A novel approach for the screening of e-cigarette aerosols using an Ames whole aerosol assay

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Introduction

Global e-cigarette use has grown significantly over the last few years, with the environment being directed by product innovation and a consumer preference for larger aerosols. The core e-cigarette design comprises a battery, microprocessor, and an e-cigarette liquid that is delivered to a coil that is heated upon activation to create an aerosol stream. E-cigarettes can be activated via puffing which triggers coil activation, or via a button which pre-heats the coil prior to puffing. Recent advances have seen the incorporation of larger, rechargeable batteries for more power, an e-liquid tank that can be refilled through standard or personalised mixtures, coil upgrades and variable and controllable voltage options, all of which are designed to facilitate an increase in aerosol generation and product performance.

In contrast to cigarette smoke, which has been extensively investigated, e-cigarette aerosols remain relatively poorly understood and characterised *in vitro*. The current understanding from the available literature suggests that e-cigarettes are significantly less harmful compared to a traditional cigarette. Some studies have demonstrated clear toxicological properties of e-cigarette test articles, whereas others have identified no activity at all. All studies appear to be in agreement that the chemical and toxicological burdens associated with e-cigarette aerosol are far lower compared to those of cigarette smoke

Aims

The aim of this study was to investigate e-cigarette use under extreme testing conditions, using a novel set of exposure parameters to assess undiluted e-cigarette aerosol *in vitro* using the bacterial reverse mutation (Ames) assay.

Materials and Methods

Aerosol Generation

A Vitrocell® VC 10 Smoking Robot was used to generate aerosol streams from a button activated e-cigarette

In this study aerosols were generated under a set of unique exposure conditions to amplify dose. Undiluted aerosol was generated by disabling the air flow (L/min) and Vacuum rate (mL/min) and by blocking the exhaust port, to divert the entire puff into the exposure module (Figure 1). Aerosols were generated to CRM No 81 puffing regimen [1].

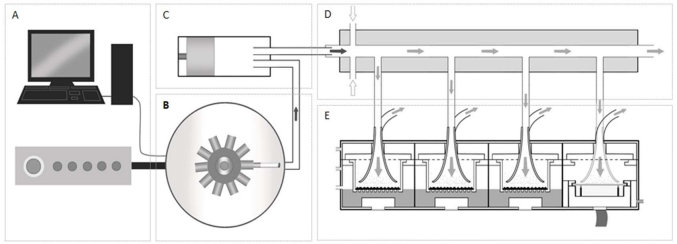

Figure 1: A schematic representation of the VC 10 smoke exposure system. A) Computer, software and controller. B) Smoking Robot carousel where e-cigarettes are loaded and puffed. C) Piston/syringe, which draws and delivers mainstream aerosol to the dilution system. D) Under normal conditions the airflow dilutes the aerosol and a vacuum sub samples into the exposure module. In this study, airflow and vacuum were disabled and the exhaust port blocked. The aerosol is diverted to the exposure module in this adapted closed system. E) Aerosol within the module is displaced by new puffs entering via positive pressure. Normally a vacuum rate constantly cycles air at a set volume (5 mL/min).

Ames Assay

Ames strains TA98 and TA100 were exclusively assessed under a number of exposure conditions.

1. Particulate trapping conditions up to 2,400 µg/plate using the plate incorporation 85 mm technique (Figure 3) [2]
2. Whole aerosol approaches using a scale-down 35 mm spread plate technique up to 360 puffs using dilute e-cigarette aerosol as per 'standard' VC 10 operating principles (Figure 3) [2-3]
3. Whole aerosol approaches using a scale-down 35 mm spread plate technique up to 900 puffs using undiluted e-cigarette aerosol and a unique set of exposure parameters (Figure 5)

E-cigarette

Vype ePen e-cigarette (Vype, Nicoventures Trading Ltd, part of the British American Tobacco Group of Companies) with blended tobacco cartomizers were used exclusively in this study, at the high voltage setting (4V). The Vype ePen is a closed modular, rechargeable device with interchangeable cartomizers.

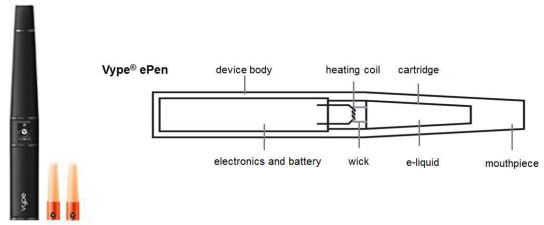

Figure 2: Actual (left) and schematic representation (right) of a Vype® ePen e-cigarette

