Application of *in vitro* approaches for the assessment of next generation tobacco and nicotine products:

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*British American Tobacco, Scientific R&D, Southampton, UK*

Symposium III: Genotoxicity of Inhaled Compounds
Annual Meeting of Genetic Toxicology Association
May 9th 2019
AGENDA

• Foundations of tobacco harm reduction
• Assessing the risk profile of e-cigarettes
• *In vitro* toolbox and examples
• Summary
FOUNDATIONS OF TOBACCO HARM REDUCTION

PREP: A product that:

results in the substantial reduction in exposure to one or more tobacco toxicants

and

can reasonably be expected to reduce the risk of one or more specific diseases or other adverse health effects

Snus is a low-toxicant smokeless tobacco, made of moist tobacco formed into portions to place under the lip.

Heat tobacco at temperatures not high enough to cause burning - the burning that causes most harm.

ENDS deliver nicotine to inhale in a vapour (glycerine and/or propylene glycol).

No Burning or No Combustion
**INCREASING ACKNOWLEDGEMENT OF THE RISK CONTINUUM MODEL**

<table>
<thead>
<tr>
<th>Cigarettes</th>
<th>THP</th>
<th>Snus</th>
<th>Vapour</th>
</tr>
</thead>
<tbody>
<tr>
<td>Position confirmed through epidemiology&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Position to be confirmed&lt;sup&gt;c&lt;/sup&gt;</td>
<td>Position to be confirmed&lt;sup&gt;*c&lt;/sup&gt;</td>
<td>Position proposed By Public Health Authorities&lt;sup&gt;*&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> Doll & Hill (1954) BMJ 1954: 1451-1455  
<sup>b</sup> Fearon et al 2017 Poster 113 GFN Conference http://bit.ly/2xRPvD  

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**“To truly protect the public, the FDA’s approach must take into account the continuum of risk for nicotine-containing products”**


Scott Gottlieb, M.D (FDA Commissioner)  
July 2017
A GROWING CONSENSUS ON E-CIGARETTE HARM REDUCTION POTENTIAL

2018: John Newton, Director for Health and Improvement:
“Our new review reinforces the finding that vaping is a fraction of the risk of smoking, at least 95% less harmful, and of negligible risk to bystanders. Yet over half of smokers either falsely believe that vaping is as harmful as smoking or just don’t know.”

Electronic cigarettes (also known as vapourisers)
“Compared to tobacco products, electronic cigarettes are significantly safer”

Nicotine without smoke: tobacco harm reduction
Promote e-cigarettes widely as substitute for smoking says new RCP report

Government of Canada
“Switching from tobacco cigarettes to vaping products will reduce a person’s exposure to many toxic and cancer-causing chemicals”

New Zealand Ministry of Health
“Smokers switching to vaping products are highly likely to reduce their health risks and for those around them”

House of Commons Science and Technology Committee
E-cigarettes
Seventh Report of Session July 2018

“E-cigarettes present an opportunity to significantly accelerate already declining smoking rates, and thereby tackle one of the largest causes of death in the UK today. They are substantially less harmful—by around 95%—than conventional cigarettes. They lack the tar and carbon monoxide of conventional cigarettes—the most dangerous components. It has also proven challenging to measure the risks from ‘second-hand’ e-cigarette vapour because it is negligible and substantially less than that of conventional cigarettes.”
EMERGING OPINIONS ON TOBACCO HEATING PRODUCTS

“...it is likely that there is a reduction in risk, though not to zero, to health for smokers who switch completely to heat-not-burn tobacco products...”

(Federal Institute for Risk Assessment)

“...Although NFDPM values for Heat Not Burn (HNB) products can formally be compared to cigarette 'tar', direct comparisons would be misleading...”

