurde daher die Umsetzbarkeit des Bridging-Ansatzes untersucht. Hierzu wurden die

*Received: 16th November 2021 – accepted: 17th February 2022

This work is licensed under a Creative Commons Attribution-NonCommercial-ShareAlike 4.0 International License (CC BY-NC-SA 4.0).
Authors who publish with this journal retain all copyrights and agree to the terms of the above-mentioned CC BY-NC-SA 4.0 license.

Aerosolemissionen sowie die *In-vitro*-Zytotoxizität von fünf THP-Varianten relativ zu einem Referenzprodukt ausgewertet. Alle Produkte wurden mit einer Referenzzigarette und mit einem handelsüblichen Vergleichsprodukt verglichen. Im Verhältnis zu Rauch war die Reduktion der freigesetzten chemischen Substanzen bei den Aerosolen aus THPs mit 94–97% innerhalb der Gruppe der untersuchten Tabakerhitzer vergleichbar. Die Aerosole wiesen in menschlichem Lungengewebe bei Exposition an der Luft-Flüssigkeitsgrenze eine ähnliche Zytotoxizität auf (p = 0,8378), waren jedoch signifikant weniger toxisch als Rauch (p = 0,04). Relativ zum THP-Vergleichsprodukt verfügten die THP-Varianten über eine geringere Zytotoxizität (p = 0,0141). Anhand der Daten zu Emissionen und Zytotoxizität konnte gezeigt werden, dass die THP-Varianten mit dem THP-Referenzprodukt, unabhängig vom Format oder Aroma, vergleichbar waren. Mit diesen Daten wurde die Umsetzbarkeit des Bridging-Ansatzes demonstriert. Sie könnten als Grundlage für eine evidenzbasierte Strategie dienen, bei der lediglich die für die Prognose der Ähnlichkeit mit einem bereits bestehenden Datensatz benötigten Daten erhoben werden. Hiermit würde die wiederholte Generierung großer Datenmengen vermieden und das Zulassungsverfahren für neue Produkte vereinfacht werden. Abschließend schätzen wir, dass zum endgültigen Nachweis der Äquivalenz dieser (und anderer) Produkte mehr Forschungsarbeit zum besseren Verständnis klinischer, chemischer und biologischer (*In-vitro*-) Daten sowie von Daten zum Konsum dieser Produkte durch den Menschen erforderlich ist. Eventuell werden weitere Studien benötigt, um zusätzliche chemische und biologische Variablen zu analysieren. Im Rahmen eines Bridging-Ansatzes sollten dann alle erhobenen Daten zu Konsumdaten in Beziehung gesetzt werden. [Contrib. Tob. Nicotine Res. 31 (2022) 1–9]

RESUME

Les produits à base de tabac chauffé (PTC) libèrent, comparativement aux cigarettes, des émissions réduites de substances toxiques. Les PTC sont en constante évolution; toutefois, des études d'innocuité et d'efficacité portant sur chaque nouvelle variante mobilisent des ressources considérables. Déjà employé dans l'industrie pharmaceutique, un processus de « triangulation » pourrait servir à démontrer l'équivalence des produits. Par conséquent, nous avons exploré l'applicabilité d'une approche par triangulation à l'évaluation des émissions d'aérosols et de la cytotoxicité *in vitro* de cinq variantes de PTC mises en regard d'un produit de référence. Tous les produits furent comparés à une cigarette de référence et à un produit de référence commercialisé. Eu égard à la fumée, les émissions réduites de substances chimiques dans les aérosols libérés par les PTC furent comparables pour les PTC analysés et oscillèrent entre 94% et 97%. Les aérosols présentèrent une cytotoxicité similaire dans les tissus pulmonaires humains exposés à l'interface air-liquide (p=0,8378) mais affichèrent une toxicité bien moindre que la fumée (p = 0,04). Par rapport au produit de référence, les variantes de PTC présentèrent une cytotoxicité moindre (p = 0,0141). Les données d'émissions et de cytotoxicité prouvèrent que les variantes de PTC étaient comparables au PTC de référence, indépendamment de l'arôme et du format de consommation. Ce recueil de données atteste la faisabilité d'une approche par triangulation et peut orienter une stratégie fondée sur des données probantes afin de récolter suffisamment de données servant à prédire la similitude par rapport à un recueil de données déjà établi. Par conséquent, il est possible d'éviter la constitution répétée de vastes recueils de données et d'assouplir les exigences d'autorisation de produits novateurs. Enfin, nous estimons que plus de travail est requis afin de comprendre les données cliniques, biologiques (*in vitro*), chimiques et relatives à la consommation humaine avant de pouvoir, de façon définitive, prouver l'équivalence de ces produits (et d'autres). Des études ultérieures seront peut-être nécessaires pour évaluer les répercussions biologiques et chimiques supplémentaires et toutes les données devront être contextualisées au regard des données relatives à la consommation humaine avant l'établissement d'un cadre de triangulation. [Contrib. Tob. Nicotine Res. 31 (2022) 1–9]

INTRODUCTION

The concept of tobacco harm reduction, whereby smoking is replaced with use of alternative reduced-risk nicotine and tobacco products, was established in 2001 (1) and is supported by several public health agencies and scientific bodies (2–4.) Tobacco heating products (THPs) – in which the tobacco is heated to temperatures (< 300 °C) well below combustion (> 900 °C) to create a cleaner and simpler tobacco smoke aerosol containing fewer, and smaller quantities of compounds (harmful and potentially harmful constituents; HPHCs) relative to cigarette smoke (5–7) – are one such category of reduced-risk product. Indeed, one THP (Philip Morris (8)) was recently (July 2020) granted reduced exposure status (9) through the Food and Drug Administration (FDA) Modified Risk Tobacco Product (MRTP) application framework (10).

