Pre-Clinical Assessment of a Dual-Temperature Operated Heated Tobacco Product

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IMPERIAL BRANDS SCIENCE

EUROTOX 2019 CONGRESS
8TH – 11TH SEPTEMBER 2019, HELSINKI, FINLAND

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1. Introduction

Heated tobacco products generate a nicotine-containing aerosol with a tobacco taste through the heating of tobacco by an electrical device. As the tobacco is heated and not burned, the aerosol generated by this type of product is expected to contain substantially lower levels of the toxicants found in cigarette smoke.

A review of the scientific literature by Public Health England recently concluded that heated tobacco products may be considerably less harmful than tobacco cigarettes but more harmful than e-vapour products [1].

The aim of this study was to chemically and biologically assess a new tobacco heating product which can generate aerosol at two different operating temperatures (**Figure 1)** and compare this response to cigarette smoke.

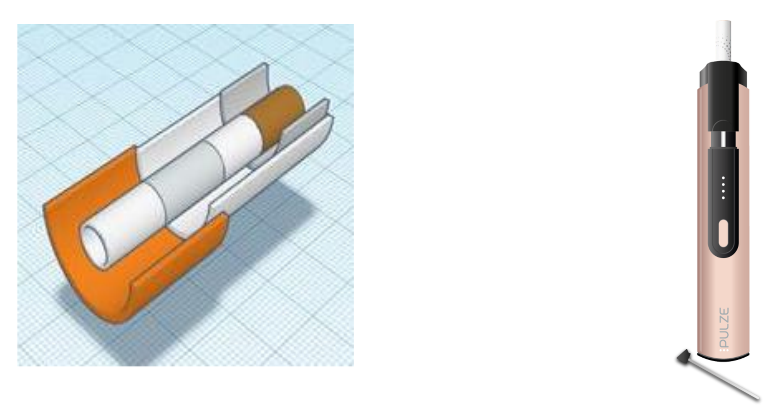


Figure 1. Anatomy of the iD stick and the Pulze heated tobacco device

2. Analytical Methods

Aerosol Generation Regime

The ISO Intense aerosol-generation method (puff volume, 55 mL; puff duration, 2s; puff interval, 30s; bell-shaped puff profile; ventilation holes blocked) was used across the study. ISO Intense was chosen based on previous research conducted by Imperial Brands on a prototype heated tobacco device [2], which showed low concentrations of toxicants and other analytes in the aerosol being formed even when a more extreme puffing regime was followed.

Aerosol Chemistry

Three replicates were measured for Pulze and 3R4F. Due to the operational characteristics of the Pulze device, a maximum of 8 puffs were obtained during the heating period under the ISO Intense smoking regime for each replicate. Approximately 10 puffs were obtained for 3R4F for each replicate. Levels of "tar", nicotine, carbon monoxide and a further 41 toxicants of notable public health interest were measured and compared between the samples. The toxicants assessed included those proposed by the World Health Organization Study Group on Tobacco Product Regulation (TobReg) [3] and the US Food and Drug Administration (FDA) [4]. A GC-MS qualitative untargeted analysis was conducted on the gas phase and total particulate matter (TPM) of Pulze under both operating temperatures. The analytes identified were further measured in the 3R4F cigarette. All analyses were conducted by Imperial Brand's ISO 17025 accredited laboratory.

***in vitro* Biological Testing**

The following regulatory in vitro toxicological assays were performed: Neutral Red uptake (NRU) for cytotoxicity in BEAS-2B cells, *Salmonella typhimurium* reverse mutation assay (Ames test) for mutagenicity in TA100 strain and in vitro micronucleus (IVM) with V79 cells (3hrs + S9) for genotoxicity. Cells were exposed to fresh smoke or fresh aerosol at the air liquid interface using the internal smoking machine 'smoke aerosol exposure in vitro system' (SAEIVS) (Burghart Tabaktechnik, Wedel, Germany) for NRU and IVM. For the Ames assay, a RM1 smoking machine (Burghart Instruments, Wedel, Germany) was connected to the impinger with bacteria suspension, and the smoke was directly bubbled through TA100. All three assays and methods were based on international OECD guidelines 129, 487 and 471, and performed in accordance with ISO 17025.

Data and Statistical Analysis

All data and statistical analysis were conducted using Microsoft Excel and GraphPad Prism.

3. Untargeted analysis

Tobacco smoke is a complex mixture of over 5700 different compounds [5]; whereas only 30 compounds were detected in the gas and TPM phase of Pulze aerosol (at both temperatures) (***Figure 2***).

