Aerosol Chemistry and *In Vitro* Evaluation of a Novel Herbal Heated Product Relative to Cigarette Smoke

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Introduction

New products are emerging that support Tobacco Harm Reduction (THR), such as products that heat but do not burn tobacco. These have been shown to produce reduced toxicant emissions and reduced *in vitro* toxicity as compared to conventional cigarettes. Here, we evaluate a novel herbal heated product (HHP) by assessing its chemical emissions and *in vitro* activity compared to a reference cigarette, 1R6F. The HHP ingredients include non-tobacco substrate consisting of Rooibos, nicotine and flavours.

Methodology

Test Articles

The 1R6F reference cigarette and a HHP were used in this study. A schematic of the HHP consumable and heating device are shown in Figure 1.

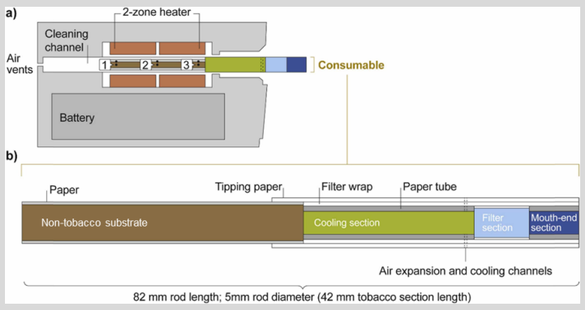

Figure 1. Schematic representation of the HHP and the consumable.

Emissions Generation

The Health Canada Intense (HCI) smoking regime was used for the 1R6F cigarette[1,2]. The HHP was puffed using a modified HCI regime (mHCI) with unblocked ventilation holes[3]. Total Particulate Matter (TPM) and Gas-Vapour Phase (GVP) were collected (Figure 2).

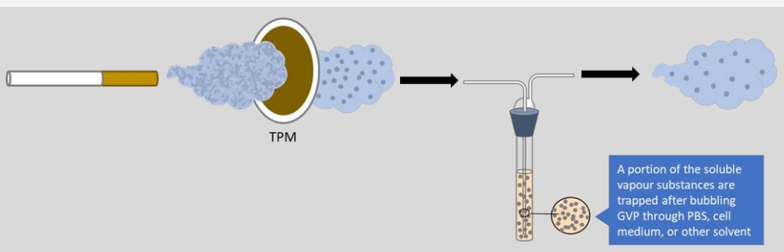

Figure 2. Schematic representation of the capturing of the aerosol emissions from 1R6F and the HHP.

Non-targeted and Targeted Emissions

Compounds in product emissions of potential interest tentatively identified by targeted screening, along with Hoffman analytes[4], were identified and quantified using GC-MS-SIM or HPLC-UV and compared to 1R6F emissions.

In Vitro Assays

The following *in vitro* assays were used to assess the genotoxic properties of the TPM+GVP from the two test articles:

- Ames test[5] (5 strains ± S9; OECD 471)
- *In vitro* micronucleus[6] (IVMN) assay (V79 cells, 3h ±S9 and 24h -S9; OECD 487)
- Neutral red uptake (NRU) assay[7,8] (BALB/c 3T3 cells, 24h -S9)

Results

Targeted Emissions

All the targeted analyte emissions measured in HHP were reduced compared to 1R6F and most analytes were within the levels found in studied tobacco heated products.

NRU

Significantly lower levels of cytotoxicity were observed with the HHP compared to 1R6F (Figure 3).

Results continued

NRU *(continued)*

No IC_{50} value could be generated from the HHP, whereas the 1R6F IC_{50} was 59.08 mg/mL.

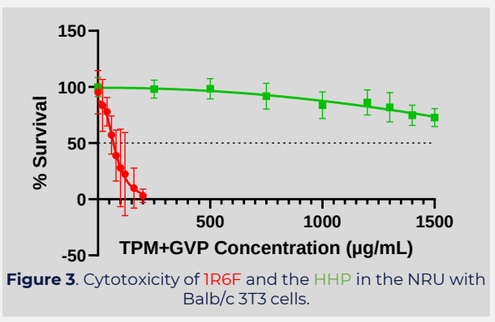

Figure 3. Cytotoxicity of 1R6F and the HHP in the NRU with Balb/c 3T3 cells.

