

In vitro substantiation of the harm reduction potential of next generation nicotine delivery products compared to traditional tobacco products

28-08-2022

Dr Fiona Chapman



## **Overview**

- Introduction to traditional tobacco and next generation nicotine delivery products (NGPs)
- An introduction to the relative risk scale for nicotine delivery products
- In vitro (geno)toxicological profiles of NGPs relative to traditional tobacco products and to each other



## **Nicotine delivery products**

### Traditional tobacco products:

- **Combustibles** (e.g., cigarettes): burn tobacco to produce smoke which is inhaled by the adult smoker
- Snus: oral products (placed between gum and lip) containing tobacco

Next generation nicotine delivery products (NGPs) offer a means of potentially reduced harm nicotine delivery to adult smokers who do not wish to quit smoking and would otherwise continue to smoke

### NGPs:

- Heated tobacco products (HTPs): reconstituted tobacco stick heated (but not burned) to produce nicotine-containing aerosol
- Electronic nicotine delivery systems (ENDS) (vape): e-liquid (base constituents + flavour concentrate ± nicotine) heated to produce an aerosol
- Oral nicotine pouches: Typically tobacco-free oral nicotine pouches

Nicotine replacement therapies (NRTs) (e.g., lozenges, patches, gum)



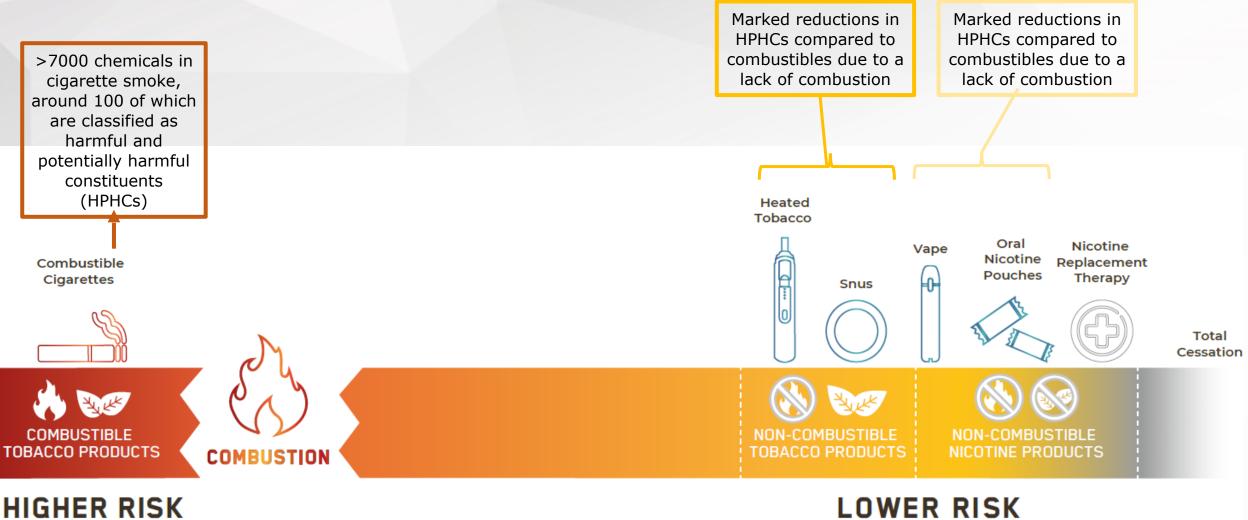






## The relative risk scale for nicotine products

• The current scientific evidence suggests that combustible cigarettes, NGPs, and nicotine replacement therapies can be placed on a relative risk (of exposure to toxicants) scale

