Bridging of a new heated tobacco product from a historical commercial comparator

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1. Introduction

New category (NC) tobacco and nicotine products such as heated tobacco products (HTPs) are characterised by rapid innovation. Bridging from a reference product is a recognised strategy for NC development[1] to enable efficient review and approval for new product iterations, while driving innovation to meet consumers' preferences and maintain product risk profile. Bridging has been adopted by BAT, as part of a published 9-step framework to assess the reduced risk potential of NC products compared to cigarettes[2]. Here, we present the *in vitro* toxicology method adopted to compare a historical commercial comparator glo device and stick (THP1.1RT) to a new iteration comprised of a new device (G400) and a new non-commercial heated tobacco stick (eHTPB2).

2. Methodology

Test Articles

The table below shows some of the main differences between each test article

Property	THP1.1RT	G200 eHTPB1	G400 eHTPB2
Heating technology	Resistive	Resistive	Induction
Operating Temperature	240 °C	250 °C	260 °C
Consumable size	KSSS	KSSS	DS
Consumable tobacco blend	Hibiscus	80:20 Hibiscus+: Hay like	80:20 Hibiscus+: Hay like

Assays

In vitro Micro nucleus (ivMN) assay

Gas-vapour phase (GVP) and aerosol collected mass (ACM) fractions were generated concurrently via trapping in ice-cold Ca^{2+} and Mg^{2+}-free PBS and Cambridge filter pads respectively. ACM was redissolved in DMSO and mixed 1:1 with the GVP phase to achieve a final concentration of 75 mg/ml of ACM equivalent. ivMN was performed using V79 cells with 3 treatment exposure regimes:

- **3h -S9**: 3h exposure (21h recovery) in absence of S9 mix (Mutazyme™)
- **3h +S9:** 3h exposure (21h recovery) in presence of S9 mix
- **24h -S9:** 24h exposure (0h recovery) in absence of S9 mix.

Cell slides were scored manually.

Real Time Cell Analysis (RTCA)

Aqueous Extracts (AqEs) were generated by bubbling whole aerosol from 4 sticks of each test product into 20 mL of complete cell culture medium. NCI-H292 cells were plated on special plates with gold electrodes and impedance was monitored in real time through a multi-plate RTCA instrument (Agilent). Cells were exposed to the test articles during the growth phase for 24h and impedance was subsequently converted to percent Normalised Cell Index * Hour as a measure of cell survival. Dose-response curves were fitted to 4-parameters variable slope curves to estimate IC_{50} values with 95% confidence intervals.

Whole aerosol exposure and MTT assay on MucilAir™ tissues

MucilAir™ tissues (Epithelix) of nasal origin were exposed to whole aerosol generated using a LM4E machine (Körber) under the CRM81 regime. In each exposure, triplicate cell culture inserts were exposed with concurrent air controls for the longest exposure length that day. After exposure cells were allowed to recover for 24h. Subsequently the MTT cytotoxicity assay was performed as per manufacturer instruction and cell viability was normalized to the air control. Data were fitted and IC_{50} values calculated as above.

