IIVS: In Vitro Exposure Systems and Dosimetry Assessment Tools for Inhaled Tobacco Products

Bethesda, MD. April 4-6, 2016

The application of dosimetry techniques for the comparison of two independent aerosol exposure systems – a case study

David Thorne*, David Azzopardi, Jason Adamson, Andrew Baxter, John McAughey, Marianna Gaça

British American Tobacco, R&D Centre, Southampton, SO15 8TL, UK

Correspondence: david_thorne@bat.com

BRITISH AMERICAN TOBACCO

Introduction

The development of whole aerosol (also termed whole smoke) exposure systems has been driven by the necessity to develop more physiologically robust methods to deliver aerosols *in vitro* to more appropriately mimic human exposure to cigarette smoke and other inhaled chemicals. The flexibility of whole aerosol exposure setups and cellular options has allowed researchers to tailor their experiments to investigate both phases of tobacco smoke with different cell types ranging from undifferentiated monolayers to fully differentiated 3D structures of the lung, yielding valuable mechanistic *in vitro* information.

The amount of whole smoke aerosol delivered within these *in vitro* exposure systems depends on the system used and the experimental set up. Each exposure system is unique in the way it dilutes and delivers the smoke aerosol to the biological system. In turn, the type of exposure module/chamber used also has an impact on aerosol delivery and deposition. This means that all studies have the potential to be very different and makes comparisons between whole smoke studies extremely difficult, and in most cases impossible.

Aims

The aim of this case study was to assess whether a consistent dosimetry approach could be used to compare cytotoxicity datasets generated on two contrasting exposure systems, the Borgwaldt RM20S and the Vitrocell® VC 10, with different experimental setups.

Materials and Methods

Study Design

Cytotoxicity data from contrasting whole smoke studies were compared using a consistent dose measure. Two different commercially available exposure systems were used to generate neutral red cytotoxicity data, with established exposure conditions and cell types [Thorne *et al.,* 2015 and Azzopardi *et al.,* 2015].

Aerosol Generation

The RM20S and the VC 10 aerosol exposure systems were used to generate dose response data **(Figure 1)**.

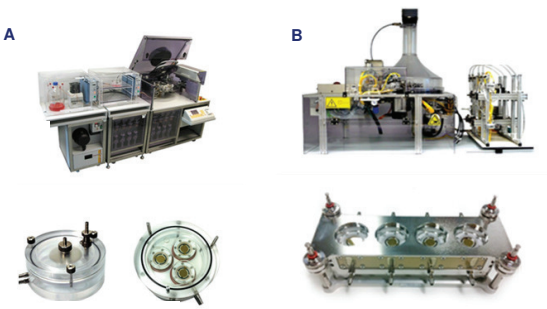

Figure 1. (A) Borgwaldt RM20S with chamber and **(B)** Vitrocell® VC 10 and module.

Cigarettes

Kentucky reference cigarettes 3R4F were used for this study and obtained from the University of Kentucky (Kentucky, USA). Prior to analysis, cigarettes were conditioned for at least 48 hours at 22 ± 1 ºC and 60 ± 3 % relative humidity in accordance with the International Organization of Standardization (ISO) guideline (ISO 3402:1999). Cigarettes were smoked exclusively at the ISO regime, defined as one 35 mL puff per 60 seconds, over 2 seconds (ISO 3308:2012).

Table 1: Key exposure system parameters and specifications.

Machine parameters	**Vitrocell® VC 10**	**Borgwaldt RM20S**
Dimensions (LxDxH)	1.5 x 0.8 x 0.85 m	2.4 x 0.8 x 1.3 m
Footprint (m^2)	Bench top	Free standing
Exposure chamber compatibility	Vitrocell® modules	BAT Perspex Chamber
Dilution principal	Flowing air (turbulent mixing)	Mechanical serial dilution
Dilution units	L/min	1:x
Range	0-12 L/min	1:2-1:4,000 (1:x)
Transit length (cm)	90	290
Time taken to puff (secs)	~8	~15-24

Table 2: Key differences in the two exposure systems and the experimental setups between studies.