All of these components were detected in the mainstream smoke and TPM of the 3R4F reference cigarette. No unique analytes attributable to iD being heated were detected.

Figure 2. *Chemical composition of Pulze aerosol and TPM, as determined by GC-MS untargeted analysis.*

Gas Phase

- Acetaldehyde
- Methanol
- Methanethiol
- Furan
- Acrolein
- Propanal
- Acetone
- Isobutyraldehyde
- Furan, 2-methyl-
- 2,3-Butanedione
- 2-Butanone
- Isovaleraldehyde
- Butyraldehyde, 2-methyl-
- 2,3-Pentanedione
- Pyrrole, 1-methyl-
- Toluene

TPM

- 2-Propanone, 1-hydroxy-
- Furfural
- 5-Methylfurfural
- Furfurylalcohol
- 2-Methylbutyric acid
- (±)-Solanone
- 3-Methylpentanoic acid
- Corylon
- Neophytadiene
- Phenol
- Furaneol
- Pyrrole-2-aldehyde
- 3-Hydroxypyridine
- 5-Hydroxymethylfurfural
- Phytol

4. Reduction in toxicants of public level interest

In comparison to the reference cigarette, the toxicant levels in the emissions generated by Pulze were substantially reduced across all chemical classes measured (***Figure 3***).

For the nine toxicants proposed by TobReg for mandated reduction in cigarette emissions [3], the overall average reduction was 95% for Pulze used in "ECO mode" (315°C) and 94% for Pulze used in "standard mode" (345°C) when compared to 3R4F levels per puff (***Figure 4***). For the abbreviated list of harmful and potentially harmful constituents (HPHCs) of smoke specified by the FDA Tobacco Products Scientific Advisory Committee (excluding nicotine) [4], the overall average reduction was 91% for Pulze used in "ECO mode" (315°C) and 90% for Pulze used in "standard mode" (345°C) when compared to 3R4F levels per puff (***Figure 4***) .

Figure 5 visually displays these differences: cigarette smoke on the left has a brown colour when captured on a filter pad; by contrast, the aerosol of Pulze used in "standard mode" (345°C) on the right is visibly different, reflecting the different chemical compositions.

Figure 3. *Percent reduction in emissions of Pulze aerosol compared to 3R4F (per puff)*

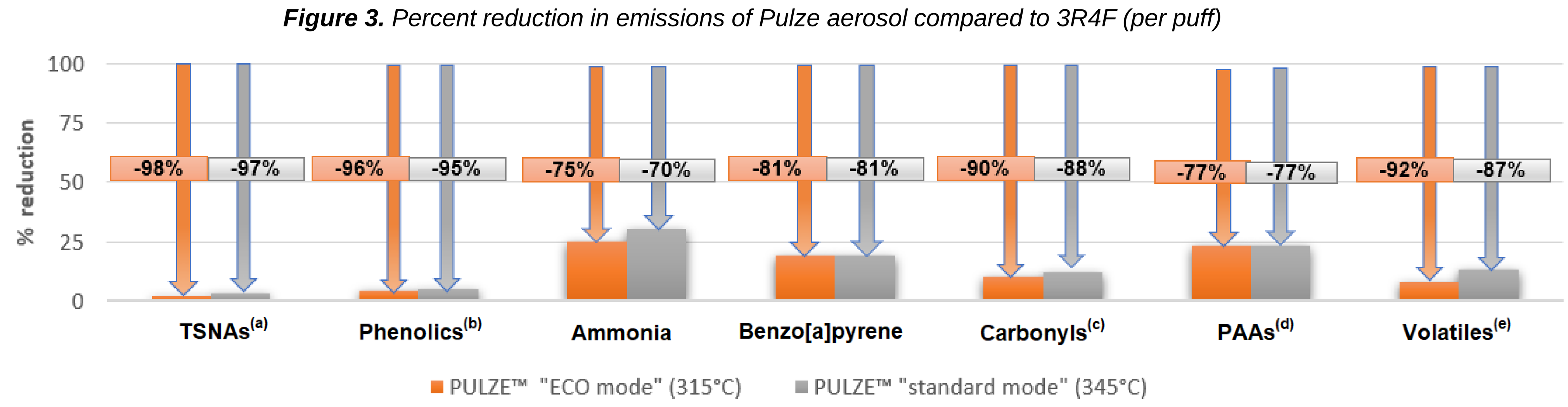


Figure 4. *Percent reduction in toxicologically relevant constituents in Pulze aerosol compared to 3R4F (per puff)*

