# The *in vitro* assessment of an electronic cigarette (Vype ePen) using a suite of pre-clinical tools

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## INTRODUCTION

There has been significant growth in the number of smokers currently using next generation products including electronic cigarettes (e-cigarettes). E-cigarette products do not contain tobacco and the toxicant delivery of these aerosols are greatly reduced in comparison to conventional tobacco products, suggesting that they could hold promise as reduced risk products. In this study we describe the in vitro assessment of a commercially available e-cigarette; Vype ePen, and compare the results relative to a reference 3R4F cigarette.

## METHODS

The products used for these studies were the 3R4F reference cigarette (University of Kentucky, USA) and an e-cigarette (Vype ePen). All test articles were generated under Health Canada Intense (HCI) smoking regime (55/2/30, vents blocked on 3R4F).

### Smoke extract production

Conventional 3R4F reference cigarettes and Vype ePen devices were smoked or activated on a Borgwaldt-KC RM20H smoking machine. Particulate matter (TPM) extracts were prepared as previously described[1]. Aerosol aqueous extract (AqE) was generated by bubbling the smoke from a single cigarette, or 10x 3 second activations (Vype ePen) in 20mL of cell culture medium.

### Ames bacterial reverse mutation assay

Product TPM exposures were conducted according to the principles of OECD 471, however utilising only *S. typhimurium* strain TA98+S9. For product whole aerosol (WA) exposures, the Ames assay was modified as previously described[2]. Briefly, TA98 was exposed to WA using a Vitrocell VR AMES 4 stainless steel module) smoke engine, in a scaled-down 35mm plate format for a period of 64 minutes.

### Cytotoxicity assay

Human bronchial epithelial cells (NCI-H292) were exposed to WA at the air-liquid interface (ALI) for a period of 1 hour, using a Borgwaldt RM20S smoking machine (Borgwaldt KC, Hamburg, Germany). Following exposure, cytotoxicity was assessed using Neutral Red Uptake as previously described[3].

### Gamma H2AX assay

Human bronchial epithelial cells (BEAS-2B) were exposed to WA at the ALI for a maximum of 3 hours[4]. Immunostaining and a high content screening (HCS) approach using the Cellomics ArrayScan® Vti platform was used to determine the frequency of DNA double strand breaks.

### Bhas42 cell transformation assay

Bhas 42 cells (v-Ha-ras-transfected Balb/c 3T3 clone A31-1-1) were treated with TPM from test products using the promoter protocol[5]. Cells were treated for 7 days to particulate matter with media change every 3 days, followed by a 7 day recovery. Plates were scored and results evaluated as previously described[6].

### Antioxidant depletion

NCI-H292 cells were exposed to AqE for a period of 4 hours. The ratio of reduced to oxidised glutathione (GSH:GSSG) was analysed in lysates using the GSH:GSSG Glo™ assay kit (Promega, UK).

### Endothelial cell migration assay

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

### Pro-inflammatory cytokine secretion

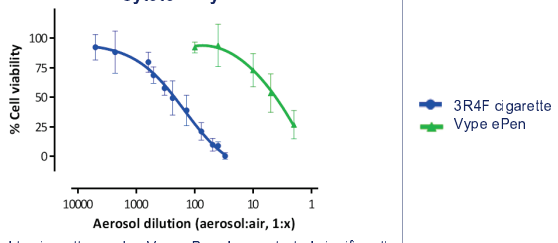
MucilAir™ primary airway epithelial cultures were exposed to whole aerosols (1:20 dilution ratio) for 4x 5 minute intervals with 30 minutes rest between each exposure, using a Borgwaldt RMS20 smoke engine. Cytokines were quantified with the Meso Scale Discovery V-plex 30 cytokine and 3-Plex MMP kits, according to manufacturer's instructions (Gaithersburg, USA).

