

Acute inhalation toxicity: *in vitro* and *ex vivo* systems

Marianna Gaça PhD

marianna_gaca@bat.com

Pre-Clinical Assessment Manager

British American Tobacco R&D

Alternative approaches for acute inhalation toxicity to address global regulatory and non-regulatory data requirements. Webinar : 26th April 2016



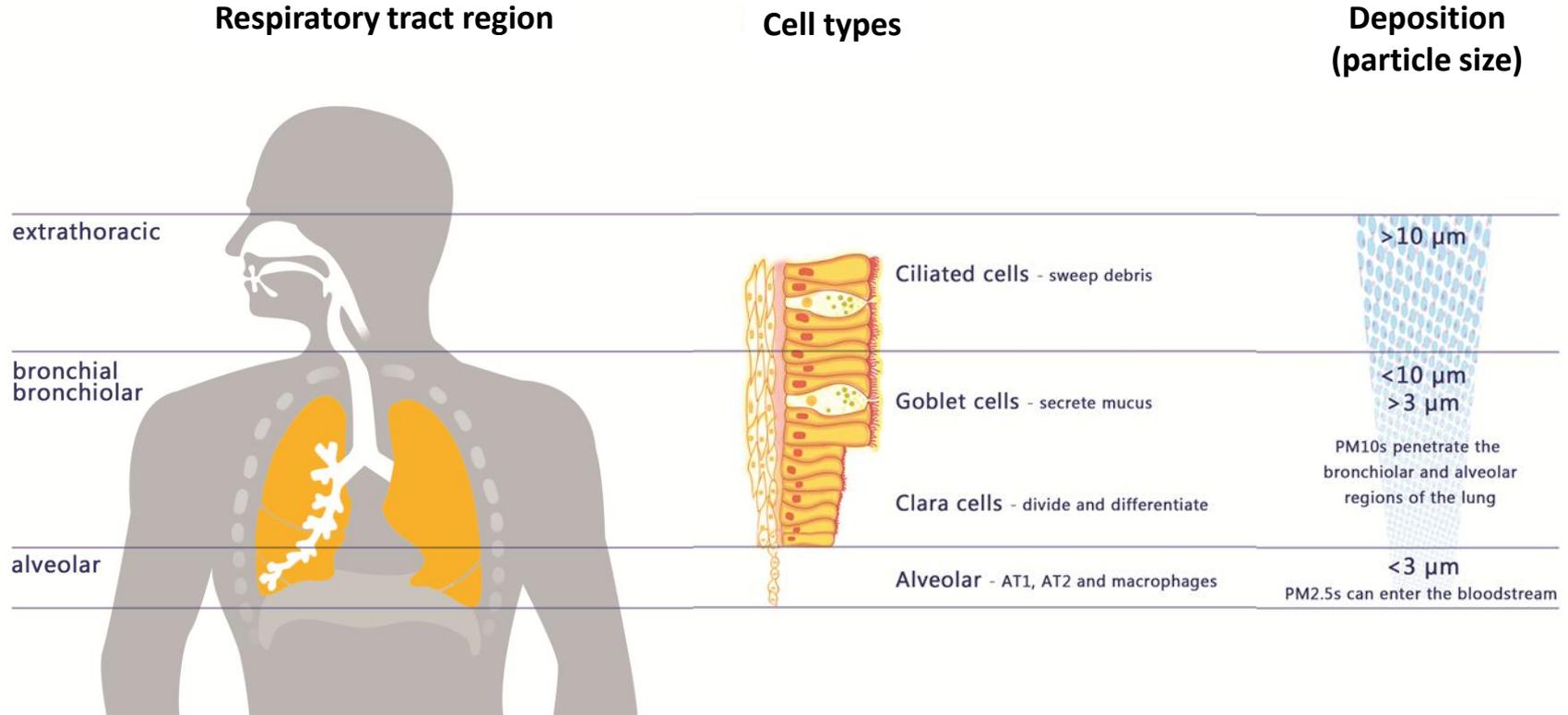
Outline

- Introduction to the lung
 - Anatomy
 - Deposition
 - *In vitro* toolbox
 - Case studies
 - Products
 - Generating aerosols
 - Example approaches
 - Summary
- 



