# Chemical and biological assessment of a Heated Tobacco Product (PULZE & iD) reveals marked reductions in aerosol toxicants and *in vitro* toxicity compared to a combustible cigarette

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## 1. INTRODUCTION

Tobacco harm reduction (THR) is the concept of providing adult smokers, who do not wish to quit nicotine and would otherwise continue to smoke, with potentially reduced harm forms of nicotine delivery[1]. Next generation nicotine delivery products (NGPs), including heated tobacco products (HTPs), generate substantially fewer and lower levels of toxicants than compared to combustible cigarettes, which is proposed to contribute to their THR potential[1]. The distinct chemical profiles of HTP aerosols and cigarette smoke has additionally been demonstrated to be reflected in toxicological outcomes[1].

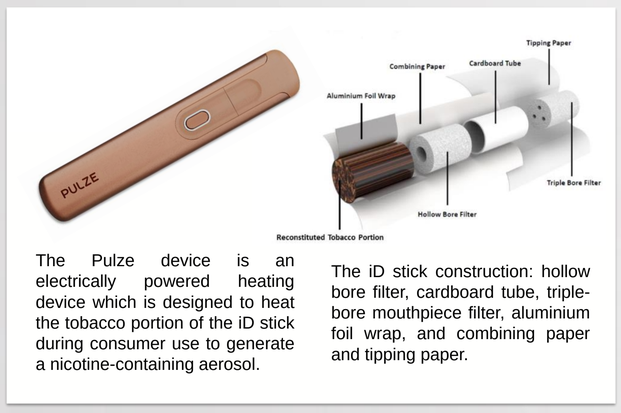
The aim of the present study was to compare the chemical composition and *in vitro* toxicological activity of a HTP aerosol (Pulze and iD) to that from conventional cigarette smoke (from the 1R6F Reference Cigarette) as HTPs form a growing category of potentially reduced harm nicotine delivery for adult smokers who do not wish to quit nicotine and would otherwise continue smoking.

## 2. METHODS

### Test Articles

- 1R6F Reference Cigarette (University of Kentucky)
- Heated Tobacco Product (HTP), (Pulze and iD sticks) (Imperial Brands PLC).

**Figure 1.** Pulze device and Rich Bronze stick configuration.




The Pulze device is an electrically powered heating device which is designed to heat the tobacco portion of the iD stick during consumer use to generate a nicotine-containing aerosol.

The iD stick construction: hollow bore filter, cardboard tube, triple-bore mouthpiece filter, aluminium foil wrap, and combining paper and tipping paper.

Both product categories were puffed according to the ISO 20778 intense regime (55ml puff volume, 30 sec puff intervals, 2 sec puff duration), although no ventilation blocking was applied to the HTP product.

### *In Vitro* Toxicology

The following *in vitro* toxicological assays were performed:

- Potential mutagenicity was determined using the *in vitro* Bacterial Reverse Mutation Test (Ames test) with *Salmonella* Typhimurium strains TA98 and TA100 (in the presence/ absence of S9 mix) in compliance with the OECD Test Guideline 471.
- Cytotoxicity was determined using the *in vitro* Neutral Red Uptake assay (NRU) with the human bronchial epithelium cell line, BEAS-2B. Cells were exposed to increasing numbers of puffs (dose levels) of fresh whole aerosol at the air liquid interface (ALI).
- Genotoxicity was determined using the *in vitro* micronucleus test (IVM) with V79 hamster lung fibroblasts. Cells were exposed to increasing dose levels of fresh aerosol at the ALI followed by a subsequent short-term incubation with S9 mix (+S9) or by direct recovery following exposure (-S9).