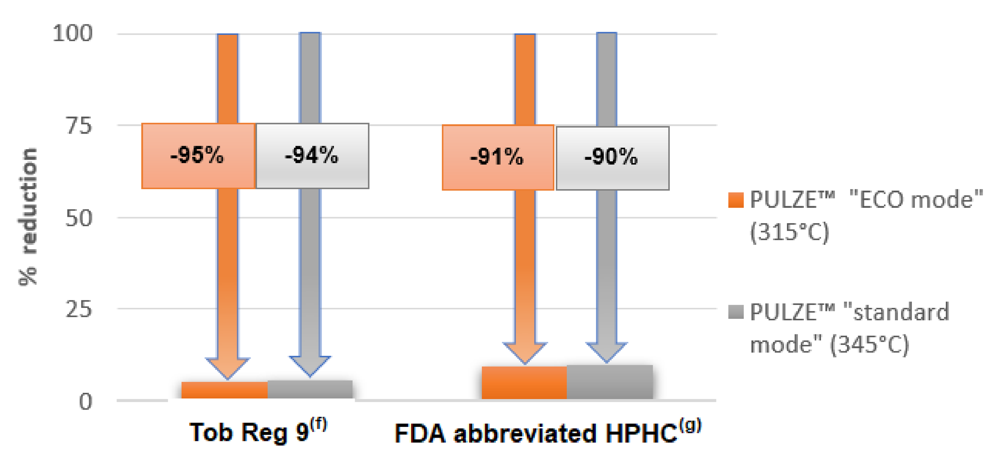
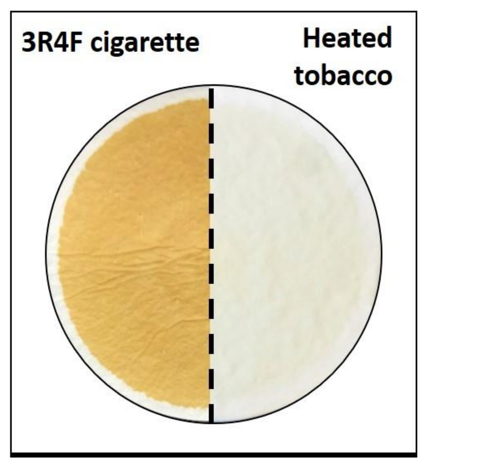


Figure 5. *Cambridge filter pad showing particulate matter collected for 3R4F Cigarette and Heated Tobacco*



5. TNCO and glycerol

Table 1 shows the yields for "tar" (NFDPM)$^{(h)}$, nicotine and carbon monoxide for Pulze under both operating temperatures and for the 3R4F reference cigarette comparator. The NFDPM produced by Pulze is principally composed of the aerosol former glycerol.

Table 1. *Standard analyte and constituent yields for Pulze and 3R4F*

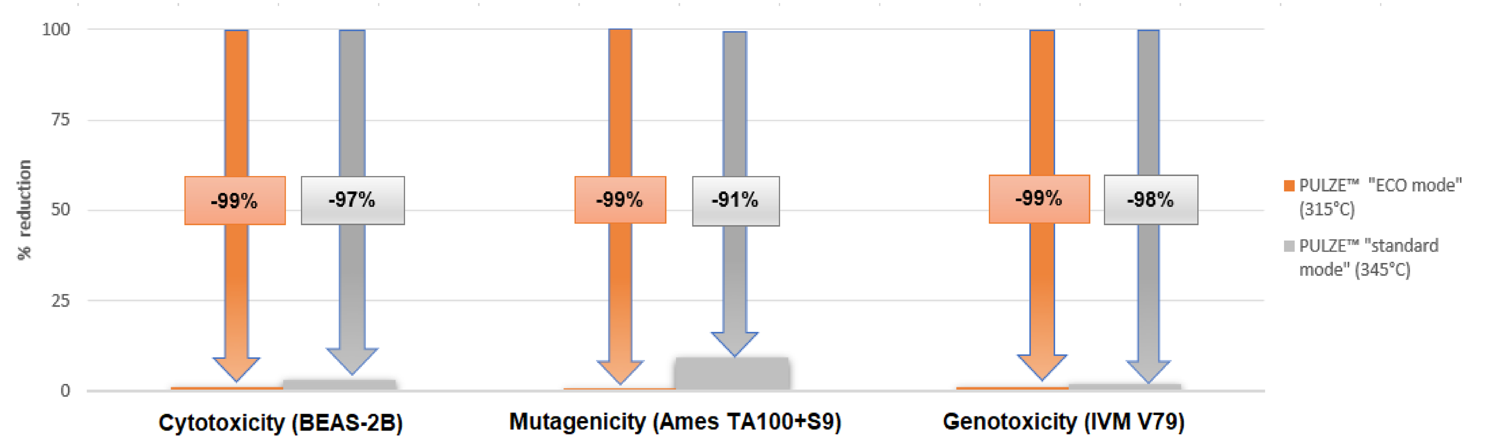
ISO Intense Parameter	3R4F cigarette smoke (mg/puff)	Pulze "ECO mode" (315°C) (mg/puff)	% difference Pulze "ECO mode" (315°C) aerosol vs. 3R4F cigarette smoke	Pulze "standard mode" (345°C) (mg/puff)	% difference Pulze "standard mode" (345°C) aerosol vs. 3R4F cigarette smoke
"tar" (NFDPM)$^{(h)}$	2.5075	1.1181	55.4098	1.4320	42.8906
Nicotine	0.1825	0.0660	63.8473	0.0807	55.7636
Carbon monoxide	3.2507	0.0288	99.1127	0.0481	98.5217
Glycerol	0.2263	0.2766	-22.2363	0.3525	-55.7837