Role of the respiratory tract

Cells in the upper and lower respiratory tract, and particle deposition



In vitro toolbox

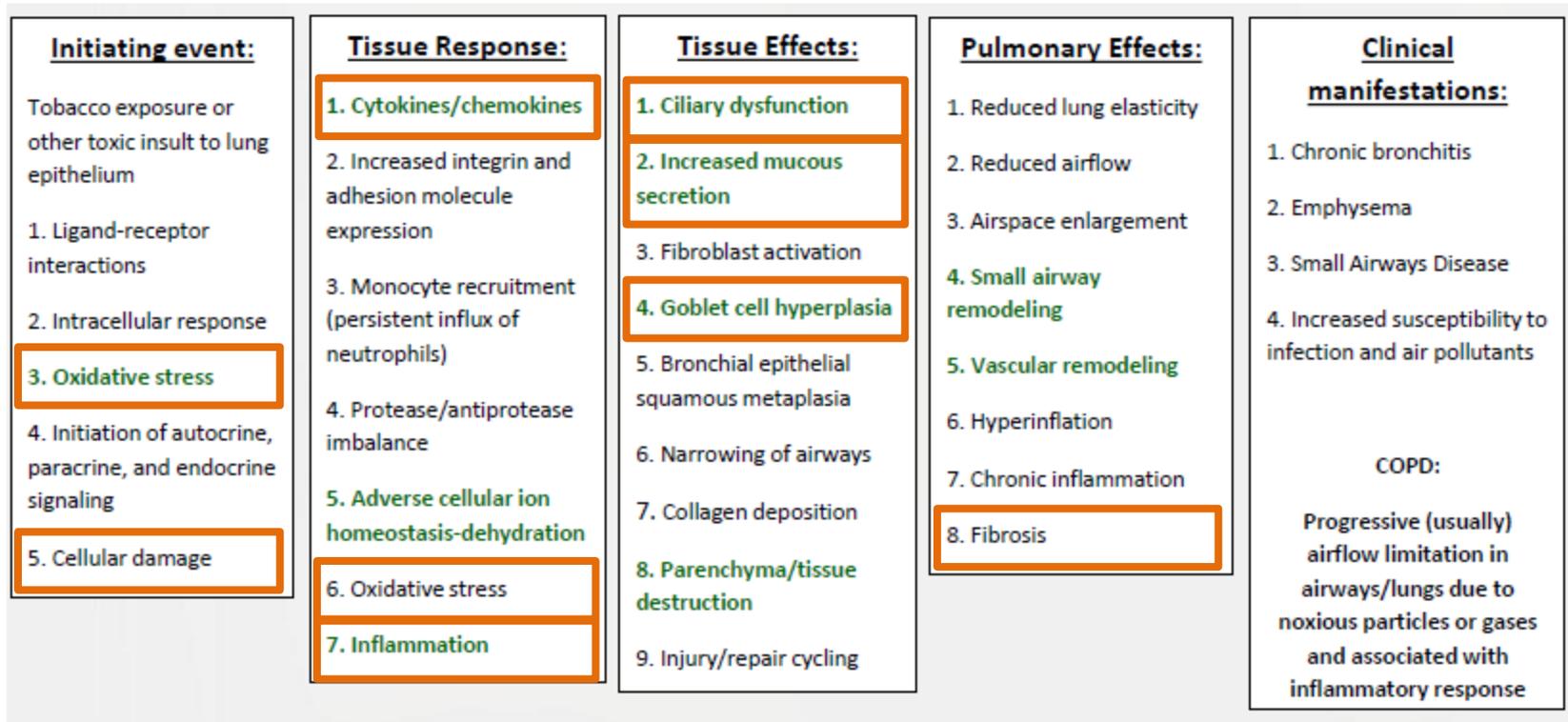
- **ESC/ iPSC**
- **Tumour derived cell lines** (e.g. H292)
 - Easy to culture and consistent results
- **Immortalised cell lines** (e.g. BEAS-2B)
 - Genetically more 'normal' than tumour-derived cells
- **Primary cells** (e.g. NHBE)
 - Retain metabolic capability and physiological characteristics
 - Donor variation
 - Limited lifespan in culture
- **3D organotypic tissue systems**
 - Retain metabolic capability and physiological characteristics
 - Donor variation
- **Lung slices**
 - Cells retain spatial orientation and intercellular interactions
 - Donor variation
 - Short lifespan after slice preparation
- **Lung-on-a-chip, microtissues**



physiological relevance

Chronic Obstructive Pulmonary Disease (COPD)

Adverse Outcome Pathway (AOP)