### Aerosol chemistry

- HTP aerosol collected mass (ACM)/ 1R6F total particulate matter (TPM) was trapped on a Cambridge filter pad using a linear 4-Channel smoking machine (LM4C Borgwaldt, Hamburg, Germany). The mass of the filter pad including the holder of the smoking machine is determined before and after use. The mass of the collected particulate phase per stick is the ACM/ TPM.
- Tobacco Specific N-nitrosamines (TSNAs) - the collected ACM/ TPM of the aerosol/ smoke produced using a linear 4-Channel smoking machine (LM4C Borgwaldt, Hamburg, Germany) was collected on Cambridge filter pads, and extracted with water/methanol.
- Gas phase - the vapour phase of the aerosol/ smoke produced using a linear 4-Channel smoking machine (LM4C Borgwaldt, Hamburg, Germany) was collected in a Tedlar bag located after the Cambridge filter pad. The sample (vapour phase) is separated by gas chromatography (GC) and detected by mass spectrometry (MS).
- Nicotine – the particle phase of the aerosol/ smoke was trapped on a Cambridge filter pad using a linear 4-Channel smoking machine (LM4C Borgwaldt, Hamburg, Germany). The filter was extracted with Propan-2-ol. An aliquot was analyzed via GC-flame ionization detection (FID).

## 3. RESULTS

### *Smoke chemistry*

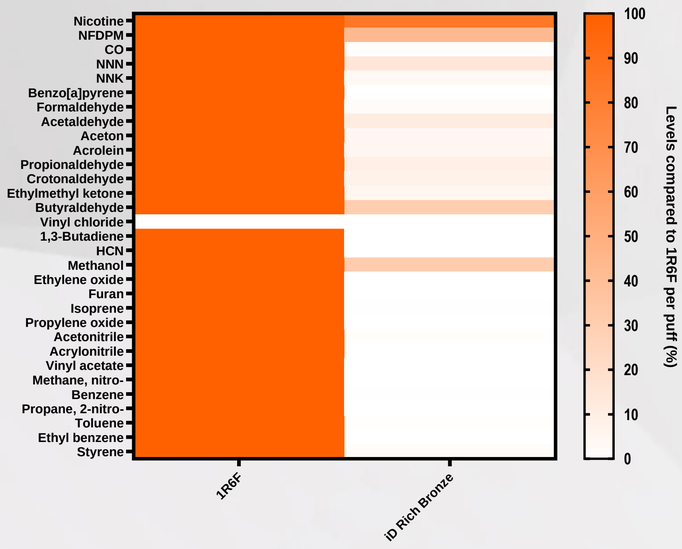



**Figure 2**. Heatmap of the levels of selected analytes in the 1R6F Reference Cigarette and iD stick, on a per puff basis. NFDPM = Nicotine-free dry particulate matter; NNN = N-nitrosonornicotine; NNK = nicotine-derived nitrosamine ketone.
Full details of the compounds in each chemical classes measured in this study can be provided by request.

**Chemical analysis** of the HTP aerosol revealed substantial reductions in toxicants present within the aerosol when compared with 1R6F cigarette smoke. The WHO 9 priority toxicants were reduced in average by 94% in Pulze aerosol per puff when compared to 1R6F smoke levels.

