

The mutagenic assessment of electronic-cigarettes and tobacco smoke using the Ames assay in strains TA98 and TA100

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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 the requirement for larger aerosols. A simple e-cigarette comprises of 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. 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 other have identified no activity at all. All studies appear to be in agreement that the toxicological burden is far lower for that of an e-cigarette compared to a traditional combustible cigarette.

Aims

The Aim of this study was to assess the mutagenicity of an e-cigarette aerosol, compared to cigarette smoke in tester strains TA98 and TA100 using two different exposure matrices, TPM/eTPM (or ACM – aerosol collected matter) and whole aerosol.

Materials and Methods

Products and Regimens

Product	Puff Regimen	Puff Volume (mLs)	Puff Frequency (secs)	Puff Duration (secs)	Puff Profile	Vent blocking	Coil pre-activation (secs)
3R4F	HCI ¹	55	30	2	Bell	100%	N/A
Vype [®] ePen	CRM 81 ²	55	30	3	Square	N/A	1

HCI T-115
CRM N° 81
N/A = not applicable

TPM and eTPM Generation

Total particulate matter (TPM) and e-cigarette total particulate matter (eTPM) were generated in the same manner. Particulates were captured on a Cambridge filter pad (CFP) and eluted in dimethyl sulfoxide (DMSO) to a stock concentration of 24/mg/ml

Whole Aerosol

A Vitrocell[®] VC 10 Smoking Robot was used to generate aerosol streams from a traditional reference cigarette (3R4F) and e-cigarette (Vype[®] ePen) (Figure 1).

Ames Assay

Two strains were exclusively tested in the study TA98 and TA100. For TPM and eTPM treatments plates were exposed up to 2,400 µg/plate using plate incorporation assay parameters. For aerosol exposures, a scaled-down spread plate air agar interface (AAI) methodology was used. Bacteria were exposed under dilution airflow conditions up to 12 L/min for 3hours and incubated for 72 hours prior to revertant analysis.

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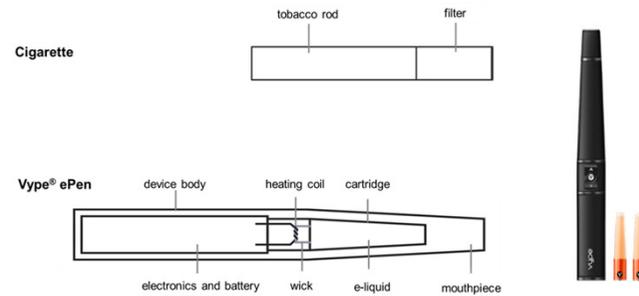


Figure 1: Schematic representation of products used in the study (3R4F and Vype ePen) and picture of actual Vype ePen product.

Characteristics	Product 3R4F	Vype [®] ePen ¹
Product category	Cigarette	e-cigarette
Manufacturer	University of Kentucky (USA)	Vype [®] (Nicoventures, UK)
Length (mm)	84	153
Diameter (mm)	8	20 (10 at mouth piece)
Nicotine content	0.7 – 2.0 mg/cig*	18 mg/mL (1.8% v/v) ²
Puff number	8-10*	250-300
Test matrix assessed	TPM and Aerosol	eTPM and Aerosol

¹ = e-cigarette, closed system modular device, operated at 4 volts with Blended Tobacco cartridges

* = dependent on smoking regimen used (ISO vs. HCI)

² = as stated on the pack

TPM = total particulate matter
ACM = aerosol collected matter

Table 1: Shows the product specifications used in the study and exposure matrices

Data Evaluation and Acceptance Criteria

- Plates were scored using an automated colony counter (Sorcerer Image Analyser, Perceptive Instruments, Haverhill, UK) and the background lawn inspected for signs of toxicity.
- Responses with positive control chemicals were compared with laboratory historical observed ranges. Observed values were comparable with historical control ranges held at Covance laboratories (Harrogate, UK) for the standard 85 mm plate assay and established ranges for the scaled-down 35 mm AAI assay.
- Data were evaluated using fold increase in revertant numbers, over the concurrent zero or air control plate counts, and analysed statistically using Dunnett's test.
- For an increase in revertant numbers to be considered as a mutagenic response, increases were required to be at least 2-fold greater than the concurrent control or statistically significant ($p < 0.05$) using Dunnett's test, and both concentration-related and reproducible over two or more independent experiments

Results

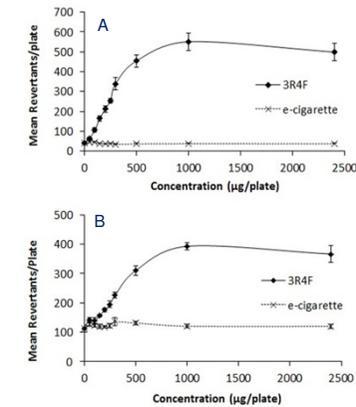


Figure 2: Response to TPM treatment in the presence of S9 metabolic activation. [A] TA98 responses to 3R4F cigarette and Vype ePen e-cigarette particulates. [B] TA100 responses to 3R4F cigarette and Vype ePen e-cigarette particulates.