Tobacco heating products are continually evolving through technical advances, but a full package of science which includes clinical studies on each new THP involve considerable time and resources. To aid regulatory approval, the FDA has published guidance for establishing "substantial equivalence" for a new tobacco product that (i) has the same characteristics as an approved base product or (ii) does not raise different questions of public health (11); however, the requirements for this framework have not been defined as yet. Demonstrating equivalence as in the Substantial Equivalents pathway could be key to demonstrating parity for new products and their iterations in future.

The concept of bioequivalence testing for the approval of drugs is well established in the pharmaceutical industry (12). For tobacco products, a "bridging" dataset of emissions and toxicological data on a new variant product might be similarly used to demonstrate equivalence to a base product, thereby easing the burden of testing and regulatory review. For equivalence, the variant product should have a similar emissions yield and exposure profile, should evoke the same type of physiological responses and be used in the same manner by the consumer (13).

In this proof-of-concept study, we have conducted emissions and toxicological testing on six THPs: a THP_base

Table 1. Test products and puffing regime.

Product	Product code/ puffing regime	Product descriptor	Variant	Blend (top flavour)	Product change from "Base"	Change classification
Reference cigarette	1R6F / HCI [1]	Combustible reference	N/A	US Blend	N/A	N/A
Commercial comparator	THS 2.2_R / HCI [2]	THP_ benchmark	THS2.2	Tobacco (Regular)	N/A	N/A
Base	THP1.0_BT / HCIm [3]	THP_base	THP1.0	Tobacco (Blended)	N/A	N/A
Variant 1	THP1.1_RT / HCIm [3]	THP_1	THP1.1	Tobacco (Rich)	Change in tobacco flavour and increased nicotine	Minor
Variant 2	THP1.1_C / HCIm [3]	THP_2	THP1.1	Citrus	Change in device aesthetics and top flavour	Minor
Variant 3	THP1.1_S / HCIm [3]	THP_3	THP1.1	Smooth	Change in device aesthetics and top flavour	Minor
Variant 4	THP1.1_F / HCIm [3]	THP_4	THP1.1	Fresh	Change in device aesthetics and top flavour	Minor
Variant 5	THP1.1_RTF / HCIm [3]	THP_5	THP1.1	Tobacco (Rich)	Change in device aesthetics, top flavour and foil wrap	Major [4]

[1] Smoke generated by HCI conditions: 55 mL puff, 30 s interval, 2 s duration, bell profile, 100% vent blocking (22). Eight puffs per consumable. Data taken from (16).
[2] Aerosol generated by HCI conditions: data taken from (7).
[3] Aerosol generated by modified HCI conditions: 55 mL puff, 30 s interval, 2 s duration, bell profile, 0% vent blocking (THP consumables are designed to have open vents to allow hot gas to escape and avoid burning (15)). Eight puffs taken per consumable.
[4] A significant change to consumable format combined with top flavour change.
Abbreviations: HCI; Health Canada Intense; THS: Tobacco Heating System; THP: Tobacco Heating Product;

(THP1.0_BT), on which an extensive chemistry, *in vitro*, and clinical foundational data set has been previously generated (6, 14–21) and five modified variants. We propose that if emissions and toxicological equivalence of the THP variants to the base THP can be demonstrated, these data might form part of an evolving bridging strategy. Such an approach can inform an evidence-based strategy in developing sufficient data to predict similarity to a base product, with an already established body of data. This would therefore avoid repetition of vast data generation and could ease authorisation requirements on newer iterations. Such bridging approaches to demonstrate THP-to-THP equivalence are currently being scientifically discussed with a view to establishing a regulatory compliant approach (13). In this case study we are comparing the chemistry as well as the biological activity across products tested, where we are trying to relate the two showing differences and similarities. This approach ensures that we can bridge product variants internally and benchmark externally against a pre-established commercial product, in the essence of FDA's substantial equivalence guidance.

Test products and study materials

The five variant THPs, THP_base, commercial MRTP-approved comparator THP (hereafter THP_benchmark/ THS2.2_R), and reference cigarette assessed in the study are summarized in Table 1. The modifications of the variant THPs were classified as minor (small changes in either top flavour, nicotine strength, or aesthetics) or major (a significant change brought about by a combination of minor changes). Unless otherwise stated, all materials and reagents were purchased from Fisher Scientific (Loughborough, UK).