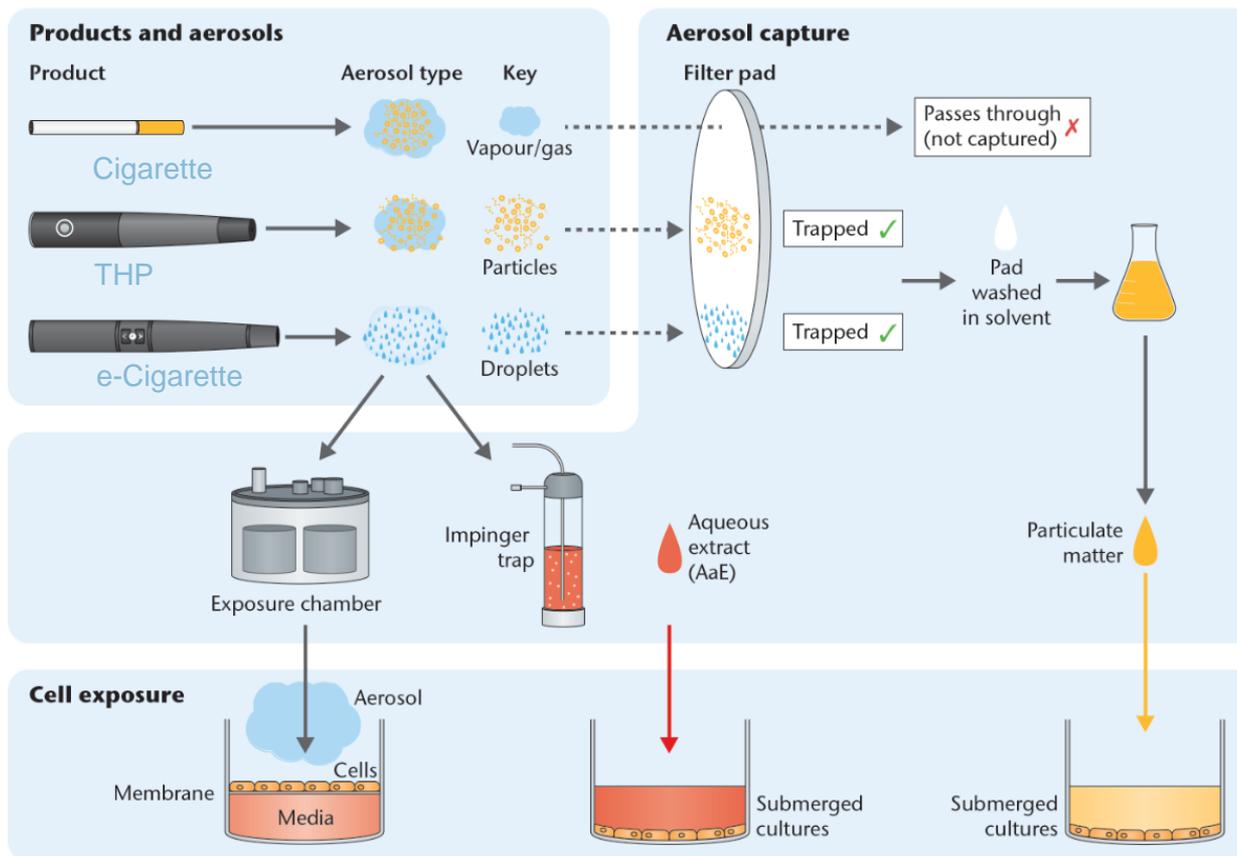


Product Diversity

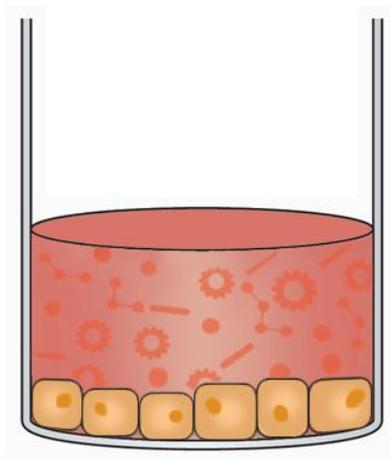
The era of next generation nicotine delivery products



How we expose cells for biological testing

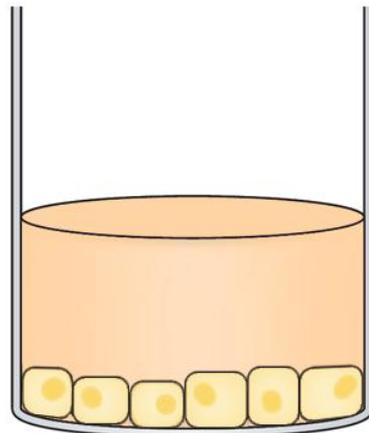


Modes of biological exposure to the test article



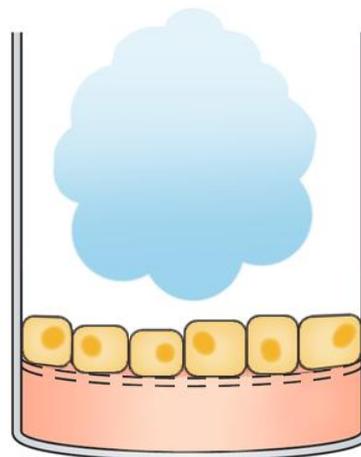
PM

Submerged exposure
to filter trapped particles
washed in solvent



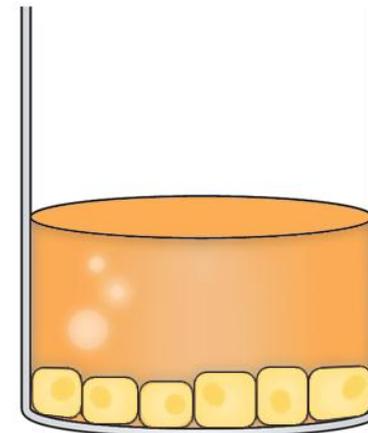
AqE

Submerged exposure
to aerosol bubbled
media or buffer



ALI

Whole aerosol or VP only
exposure at the air-liquid
(air-agar) interface



e-liquid

Submerged exposure
to unaltered e-liquid
or its ingredients

What are the associated challenges and dose implications with each mode of exposure?

In vitro air-liquid interface (ALI) exposure systems

BAT's approach



BRITISH AMERICAN
TOBACCO

Industrial Toxicology 2010; 22(14): 1174-1183

informa
healthcare

ORIGINAL ARTICLE

Evaluation of precision and accuracy of the Borgwaldt RM20S[®] smoking machine designed for *in vitro* exposure

Narvesen Kaur¹, Marlene Larocque¹, Jean-Philippe Roy¹, Jean-Louis Cahouf¹, Issam Adamson¹, Graham Erington¹, Karen C. Waldron¹, Mariana Goya², and Andri Miorci¹

¹Department of Chemistry, University of Montreal, Montreal, Quebec, Canada, ²Preparatory Tobacco Canada Ltd., Montreal, Quebec, Canada, and ³British American Tobacco Global Center, Hillside, Northborough, MA

Volume 11 | Chemistry Central Journal 2011, 1:10
http://dx.doi.org/10.1039/c1cc00001a

Chemistry Central
Journal

RESEARCH ARTICLE

Open Access

Assessment of an *in vitro* whole cigarette smoke exposure system: The Borgwaldt RM20S 8-syringe smoking machine

Issam Adamson, David Atzparad, Graham Erington, Colin Dickens, John McHughy and Mariana D Goya¹

Research Article

Quantification of Cigarette Smoke Particle Deposition *In Vitro* Using a Triplicate Quartz Crystal Microbalance Exposure Chamber

Issam Adamson, David Thorne, John McHughy, Deborah Dillon, and Clive Meredith

British American Tobacco Group BATS, Regent Park Road, Southeyton SA1 0PL, UK
Correspondence should be addressed to: Issam Adamson, issam_adamson@bats.com

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Published Online: 15 December 2010

Toxicology in Vitro 2011, 25(12): 1702-1708

Contents lists available at ScienceDirect

Toxicology in Vitro

Journal homepage: www.elsevier.com/locate/toxinvit

Evaluation of an air-liquid interface cell culture model for studies on the inflammatory and cytotoxic responses to tobacco smoke aerosols

David Atzparad, Linsey E. Harwell, Geoff Foss-Smith, Katherine Hewitt, Nathan Anquith, Sarah Cooke, Gary Phillips¹

¹British American Tobacco (Investments) Limited, Global R&D, Regent Park Road, Southeyton SA1 0PL, UK

A



BORGWALDT
KÖRBER SOLUTIONS

RM20S Smoking Machine



BAT chamber with QCMs

B



VITROCELL
SYSTEMS

VC 10 Smoking Robot



6/4 module with QCMs

Volume 11 | Chemistry Central Journal 2011, 1:10
http://dx.doi.org/10.1039/c1cc00001a

