

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

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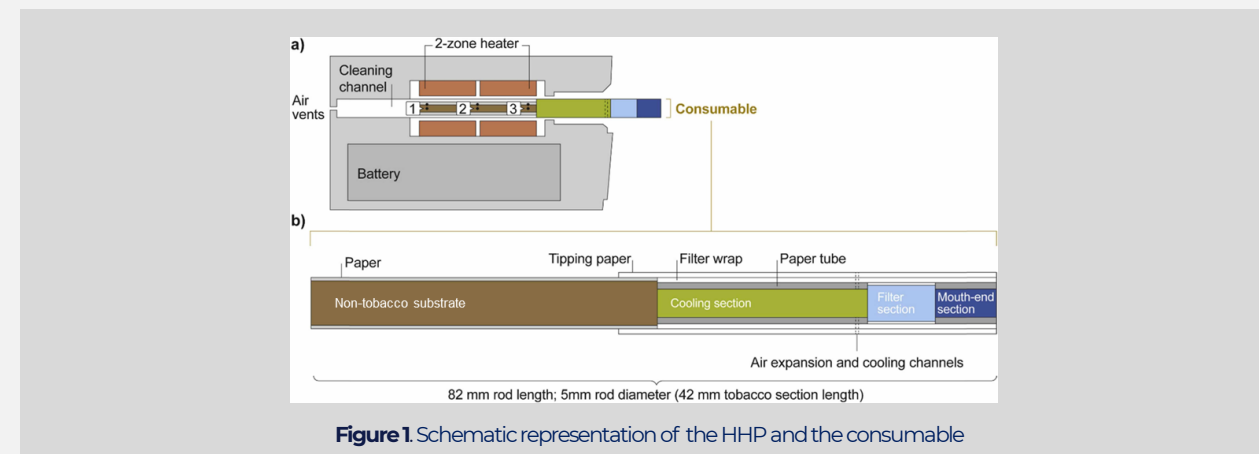
## Introduction

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

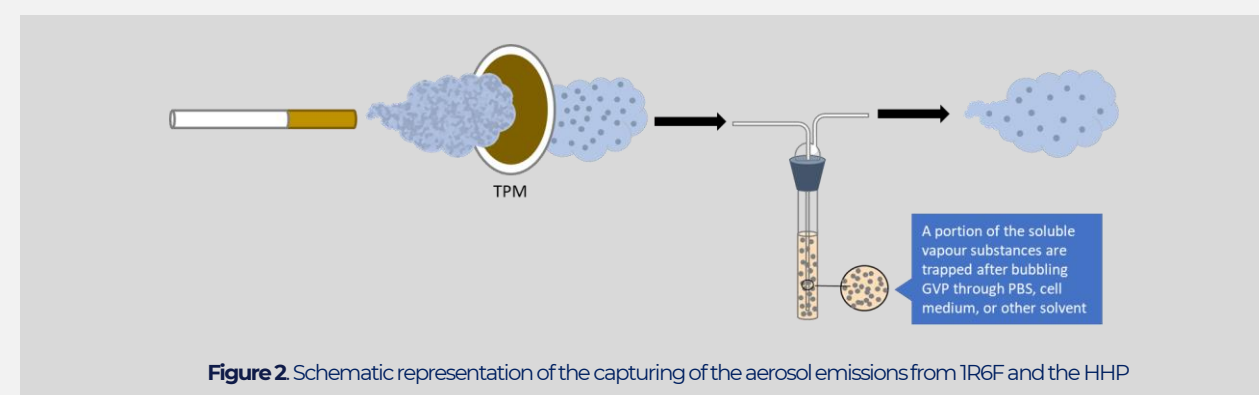
### Test Articles

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.



### Emissions Generation

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



### Non-targeted and Targeted Emissions

Compounds in product emissions of potential interest tentatively identified by targeted screening, along with Hoffman analytes,<sup>4</sup> 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<sup>5</sup> (5 strains  $\pm$  S9; OECD 471)
- *In vitro* micronucleus<sup>6</sup> (IVMN) assay (V79 cells, 3h  $\pm$  S9 and 24h -S9; OECD 487)
- Neutral red uptake (NRU) assay<sup>7,8</sup> (BALB/c 3T3 cells, 24h -S9)