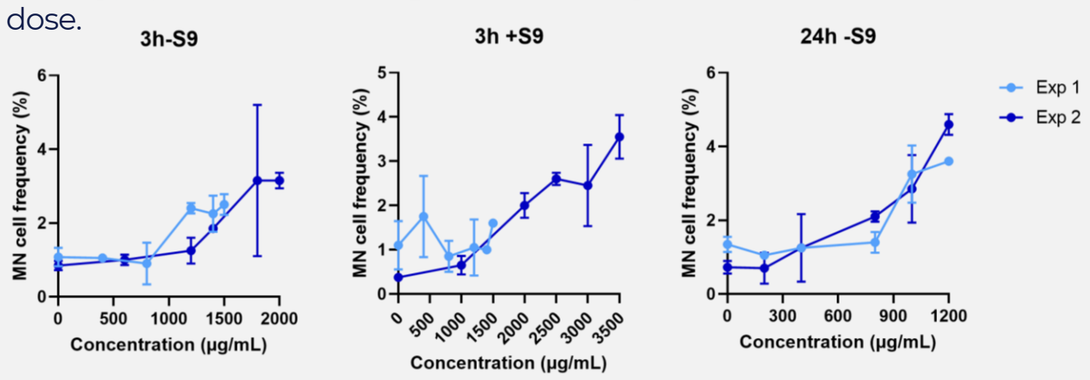
ToxTracker™

AqEs as generated above were tested in-house in the ToxTracker assay according to the manufacturer instructions (Toxys), both in presence and absence of S9 rat liver extract for metabolic activation. Potency was estimated by means of benchmark dose (BMD) analysis using the PROAST (v70.3) package in R software (v.4.2.1). The lower and upper bound of the benchmark dose (BMDL and BMDU, respectively) were calculated to estimate the BMD interval and enable comparison between products.

3. Results

Preliminary Genotoxicity Assessment of eHTPB1 on G200 device

The genotoxicity of the GVP+ACM extracts of eHTPB1 (KSSS stick format prototype) were tested using the ivMN assay up to maximum achievable dose.



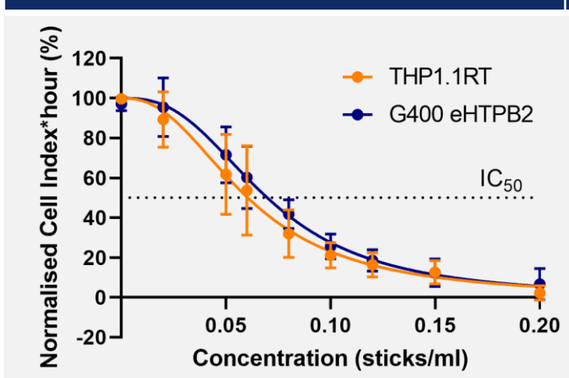
Previous reference products were tested at concentrations up to 250 µg/ml and showed negative responses[3], similar to eHTPB1 at the same concentration.

Cytotoxicity Assessment of G400 with eHTPB2 versus THP1.1RT

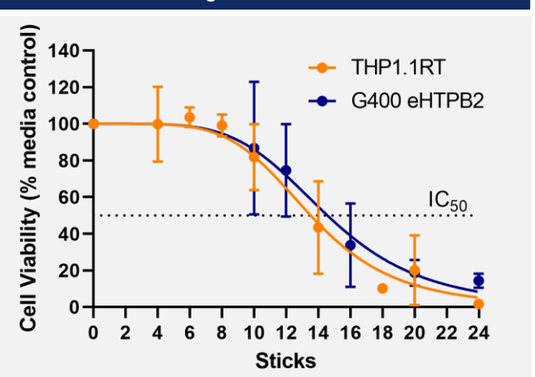
2D assessment: RTCA assay exposing a monolayer of NCI-H292 cells to AqEs of the HTP products.

3D assessment: MTT assay on MucilAir tissues exposed to whole aerosol of the HTP products