Chemistry Central
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RESEARCH ARTICLE

Open Access

Characterisation of a Vitrocell[®] VC 10 *in vitro* smoke exposure system using dose tools and biological analysis

David Thorne¹, Juanne Kilford¹, Rebecca Payne¹, Issam Adamson¹, Alan Scott¹, Annette Dalrymple¹, Clive Meredith¹ and Deborah Dillon¹

Contents lists available at ScienceDirect

Toxicology in Vitro

Journal homepage: www.elsevier.com/locate/toxinvit

An inter-machine comparison of tobacco smoke particle deposition *in vitro* from six independent smoke exposure systems

J. Adamson¹, D. Thorne¹, C. Erington¹, W. Fields¹, X. Li¹, S. Payne¹, E. Krebs¹, A. Dalrymple¹, K. Fowler¹, D. Dillon¹, F. Xie¹, C. Meredith¹

¹British American Tobacco Group BATS, Northborough, MA 01532, USA
²British American Tobacco (Investments) Limited, Regent Park Road, Southeyton, SA1 0PL, UK
³British American Tobacco (Investments) Limited, Global R&D, Regent Park Road, Southeyton, SA1 0PL, UK
⁴British American Tobacco (Investments) Limited, Global R&D, Regent Park Road, Southeyton, SA1 0PL, UK
⁵British American Tobacco (Investments) Limited, Global R&D, Regent Park Road, Southeyton, SA1 0PL, UK

Contents lists available at ScienceDirect

Mutation Research: Genetic Toxicology and Environmental Mutagenesis

Journal homepage: www.elsevier.com/locate/mutres
Contents lists available at ScienceDirect
Mutation Research: Genetic Toxicology and Environmental Mutagenesis

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

Juanne Kilford¹, David Thorne¹, Rebecca Payne¹, Annette Dalrymple¹, Julie Clements¹, Clive Meredith¹, Deborah Dillon¹

¹British American Tobacco Group BATS, Northborough, MA 01532, USA
²British American Tobacco (Investments) Limited, Regent Park Road, Southeyton, SA1 0PL, UK

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Toxicology in Vitro

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Development of an *in vitro* cytotoxicity model for aerosol exposure using 3D reconstructed human airway tissue; application for assessment of e-cigarette aerosol

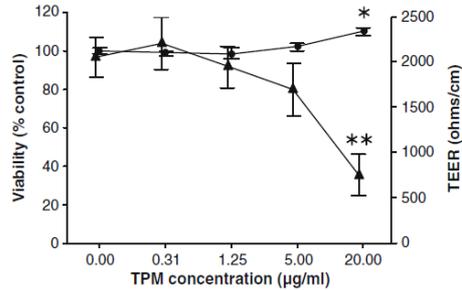
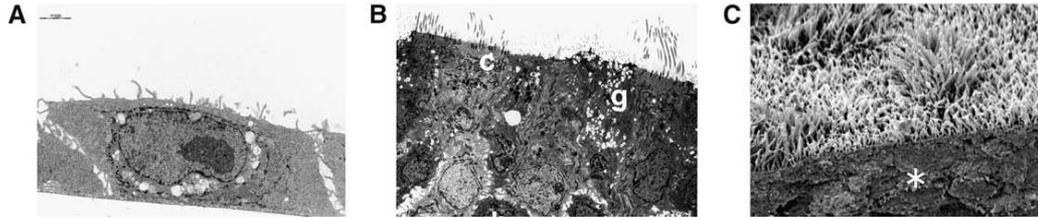
Leslie Neilson¹*, Courtney Mankus¹, David Thorne¹, George Jackson¹, Jason DeWay¹, Clive Meredith¹

¹British American Tobacco (Investments) Limited, Regent Park Road, Southeyton, SA1 0PL, UK
²British American Tobacco (Investments) Limited, Global R&D, Regent Park Road, Southeyton, SA1 0PL, UK

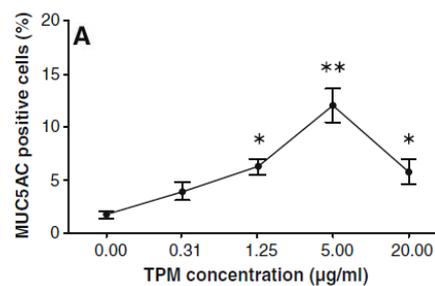
TPM increases mucous secreting cell numbers *in vitro*

A potential model of goblet cell hyperplasia

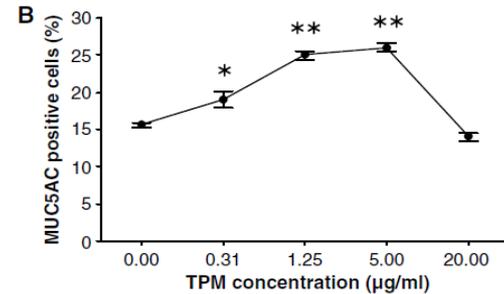
Transmission (A and B) and scanning (C) electron micrographs of HBEC ALI cultures at day 1 (A) and day 28 (B and C)



