Introduction
Changes in societal and regulatory pressures have greatly altered the use of tobacco and nicotine products in recent years. Next generation products, such as e-cigarettes and tobacco heating devices are becoming increasingly popular. As consumers change their smoking behaviour, in vitro testing methodologies need to evolve in parallel, to compare biological outcomes from cigarette smoking to that of these emerging nicotine and tobacco product categories.

Standard smoking puffing parameters, regimes and in vitro exposure systems need to be altered to suit the new way of using tobacco products, which can pose several technical challenges. The differing chemical properties of e-cigarettes compared to cigarette aerosols, such as osmolality, viscosity, hygroscopicity and volatilisation, means aerosols transit through smoking engines differently. The reduced number of toxicants present in e-cigarette aerosols compared to cigarette smoke means that there is a significant difference in biological potency when comparing aerosols using traditional techniques.

In vitro e-cigarette aerosol testing has adopted many of the same principles used for cigarette smoke testing, such as exposure time and dilution principles, in order to draw comparisons between the two categories [1]. So ultimately, e-cigarette testing has been conducted on in vitro assays optimised for the assessment of cigarette smoke and not e-cigarette aerosol, which is significantly different in terms of its chemical and physiological composition. Therefore, in vitro testing of e-cigarette aerosols needs to evolve beyond basic cigarette to e-cigarette comparisons, using traditional established techniques [1]. Assays and techniques need to be modified and adapted to factor in changing aerosols and lower chemical and toxicant burdens.

In this study, the adaptation of a Vitrocell VC 10 smoking robot to generate and deliver undiluted cigarette and e-cigarette aerosols to in vitro respiratory cultures is described. Aerosol concentration was a fixed parameter, with duration of exposure (mins) being the controlling variable. Assessment of biological responses and nicotine dosimetry enabled the comparison of products in the exposure system. Finally, this study further assessed the effect of adding a flavour compound to the e-cigarette aerosol to investigate the sensitivity of the method and as a benchmark aerosol control for this and future e-cigarette studies.

Approach and Methods

Products

Exposure Regimens
- Cigarette were smoked to either ISO or HCl [1, 2]
- E-cigarettes were vapoured to CRM81 [5]

Exposure Setup

Figure 4. Pictorial and schematic representation of Vype eBox device, size, dimensions and controls.

Figure 1. Pictorial and schematic representation of Vype eBox device, size, dimensions and controls.

Figure 2. Schematic representation of moan connectors made to the VC 10 Smoking Robot. In an alternative approach, the two diluting air flow ports were blocked (X) such that undiluted aerosol was sampled into the module at 6 mL/min/well (Y). The first three positions in the module were used for cell exposure and the forth position was used to assess dose.

Results

Effect of exposure regime on cytotoxicity

A

B

C

References

1. An approach to testing undiluted e-cigarette aerosol in vitro using 3D reconstituted human airway epithelium. Bishop et al., 2018. Toxicology In Vitro xxx


3. Extreme testing of undiluted e-cigarette aerosol demonstrates non-mutagenic responses in five bacterial tester strains using an Ames air-agar-interface technique. Thorne et al., 2018 Mutation Research xxx