## REFERENCES

1. The use of a novel tobacco-substitute sheet and smoke dilution to reduce toxicant yields in cigarette smoke. KG McAdam et al. Food Chem Toxicol. (2011) 49(8):1684-96.
2. A method for assessment of the genotoxicity of mainstream cigarette-smoke by use of the bacterial reverse-mutation assay and an aerosol-based exposure system, J. Kilford et al, Mutat. Res. (2014) 769 : 20–28.
3. Evaluation of an air–liquid interface cell culture model for studies on the inflammatory and cytotoxic responses to tobacco smoke aerosols. D. Azzopardi et al. Toxicol in vitro. (2015) 29 (7) : 1720–1728
4. γH2AX as a novel endpoint to detect DNA damage: applications for the assessment of the *in vitro* genotoxicity of cigarette smoke. C. Garcia-Canton et al. Toxicol In vitro. (2012) 26(7) : 1075-86.
5. An international validation study of a Bhas 42 cell transformation assay for the prediction of chemical carcinogenicity. A. Sakai et al. Mutat. Res (2011) 725 : 57-77.
6. Cigarette smoke-induced morphological transformation of Bhas 42 cells *in vitro*. D Weisensee et al. ATLA (2013) 41: 181-189.

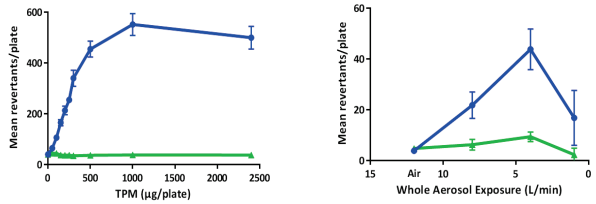
## RESULTS

### Cytotoxicity



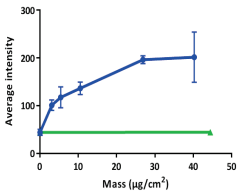

Compared to cigarette smoke, Vype ePen demonstrated significantly lower cytotoxicity.

### Mutagenicity



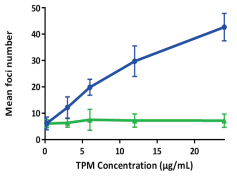

Vype ePen did not induce mutations, whereas cigarette smoke demonstrated a positive dose response.

### DNA Double Strand Breaks



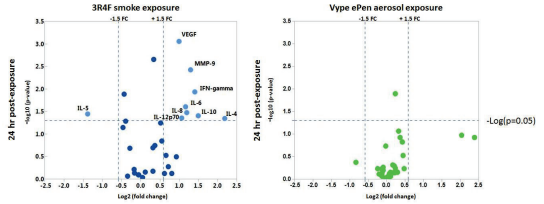

Vype ePen did not induce DNA double strand breaks across all doses, whereas cigarette smoke demonstrated a positive dose response.

### Cell Transformation



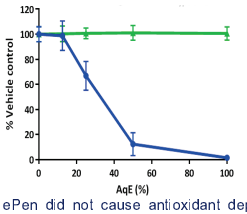

Vype ePen did not induce transformation of initiated Bhas cells, whereas cigarette smoke demonstrated a positive dose response.

### Pro-Inflammatory Cytokine Secretion



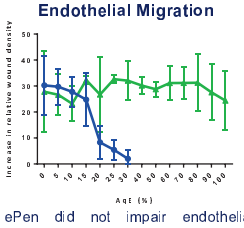

Cytokine expression was up-regulated (FC>1.5 p-value<0.05) following 3R4F smoke exposure with no significant increase in mediator release in response to Vype ePen exposure

### Antioxidant Depletion




Vype ePen did not cause antioxidant depletion, whereas cigarette smoke demonstrated a positive dose response.

### Endothelial Migration




Vype ePen did not impair endothelial cell migration, whereas cigarette smoke demonstrated a positive dose response

## CONCLUSIONS

- Under these test conditions using a number of *in vitro* assays, e-cigarette Vype ePen demonstrated reduction in responses compared to a reference 3R4F cigarette, indicating a potential to be reduced risk versus cigarettes
- Additional research including clinical and population studies are needed to substantiate disease relevant risk reduction in populations



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