(National Institute for Public Health and the Environment)

“...levels of major carcinogens are markedly reduced in HNB emissions in relation to cigarettes...”
ASSESSING THE RISK PROFILE - A THREE-STEP SCIENTIFIC JOURNEY

01 EMISSIONS
› Science showing no combustion
› Untargeted emissions
› Targeted emissions
› Environmental emissions

02 EXPOSURE
› Puffing behaviour
› Average daily consumption
› Clinical PK
› Clinical BoE

03 RISK
› Clinical BoBE
› Risk perception
› Post-market surveillance

Reduced toxicity in lab models

in vitro regulatory toxicology  in vitro disease models  in vitro systems biology

**IN VITRO TOOLBOX: LUNG**

- 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)

<table>
<thead>
<tr>
<th>Initiating event:</th>
<th>Tissue Response:</th>
<th>Tissue Effects:</th>
<th>Pulmonary Effects:</th>
<th>Clinical manifestations:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tobacco exposure or other toxic insult to lung epithelium</td>
<td>1. Cytokines/chemokines</td>
<td>1. Ciliary dysfunction</td>
<td>1. Reduced lung elasticity</td>
<td>1. Chronic bronchitis</td>
</tr>
<tr>
<td>1. Ligand-receptor interactions</td>
<td>2. Increased integrin and adhesion molecule expression</td>
<td>2. Increased mucous secretion</td>
<td>2. Reduced airflow</td>
<td>2. Emphysema</td>
</tr>
<tr>
<td>4. Initiation of autocrine, paracrine, and endocrine signaling</td>
<td>5. Adverse cellular ion homeostasis-dehydration</td>
<td>5. Bronchial epithelial squamous metaplasia</td>
<td>5. Vascular remodeling</td>
<td>COPD:</td>
</tr>
<tr>
<td></td>
<td>7. Inflammation</td>
<td>7. Collagen deposition</td>
<td>7. Chronic inflammation</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>8. Parenchyma/tissue destruction</td>
<td>8. Fibrosis</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>9. Injury/repair cycling</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

HP Behrsing, Institute for In Vitro Sciences - COPD Workshop December 2014
E-CIGARETTES HAVE EVOLVED RAPIDLY

Development of e-liquids
GENERATING THE TEST ARTICLE: E:CIGARETTES

- **E-liquid**: Submerged exposure to unaltered e-liquid or its ingredients.
- **Particulate Matter (PM)**: Submerged exposure to filter trapped particles washed in solvent.
- **Aqueous Extract (AqE/GVP)**: Submerged exposure to aerosol bubbled media or buffer.
- **Aerosols**: Whole aerosol or VP only exposure at the air-liquid/agar interface.
IN VITRO ALI EXPOSURE SYSTEMS

BAT’S APPROACH
**IN VITRO DOSIMETRY**

- Tools and technologies to assess test articles
- Chemical composition at source and at exposure interface

<table>
<thead>
<tr>
<th>e-Liquids</th>
<th>PM(^{[1]})</th>
<th>AqE(^{[2]})</th>
<th>Aerosol</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Quantitation/dosimetry</strong></td>
<td>Nicotine</td>
<td>Tar Nicotine</td>
<td>OD @320 nm CO Nicotine Carbonyls</td>
</tr>
</tbody>
</table>

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**NICOTINE**
- Cross-category marker
- In situ of exposure
- QCM surface (CFP), PBS, cell culture insert
- Conversion of dilution to delivered nicotine

**QCM**
- Real time data generation
- In situ of exposure

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1. ISO 4387:2000
3. Adamson et al. *Chemistry Central*, 2016 **10**: 74
4. Adamson et al. *Applied In Vitro Toxicology* 2017 **3**:14-27
<table>
<thead>
<tr>
<th></th>
<th>Visual</th>
<th>Description</th>
<th>Aerosol generation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reference cigarette</td>
<td><img src="image1.png" alt="Image of a traditional cigarette" /></td>
<td>US blend (3R4F) reference cigarette from the University of Kentucky, US</td>
<td>Combustion and pyrolysis of tobacco</td>
</tr>
<tr>
<td>Vype e-Pen</td>
<td><img src="image2.png" alt="Image of an e-Pen" /></td>
<td>A closed system vaping device</td>
<td>Heating of a tobacco-free, flavoured nicotine solution</td>
</tr>
</tbody>
</table>
These qualities do not necessarily mean this product produces less adverse health effects than tobacco products.