### *Ames test*

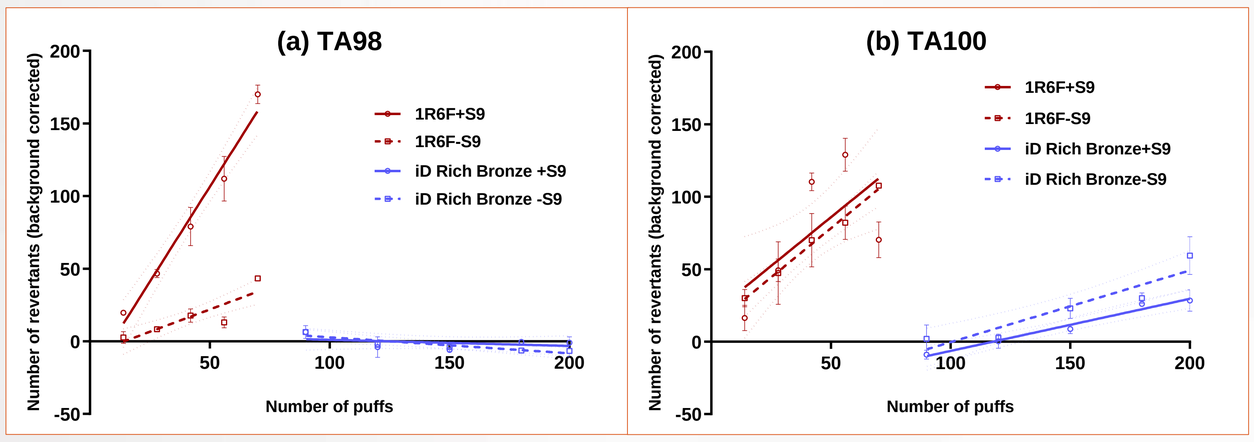



**Figure 4**: Average (background corrected) number of revertant colonies per plate for TA98 (a) or TA100 (b) strains exposed to increasing numbers of puffs of 1R6F Reference Cigarette smoke or Pulze & iD stick aerosol, +/-S9. n = 3; error bars represent SEM. Linear regression analysis was applied to the data (solid trendlines) and the slope calculated; dotted lines represent the 95% confidence intervals about the slope.

**The Ames assay demonstrated reductions in HTP aerosol mutagenic potential compared to the 1R6F reference cigarette**

- Smoke generated from the 1R6F reference cigarette caused a statistically significant and reproducible increase in the number of revertants for TA98 and TA100 ±S9 mix, and was therefore classed as mutagenic under the test conditions.
- The Pulze & iD stick aerosol was not classified as mutagenic under the test conditions in TA98 (± S9 mix).
- In TA100 (± S9 mix), the Pulze & iD stick aerosol was classed as mutagenic under the test conditions, however, when compared to the 1R6F reference cigarette, mutagenic potential was reduced by a factor of between 4-9 (dependent on the replicate).

### *Neutral Red Uptake assay*

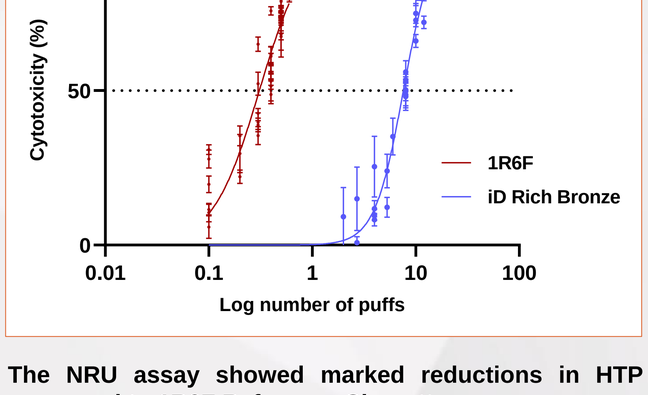



**Figure 3**: Percentage cytotoxicity induced in the neutral red uptake assay (Beas-2B cells) with exposure to increasing numbers of puffs (log scale) of 1R6F Reference Cigarette whole smoke, or Pulze & iD stick whole aerosol. Fifty percent cytotoxicity ($EC_{50}$) is marked with a black dotted line. n = 3; error bars represent standard error of the mean (SEM).

**The NRU assay showed marked reductions in HTP aerosol cytotoxicity compared to 1R6F Reference Cigarette**

- The data indicate that the aerosol from Pulze & iD was 25-fold less cytotoxic than smoke from 1R6F Reference Cigarette