Analytical measurements

For the bridging dataset, we evaluated emissions and cytotoxicity. Emissions measurements were conducted on the THP_base and five variants for toxicants identified by the World Health Organisation Study Group on Tobacco Harm Reduction (TobReg9). (23). Historical data were compiled for cigarette smoke (16) and the THP_benchmark as a commercial comparator (7). All emissions analyses were conducted at an independent accredited laboratory, Labstat International ULC (Kitchener, ON, Canada) following the TobReg9 recommendations (23). Five replicates were performed per analysis. All analytical approaches, together with the limits of detection and quantification for each analyte, have been previously documented (16).

For cytotoxicity testing, human bronchial epithelial cells (NCI-H292; American Type Culture Collection, Teddington, Middlesex, UK) were grown and maintained in sterile-filtered RPMI-1640 medium for cell culture. The medium was supplemented with 10% foetal bovine serum (GE Healthcare Life Sciences, Hatfield, Hertfordshire, UK), 2 mM glutamine, 50 U/mL penicillin and 50 mg/mL streptomycin. Twelve-well cell culture plates containing Transwells™ were seeded with 500 µL of cell suspension (~ 2×10^5 cells). Cultures were incubated for 72 h at 37 °C in humidified 5% carbon dioxide before experimental exposure. Cells were exposed at the air-liquid interface to THP whole aerosol (WA) generated by a Borgwaldt LM4E vaping machine or 1R6F reference cigarette whole smoke (WS) generated by a Borgwaldt RM20D smoking machine as previously described (24, 25).

A full cytotoxicity dataset was generated over 1–180 puffs of undiluted WA from the THPs (THP_base, n = 9; variant

Table 2. Comparison of analyte emissions among products [1].

Analyte	Units	1R6F [2]	THP_ benchmark [3]	THP_base	THP variant				
					1	2	3	4	5
Acetaldehyde	µg/consumable	1859 (169)	219 (31)	122.6 (5.55)	128.6 (7.89)	131.8 (6.49)	126.6 (12.62)	123.6 (6.19)	83.41 (3.95)
Acrolein	µg/consumable	148 (22)	11.3 (24)	1.69 (0.13)	1.70 (0.19)	2.10 (0.21)	1.99 (0.23)	1.75 (0.15)	1.24 (0.08)
Benzene	µg/consumable	76 (5.8)	0.649 (0.074)	0.06 (0.02)	0.09 (0.03)	0.06 (0.02)	0.04 (0.01)	0.07 (0.03)	0.06 (0.02)
Benzo[*a*]pyrene	ng/consumable	11.4 (1.7)	NQ	0.45 (0.15)	0.31 (0.06)	0.53 (0.06)	0.43 (0.05)	0.40 (0.05)	NQ
1,3-Butadiene	µg/consumable	114 (4)	0.294 (0.042)	BDL	BDL	BDL	BDL	BDL	BDL
Formaldehyde	µg/consumable	68.4 (3.9)	5.53 (0.69)	2.73 (0.96)	2.38 (0.48)	2.81 (1.56)	2.65 (1.11)	2.77 (1.22)	1.51 (0.28)
NNK	ng/consumable	208 (7)	6.7 (0.6)	7.46 (0.54)	5.08 (0.63)	10.01 (0.76)	5.63 (1.18)	6.46 (1.16)	6.27 (0.84)
NNN	ng/consumable	191 (8)	17.2 (1.25)	24.4 (1.96)	16.5 (1.5)	26.92 (1.72)	0.04 (0.01)	22.30 (3.16)	13.31 (1.45)
CO	mg/consumable	29.4 (0.6)	0.532 (0.068)	BDL	BDL	BDL	BDL	BDL	BDL
Glycerol	mg/consumable	1.36 (0.05)	4.63 (0.83)	2.12 (0.24)	1.94 (0.35)	2.81 (0.49)	2.25 (0.37)	2.38 (0.26)	3.95 (0.29)
TPM	mg/consumable	45.5 (2.2)	10.3 (0.9)	26.16 (1.49)	27.12 (0.42)	27.66 (1.25)	26.34 (1.29)	25.74 (1.84)	26.11 (1.07)
Nicotine	mg/consumable	2.0 (0.08)	1.32 (0.16)	0.38 (0.03)	0.38 (0.04)	0.46 (0.03)	0.38 (0.03)	0.38 (0.02)	0.79 (0.02)
TAY	mg/consumable	80.53	17.02	28.79	29.57	31.07	29.10	28.63	30.94
TAY	mg/puff	10.07	2.13	3.60	3.70	3.88	3.64	3.58	3.87
TAY [4]	mg	32.1	181.05	306	314.5	329.8	309.4	304.3	329
% reduction relative to (1R6F)	%	N/A	93.24	95.41	96.36	94.82	97.35	95.68	97.07

[1] Data are given as mean (±SD) unless stated otherwise. [2] Data taken from (16). [3] Data taken from (7).
[4] Total analyte yields (TAY) calculated based on a per-puff basis for cigarette smoke and THP.
Cigarette smoke TAY based on 3 puffs (10.7 × 3 = 32.1 mg); THP TAY based on 85 puffs (mg/puff × 85)
Abbreviations: NNK: 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone; NNN: *N*-nitrosonornicotine; CO: carbon monoxide; TPM: total particulate matter; NQ: not quantifiable; BDL: below detection limit; N/A: not applicable

THPs, n = 3) or 0–8 puffs of undiluted 1R6F WS (n = 3), which evoked a maximum cytotoxic response. On each exposure day, we carried out a sham air control (blank) in which filtered laboratory air was puffed onto the cells; the number of puffs corresponded to the highest puff number used on that day. After exposure, all chambers including the sham air control were left for 5–10 min to allow any aerosol to settle before the cells were processed to recovery plates for 24 h. A set of negative controls were also carried out on each exposure day: 3 media-submerged inserts (incubator controls); and 3 inserts at the air-liquid interface (ALI) prepared by removing the apical media and replacing the chamber back in the incubator (ALI controls). Treatment of cells with 350 mM sodium dodecyl sulphate (SDS) served as a positive control.