TPM affects cell viability (●) and TEER (▲)
*p<0.005 and ** p<0.001

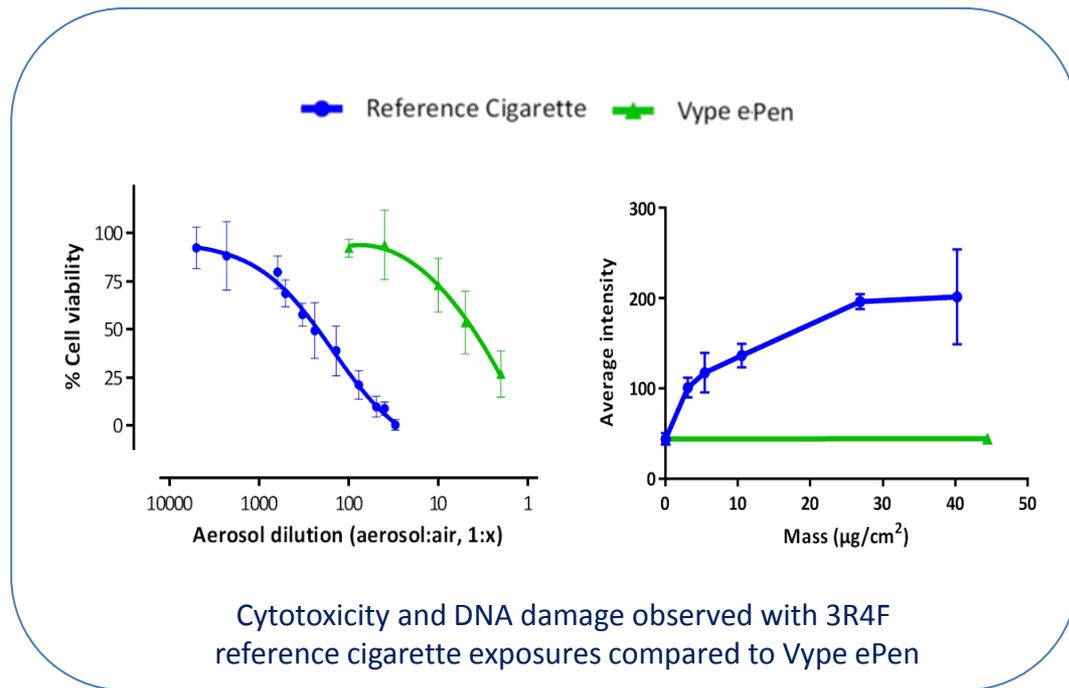
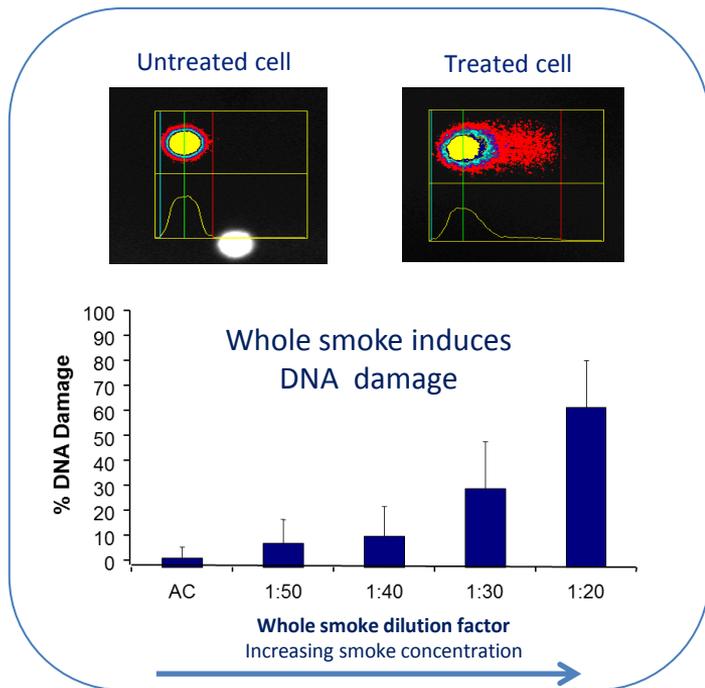


TPM increases % of MUC5AC positive cells as measured by flow cytometry (A)
and immunocytochemistry (B). *p<0.005 and ** p<0.001

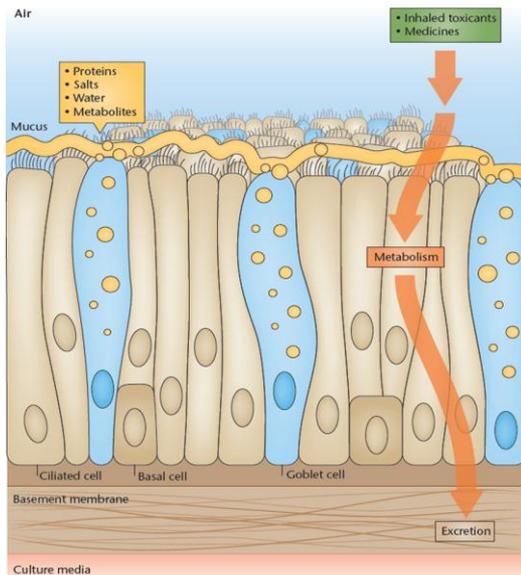


Continuous cell lines exposed to whole aerosol

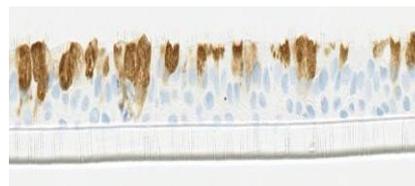
Key events modelled *in vitro*



Commercially available 3D organotypic models for inhalation toxicology

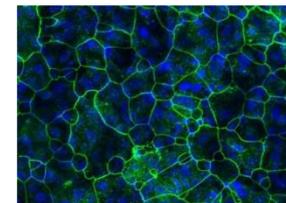
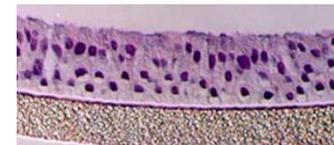