E-CIGARETTE HAS REDUCED LEVELS OF TOXICANTS RELATIVE TO CIGARETTES


Substantial reductions in toxicant levels observed versus cigarettes for all regulatory lists under these test conditions.
E- LIQUID SCREENING USING *IN VITRO*

- Market survey of 30 e-liquids: cytotoxicity in human lung epithelial cells
- IC50 achieved at a range of concentrations demonstrating a broad range of toxicity responses across the test samples
- A high throughput *in vitro* screen for primary safety assessment of e-liquids

These qualities do not necessarily mean this product produces less adverse health effects than tobacco products.
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E-Cigarettes gave no response even at 900 puffs.

Thorne et al. (2016) *Mutation Research* **812**: 29-38

Exposure to reference cigarette smoke caused mutations in a dose dependent manner; e-cigarettes gave no response.

These qualities do not necessarily mean this product produces less adverse health effects than tobacco products.
Substantial reductions in responses in tests relevant to oxidative stress$^a$–$^d$, endothelial cell wound healing$^e$, genotoxicity$^f$, tumour promotion$^g$ and cytotoxicity vs. cigarettes

These qualities do not necessarily mean this product produces less adverse health effects than tobacco products

Taylor M et al. (2016). Toxicology Mechanisms and Methods, 26: 465-476
Breheny, et al. (2017). Environmental and Molecular Mutagenesis, 58(4), 190–198
**Comparing transcriptional perturbations in Mucilair™**

48,854 genes & RNA features screened
- 3R4F: 8197 significant genes & RNA features
- Vype ePen: 49 significant genes & RNA features
- Vype ePen**: 113 significant genes & RNA features

RNA-seq data mapped onto 131 pathway-focussed gene sets with specific biological function and disease processes.

Toxicogenomics – RNA-seq differential gene expression

**X2 nicotine dose
Haswell et al. (2017) Scientific Reports 7:888

Gene enrichment analysis: heatmap indicating fold change for RNAs significant at pFDR<0.05

These qualities do not necessarily mean this product produces less adverse health effects than tobacco products.
SWITCHING TO VYPE FOLLOWING 2 WEEKS 3R4F EXPOSURE REVERSED BIOLOGICAL EFFECTS IN VITRO

INFLAMMATORY CYTOKINE EXPRESSION WAS GREATLY REDUCED COMPARED TO 4 WEEK 3R4F EXPOSURE

These qualities do not necessarily mean this product produces less adverse health effects than other tobacco products

IOM (2012) Scientific standards for studies on MRTPs
Oxidative Stress Leading to Hypertension
https://aopwiki.org/aops/149

EGFR Activation Leading to Decreased Lung Function
https://aopwiki.org/aops/148

Lowe et al. (2017) Applied In Vitro Toxicology 3(1): 131 – 148
SCIENTIFIC PUBLICATIONS ON OUR E-CIGARETTES

The world’s largest published dataset on a single vapour product
SUMMARY

*in vitro* holds promise as a part of a scientific assessment for e-cigarettes

We have demonstrated significant reductions in toxicity and biological activity *in vitro* when exposed to e-cigarette aerosol versus tobacco smoke

- Extrapolation to human requires dose assessments
- Appropriate exposures to appropriate matrix
- Validation and qualification – fit for purpose
- New tools to support screening
- Systems biology for global assessments; supports biomarker discovery *in vitro* and in the clinic: AOPs
THANK YOU

BAT
Andrew Baxter
Chris Proctor
David Smart
Ian Crooks
Linsey Haswell
Stuart Meredith
Anisha Banerjee
Chuan Liu
Emma Cheung
Ivan Verrastro
Nicole East
Simone Santopietro
Anya Terry
Damien Breheny
Emmanuel Minet
James Murphy
Oscar Camacho
Stela Bozhilova
Annette Dalrymple
David Thorne
Frazer Lowe
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