Exposure system	**Vitrocell® VC 10**	**Borgwaldt RM20S**
Exposure chamber	Vitrocell® 6/4 CF	Perspex
Exposure matrix	Whole smoke	Whole smoke
Dilution principal	Flowing air (turbulent mixing)	Mechanical serial dilution
Dilution units	L/min	1:x
Cell type	BALB/c	NCI-H292
Cigarettes	3R4F	3R4F
Exposure time (mins)	180	30
Dilution range	1-10 L/min	1:5-1:250 1:x
Vacuum	5 mL/min	N/A
Exposure regimen and cigarette conditioning	ISO	ISO
Cytotoxicity assay	NRU	NRU
Dosimetry measures	Deposited mass - QCM (µg/cm^2)	Deposited mass - fluorescence spectroscopy (µg/cm^2)

Cytotoxicity – Neutral Red Uptake Assay

Cells (BALB/c and NCI-H292) were exposed at the ALI to freshly generated cigarette smoke. Neutral Red dye was released by the addition of Neutral Red de-stain solution (ethanol: acetic acid: distilled water; (50:1:49)) and were measured by absorbance at 540 nm. In each case, data was compared to air controls obtained on the same day as exposure for a background subtraction.

Dosimetry characterisation

Quartz crystal microbalances (QCM) were used in this study to assess whole smoke particle deposition (µg/cm^2) *in situ* for the VC 10. Deposition within the RM20S system was determined using fluorescence spectroscopy and converted to µg/cm^2 using a particulate matter standard curve. Deposited mass was assessed using fluorescence spectroscopy has been demonstrated to have a strong correlation with deposited mass obtained using QCM technology (R^2=97.4%) [Adamson *et al.,* 2012].

www.bat-science.com

@BAT_Sci

Results

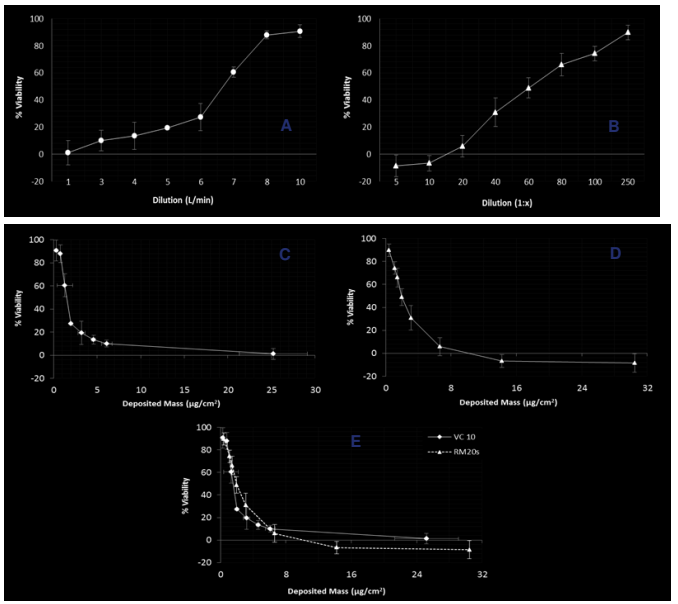

Figure 2. Cell viability data from the two independent exposure systems. **(A)** VC 10 data presented as a function of diluting airflow (L/min). **(B)** RM20S data presented as a function of smoke:air ratio (1:x), **(C-E)** smoke data presented as a function of deposited mass and directly compared between exposure setups.

Conclusions

- This case study has demonstrated that dosimetry techniques can be used to bridge the gap between diverse aerosol exposure systems, facilitate the comparison of data across laboratories and provide a means by which to align data from different exposure setups.
- This case study has demonstrated for the first time that *in vitro* biological data generated on two independent aerosol exposure systems, under contrasting experimental designs, with different cell types, can be directly compared using a consistent dosimetry approach.
- The EC_{50} is almost identical for both systems, which implies, that it does not matter how the smoke aerosol is delivered, rather, it is more important to understand what dose is reaching the ALI cellular system.

