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. Product investigations were conducted using the Kentucky reference cigarette (3R4F), a tobacco heating product (THP), a hybrid product (HYB) and a myblu® e-cigarette (Tobacco Flavour, 1.6%). Smoke/aerosol was produced using Health Canada Intensive Method for 3R4F and THP and Coresta Recommended Method N°81 for HYB and myblu®. Product smoke/aerosols were tested in established regulatory in vitro toxicity assays.

2. MATERIALS

2.1. Testing Methods

- Kentucky 3R4F Reference Cigarette
- Commercially available tobacco heating product (THP), German market
- Commercially available hybrid product (HYB), Romanian market
- E-vapour product: myblu® device and pod (16% w/w nicotine; tobacco flavour), UK market

NGP product formats are shown in Figure 1

2.2. Smoke and Aerosol Generation

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

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 487; and in vitro micronucleus (VM) with V79 (3hrs + 9hr) for genotoxicity in compliance with OECD test Guideline 487. Cells were exposed to smoke or aerosol by the air liquid interface using the internal smoking machine 'smoke aerosol exposure in vitro system' (SAEIVIS) (Burghart Tabakbeschled, 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 Characterisation

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® (Figure 1).

Figure 1: Next generation product form factor

3.2. In vitro Toxicology

Cytotoxicity (NRU)

All NGP aerosols demonstrated marked cytotoxicity reductions compared to cigarette smoke on a per puff basis (Figure 4). myblu® displayed low levels of cytotoxicity compared to other test articles. The puff specific cytotoxicity can be described as: 3R4F > HYB > myblu® > THP

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® aerosol produced a significant number of revertants, up to 305 puffs in the presence of S9 mix compared to ambient air (Figure 5).

Genotoxicity (IVM)

Both 3R4F smoke and THP aerosol induced reproducible and statistically significant increases in micronucleus frequencies, with 3R4F smoke inducing significant genotoxicity after 1 puff. When comparing micronucleus frequencies over background levels (ECM2N) from THP aerosol compared to 3R4F smoke, a 30 fold lower genotoxicity was observed for the THP (Figure 6). HYB and myblu® aerosol, up to 100 puffs, did not induce any statistically significant increases in MN frequency, compared to negative control.

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. THP 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® demonstrated significantly reduced toxicity. This data contributes to the growing body of evidence that myblu® is a potential reduced harm product compared to conventional cigarettes.

REFERENCES


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