The *in vitro* assessment of an e-cigarette (Vype ePen)

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INTRODUCTION

Cigarette smoking is an identified cause of a number of human diseases. We are developing a range of products including electronic cigarettes (e-cigarettes) which deliver lower yields of aerosol toxicants than cigarette smoke. As part of an integrated testing strategy to allow us to compare the relative biological effects of new categories of nicotine delivery products with those of traditional cigarettes, we have developed a suite of *in vitro* assays to model smoking related disease processes.

The aim of this study was to compare the responses induced by aerosols from a commercially available e-cigarette (Vype ePen, Nicoventures, UK) with those from aerosols of a reference 3R4F cigarette in this suite of *in vitro* assays.

METHODS

Generation of test matrices

Three different test matrices were used for *in vitro* assessments: total particulate matter (TPM)/aerosol collected matter (ACM), whole aerosol (WA), and aqueous aerosol extract (AqE). These were produced according to the puffing regimes detailed in Table I.

Table I: Aerosol generation regimens

Product	Puff Regimen	Puff Volume (mL)	Puff Frequency (secs)	Puff Duration (secs)	Puff Profile	Vent blocking	Coil pre-activation (secs)
3R4F	HCI[1]	55	30	2	Bell	100 %	N/A
ePen	CRM[2]	55	30	3	Square	N/A	0

[1] = HCI T-115 [20]; [2] = CRM Nº 81 [21]; N/A = metric not applicable

TPM/ACM: Approximately 150 mg of TPM or ACM were collected on 44 mm Cambridge filter pads (Whatman, Maidstone, UK). DMSO (Sigma-Aldrich, UK) was used to elute the TPM or ACM from the pads to a stock concentration of 24 mg/mL. The extracts were stored in single-use volumes at -80ºC until required.

AqE: AqE from both test products were produced by bubbling 10 puffs from each product through 20 mL of non-supplemented DMEM/F12 medium (Gibco, New York, USA) in a glass impinger. Samples were used within 3 hours of production.

WA: a Vitrocell Smoking Robot VC10® (Vitrocell Systems, Waldkirch, Germany) was used for the Ames assay, as previously described[1]. A Borgwaldt RM20S exposure system was used for the cytotoxicity assay, as detailed previously[2]. Deposited particulate mass was measured, and estimated nicotine deposition was calculated using previously-published methods[2].

Ames bacterial reverse mutation assay

Particulate matter exposures were conducted according to the principles of OECD 471, however utilising only *S. typhimurium* strain TA98+S9. For product WA exposures, the Ames assay was modified as previously described[1].

Cytotoxicity assay

Human bronchial epithelial cells (NCI-H292) were exposed to WA at the air-liquid interface (ALI) for 1 hour, using a Borgwaldt RM20S smoking machine (Borgwaldt KC, Hamburg, Germany). Following exposure, cytotoxicity was assessed using the Neutral Red Uptake assay as previously described[2].

Oxidative stress and apoptosis

NCI-H292 cells were assessed for oxidative stress and apoptosis following exposure to AqE from both products as previously described[3].

Endothelial cell migration assay

Human umbilical vein endothelial cells (HUVEC) were grown to confluency, "wounded" using a pipette tip and exposed to AqE for 22 hours in 24 well ImageLock plates (Essen Instruments, MI, USA). Cell migration was quantified by measuring the closure of the "wound" using an Incucyte time lapse video camera and software (Essen Instruments, MI, USA).

RESULTS

ePen showed no activity in any assay apart from WA cytotoxicity, while 3R4F induced a dose-related response across all tests.