At 24 h after exposure, neutral red uptake (NRU) assay (15, 26) was used to assess cell viability. Cells were washed twice with sterile PBS and incubated with neutral red dye for 3 h, and then washed twice with PBS to remove excess dye. Uptake of dye by viable cells was determined by lysing the cells with 500 µL of de-stain solution and quantifying 100 µL aliquots on a microplate spectrophotometer at 540 nm using a reference filter of 630 nm. Cell viability was reported as a percentage of the air control.

Data analysis

Data were processed in GraphPad Prism 8 or Microsoft Excel. All cytotoxicity data were normalised to the air control response. Emissions data were compared using GraphPad Prism (version 8) and percentage reductions were compared across all products. Total analyte yields (TAY), which is the accumulation of all measured analytes, were calculated in Microsoft Excel. TAY was based on a per-puff basis for cigarette smoke and THP. Cigarette smoke TAY was based on 3 puffs (10.7 × 3 = 32.1 mg); THP TAY was based on 85 puffs (mg/puff × 85). Biological responses to undiluted WA were compared by one-way ANOVA ($p < 0.05$ was considered significant) of the generated IC_{50}s. Three cell samples were used per treatment condition. To compare the THPs, a single 85-puff dose was selected from the THP baseline data; this dose represented the first dose beyond the half-maximal inhibitory concentration (IC_{50}) and ensured that comparisons were made for an active dose. Cigarette smoke data were used to contextualise the THP responses.

Emissions

THP emissions were measured for 12 analytes (Table 2). In all cases, levels in the five variant THPs were comparable

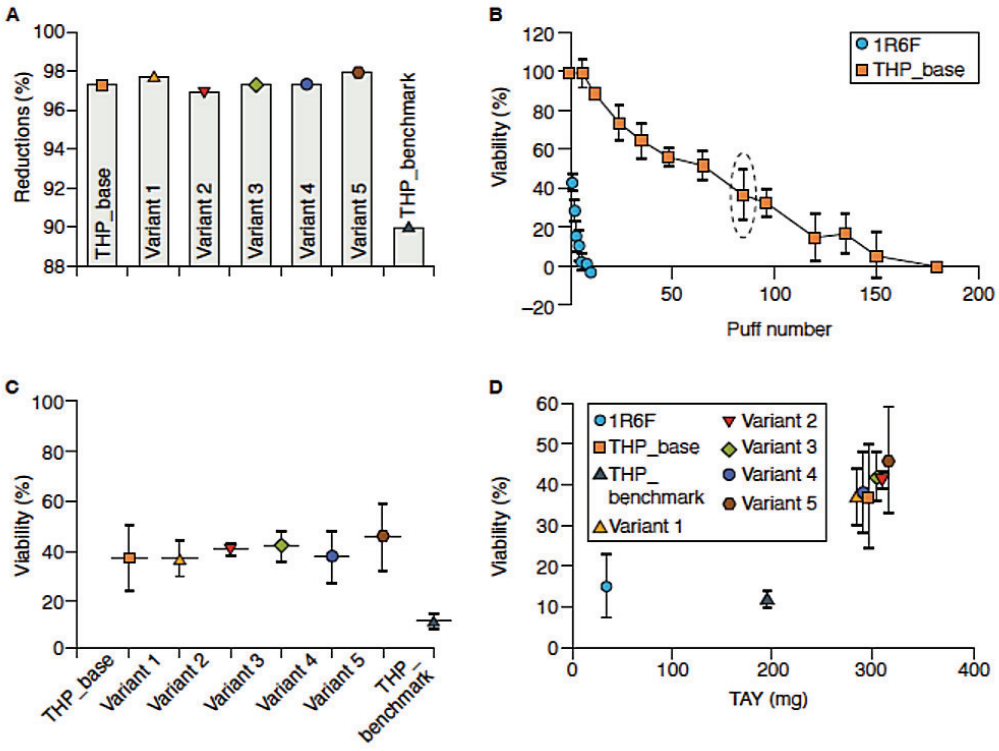


Figure 1. Emissions and toxicology bridging data. (a) Percentage reductions in emissions of selected TobReg9 analytes for THP variants relative to a reference cigarette. (b) Viability of human lung cells exposed to cigarette smoke and THP aerosol. (c) Viability of cells exposed to 85 puffs of aerosol from THP_base, variants, and the MRTP-approved commercial THP_benchmark. (d) Viability data for reference cigarette, THP_benchmark, THP_base and variants contextualised against total analyte yields.

to the THP_base. Relative to cigarette smoke, percentage reductions were between 94% and 97% for all six THPs (Table 2 and Figure 1a). All six products were compared by a one-way ANOVA and were deemed comparable ($p = 0.5508$).