Results

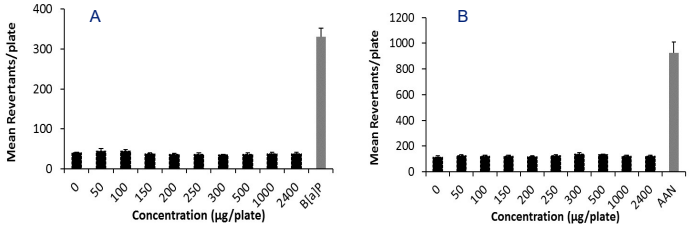

Figure 3: A non-mutagenic response following a 72 hour plate incorporation technique. A) TA98 + S9; B) TA100 + S9 [2]

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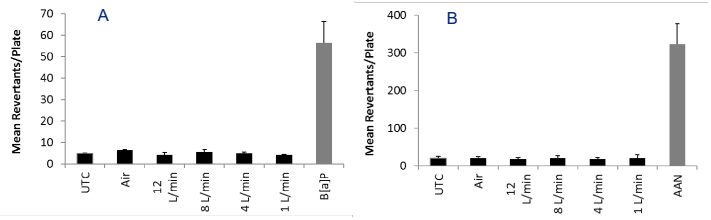

Figure 4: Showing a non-mutagenic response following a spread-plate scaled-down 35 mm whole aerosol technique using standard dilution techniques. A) TA98 + S9; B) TA100 + S9 [2-3]

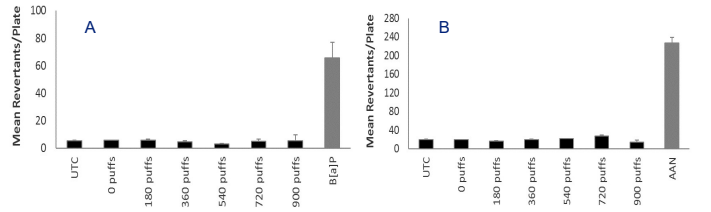

Figure 5: A non-mutagenic response following a spread-plate scaled-down 35 mm whole aerosol technique using undiluted e-cigarette aerosol and a unique set of exposure parameters. A) TA98 + S9; B) TA100 + S9

Conclusions

- Non-mutagenic responses were observed in tester strains TA98 and TA100 in response to particulate matter and whole aerosol techniques.
- Using a set of unique exposure parameters, undiluted e-cigarette aerosol was assessed up to 900 puffs and no mutagenic activity was observed, confirming the non-mutagenic responses observed in TPM and traditional whole aerosol approaches.
- Undiluted e-cigarette aerosol approaches offer an advantage over standard principles, as the e-cigarette aerosol is not diluted prior to delivery to the bacterial suspension.
- Using undiluted e-cigarette aerosol may provide a quicker and more cost-effective means to screen e-cigarette aerosols.

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Abstract

The *in vitro* mutagenic potential of the aerosol from a commercially available, electronic-cigarette (e-cigarette; Vype® ePen) was assessed using the Ames assay. Aerosol generated from the e-cigarette was trapped on a Cambridge filter pad, eluted in DMSO and compared to cigarette smoke total particulate matter (TPM), which was generated in the same manner. Fresh e-cigarette and cigarette smoke aerosols were generated on a Vitrocell® VC 10 smoking robot (Vitrocell Systems GmbH) and compared following direct aerosol exposure using a modified scaled-down 35 mm air agar interface (AAI) methodology.

E-cigarette aerosol collected matter (ACM) was found to be negative in the 85 mm Ames assay in strains TA98 and TA100 when conducted to OECD TG471, at concentrations up to 2400 µg/plate. Freshly generated e-cigarette aerosol was also found to be negative in both strains after an AAI aerosol exposure, when tested between 1-12 L/min dilution for up to 3-hours (360 puffs). In contrast, cigarette smoke TPM and aerosol from 3R4F reference cigarettes were found to be mutagenic in both tester strains, under comparable test conditions to those of e-cigarettes. To confirm these negative findings with e-cigarette exposure, further studies investigated extreme exposures up to 900 puffs. Some evidence of thinning of the background lawn and a marked reduction in revertants in TA98 and TA100 were observed following 900 puffs of undiluted e-cigarette aerosols. The data demonstrate that e-cigarette aerosols remained non-mutagenic under extreme testing conditions and confirms the original conclusions.

This novel whole aerosol approach could be used in a targeted manner to support e-cigarette assessments, and may even be useful in understanding the nature of the product in its extremes. They next key step is to contextualise the findings against human consumption data, to ensure that these products are being tested within the constraints of their manufacturers' specifications and intended use. Furthermore, *in vitro* dosimetry approaches should be considered to draw more accurate comparisons between cigarette smoke, e-cigarette aerosol exposures and human use.

Key Words

Ames; TA98; TA100; E-cigarette, Cigarette smoke; Aerosol exposure; TPM; ACM; VC 10

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