The assessment of yH2AX induction from conventional and electronic cigarette aerosols

David Thorne, Sophie Larad, Carolina Garcia-Canton, Clive Meredith and Marianna Ga

British American Tobacco, R&D Centre, Southampton, SO15 8TL, UK
Correspondence: david_thorne@bat.com

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 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 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 optimise the yH2AX assay using high content screening approaches with the Vitrocell® VC 10 ALI exposure system. In contrast to previous studies, this study has used applied dosimetry approaches up front for the comparison of cigarette and e-cigarette aerosols (µg/cm² and nicotine delivery).

Materials and Methods

Aerosol Generation

A Vitrocell® VC 10 Smoking Robot was used to generate aerosol streams from a traditional reference cigarette and two e-cigarette variants (Vype® eStick and ePen).

yH2AX genotoxicity

Human bronchial epithelial cells (BEAS-2B) were obtained from the American Type Culture Collection (ATCC), BEAS-2Bs were maintained at 37°C in an atmosphere of 5% CO₂ in air in Bronchial Epithelial Cell Growth Medium (BEGM). BEGM consisted of Bronchial Epithelial Basal Medium with a SingleQuots kit containing growth factors, cytokines and other supplements. H2AX intensity was determined using a Cellomics Arayscan VTI platform combined with the Target Activation Bioapplication software [1]. Two different nuclear stains were measured. Nuclear DNA staining with Hoechst dye was assessed in channel 1 to identify viable cell nuclei and channel 2 measured the phosphorylated form of the histone 2AX, whose fluorescence intensity is directly proportional to the number of double strand breaks [2].

Aerosol products

Three aerosol products were selected for this study:

1. 3R4F reference cigarette smoke (University of Kentucky, USA).
2. A commercially available cigalike e-cigarette (Vype® eStick), puff activated, fixed voltage.
3. A commercially available closed modular dual voltage e-cigarette (Vype® ePen) with blended tobacco flavour e-liquid formulation.

Results

Figure 1: Vype® eStick, a cigalike product (left) and Vype® ePen, a closed modular device with dual voltage (right).

Figure 2: A schematic representation of the three aerosol products used in the study

Table 1: Shows the product specifications for a 3R4F cigarette compared to a cigalike and a closed modular e-cigarette device, eStick and ePen respectively.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Product</th>
<th>eStick</th>
<th>ePen</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aerosol</td>
<td>Ref cig smoke</td>
<td>eStick vapour</td>
<td>eStick vapour</td>
</tr>
<tr>
<td>Manufacturer</td>
<td>University of Kentucky</td>
<td>Vype/Nicoventure, UK</td>
<td>Vype/Nicoventure, UK</td>
</tr>
<tr>
<td>Length (mm)</td>
<td>14</td>
<td>14</td>
<td>157</td>
</tr>
<tr>
<td>Diameter (mm)</td>
<td>8</td>
<td>8</td>
<td>20.10</td>
</tr>
<tr>
<td>Nicotine content</td>
<td>1.7 - 2.0 mg/g</td>
<td>11.3 mg (0.5% v/v)</td>
<td>27 mg/ (1% v/v)</td>
</tr>
<tr>
<td>Puff number</td>
<td>9.19</td>
<td>100.10</td>
<td>250.00</td>
</tr>
<tr>
<td>Voltage options (%)</td>
<td>N/A</td>
<td>100</td>
<td>Yes (3.6 m C)</td>
</tr>
<tr>
<td>Voltage used in (V)</td>
<td>N/A</td>
<td>3.7 nomal</td>
<td>4.1</td>
</tr>
<tr>
<td>Cartridge used</td>
<td>N/A</td>
<td>Optic favoured</td>
<td>Filled Tobacco</td>
</tr>
<tr>
<td>Refillable?</td>
<td>N/A</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Exposure regimen</td>
<td>HCl</td>
<td>CCM® N° 81</td>
<td>CRM® N° 81</td>
</tr>
<tr>
<td>Fix coil activation</td>
<td>N/A</td>
<td>1/2 second</td>
<td>1/2 second</td>
</tr>
</tbody>
</table>