Cell viability

Regarding cytotoxicity as assessed using NRU viability in human lung epithelial cells, undiluted cigarette smoke demonstrated full cytotoxicity within 10 undiluted puffs, whereas all THPs elicited a full toxic response only after delivery of 180 undiluted puffs (Figure 1b). On this basis, the THP response was deemed ~ 95% less toxic relative to cigarette smoke (16). To compare the THPs in more detail, we selected a dose point that was in the toxic range of all products (85 puffs) (this dose represented the first dose beyond the half-maximal inhibitory concentration (IC_{50})). The five THP variants and the THP_base showed no significant difference in toxicity ($p = 0.8378$) (Figure 1c), suggesting that the top flavours and innovations in the variants had no impact on overall toxicity. As compared with the THP_benchmark, the THP_base and variant THPs were all deemed significantly less toxic relative to cigarette smoke ($p = 0.0141$).

Lastly, when the cytotoxicity data were presented as a function of total analyte yields (TAY; i.e., the accumulation of all analytes measured), the THP_base and five variants were grouped together, whereas the cigarette data sat independently from those of the THPs (Figure 1d). Interestingly, cigarette smoke reached a cytotoxicity of 80% at a TAY of 20 mg. By contrast, at 50%–60% toxicity, the THPs all delivered a TAY of approximately 300 mg. These data suggest that the cigarette smoke contains chemicals that are more toxic, and a much smaller dose is required to achieve greater cytotoxicity relative to the THP aerosols. This is consistent with the observed cytotoxicity curves (Figure 1b) and the measured emissions, which showed that the THPs are approximately 94% less toxic than a combustible product (21).

The development and the availability of alternative tobacco and nicotine products are fast evolving. New products such as THPs are becoming more acceptable to the consumer and their use is growing globally. As consumer popularity increases, so does innovation and product evolution. This leads to shorter product life cycles, with continued requirement to appropriately assess their safety and quality. As part of a toxicological assessment, products undergo careful risk assessments of their chemical content and emissions, and thresholds of toxicological concern (TTC) are derived. The device components and the potential for degradation and leachable by-products are also assessed (27). Only when the product meets these standards (including battery performance) is the product launched. However, conducting such risk assessments using TTCs is time-consuming, and it is almost impossible to conduct a complete chemical, biological, and clinical review of each new product variant.

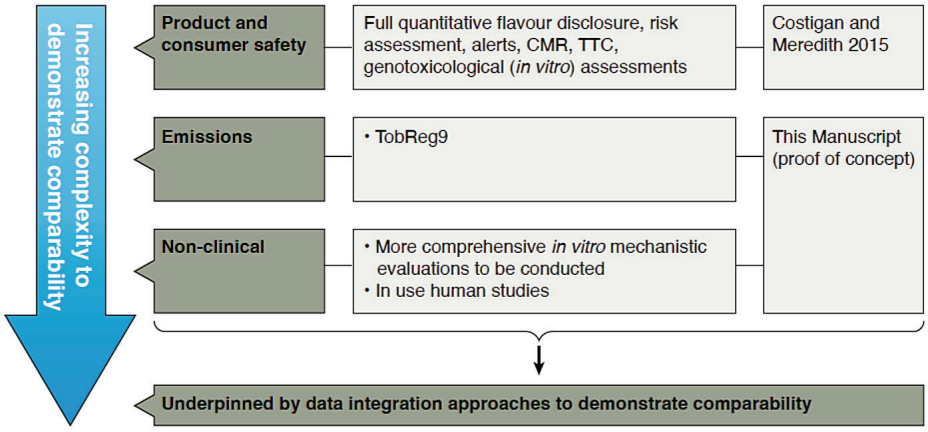


Figure 2. A schematic representation of a proposed future bridging process to leverage data from multiple sources.

Thus, as used in the pharmaceutical industry (12), a practical testing strategy is required to demonstrate the 'equivalence' of product variants based on an original product with a comprehensive science dataset.

As a first towards demonstrating the feasibility of an equivalence or a bridging approach, in this study we investigated whether five THP variants, relative to the THP_base with a foundational dataset (6, 14–21), maintain a similar percentage reduction in their chemical and *in vitro* toxicological profile when compared with cigarette smoke. The five THP variants were each classified as having minor (e.g., different top flavours or nicotine strengths) or major (device innovations) changes from the base variant.

Chemical analysis based on the TobReg9 mandated toxicant list (23) and a physiologically relevant aerosol exposure for cytotoxicity assessment, demonstrated that five variant THPs were all comparable to the THP_base, irrespective of innovation to consumable format or top flavour. Relative to cigarette smoke, the reductions in THP aerosol emissions were significantly lower ($p < 0.001$) and were also comparable among all variants at 94%–97% (Table 1 and Figure 1a). *In vitro* toxicological analysis of the THP_base aerosol *versus* cigarette smoke showed an approximately 95% reduction in cell cytotoxicity. The THP_base and five variant THP aerosols showed comparable cytotoxicity using an aerosol-based air-liquid interface approach ($p = 0.8378$) and were significantly less cytotoxic than cigarette smoke ($p = 0.04$). When the emissions analysis was combined with the toxicology data, the THP variants showed an approximate 94% reduction relative to the reference combustible product (16). When compared with the THP_benchmark, the THP_base and variant THPs all showed significantly lower toxicity ($p = 0.0141$).

