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

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CONFLICT OF INTEREST

I declare that this work was fully funded by British American Tobacco (Investments) Ltd and that myself and my co-workers were full time employees of British American Tobacco (Investments) Ltd for the duration of the research.
AGENDA

• Foundations of tobacco harm reduction

• Consumer safety assessment of vaping products

• *In vitro* approaches to assessing vaping products
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

TOXICANTS FROM COMBUSTED TOBACCO ARE RESPONSIBLE FOR SMOKING RELATED DISEASES

“It’s the other chemical compounds in tobacco, and in the smoke created by setting tobacco on fire, that directly and primarily cause the illness and death, not the nicotine.”

Dr. Scott Gottlieb, US FDA commissioner, July 2017

“Nicotine is not, however, in itself a highly hazardous drug... it is inherently unlikely that nicotine inhalation itself contributes significantly to the mortality or morbidity caused by smoking. The main culprit is smoke and, if nicotine could be delivered effectively and acceptably to smokers without smoke, most if not all of the harm of smoking could probably be avoided”

UK Royal College of Physicians, Nicotine without smoke: Tobacco harm reduction (2016)
“To truly protect the public, the FDA’s approach must take into account the continuum of risk for nicotine-containing products*”

WE HAVE ESTABLISHED A FRAMEWORK TO EVALUATE REDUCED RISK POTENTIAL

<table>
<thead>
<tr>
<th>Purpose</th>
<th>Type of study</th>
<th>Vaping</th>
<th>THP &amp; Hybrid</th>
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<tbody>
<tr>
<td>Population risk reduction</td>
<td>Post-market surveillance</td>
<td>In preparation</td>
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<td>Consumer perception study</td>
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<td>Individual risk reduction</td>
<td>Systems science</td>
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<td></td>
<td>Biomarker of effect study</td>
<td>In preparation</td>
<td>In progress</td>
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<td>Toxicant exposure reduction</td>
<td>In vitro models of disease</td>
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<td>Exposure &amp; pharmacokinetic studies</td>
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<td>Computational toxicology</td>
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<td>Stewardship science</td>
<td>In vitro regulatory toxicology</td>
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<td></td>
<td>Chemical &amp; physical characterisation</td>
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<td></td>
<td>Product design stability</td>
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</table>
VAPOUR PRODUCT RISK ASSESSMENT

Stewardship covers all aspects of the product from ingredient selection to product performance

Costigan and Meredith (2015) Reg Tox Pharm. 72: 361–369
IN SILICO RISK ASSESSMENTS

▪ An initial Hazard Evaluation to exclude any ingredients with significant toxicological alerts, including reproductive/developmental toxicity.

▪ This evaluation is performed via consultation of regulatory websites for information on formal classification (ECHA or other authorities) and toxicological databases for scientific publications.

▪ Ingredients that have a formal harmonised classification by ECHA for reproductive toxicity (1A, 1B, 2 or Lact.) are automatically excluded.

▪ Any ingredients that are not formally classified as reproductive toxicants but for which the hazard evaluation revealed the existence of scientific studies indicating potential reproductive toxicity are then subjected to a more in-depth quantitative risk assessment (QRA).
QUANTITATIVE RISK ASSESSMENTS

▪ QRAs look at all the scientific evidence of any potential reproductive toxicity *in-vivo* and determine if:

▪ The toxicology evidence supports the initial alert for reproductive toxicity
  ▪ A supportable level of the ingredient can be derived that does not pose a risk after chronic consumption, or
  ▪ No supportable level can be derived and the ingredient is not permitted for use.

▪ The toxicology evidence disproves the alert.
COMPARISON OF THE CHEMICAL COMPOSITION OF CIGARETTE SMOKE AND THE VAPOUR FROM VYPE e-Pen*


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

= level in vapour is greater than 90% reduced compared to smoke
E-CIGARETTE HAS REDUCED TOXICITY RELATIVE TO CIGARETTES*
OECD TG 471: BACTERIAL REVERSE MUTATION TEST, S. TYPHIMURIUM TA98

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.

Thorne et al. (2016) Mutation Research 812: 29-38
Thorne et al. (2018) Mutation Research 828: 46-54
IN VITRO DISEASE MODELS*
REDUCED IN VITRO TOXICITY

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

Taylor et al. (2016) Toxicology Mechanisms and Methods 26: 465-476
Thorne et al. (2017) Toxicology Letters 265:170–178
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

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

**X2 nicotine dose
Haswell et al. (2017) Scientific Reports 7:888
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
INNOVATIONS PROVIDING GREATER SATISFACTION WITH LESS NICOTINE, MORE AEROSOL AND LOWER TOXICANTS*

- **ePen2**
  - Core flavours

- **ePen3**
  - Improved flavour collections

- **iSwitch**
  - Increase particle size
  - PureTech atomizer = no burnt taste

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

95% less toxicants (cf 3R4F reference cigarette measuring WHO TobReg 9)

99% less toxicants

99.8% less toxicants

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In extremis in vitro testing for cytotoxicity shows all vaping products are much less cytotoxic than cigarette smoke, with iSwitch the least cytotoxic.

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Comparing transcriptional perturbations in Mucilair™: iSwitch*
Differential gene expression by RNA-seq

Volcano plots demonstrating differential gene expression in response to 1R6F (left panel) and e-cig (right panel). Data for (A) 24 hrs (B) 48 hrs and (C) Adjusted for time have been combined in this figure. Volcano plots were generated using a fold change of [FC]>1.5 and an adjusted p-value significance threshold of p<0.05

<table>
<thead>
<tr>
<th>Comparisons</th>
<th>Number of significant genes</th>
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<tbody>
<tr>
<td>Air vs 1R6F (24 hrs)</td>
<td>5603</td>
</tr>
<tr>
<td>Air vs iSwitch (24 hrs)</td>
<td>0</td>
</tr>
<tr>
<td>Air vs 1R6F (48 hrs)</td>
<td>2180</td>
</tr>
<tr>
<td>Air vs iSwitch (48 hrs)</td>
<td>0</td>
</tr>
<tr>
<td>Air vs 1R6F combined</td>
<td>6045</td>
</tr>
<tr>
<td>Air vs iSwitch combined</td>
<td>0</td>
</tr>
</tbody>
</table>

Table showing the number of significant genes identified for each contrast performed when selecting genes (at adjusted p-value < 0.05 and fold change >1.5)

*These qualities do not necessarily mean this product produces less adverse health effects than tobacco products
VYPE – iSWITCH: 5 DAY CONFINED SWITCHING STUDY*

- 14 BoEs for cigarette smoke toxicants measured
- All BoEs significantly reduced for subjects switching to iSwitch**
- In most cases BoEs reduced to levels similar to nicotine

**o-Toluidine significant after one inexplicably high value removed from cessation arm (sensitivity test)

*These qualities do not necessarily mean this product produces less adverse health effects than tobacco products
SCIENTIFIC PUBLICATIONS ON OUR E-CIGARETTES

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

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

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

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 2017
“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.”

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.”
SUMMARY

• Scientific framework established to substantiate risk reduction potential of Potentially Reduced Risk Tobacco and Nicotine Products (PRRPs)
• Our PRRPs operate in a different manner to cigarettes producing simpler and cleaner aerosols than smoke
• Pre-clinical and clinical assessment shows that our PRRPs have the potential to be reduced risk relative to cigarettes
• *In vitro* toxicology and biology can play an important role in assessing PRRPs
THANK YOU

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