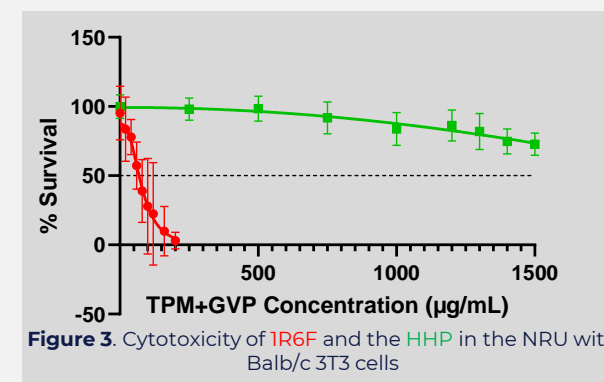
## Results

### 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

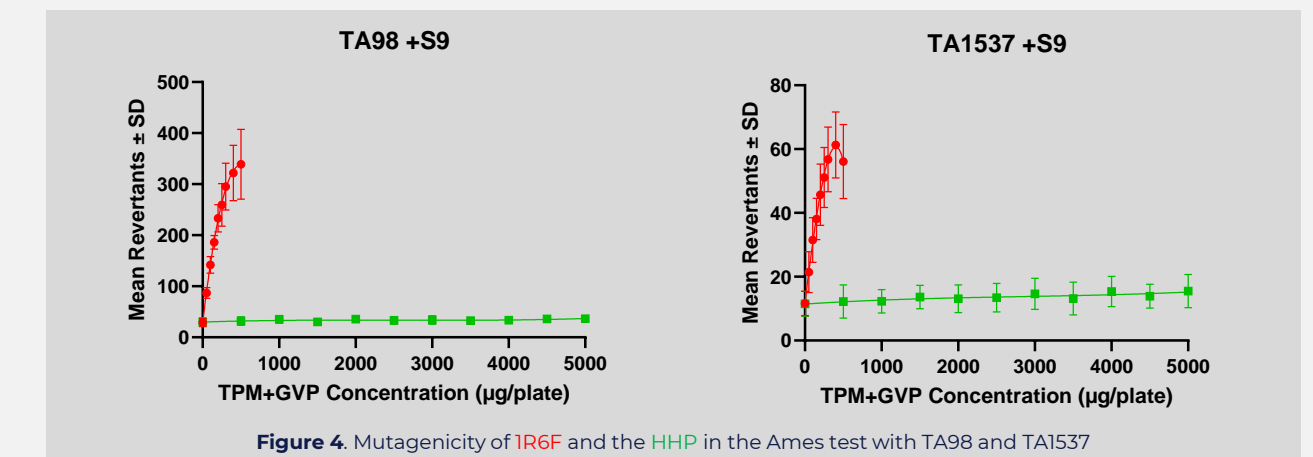
- Significantly lower levels of cytotoxicity were observed with the HHP compared to 1R6F (Figure 3)
- No IC<sub>50</sub> value could be generated from the HHP, whereas the 1R6F IC<sub>50</sub> was 59.08 mg/mL



### Ames test

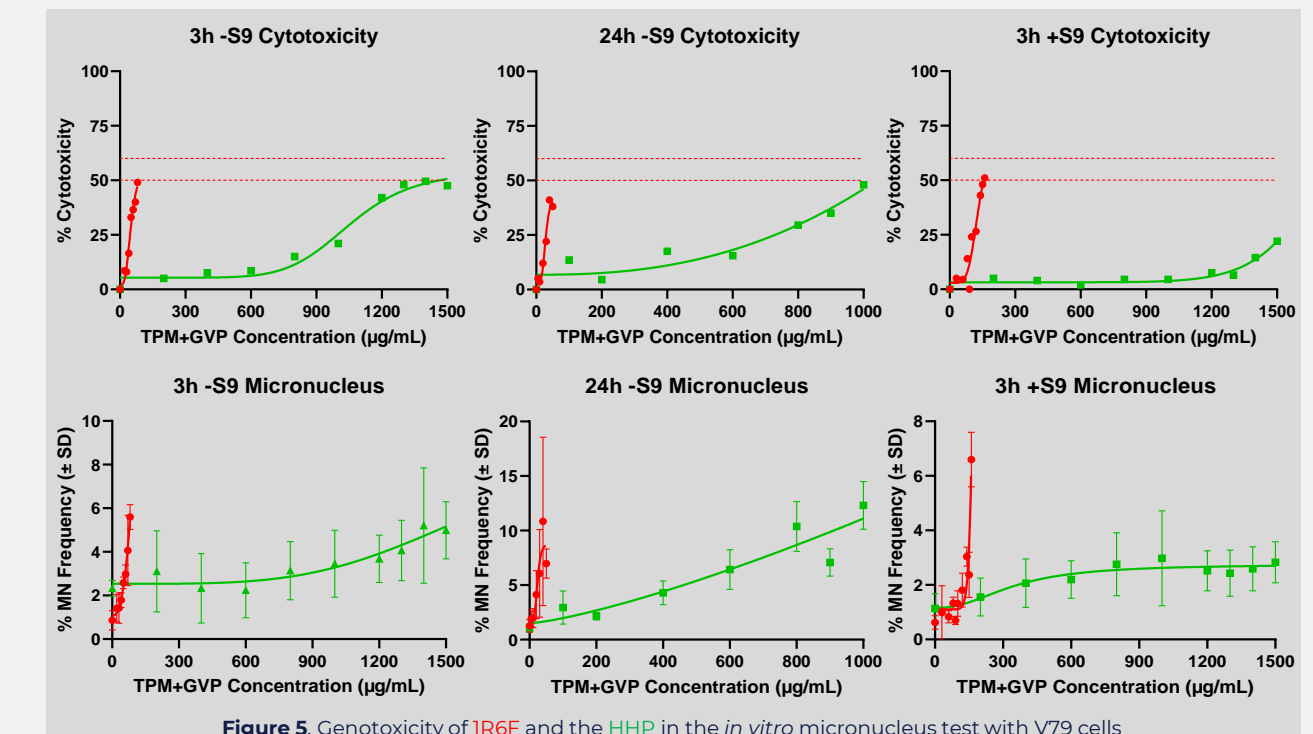
- 1R6F smoke induced reproducible, statistically significant, concentration-dependant increases in revertant colonies in strains TA98, TA100 and TA1537 +S9 (Figure 4)
- There was an indication of 1R6F weak mutagenicity in some remaining strains, but these were not reproducible
- The HHP did not induce reproducible increases in revertants that exceeded 2-fold the vehicle control in any strain or treatment condition

### Ames test (continued)



### In vitro micronucleus test

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



## Conclusion

The results from these *in vitro* studies show 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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