For a more informative analysis, the emissions data summarized as Total Analyte Yields (TAY) and were plotted against viability (Figure 1d), which showed that the THP_base and variant THPs were grouped together, well apart from the reference cigarette. Thus, cigarette smoke is clearly different from THP aerosol; it is more toxic and requires much lower doses to achieve cytotoxicity. For cigarette smoke, a TAY of only 20 mg was required to achieve 80% toxicity; for THP aerosol, by contrast, a TAY of 300 mg was required to achieve 50%–60% toxicity. Based on TAY calculations from TobReg9 in this study, the chemicals in cigarette smoke are more toxic at lower concentrations as compared with those in THP aerosol. This observation of reduced chemical and toxicological profiles relative to cigarette smoke is consistent with previous reports (7, 15–18).

A bridging framework will help to support product testing to inform an evidence-based strategy to predict similarity of future product variants to a base product, with an already established body of data. This would therefore avoid repetition of vast data generation and could ease authorisation requirements of newer products. The data presented will help inform potential next steps for a practical bridging framework especially considering the rapidly evolving product landscape, where it will become impossible to assess every variant under a "complete" testing strategy. Nevertheless, the small dataset presented here shows that the five THP variants assessed were all comparable (or "bridgeable back") to the THP_base product foundational dataset and that the introduction of flavour and/or device innovations did not adversely affect the toxicological impact of the product, under these test conditions using this one approach.

This approach should be caveated in that, only a limited selection of analytes were measured in this proof-of-concept study and therefore the reductions and calculations are only based on a small subset of the total emissions and does not represent the total chemical profile. In addition, the chemicals driving the response may not be captured in this analysis, being based on an accumulation of analytes, the value placed on mg substances may be more heavily weighted compared to those in the ng range. More work is required to understand chemical, biological (*in vitro*), human consumption, and clinical data before the equivalence of these products can be definitively demonstrated. Future studies may need to assess additional chemical and biological outputs as well as test articles to those presented here, and all data will need to be contextualised against

human consumption data in terms of a bridging framework. Figure 2 demonstrates the proposed process in which an expanded bridging framework could follow. This process is evolving and may change as bridging concepts for THPs become more defined. Specifically, data should leverage where possible, genetic toxicological assessments (conducted as part of a stewardship approach in this example), a more comprehensive emissions panel (which included untargeted analysis), combined with *in vitro* disease and mechanistic screening approaches and human in use data. Finally, to make sense of the growing complexity, bioinformatical approaches should be employed and appropriate competitors such as cigarette smoke and a commercial reference product. We have left this part absent in the framework as this could be project and study specific. Finally, although this study is specific to tobacco heating products, we believe in working towards bridging strategies for new generation products including THPs and electronic nicotine delivery systems.

In conclusion, this study demonstrates the feasibility of a preclinical equivalence concept for tobacco heating products across five variants with shifting base flavour (tobacco, fresh, smooth, and citrus) and combinations of changes (such as foil wrap consumable size and flavour change). The outcomes articulated here are predicated on a sound and robust stewardship approach to ensure product and consumer safety are maintained, in line with published approaches (27). The data presented here is only a small data package to demonstrate the concept of bridging between products and represents the start of a preclinical weight of evidence journey. Work is required to establish a full bridging framework that incorporates additional chemical and *in vitro* biological data, coupled with human use, and ultimately linked to clinical outcomes. Furthermore, THP responses should be contextualised against cigarette smoke and benchmarked against a commercial comparator (or multiple comparators to give a wider commercial perspective). For the latter we have benchmarked against one that has already been authorised by regulators in the USA.

DECLARATIONS

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

CONSENT TO PUBLISH

The authors have all approved the final version. The data had not been published elsewhere, in part or its entirety.

AVAILABILITY OF DATA AND MATERIALS

Not applicable.

COMPETING INTERESTS

All authors are (or were at the time of study conduct) employees of British American Tobacco.

FUNDING

The work was fully funded by British American Tobacco.

AUTHORS' CONTRIBUTIONS

DT, TJ, and MG designed the study. TJ, DT, JF, and AB participated in its execution. TJ, DT, and JF wrote the manuscript. DT, TJ, and JF conducted analyses. MG gave technical oversight, all authors approved the final version.