Ames test

- 1R6F smoke induced reproducible, statistically significant, concentration-dependant increases in revertant colonies in strains TA98, TA100 and TA1537 +S9 (Figure 4).
- There was an indication of 1R6F weak mutagenicity in some remaining strains, but these were not reproducible.
- The HHP did not induce reproducible, dose-related increases in revertant number in any strain or treatment condition.

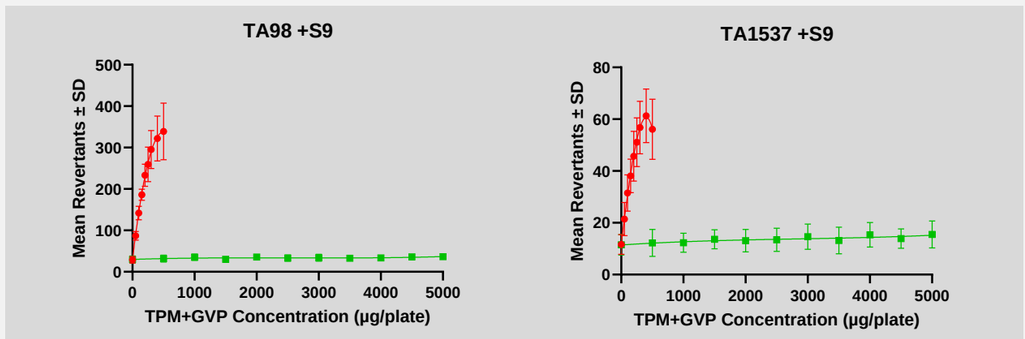

Figure 4. Mutagenicity of 1R6F and the HHP in the Ames test with TA98 and TA1537

In vitro micronucleus test

- 1R6F smoke induced reproducible, statistically significant, dose dependent increases in all treatment conditions (Figure 5).
- The HHP aerosol also induced genotoxicity, however this was significantly less ($p \leq 0.05$) than 1R6F smoke.

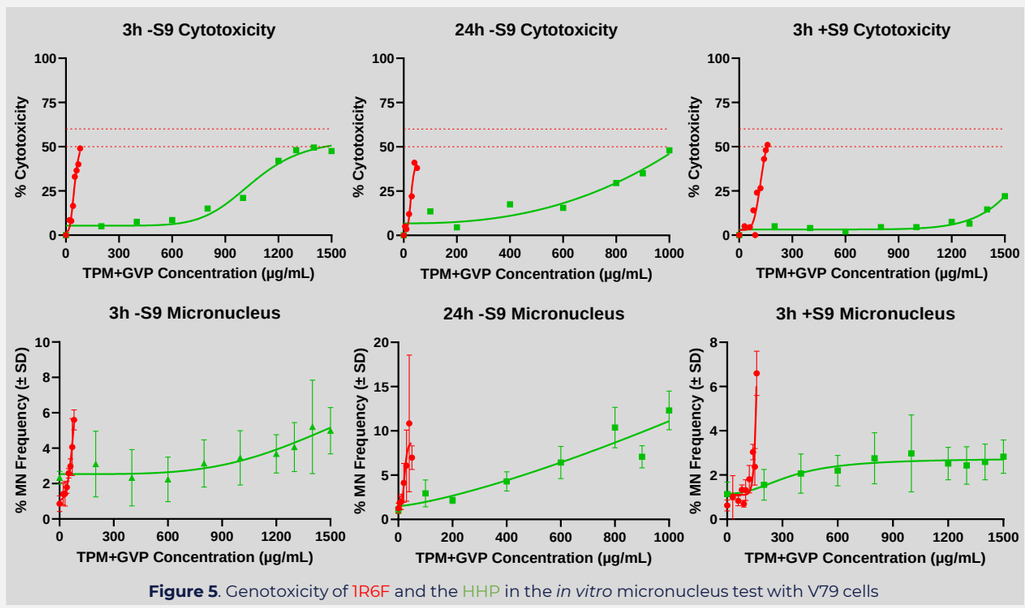

Figure 5. Genotoxicity of 1R6F and the HHP in the *in vitro* micronucleus test with V79 cells

Conclusion

The results from these *in vitro* studies show that that the aerosol from the HHP has the potential to be less mutagenic, genotoxic, and cytotoxic than cigarette smoke

Following a weight of evidence approach, the results of these studies suggest that this HHP has the potential to be reduced risk relative to cigarettes. However, additional studies are needed to determine the potential utility of these products for tobacco harm reduction

Contact
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References

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