Conclusions

- This study demonstrates that the in vitro yH2AX assay can be used for the assessment of aerosol genotoxicity from cigarette smoke and e-cigarette vapours.
- That under these test conditions, e-cigarette aerosols from a cigalike and closed modular device produced no DNA damage or cytotoxicity above background levels, compared to cigarette smoke, which was positive for cytotoxicity and genotoxicity.
- Studies such as this, will become important when determining whether e-cigarette aerosols are in fact less biologically active when compared to cigarette smoke, as the literature and this data suggests.
- Finally, this study has highlighted the fundamental importance of applying dosimetric approaches to quantify exposure, which will in turn facilitate more accurate interpretations of the resulting data and to enable the data to be presented in a format that appropriately allows cross-study and cross-system comparisons.

References

Related Publications


•Garcia-Canton, C., Errington G., Anadon, A., Meredith, C. Characterisation of an aerosol exposure system to evaluate the genotoxicity of whole mainstream cigarette smoke using the in vitro γH2AX assay. BMC Pharmacology and Toxicology 2014; 15:41


•Breheny, D., Cunningham, F., Killford, J., Payne, R., Dillon, D., Meredith, C. Application of a modified gaseous exposure system to the in vitro toxicological assessment of tobacco smoke toxicants. Environmental and Molecular Mutagenesis 2014; 55: 662-672

•Garcia-Canton, C., Anadon, A., Meredith, C. Assessment of the in vitro γH2AX assay by High Content Screening as a novel genotoxicity test. Mutation Research 2013; 757: 158-166


•Garcia-Canton, C., Anadon, A., Meredith, C. γH2AX as a novel endpoint to detect DNA damage: Applications for the assessment of the in vitro genotoxicity of cigarette smoke. Toxicology In Vitro 2012; 26: 1075-1086

British American Tobacco
R&D Centre
Southampton SO15 8TL
United Kingdom
Tel +44 (0)2380 58 8808
www.bat.com

Abstract No: 3031
Poster No: P173
Meeting: SOT Meeting 2016
Date: 13-17th March
Location: New Orleans, United States

The assessment of γH2AX induction from conventional and electronic cigarette aerosols

David Thorne, Sophie Larard, Carolina Garcia-Canton, Clive Meredith and Marianna Ga a

British American Tobacco, Research and Development, Southampton, SO15 8TL, United Kingdom.

Correspondence: david_thorne@bat.com

Abstract

Exposure systems have been used to assess cigarette smoke aerosols for many years, using a variety of in vitro endpoints. These systems produce a more physiologically relevant test matrix compared to traditional methods, and as a result in vitro aerosol techniques are widely being developed. Of particular interest are genotoxicity assays such as the γH2AX assay which may be used to detect DNA damage, a possible precursor to cancer. The γH2AX assay detects DNA double strand breaks (DSB), via phosphorylation of the H2AX histone protein which occurs at the site of the DSB. This study describes the method development and optimisation of the γH2AX assay for the assessment of conventional and electronic cigarette (e-cigarette) aerosols.

The γH2AX assay was adapted for use with the Vitrocell® VC 10 air liquid interface (ALI) aerosol exposure system. Exposures were defined in this study as a dosimetric measure rather than routine diluting airflows (L/min). Using quartz crystal microbalance technology, exposures were based on a gravimetric dose measurements (µg/cm²) obtained in situ.

Cytotoxicity was assessed using total cell counts.

Conventional 3R4F reference cigarettes demonstrated a dose dependent correlation for cytotoxicity and γH2AX induction with increased exposure, at 0, 3, 5, 10, 20 and 40 µg/cm². Doses at 20 and 40 150 µg/cm² were excluded from analysis due to high cytotoxicity. E-cigarette aerosol generated from commercially available product remained negative for cytotoxicity and γH2AX induction, despite dosing to more than 150 fold higher than the equivalent cigarette smoke exposure. All negative controls remained unaffected and the positive control chemical etoposide produced a strong positive increase in γH2AX induction.

This study demonstrates a novel approach incorporating dosimetry as an important consideration for in vitro product testing. It further demonstrates the successful adaptation to the ALI for the assessment of conventional and e-cigarette aerosols and may prove useful as a rapid high content screening technique for product assessment.