MucilAir™



SmallAir™

EpiAirway™



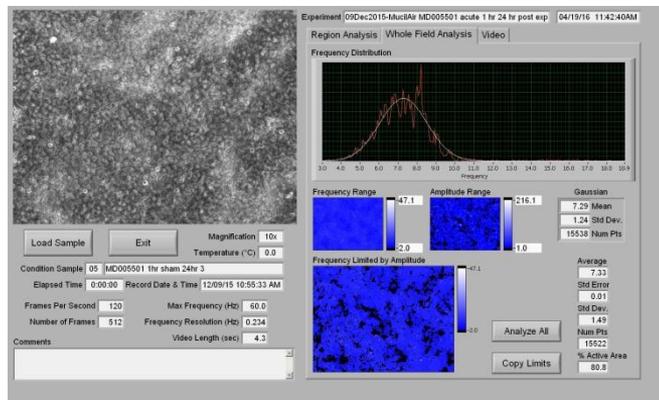
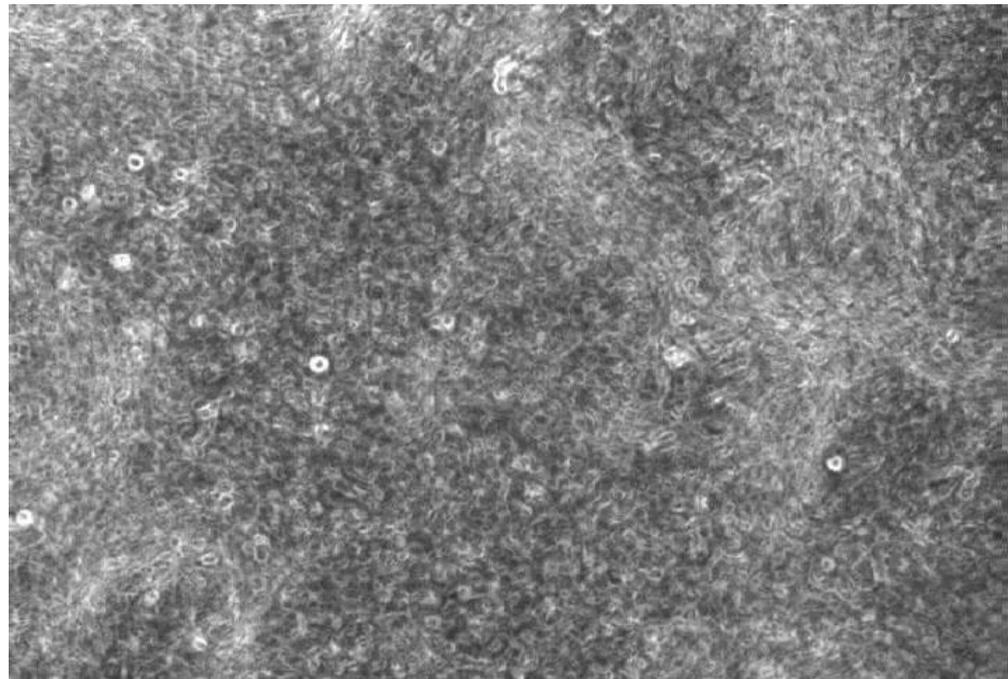
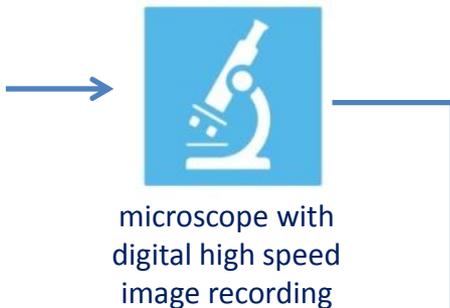
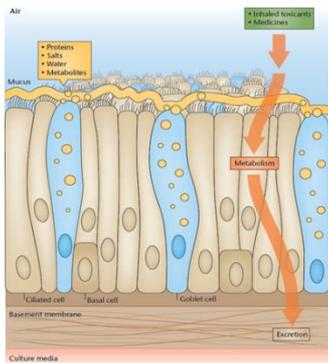
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www.epithelix.com

www.mattek.com

Functional endpoint analysis

Ciliary beat frequency (CBF)



EpiAirway™ and e-cigarette testing

Toxicology in Vitro 29 (2015) 1952–1962

Contents lists available at ScienceDirect

Toxicology in Vitro

journal homepage: www.elsevier.com/locate/toxinvit



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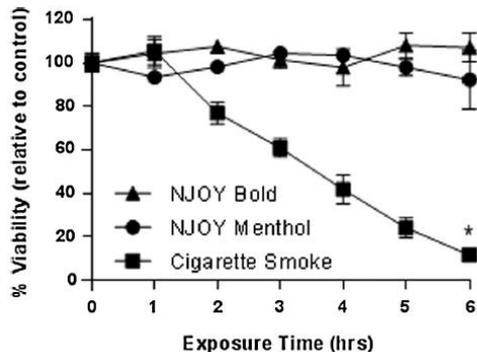


Development of an *in vitro* cytotoxicity model for aerosol exposure using 3D reconstructed human airway tissue; application for assessment of e-cigarette aerosol

Louise Neilson^{a,*}, Courtney Mankus^b, David Thorne^a, George Jackson^b, Jason DeBay^b, Clive Meredith^a

^a British American Tobacco, Group Research and Development, Regents Park Road, Southampton, Hampshire SO15 8TL, United Kingdom