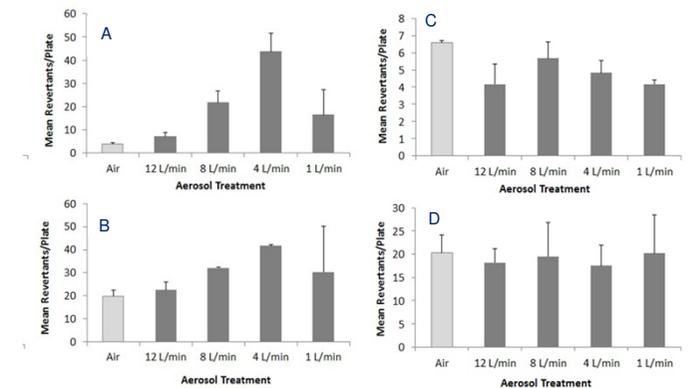


Figure 3: Response to whole aerosol treatment in the presence of S9 metabolic activation. [A – B] TA98 and TA100 responses to 3R4F cigarette smoke respectively. [C - D] TA98 and TA100 responses to Vype ePen aerosol respectively.

Conclusions

- This study demonstrates, compared to cigarette smoke, Vype[®] ePen e-cigarette particulates and aerosols were deemed negative under the test conditions assessed.
- Conversely, 3R4F cigarette smoke TPM and freshly generated whole smoke were clearly positive.
- In the case of freshly generated cigarette smoke, a positive response in both strains was observed within 24 minutes, whereas e-cigarette aerosols remained negative up to 3 hours.
- Future investigations should consider extended exposure conditions and additional tester strains.

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Related Publications

- Jennifer Margham, Kevin McAdam, Mark Forster, Chuan Liu, Christopher Wright, Derek Mariner, and Christopher Proctor. **Chemical Composition of Aerosol from an E-Cigarette: A Quantitative Comparison with Cigarette Smoke.** Chemical Research in Toxicology, 2016. DOI: 10.1021/acs.chemrestox.6b00188
- Mark Taylor, Tony Carr, Oluwatobiloba Oke, Tomasz Jaunky, Damien Breheny, Frazer Lowe & Marianna Gaça. **E-cigarette aerosols induce lower oxidative stress in vitro when compared to tobacco smoke.** Toxicology Mechanisms and Methods, 26:6, 465-476, DOI: 10.1080/15376516.2016.1222473.
- David Azzopardi, Kharishma Patel, Tomasz Jaunky, Simone Santopietro, Oscar M. Camacho, John McAughy & Marianna Gaça. **Electronic cigarette aerosol induces significantly less cytotoxicity than tobacco smoke.** Toxicology Mechanisms and Methods, 26:6, 477-491. DOI: 10.1080/15376516.2016.1217112.

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Abstract

Salmonella typhimurium strains TA98 and TA100 were used to assess the mutagenic potential of a commercially available rechargeable, dual voltage, closed system modular electronic-cigarette (Vype® ePen, Nicoventures UK). Results obtained, were compared to a Kentucky reference (3R4F) cigarette. Two different test matrices were assessed. 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), generated in the same manner. Fresh e-cigarette and cigarette smoke aerosols were generated on the Vitrocell® VC 10 smoking robot and compared using a modified scaled-down 35 mm air agar interface (AAI) methodology. E-cigarette TPM (eTPM) was found to be negative in the 85 mm Ames assay in strains TA98 and TA100 conducted in accordance with OECD 471. Freshly generated e-cigarette aerosol was also found to be negative in both strains following a 3 hour AAI aerosol exposure. Positive control responses were observed in both strains, using benzo[a]pyrene and 2-aminoanthracene for TA98 and TA100 respectively. In contrast, cigarette smoke TPM and whole aerosol from 3R4F reference cigarettes were found to be mutagenic in both tester strains, under comparable test conditions to that of e-cigarette exposure.

Currently, limited information exists on the mutagenic activity of captured e-cigarette particulates and whole aerosol AAI approaches. Regulatory standard product testing approaches as used in this study will become important when determining whether e-cigarette aerosols are less biologically active when compared to cigarette smoke, as suggested by the literature and data presented here.

Key Words

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

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