

Next Generation Products induce lower biological activity than combusted cigarettes: A comparison of aerosol chemistry & *in vitro* toxicity



1. INTRODUCTION

Combustible tobacco products like cigarettes are known to cause serious disease in smokers, including lung cancer, heart disease and emphysema. A range of next generation products (NGPs), which do not involve combustion, are commercially available and there is a growing belief that NGPs may be a less harmful alternative to combustible tobacco products. The aim of the study was to compare the chemical and *in vitro* toxicological activity of NGP aerosols to that of cigarette smoke. Products investigated were the Kentucky reference cigarette (3R4F), a tobacco heating product (THP), a hybrid product (HYB) and a *myblu*TM e-cigarette (Tobacco Flavour; 1.6% Nicotine). Smoke/aerosol were produced using Health Canada Intense method for 3R4F and THP and Coresta Recommended Method N°81 for HYB and *myblu*TM. Product smoke/aerosols were tested in established regulatory *in vitro* toxicology assays.

2. MATERIALS

2.0 Test Articles

- Kentucky 3R4F Reference Cigarette
- Commercially available tobacco heating product (THP), German market
- Commercially available hybrid product (HYP), Romanian market
- E-vapour product: *myblu*TM device and pod (1.6% [w/w] nicotine; tobacco flavour), UK market

NGP product formats are shown in Figure 1

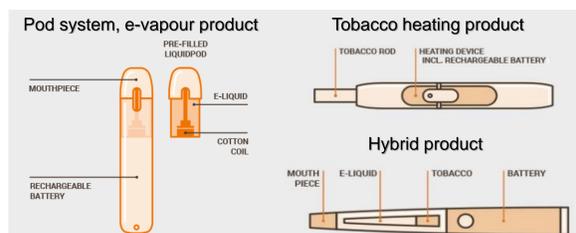


Figure 1: Next generation product formats

2.1 Smoke and Aerosol Generation

Test product aerosol/smoke was generated using the following regimes (Table 1).

Test product	Sampling blocks for chemistry testing	Smoking regime	Puff volume (ml)	Puff duration (s)	Puff interval (s)	Ventilation blocking	Puff profile	Smoking machine
3R4F	-	Health Canada Intense ^[1]	55	2	30	Yes	Bell	Linear
HTP	-	Health Canada Intense ^[1]	55	2	30	N/A	Square (emissions) Bell (<i>in vitro</i> toxicology)	Linear
HYB	4 blocks, each 50 puffs	Coresta Recommended Method N°81 ^[2]	55	3	30	N/A	Square	Linear
<i>myblu</i> TM	3 blocks, each 50 puffs	Coresta Recommended Method N°81 ^[2]	55	3	30	N/A	Square	Linear

2.2 Smoke and Aerosol Characterization

Internal accredited laboratory quantified the following emissions for all test articles: tar, nicotine, carbon monoxide and WHO TobReg 9 analytes. WHO TobReg 9 analytes consist of the following: acetaldehyde, acrolein, 1,3-butadiene, benzene, benzo-a-pyrene, carbon monoxide, formaldehyde, 3-(1-Nitrosopyrrolidin-2-yl)pyridine (NNN) and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK)^[3]. All methods used are established and validated only for cigarette and cigarette smoke applying ISO smoking regime (35/2/60-bell shape) and are accredited according ISO 17025.

2.3 *In vitro* Toxicology

The following regulatory *in vitro* toxicological assays were performed: Neutral red uptake (NRU) for cytotoxicity in BEAS-2B cells, following standard assay protocols in accordance with ISO 17025; Salmonella typhimurium reverse mutation assay (Ames test) for mutagenicity in TA98 and TA100 in compliance with OECD test Guideline 471; and *in vitro* micronucleus (IVM) with V79 (3hrs + S9) for genotoxicity in compliance with OECD test Guideline 487. Cells were exposed to smoke or 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 and using the smoking machine RM1 (Burghart Instruments, Wedel, Germany) for the Ames assay.