^b MarTek Corporation, 200 Homer Avenue, Ashland, MA 01721, United States



Comparison of cytotoxicity after cigarette and e-cigarette exposure

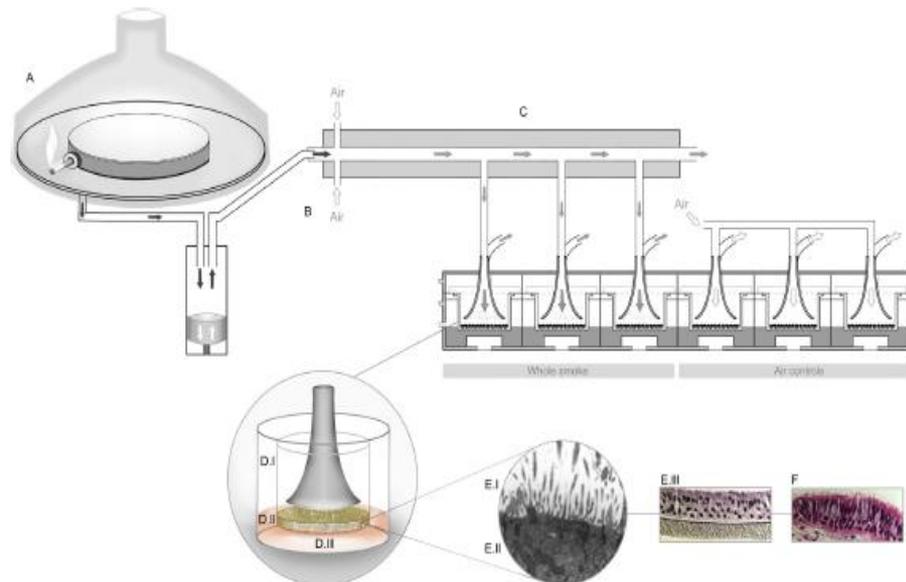
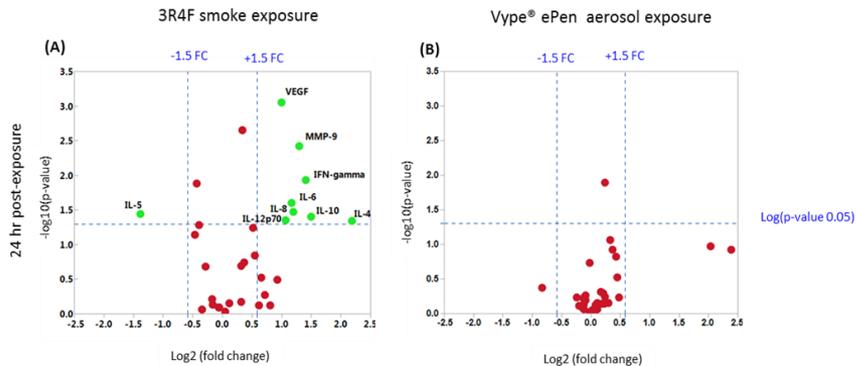
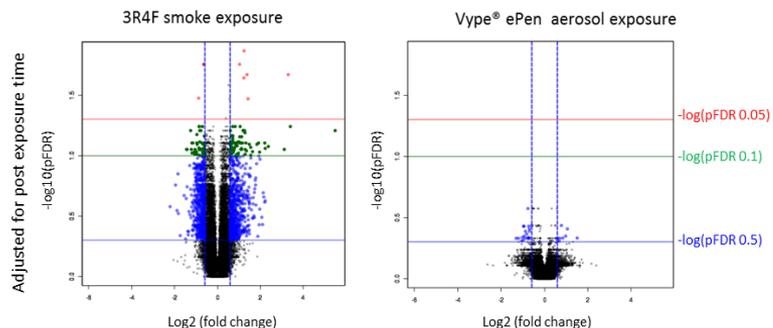


Fig. 1. Schematic representation of the VITROCELL® VC 01 Smoking Robot, mammalian 12/6 CF stainless-steel exposure module, and EpiAirway™ tissue model. (A) VC 01 single port smoking robot, enclosed in a ventilation hood with a piston/syringe that draws and delivers smoke or aerosol to the dilution bar. (B) Dilution bar, where smoke or aerosol is diluted, mixed, and delivered to the exposure module. Diluted smoke/aerosol within the dilution bar transmits to exhaust. (C) 12/6 CF stainless-steel exposure module, where EpiAirway™ inserts are housed during exposure. (D.I) Culture insert on which EpiAirway™ tissue culture is supported at the air-liquid interface with smoke/aerosol distributing “trumpet” sitting 2 mm above the surface of the tissue. (D.II) Fresh culture media (AIR-100 maintenance media) basally feeding human airway epithelium. Transmission electron micrograph (magnification $\times 20,000$) showing (E.I) cilia and (E.II) tight junctions. Haematoxylin and eosin stained cross-sections (magnification $\times 360$) of (E.III) pseudostratified mucociliary morphology of EpiAirway™ tissue and (F) excised human bronchial epithelium for comparison.

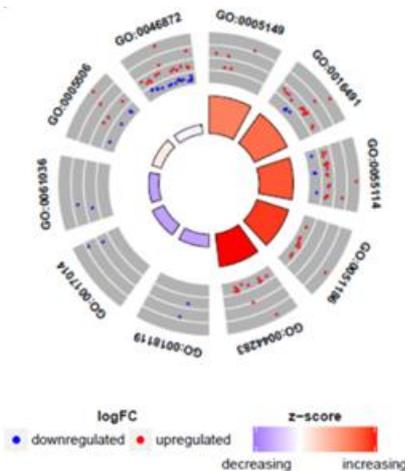
Comparing perturbations in MucilAir™



The differential level of 33 secreted cytokines in the culture media of MucilAir™ exposed to 3R4F (A) and Vype ePen (B)



Differential expression of 44,184 sequenced RNAs (Log₂ fold change -X axis and the Log₁₀ pFDR -Y axis) Air vs 3R4F (A) and air vs Vype® ePen (B) over a period of 48hrs post exposure recovery



GO ID	Term
GO:0005149	Interleukin-1 receptor binding
GO:0016491	Oxidoreductase activity
GO:0055114	Oxidation-reduction process
GO:0051186	Cofactor metabolic process
GO:0044283	Small molecule biosynthetic process
GO:0018119	Peptidyl-cysteine S-nitrosylation
GO:0017014	Protein nitrosylation
GO:0061036	Positive regulation of cartilage development
GO:0005506	Iron ion binding
GO:0046872	Metal ion binding