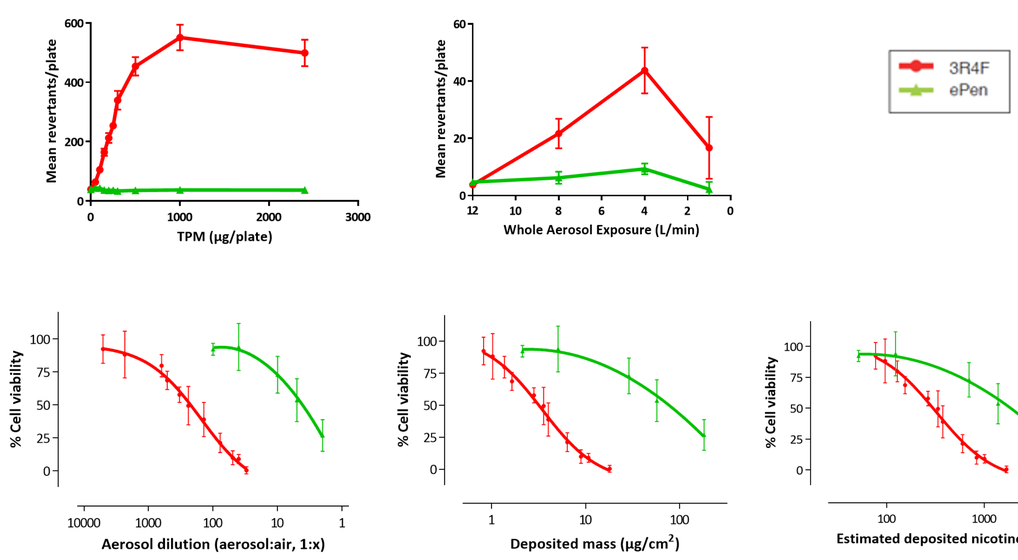

Figure 1. Genotoxic and cytotoxic effects of whole aerosol (WA) and total particulate matter (TPM)/ aerosol collected matter (ACM) from a 3R4F reference cigarette and e-cigarette, respectively. (**a**,**b**) Mutagenic response of *S. typhimurium* TA98 exposed to TPM/ACM (**a**) and WA (**b**) in the Ames test. (**c**) Cytotoxic response of lung epithelial H292 cells exposed to WA in a cell viability assay. Data expressed as a function of aerosol dilution, (**d**) deposited mass, and **(e)** deposited nicotine. Data shown are mean ± S.D. (n=3 (a, b); n≥6 (c, d, e)).

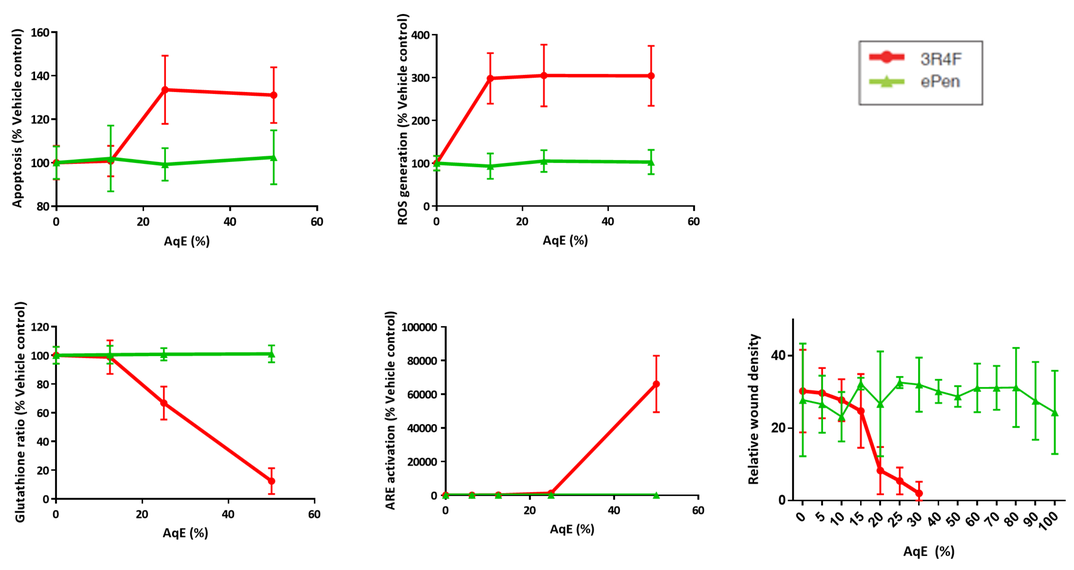

Figure 2. *In vitro* biological effect of exposure to AqE from a 3R4F reference cigarette, and an e-cigarette. Apoptotic response (**a**), generation of intracellular oxidant species (**b**), GSH:GSSG ratio (**c**), and ARE activation (**d**) in lung epithelial H292 cells. (**e**) Wound healing rates in HUVEC monolayers. Data shown are mean ± S.D. (n=5)

CONCLUSIONS

- ePen showed little or no activity in any of the *in vitro* assays where it was assessed, and was significantly less active than the 3R4F reference cigarette across all studies.
- These studies indicate that ePen use has the potential to be reduced risk compared to cigarette smoking.

References

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