2.6 Data and statistical analysis

All data and statistical analysis were conducted using Microsoft Excel and GraphPad Prism. Statistically significant differences between samples were calculated using ANOVA with posthoc Dunnett test. All differences were considered statistically significant with a p-value ≤ 0.05.

3. RESULTS

3.1 Smoke and Vapour Characterization

Cambridge Filter Pads

Particulate phase emissions of 5x 3R4F sticks compared to (A) 5x THP sticks (10 puffs per stick), (B) one block of 50 puffs for HYB and (C) one block of 50 puffs for *myblu*TM (Image 1).

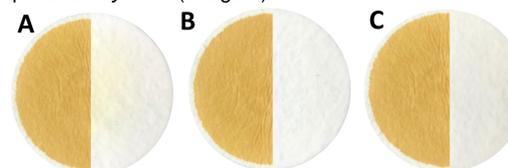


Image 1: Cambridge filter pad showing particulate matter collected for (A) THP aerosol (B) HYB aerosol and (C) *myblu*TM aerosol compared to 3R4F particulate emissions

TNCO

Mean "tar", nicotine and carbon monoxide levels for test articles are shown in Figure 2. The average nicotine yield per puff was 175.5µg/puff for 3R4F, 106µg/puff for HTP, 22.9µg/puff for HYB and 85.4µg/puff for *myblu*TM.

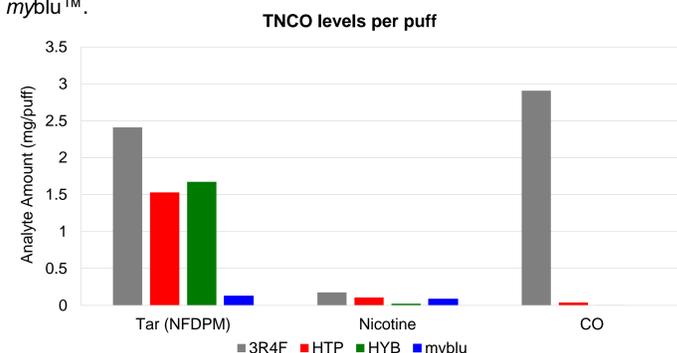


Figure 2: Tar (Nicotine free dry particulate matter), nicotine and carbon monoxide delivery per puff for 3R4F, HTP, HYB and *myblu*TM

* "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. "Tar" collected from NGPs is referred to as "nicotine-free dry particulate matter or NFDPM".

WHO TobReg

Emissions analysis of all the NGP aerosols tested revealed substantial reductions in aerosol constituents when compared with 3R4F smoke (Figure 3).

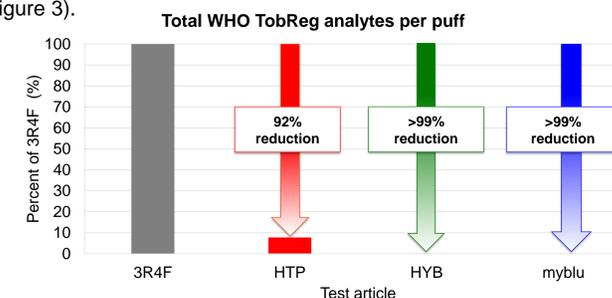


Figure 3: Percent reduction in emissions of NGP aerosol compared to 3R4F, for TobReg priority toxicants (per puff).

3.2 *In vitro* toxicity

Cytotoxicity (NRU)

All NGP aerosols demonstrated marked cytotoxicity reductions compared to cigarette smoke on a per puff basis (Figure 4). *myblu*TM displayed low levels of cytotoxicity compared to the other test articles.