ABBREVIATIONS	
ALI	Air liquid interface
ANOVA	Analysis of variance
CMR	Carcenogenic Mutagenic and Reprotoxic chemicals
HCI	Health Canada Intense Cigarette Smoking Regime
HCIm	Modified Health Canada Intense Smoking Regime for THPs (vents not blocked)
PRRPs	Potentially reduced risk products (nicotine) products
NRU	Neutral Red Uptake assay
LM4E	Linear smoking Machine for vaporizers
RM20D	Borgwaldt RM20D Smoking Machine
THP	Tobacco Heating Product
THS	Tobacco Heating System
UPLC-MS/MS	Ultra high performance liquid chromatography-tandem mass spectrometry
1R6F	University of Kentucky reference cigarette

REFERENCES

1. Institute of Medicine Committee to Assess the Science Base for Tobacco Harm Reduction: Clearing the Smoke: Assessing the Science Base for Tobacco Harm Reduction; *edited by* K. Stratton, P. Shetty, R. Wallace, and S. Bondurant, National Academy Press, Washington, DC, USA, 2001.
2. Royal College of Physicians: Nicotine Without Smoke: Tobacco Harm Reduction; Royal College of Physicians, London, UK, 2016. Available at https://www.rcplondon.ac.uk/projects/outputs/nicotine-without-smoke-tobacco-harm-reduction (accessed March 2022).
3. McNeill, A., L.S. Brose, R. Calder, L. Bauld, and D. Robson: Evidence Review of E-Cigarettes and Heated Tobacco Products 2018: A Report Commissioned by Public Health England; Public Health England, London, UK, 2018. Available at https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/684963/Evidence_review_of_e-cigarettes_and_heated_tobacco_products_2018.pdf (accessed February 2022).
4. The National Academies of Sciences, Engineering, Medicine: Consensus Study. Public Health Consequences of E-Cigarettes; National Academies Press, Washington, DC, USA, 2018. DOI: 10.17226/24952
5. Takahashi, Y., Y. Kanemaru, T. Fukushima, K. Eguchi, S. Yoshida, J. Miller-Holt, and I. Jones: Chemical Analy-

