1. INTRODUCTION

Combustible cigarettes are known to cause serious diseases in smokers, including lung cancer, heart disease and emphysema. A range of next generation products (NGPs), which do not involve combustion or do not contain tobacco, are commercially available and there is a growing understanding that these NGPs may be a less harmful alternative to combustible cigarettes. TT21C technologies can be used to assess the harm reduction potential of NGPs using human-derived cellular systems and biological endpoints. The harm reduction potential of three different NGPs was compared to a reference cigarette (3R4F) in a series of in vitro assays. Phosphate buffered saline (PBS) extracts were used within in vitro assays where direct exposure to smoke/aerosol was not possible.

2. SAMPLE GENERATION AND QUANTIFICATION

2.1 Test Articles

- Kentucky 3R4F Reference Cigarette
- Commercially available heated tobacco product (THP)
- Commercially available next generation product (NGP)
- E-vape product: myblu™ device and pod (1.6% [w/v] nicotine; tobacco flavour)

2.2 Smoke and Aerosol Extract Generation

Smoke and aerosol was generated using the following regimes:
- 3R4F smoke and THP aerosols were generated using ISO Intense regime 20778 (55m puff volume, 3 s puff duration, 30 s puff interval).
- HYB and myblu aerosols were generated using CORESTA Recommended method N81 (55mL puff volume, 3 s puff duration, 30 s puff interval).

Smoke and aerosols were generated with a Vitrobot VC10 (Vitrobot, Munich, Germany) smoking machine. Smoke or aerosol extracts were prepared by bubbling the smoke aerosol 3 in to 3 in incubator, each containing 10 mL PBS. A total stock solution of 30mL per test article was used: 8.8 puffs per mL for THP (total 54 puffs) and 4 puffs per mL for NGPs (total 120 puffs).

Nicotine and carbonyl trapped in fresh bubbled PBS samples were quantified using LC-MS/MS and LC-GAD methods respectively. For nicotine measurement, the internal standard nicotine-d4 was used. For carbonyl determination DNPH (2,4-Dinitrophenylhydrazine) was used and the carbonyl DNPH derivatives were quantified.

Quantification of nicotine and carbonyls in aerosol or smoke bubbled PBS (bPBS) extracts are shown in Table 1.

3. IN VITRO TOXICITY ASSAYS

3.1 Regulatory toxicity

The following regulatory in vitro assays were performed on the PBS extracts in accordance with ISO 17025: Neutral red uptake (NRU) in HepG2 and BEAS-2B cells in compliance with in compliance with OECD TG 129; Ames assay in TA98 (+S9) and TA100 (+S9) in compliance with OECD TG 471; and in vitro micronut (IVM) with V79 (3hrs +/- S9, 24hrs – S9) in compliance with OECD TG 487.

- There was a clear cytotoxicity effect for 3R4F, a reduced effect for THP and no effect for HYB under the test conditions (n=3).
- There was marked mutagenicity for 3R4F with TA98(+S9) which was reduced for THP (~3 times lower), weak for HYB and no effect was observed for myblu (n=3) under the test conditions. With TA98+-S9 a mutagenic response was observed for 3R4F only.
- None of the bPBS extracts elicited any effects in the IVMM assay.

3.2 Cellular Transformation Assay (CTA)

The BHAS 42 CTA assay was conducted on the PBS extracts by BioReliance according to OECD draft Guidelines (2016).1
- 3R4F showed extensive cytotoxicity at concentrations >1%, THP, HYB and myblu demonstrated little/no cytotoxic effect at the highest concentration (5%) (Figure 1).
- Only 3R4F was positive for promoting activity (*p≤0.05 [ANOVA, Dunnett’s post-hoc]; statistically significant increase) (Figure 1).

3.3 Endothelial cell migration

Human umbilical vein endothelial cells (HUVECs) were exposed to up to 10% PBS extracts for 30 hours and scratched with methodology previously described by Rudder et al.2 The RWD50 (10% relative wound density) is defined as the time point at which 50% of the initial scratch wound area is occupied by migrating cells. RWD50 plotted against the concentration of bPBS extract followed a linear model and is defined as cRWD50.

- 3R4F displayed significant inhibitory activity on HUVEC migration at concentrations >1.4% (p≤0.05), resulting in a significant increase in cRWD50 after treatment (Figure 2).
- THP and HYB samples showed lower migration activity over control indicating no inhibition of the wound healing activity. A Dunnett’s test with each individual concentration confirmed the effects (no response was observed at PBS concentrations >5% (p≤0.01).
- The myblu extract did not show any significant inhibition up to a maximum tested concentration of 10%.

4. CONCLUSIONS

This study demonstrates the suitability of aerosol bubbled PBS as an exposure media in established in vitro assays.

The generated results can be used as part of a weight of evidence approach to substantiate the harm reduction potential for NGPs for adult smokers. Under the study conditions, the ranking of harm for the products could be defined as 3R4F>THP>HYB>myblu. It should be noted that large differences in biological response were observed between 3R4F and THP under the test conditions. Further studies, including clinical studies, are required to validate the findings in the presented work and to establish the ranking of potential harm.

REFERENCES