6. Toxicological response *in vitro*

All in vitro tests were performed with fresh generated whole aerosol. Cytotoxicity and genotoxicity was detectable for both tested products. However, Pulze aerosol has been found to be 99% less cytotoxic, mutagenic and genotoxic than 3R4F smoke when used in "ECO mode" (315°C) , and be 97% less cytotoxic, 91% less mutagenic and 98% less genotoxic than 3R4F smoke when used in "standard mode" (345°C) **(Figure 6).**

No mutagenicity was detected for Pulze used in "ECO mode" with up to 126 puffs.

Figure 6. *Percent reduction of in vitro biological response of Pulze aerosol compared to 3R4F (per puff)*



Legend

(a) TSNAs: NNN, NNK, NAT, NAB;
(b) Phenolics: hydroquinone, resorcinol, catechol, phenol, p-cresol, m-cresol, o-cresol;
(c) Carbonyls: formaldehyde, acetaldehyde, acetone, acrolein, propionaldehyde, crotonaldehyde, ethylmethyl ketone, butyraldehyde;
(d) PAAs: 1-aminonaphthalene, 2-aminonaphthalene, 3-aminobiphenyl, 4-aminobiphenyl;
(e)Volatiles: 1,3-butadiene, HCN, methanol, ethylene oxide , furan, isoprene, propylene oxide, acetonitrile, acrylonitrile, vinyl acetate, methane, nitro-, benzene, propane, 2-nitro-, toluene, ethyl benzene, styrene;
(f) TobReg 9: acetaldehyde, acrolein, 1,3-butadiene, benzene, B[a]P, CO, formaldehyde, NNN, NNK;
(g) FDA abbreviated HPHC: TobReg 9 analytes + 1-aminonaphthalene, 2-aminonaphthalene, 4-aminobiphenyl, acrylonitrile, ammonia, crotonaldehyde, isoprene, toluene;
(h) "Tar" refers to the residue from cigarette smoke when a cigarette is burned and is the raw anhydrous nicotine-free condensate of smoke. "Tar" is calculated using the following formula: Tar = Total Particulate Matter – Nicotine – Water. Here we refer to the "tar" collected from the Pulze as "nicotine-free dry particulate matter or NFDPM".

This work was supported by Imperial Brands plc. Imperial Brands plc is the manufacturer of Pulze and iD sticks used in this study.

7. Conclusions

- Pulze used with the iD stick produces a greatly simplified aerosol compared to cigarettes.
- Under the conditions tested, the level of toxicants were slightly more reduced when Pulze is used "ECO mode" (315°C) than "standard mode" (345°C).
- This is also reflected in the biological response of Pulze aerosol, which has been found to be 99% less cytotoxic, mutagenic and genotoxic than 3R4F smoke when used in used "ECO mode" (315°C), and be 97% less cytotoxic, 91% less mutagenic and 98% less genotoxic than 3R4F smoke when used in "standard mode" (345°C).
- This data contributes to the growing body of evidence that new generation products such as Pulze may offer the potential for substantially reduced exposure to toxicants when used as an alternative to cigarettes.

References

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