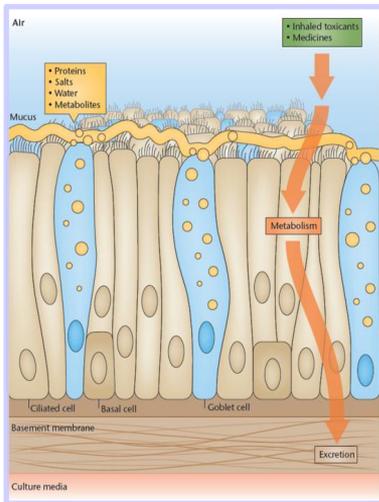
3R4F exposure gene ontology enrichment identified perturbations in oxidative stress response, inflammation and xenobiotics metabolism response pathways



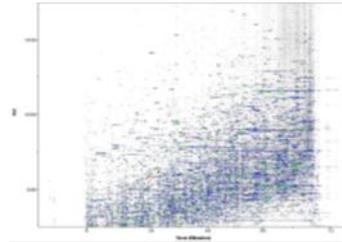
In vitro airway surface liquid (ASL) provides a rich source of information to study tissue homeostasis

Good similarity between protein profiles from clinical samples and 3-D *in vitro* lung models

Characterisation of proteins in *in vitro* airway surface liquid

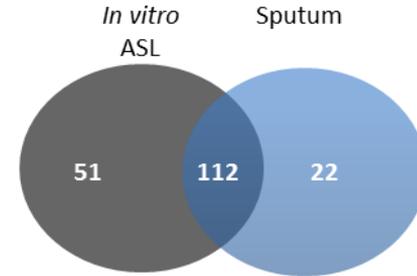


LC-MS/MS



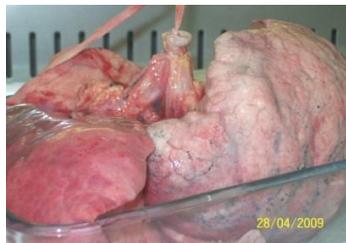
+ Database search

Comparison of *in vitro* ASL samples with healthy sputum



A strong overlap of 112 common proteins

Precision cut lung slices (PCLS)



Tissue is stabilised by perfusion of agarose through the airways, prior to slicing



Cores are taken through the tissue



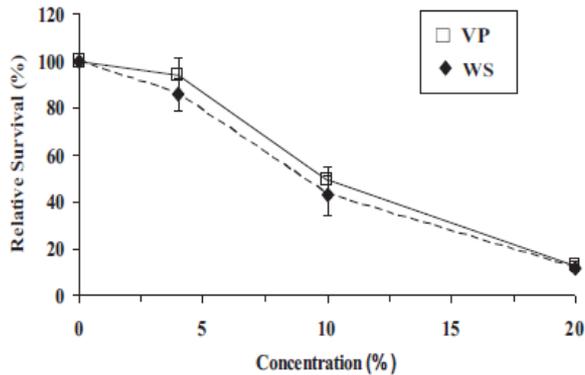
Slices are placed on a mesh in culture medium



Precision-cut slices generated using a Krumdieck tissue slicer



An *ex vivo* approach to the differential parenchymal responses induced by cigarette whole smoke and its vapour phase



Toxicity of cigarette smoke to the lung slices. Rat lung slices were subjected to a 30 min/day exposure of diluted cigarette whole smoke, (WS) or vapor phase, (VP) for 3 consecutive days. Lung slices were harvested for MTT assay 24 h post last exposure. Relative survival rates of smoke-exposed groups were obtained by comparing to the air-exposed group.

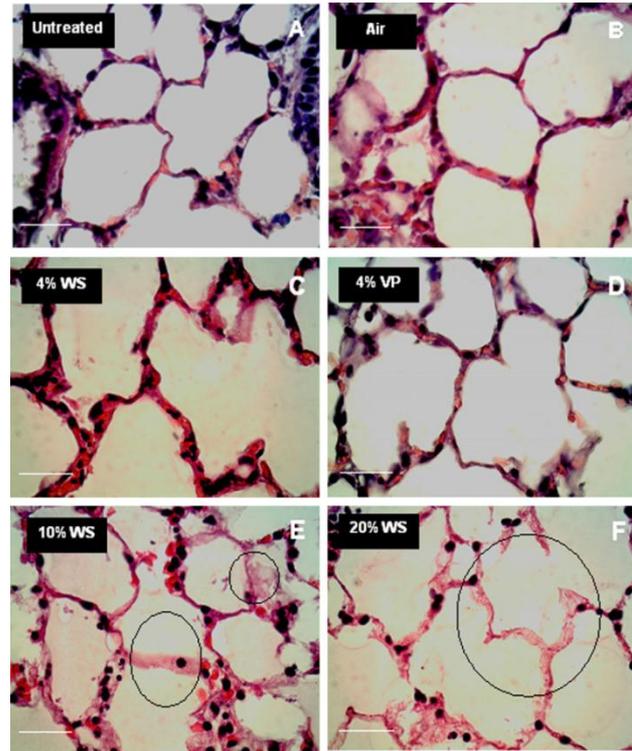


Fig. 2. Alveolar structure of cultured rat lung slices exposed to cigarette smoke. Lung slice left untreated (A), exposed to air (B), 4, 10, 20% whole smoke, WS (C, E, and F) or to 4% vapor phase, VP (D) were harvested for histology processing 24 h post last exposure. Three consecutive days of exposure were performed as described earlier. Bar represents 20 nm and circles indicate alveolar septum damage.



Acute inhalation toxicity:

in vitro and *ex vivo* systems- considerations



- Different complexity - model specific aspects of disease processes
- Dosimetry - what are the cells exposed to?
- Exposure systems - acute/short term exposure - longer term
- Validation and qualification - fit for purpose?
- Support biomarker discovery *in vitro* and in the clinic-AOPs

Contributions and thanks

BAT

Andrew Baxter
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David Thorne
Emmanuel Minet

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Linsey Haswell
Louise Neilson
Sarah Corke
Simone Santopietro
Tomasz Jaunky



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