The puff specific cytotoxicity can be described as: 3R4F > HTP > *myblu*TM ≥ HYB

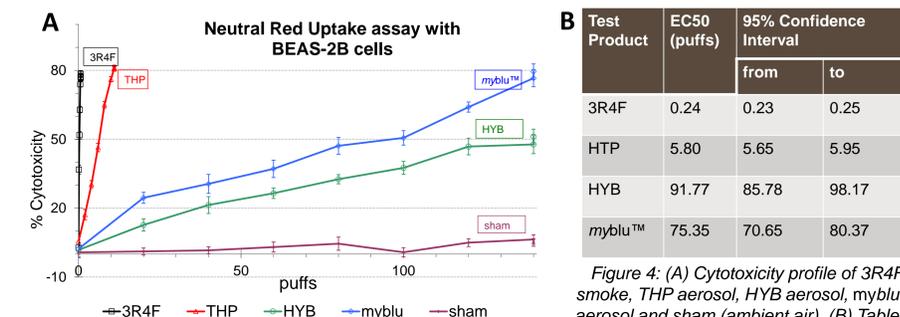


Figure 4: (A) Cytotoxicity profile of 3R4F smoke, THP aerosol, HYB aerosol, *myblu*TM aerosol and sham (ambient air). (B) Table of EC50 values for each test product

Mutagenicity (Ames)

3R4F smoke was highly mutagenic in the Ames test. In TA100+S9, 3R4F smoke showed marked mutagenicity, which was reduced in THP (approximately 7 fold) (Figure 3). In TA98 (data not shown), only 3R4F smoke produced a positive, mutagenic response. Neither HYB or *myblu*TM aerosol produced a significant number of revertants, up to 300 puffs in the presence of S9 mix compared to ambient air (Figure 5).

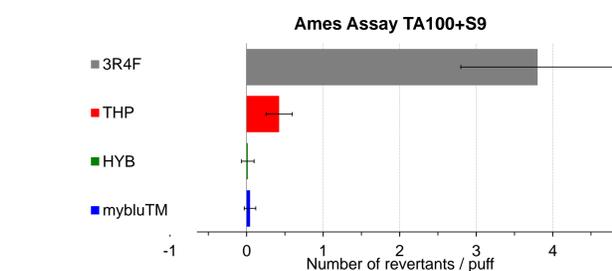


Figure 5: The number revertants per product puff for 3R4F smoke and THP, HYB and *myblu*TM aerosol in TA100+S9 (p value ≤ 0.05)

Genotoxicity (IVM)

Both 3R4F smoke and THP aerosol induced reproducible and statistically significant increased in micronucleus frequencies, with 3R4F smoke inducing significant genotoxicity after 1 puff. When comparing micronucleus frequencies over background levels (ECMN3) from HTP aerosol compared to 3R4F smoke, a 30 fold lower genotoxicity was observed for the HTP (Figure 6).

HYB and *myblu*TM aerosol, up to 100 puffs, did not induce any statistically significant increases in MN frequency, compared to negative control.

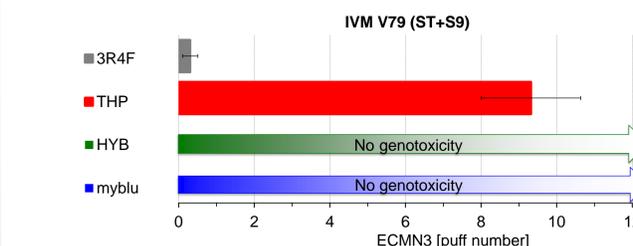


Figure 6: The micronucleus frequencies over background for 3R4F smoke THP, HYB and *myblu*TM aerosol (p value ≤ 0.05)

4. CONCLUSIONS

- The regulatory assays described above form part of a core battery of tests, to determine the potential hazard of cigarettes and NGP products.
- As expected, there are clear cytotoxic, mutagenic and genotoxic effects observed for 3R4F smoke. HTP produced some responses but to a much lesser degree.
- There are marked reductions in the emissions and *in vitro* toxicity of NGP products compared to 3R4F cigarettes.
- Under the test conditions *myblu*TM demonstrated significantly reduced toxicity. This data contributes to the growing body of evidence that *myblu*TM is a potential reduced harm product compared to conventional cigarettes.