2D – RTCA on NCI-H292 cells



3D – MTT Assay on MucilAir tissues

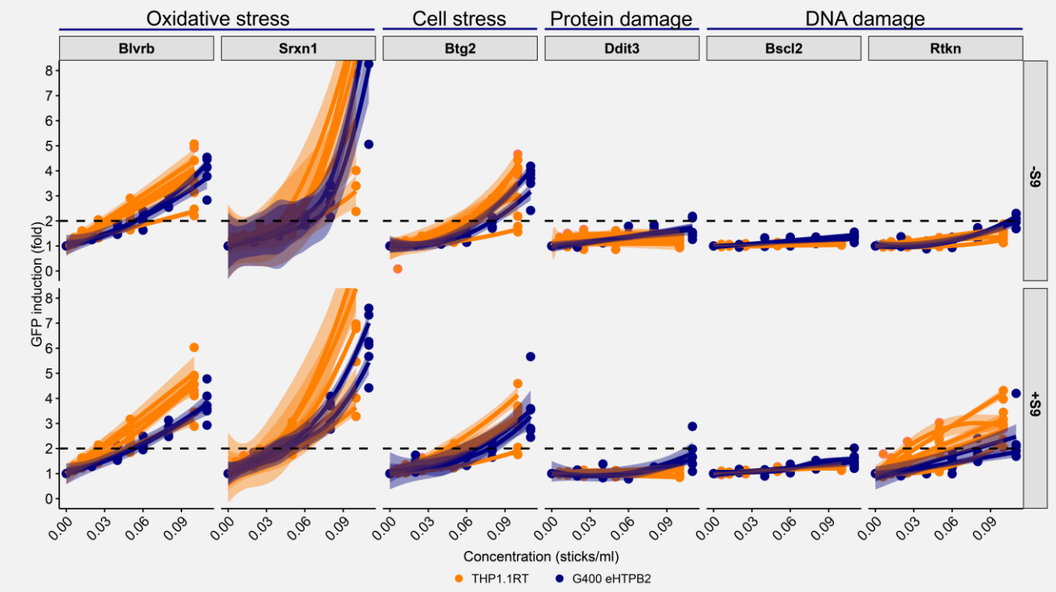


In both cases IC_{50} intervals were comparable for the two products. Table shows calculated IC_{50} values and the 95% confidence interval.

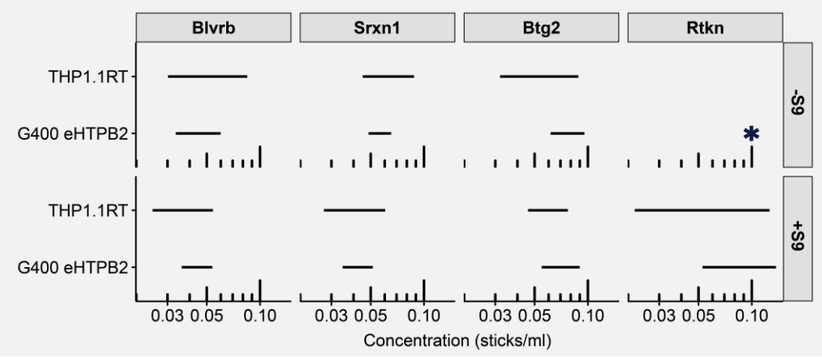
Product	2D	3D
THP1.1RT	0.0605 (0.0526 ~ 0.0677)	13.53 (12.19 ~ 15.03)
G400 eHTPB2	0.0698 (0.0641 ~ 0.0755)	14.57 (12.87 ~ 16.52)

ToxTracker Assessment of G400 with eHTPB2 versus THP1.1RT

ToxTracker assessment was repeated for 5 batches of the THP1.1RT reference products and 2 batches of the test product G400 eHTPB2 to quantify variability.



Range of the BMDL and BMDU values calculated across all batches for each product. BMD analysis was performed for each batch separately. Graph below represents the distance between the smallest BMDL and highest BMDU calculated for each product.



* 1 out of 2 batches was positive for this endpoint however BMD range could not be accurately calculated

4. Conclusion

We describe the *in vitro* toxicological assessment as part of a Bridging strategy to evaluate device and consumable changes in HTP product iterations compared to an established reference product.

Assessing complexity	Assessing mechanisms	An evolving strategy
Device and consumable changes tested did not induce change in cytotoxicity	Major product changes did not affect gene induction linked to genotoxicity	Bridging is a process continuously improved and refined

Acknowledgements

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References

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3. Thorne et al. (2020). The genotoxicological assessment of a tobacco heating product relative to cigarette smoke using the in vitro micronucleus assay. Toxicology Reports, 7, 1010-1019.

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