### *Micronucleus assay*

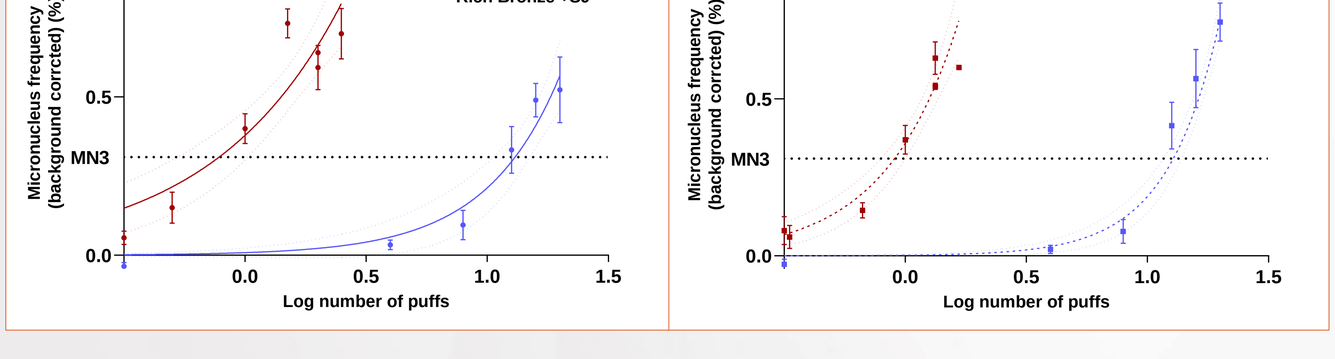



**Figure 5**: Background subtracted micronucleus frequency in V79 cells following exposure to increasing puffs (log scale) of 1R6F whole smoke or Pulze & iD stick whole aerosol in either the presence (a) or absence (b) of S9. ECMN3 analysis was carried out using non-linear regression analysis (solid lines for each test item) to indicate the puff-wise exposure required to induce a MN frequency three times that of background levels for each test article. Error bars represent SEM; n = 2.

**The *In Vitro* Micronucleus assay indicated marked reductions in HTP aerosol genotoxicity compared to 1R6F Reference Cigarette smoke**

- Dose dependent, reproducible and statistically significant increases in micronucleus frequencies were observed independent of the metabolic activation status.
- However, the data shows that the aerosol from Pulze & iD was 16.7-fold less genotoxic in the presence and 14.3-fold less genotoxic in the absence of S9, compared to 1R6F Reference Cigarette smoke.

## 4. CONCLUSIONS

- Chemical analysis of the Pulze & iD stick aerosol revealed substantial reductions in the numbers and levels of toxicants compared to 1R6F smoke (on average 94% reduction in WHO TobReg 9 analytes). This translated to substantial reductions in *in vitro* toxicological outcomes for Pulze & iD Rich stick aerosol compared to 1R6F smoke under the conditions of the test.
- The Pulze aerosol demonstrated marked reductions in cytotoxicity compared to 1R6F cigarette smoke on a per puff basis (96%). As anticipated, 1R6F cigarette smoke was highly mutagenic and genotoxic, whereas for the HTP aerosol, there were marked reductions in mutagenicity and genotoxicity under the conditions of the tests.
- The data shows clear differences between 1R6F cigarette smoke and HTP aerosol emissions and *in vitro* toxicity. These findings contribute to the weight of evidence for the tobacco harm reduction potential of HTPs.

## REFERENCES

[1] O'Leary, R., & Polosa, R. (2020). Tobacco harm reduction in the 21st century. Drugs Alcohol Today 20 (3): 219–234. [2] Chapman, F., Sticken, E. T., Wieczorek, R., Pour, S. J., Dethloff, O., Budde, J., ... & Stevenson, M. (2023). Multiple endpoint in vitro toxicity assessment of a prototype heated tobacco product indicates substantially reduced effects compared to those of combustible cigarette. *Toxicology in Vitro*, *86*, 105510. [3] ISO 20778 (2018b). Cigarettes–Routine Analytical Cigarette Smoking Machine–Definitions and Standard Conditions with an Intense Smoking Regime. Geneva: International Organization for Standardization. [4] OECD, 2016. Test No. 487. In vitro Mammalian Cell Micronucleus Test, OECD Guidelines for the testing of chemicals, Section 4: Health effects. OECD Publishing [5] OECD, 1997. Test No. 471: Bacterial Reverse Mutation Test, OECD Guidelines for the Testing of Chemicals, Section 4: Health Effects. OECD Publishing