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

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

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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

<table>
<thead>
<tr>
<th>Respiratory tract region</th>
<th>Cell types</th>
<th>Deposition (particle size)</th>
</tr>
</thead>
<tbody>
<tr>
<td>extrathoracic</td>
<td>Ciliated cells - sweep debris</td>
<td>&gt;10 μm</td>
</tr>
<tr>
<td>bronchial bronchiolar</td>
<td>Goblet cells - secrete mucus</td>
<td>&lt;10 μm &gt;3 μm</td>
</tr>
<tr>
<td>alveolar</td>
<td>Clara cells - divide and differentiate</td>
<td>&lt;3 μm</td>
</tr>
<tr>
<td></td>
<td>Alveolar - AT1, AT2 and macrophages</td>
<td>PM10s penetrate the bronchiolar and alveolar regions of the lung</td>
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<tr>
<td></td>
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<td>PM2.5s can enter the bloodstream</td>
</tr>
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**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**
Chronic Obstructive Pulmonary Disease (COPD)

Adverse Outcome Pathway (AOP)

Initiating event:
- Tobacco exposure or other toxic insult to lung epithelium
- 1. Ligand-receptor interactions
- 2. Intracellular response
- 3. Oxidative stress
- 4. Initiation of autocrine, paracrine, and endocrine signaling
- 5. Cellular damage

Tissue Response:
1. Cytokines/chemokines
2. Increased integrin and adhesion molecule expression
3. Monocyte recruitment (persistent influx of neutrophils)
4. Protease/antiprotease imbalance
5. Adverse cellular ion homeostasis-dehydration
6. Oxidative stress
7. Inflammation

Tissue Effects:
1. Ciliary dysfunction
2. Increased mucous secretion
3. Fibroblast activation
4. Goblet cell hyperplasia
5. Bronchial epithelial squamous metaplasia
6. Narrowing of airways
7. Collagen deposition
8. Parenchyma/tissue destruction
9. Injury/repair cycling

Pulmonary Effects:
1. Reduced lung elasticity
2. Reduced airflow
3. Airspace enlargement
4. Small airway remodeling
5. Vascular remodeling
6. Hyperinflation
7. Chronic inflammation
8. Fibrosis

Clinical manifestations:
1. Chronic bronchitis
2. Emphysema
3. Small Airways Disease
4. Increased susceptibility to infection and air pollutants

COPD:
Progressive (usually) airflow limitation in airways/lungs due to noxious particles or gases and associated with inflammatory response

Product Diversity

The era of next generation nicotine delivery products
How we expose cells for biological testing

**Products and aerosols**

- **Cigarette**
  - Aerosol type: Vapour/gas
  - Key: Particles
  - Key: Droplets
- **THP**
- **e-Cigarette**

**Aerosol capture**

- Filter pad: Passes through (not captured)
- Filter pad: Trapped
- Filter pad: Trapped
- Filter pad: Trapped
- Filter pad: Pad washed in solvent
- Filter pad: Particulate matter

**Cell exposure**

- **Aerosol**
  - Membrane
  - Cells
  - Media
  - Submerged cultures
  - Submerged cultures
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**

**A**

RM20S Smoking Machine

**B**

VC 10 Smoking Robot

6/4 module with QCMs

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**Research Article**

**Title:** Evaluation of precision and accuracy of the Borgwaldt RM20S smoking machine designed for in vitro exposure

**Authors:** [Details of authors]

**Abstract:**

This research evaluated the precision and accuracy of the Borgwaldt RM20S smoking machine (SM) designed for in vitro exposure. Several parameters were assessed to ensure the machine's reliability for research purposes.

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**Research Article**

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

**Authors:** [Details of authors]

**Abstract:**

This study aimed to characterise the Vitrocell® VC 10 in vitro smoke exposure system (S) using dose tools and biological analysis. The objective was to understand the effectiveness of the system in simulating real-world exposure conditions.

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**Research Article**

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

**Authors:** [Details of authors]

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This investigation focused on quantifying the deposition of cigarette smoke particles in vitro using a tripple quartz crystal microbalance exposure chamber. The method promises to improve the accuracy of smoke exposure simulations.

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TPM increases mucous secreting cell numbers \textit{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 (▲)

* \textit{p}<0.005 and ** \textit{p}<0.001

TPM increases % of MUC5AC positive cells as measured by flow cytometry (A) and immunocytochemistry (B). * \textit{p}<0.005 and ** \textit{p}<0.001
Continuous cell lines exposed to whole aerosol

Key events modelled in vitro

Whole smoke induces DNA damage

Cytotoxicity and DNA damage observed with 3R4F reference cigarette exposures compared to Vype ePen

D Thorne, J Wilson, T.S Kumaravel, ED Massey, M McEwan
Mutation Research 2008, 673(1):3-8
Commercially available 3D organotypic models for inhalation toxicology

MucilAir™

SmallAir™

EpiAirway™

EpiAlveolar™

www.epithelix.com

www.mattek.com
Functional endpoint analysis
Ciliary beat frequency (CBF)

microscope with
digital high speed
image recording

Analysis software measure CBF
EpiAirway™ and e-cigarette testing

Comparison of cytotoxicity after cigarette and e-cigarette exposure
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

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**

Comparison of **in vitro** ASL samples with healthy sputum

A strong overlap of 112 common proteins

Haswell et al. Society of Toxicology meeting 2014 (Abstract # 1530)
Haswell et al Society of Toxicology meeting 2016 (Abstract # 3041)
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.
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**
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Sarah Corke
Simone Santopietro
Tomasz Jaunky