sis and *In Vitro* Toxicological Evaluation of Aerosol From a Novel Tobacco Vapor Product: A Comparison With Cigarette Smoke; Regul. Toxicol. Pharmacol. 92 (2018) 94–103. DOI: 10.1016/j.yrtph.2017.11.009
6. Eaton, D., B. Jakaj, M. Forster, J. Nicol, E. Mavropoulou, K. Scott, C. Liu, K. McAdam, J. Murphy, and C.J. Proctor: Assessment of Tobacco Heating Product THP1.0. Part 2: Product Design, Operation and Thermophysical Characterisation; Regul. Toxicol. Pharmacol. 93 (2018) 4–13. DOI: 10.1016/j.yrtph.2017.09.009
7. Schaller, J.-P., D. Keller, L. Poget, P. Pratte, E. Kaelin, D. McHugh, G. Cudazzo, D. Smart, A.R. Tricker, L. Gautier, M. Yerly, R. Reis Pires, S. Le Bouhellec, D. Ghosh, I. Hofer, E. Garcia, P. Vanscheeuwijck, and S. Maeder: Evaluation of the Tobacco Heating System 2.2. Part 2: Chemical Composition, Genotoxicity, Cytotoxicity, and Physical Properties of the Aerosol; Regul. Toxicol. Pharmacol. 81 (2016) S27–S47. DOI: 10.1016/j.yrtph.2016.10.001
8. Smith, M.R., B. Clark, F. Lüdicke, J.P. Schaller, P. Vanscheeuwijck, J. Hoeng, and M.C. Peitsch: Evaluation of the Tobacco Heating System 2.2. Part 1: Description of the System and the Scientific Assessment Program; Regul. Toxicol. Pharmacol. 81 (2016) S17–S26. DOI: 10.1016/j.yrtph.2016.07.006
9. U.S. Food and Drug Administration (FDA): Modified Risk Orders. IQOS System Holder and Charger; FDA, Silver Spring, MD, USA, 2020. Available at https://www.fda.gov/tobacco-products/advertising-and-promotion/modified-risk-orders (accessed February 2022).
10. U.S. Food and Drug Administration (FDA): Guidance for Industry. Modified Risk Tobacco Product Applications. Draft Guidance; FDA, Silver Spring MD, USA, 2012. Available at https://www.fda.gov/media/83300/download (accessed February 2022).
11. U.S. Food and Drug Administration (FDA): Section 905(j) Reports: Demonstrating Substantial Equivalence for Tobacco Products. Guidance for Industry and Food and Drug Administration Staff; FDA, Silver Spring, MD, USA, 2011. Available at https://www.fda.gov/-regulatory-information/search-fda-guidance-documents/section-905j-reports-demonstrating-substantial-equivalence-tobacco-products (accessed February 2022)
12. Midha, K.K. and G. McKay: Bioequivalence: Its History, Practice, and Future; AAPS J. 11 (2009) 664–670. DOI 10.1208/s12248-009-9142-z
13. Schlage, W.K., B. Titz, A. Iskandar, C. Poussin, M. Van der Toorn, E.T. Wong, P. Pratte, S. Maeder, J.P. Schaller, P. Pospisil, S. Boué, G. Vuillaume, P. Leroy, F. Martin, N.V. Ivanov, M.C. Peitsch, and J. Hoeng: Comparing the Preclinical Risk Profile of Inhalable Candidate and Potential Candidate Modified Risk Tobacco Products: A Bridging Use Case; Toxicol. Rep. 7 (2020) 1187–1206. DOI: 10.1016/j.toxrep.2020.09.004
14. Proctor, C.: Assessment of Tobacco Heating Product THP1.0. Part 1: Series Introduction; Regul. Toxicol. Pharmacol. 93 (2018) 1–3. DOI: 10.1016/j.yrtph.2017.09.010
15. Jaunky, T., J. Adamson, S. Santopietro, A. Terry, D. Thorne, D. Breheny, C. Proctor, and M. Gaça: Assessment of Tobacco Heating Product THP1.0. Part 5: *In Vitro* Dosimetric and Cytotoxic Assessment; Regul. Toxicol. Pharmacol. 93 (2018) 52–61. DOI: 10.1016/j.yrtph.2017.09.016
16. Forster, M., S. Fiebelkorn, C. Yurteri, D. Mariner, C. Liu, C. Wright, K. McAdam, J. Murphy, and C. Proctor: Assessment of Novel Tobacco Heating Product THP1.0. Part 3: Comprehensive Chemical Characterisation of Harmful and Potentially Harmful Aerosol Emissions; Regul. Toxicol. Pharmacol. 93 (2018) 14–33. DOI: 10.1016/j.yrtph.2017.10.006
17. Thorne, D., D. Breheny, C. Proctor, and M. Gaça: Assessment of Novel Tobacco Heating Product THP1.0. Part 7: Comparative *In Vitro* Toxicological Evaluation; Regul. Toxicol. Pharmacol. 93 (2018) 71–83. DOI: 10.1016/j.yrtph.2017.08.017
18. Taylor, M., D. Thorne, T. Carr, D. Breheny, P. Walker, C. Proctor, and M. Gaça: Assessment of Novel Tobacco Heating Product THP1.0. Part 6: A Comparative *In Vitro* Study Using Contemporary Screening Approaches; Regul. Toxicol. Pharmacol. 93 (2018) 62–70. DOI: 10.1016/j.yrtph.2017.08.016
19. Murphy, J., C. Liu, K. McAdam, M. Gaça, K. Prasad, O. Camacho, J. McAughey, and C. Proctor: Assessment of Tobacco Heating Product THP1.0. Part 9: The Placement of a Range of Next-Generation Products on an Emissions Continuum Relative to Cigarettes via Pre-Clinical Assessment Studies; Regul. Toxicol. Pharmacol. 93 (2018) 92–104. DOI: 10.1016/j.yrtph.2017.10.001
20. Gee, J., K. Prasad, S. Slayford, A. Gray, K. Nother, A. Cunningham, E. Mavropoulou, and C. Proctor: Assessment of Tobacco Heating Product THP1.0. Part 8: Study to Determine Puffing Topography, Mouth Level Exposure and Consumption Among Japanese Users; Regul. Toxicol. Pharmacol. 93 (2018) 84–91. DOI: 10.1016/j.yrtph.2017.08.005
21, Forster, M., J. McAughey, K. Prasad, E. Mavropoulou, and C. Proctor: Assessment of Tobacco Heating Product THP1.0. Part 4: Characterisation of Indoor Air Quality and Odour; Regul. Toxicol. Pharmacol. 93 (2018) 34–51. DOI: 10.1016/j.yrtph.2017.09.017
22. Health Canada: Official Method T-115 – Determination of "Tar", Nicotine and Carbon Monoxide in Mainstream Tobacco Smoke; Health Canada, Ottawa, Canada, 1999.
23. World Health Organization (WHO): Report on the Scientific Basis of Tobacco Product Regulation – TRS 1015; Study Group on Tobacco Product Regulation, World Health Organization, Geneva, Switzerland, 2019.
24. Adamson, J., T. Jaunky, D. Thorne, and M.D. Gaça: Characterisation of the Borgwaldt LM4E System for *In Vitro* Exposures to Undiluted Aerosols From Next Generation Tobacco and Nicotine Products (NGPs); Food Chem. Toxicol. 113 (2018) 337–344. DOI: 10.1016/j.fct.2018.02.005
25. Bishop, E., L. Haswell, J. Adamson, S. Costigan, D. Thorne, and M. Gaça: An Approach to Testing Undiluted E-Cigarette Aerosol *In Vitro* Using 3D Reconstituted Human Airway Epithelium; Toxicol. In Vitro 54 (2019) 391–401. DOI: 10.1016/j.tiv.2018.01.010

26. Azzopardi, D., L.E. Haswell, G. Foss-Smith, K. Hewitt, N. Asquith, S. Corke, and G. Phillips: Evaluation of an Air-Liquid Interface Cell Culture Model for Studies on the Inflammatory and Cytotoxic Responses to Tobacco Smoke Aerosols; Toxicol. In Vitro 29 (2015) 1720–1728. DOI: 10.1016/j.tiv.2015.06.016
27. Costigan, S. and C. Meredith: An Approach to Ingredient Screening and Toxicological Risk Assessment of Flavours in E-Liquids; Regul. Toxicol. Pharmacol. 72 (2015) 361–369. DOI: 10.1016/j.yrtph.2015.05.018

Corresponding author:

Tomasz Jaunky, MSc.
British American Tobacco, R&D
Southampton, Hampshire SO15 8TL
UK
E-Mail: Tomasz_Jaunky@bat.com