References

- Thorne *et al.,* 2015 . Inhalation Toxicology 27: 1091-7691
- Azzopardi *et al.,* 2015. Toxicology In Vitro 29: 1720-1728
- Adamson *et al.,* 2012. Chemistry Central Journal: 6:98

Related Publications

- Azzopardi, D., Haswell, L., Foss-Smith, G., Hewitt, K., Asquith, N., Corke, S., Phillips, G. **Evaluation of an air–liquid interface cell culture model for studies on the inflammatory and cytotoxic responses to tobacco smoke aerosols.** Toxicology In Vitro 2015; 29: 1720-1728

- Thorne, D., Dalrymple, A., Dillon, D., Duke, M., Meredith, C. **A comparative assessment of cigarette smoke aerosols using an in vitro air–liquid interface cytotoxicity test.** Inhalation Toxicology 2015; 27: 1091-7691

- 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

- Adamson, J., Thorne, D., Errington, G., Fields, W., Li, X., Payne, R., Krebs, T., Dalrymple, A., Fowler, K., Dillon, D., Xie, F., Meredith, C. **An inter-machine comparison of tobacco smoke particle deposition *in vitro* from six independent smoke exposure systems.** Toxicology In Vitro 2014; 28: 1320-1328

- Adamson, J., Thorne, D., Dalrymple, A., Dillon, D., Meredith, C. **Cigarette smoke deposition in a Vitrocell® exposure module: real-time quantification *in vitro* using quartz crystal microbalances.** Chemistry Central Journal 2013; 7:15

- Thorne, D and Adamson, J. **A review of in vitro cigarette smoke exposure systems.** Experimental and Toxicologic Pathology 2013 ; 65: 1183-1193

- 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

- Adamson, J., Hughes, S., Azzopardi, D., McAughey, J., Gaça, M. **Real-time assessment of cigarette smoke particle deposition *in vitro*.** Chemistry Central Journal 2012; 6:98

British American Tobacco
R&D Centre
Southampton SO15 8TL
United Kingdom
Tel +44 (0)2380 58 88088
www.bat.com

BRITISH AMERICAN TOBACCO

The application of dosimetry techniques for the comparison of two independent aerosol exposure systems – a case study

David Thorne*, David Azzopardi, Jason Adamson, Andrew Baxter, John McAughey, Marianna Gaça

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

Correspondence: david_thorne@bat.com

Abstract

Whole smoke/aerosol exposure techniques have been employed for the assessment of tobacco smoke aerosols for many years and are widely considered a more physiologically comparable test system compared to that of traditional submerged particulate exposure techniques. Whole aerosol systems offer the advantage of exposing cell cultures to a test matrix that is consistent to what the human smoker receives. Cells are often raised to the air-liquid interface to facilitate exposure and a plethora of cell systems can be used to create unique experimental setups, more closely mimicking that of the human lung. However these whole aerosol exposure systems are based on different dilution technologies and principles, which means, that studies have the potential to be very unique, depending on the exposure system, the module/chamber, cell type and biological end-point employed. To further compound this, whole smoke aerosol data can be presented in many ways, often dependent upon the machine used and the dilution principle associated with the system, resulting in the inability to compare data between systems and laboratories.

This aim of this case study was to assess whether a consistent dosimetry approach could be used to yield further insight into the charaterisation of *in vitro* aerosol exposure systems and whether two cytotoxicity datasets generated on contrasting exposure systems, the Bogwaldt RM20S and the Vitrocell® VC 10 with different experimental setups could be directly compared.

The cytotoxicity data between the two systems, the RM20S and the VC 10 at first seem incomparable, with little read across between the data sets. Expressing each dataset as a function of dose using µg/cm^2 has allowed comparisons to be drawn where previously there were no commonalities between the studies. This case study demonstrates the importance of dosimetry techniques and how they can be used to align data between two completely different exposure systems and setups, to facilitate comparisons. Additionally dose tools may now also provide the link between *in vitro*, *in vivo* and human dosimetry studies and aid in the comparison of data across different tobacco categories.

www.bat-science.com

@BAT_Sci