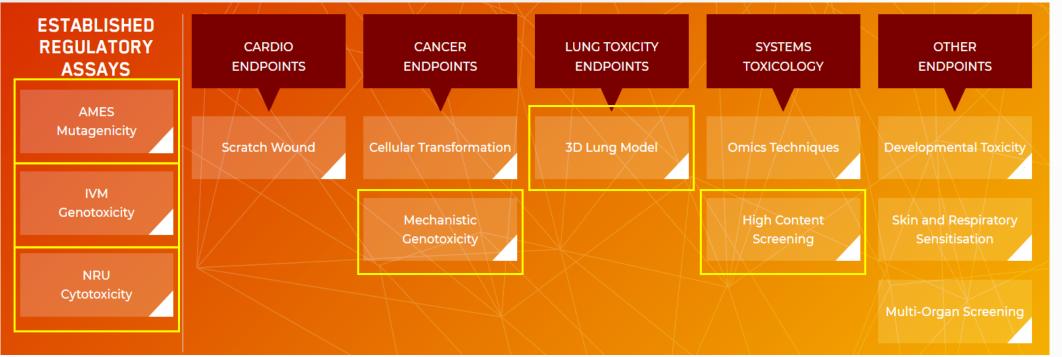


FEWER TOXICANTS

MORE TOXICANTS

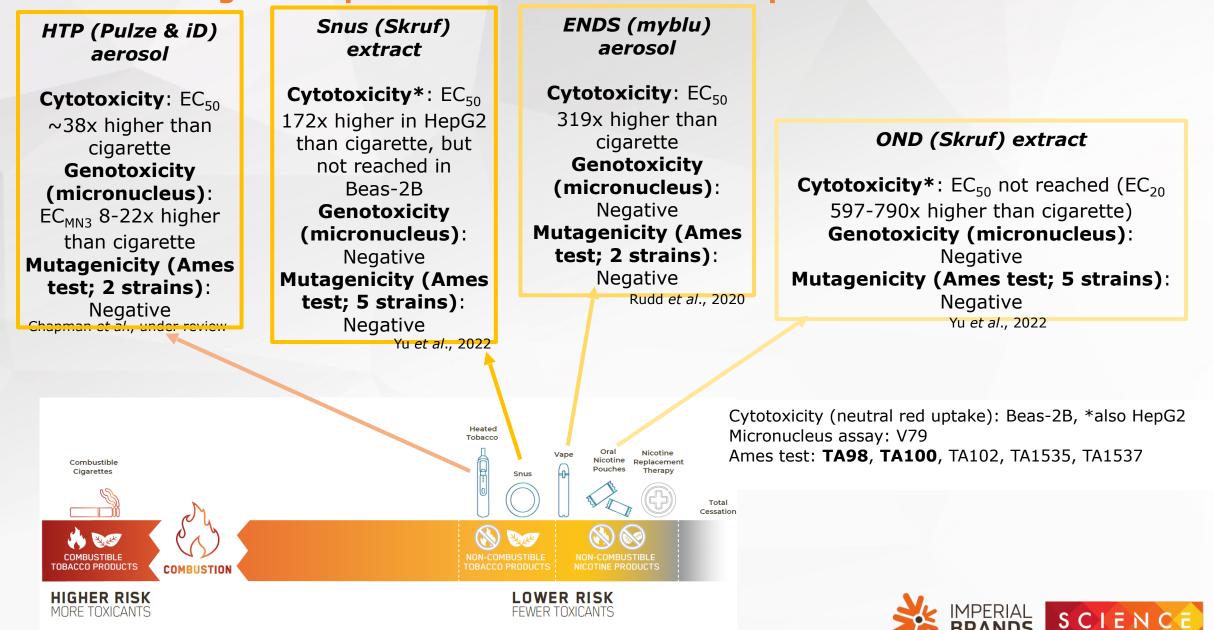
## In vitro toxicological assessment of NGPs

- Novel NGPs require screening for their potential toxicological effects, relying solely on chemical analysis
  is not sufficient
- In vitro assessments can also contribute to understanding the reduced risk potential of NGPs compared to combustible cigarettes
  - Endpoints of interest include those which assess mechanisms of smoking-related diseases
- The below testing framework therefore includes both regulatory assays and newer methodologies

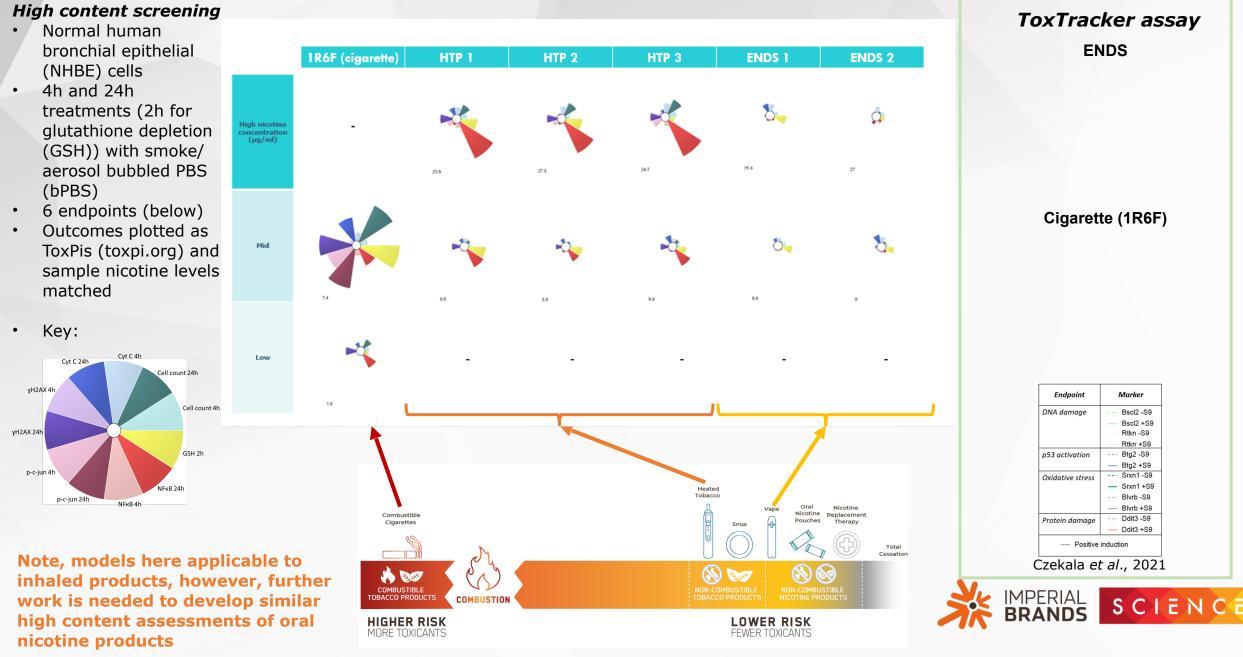




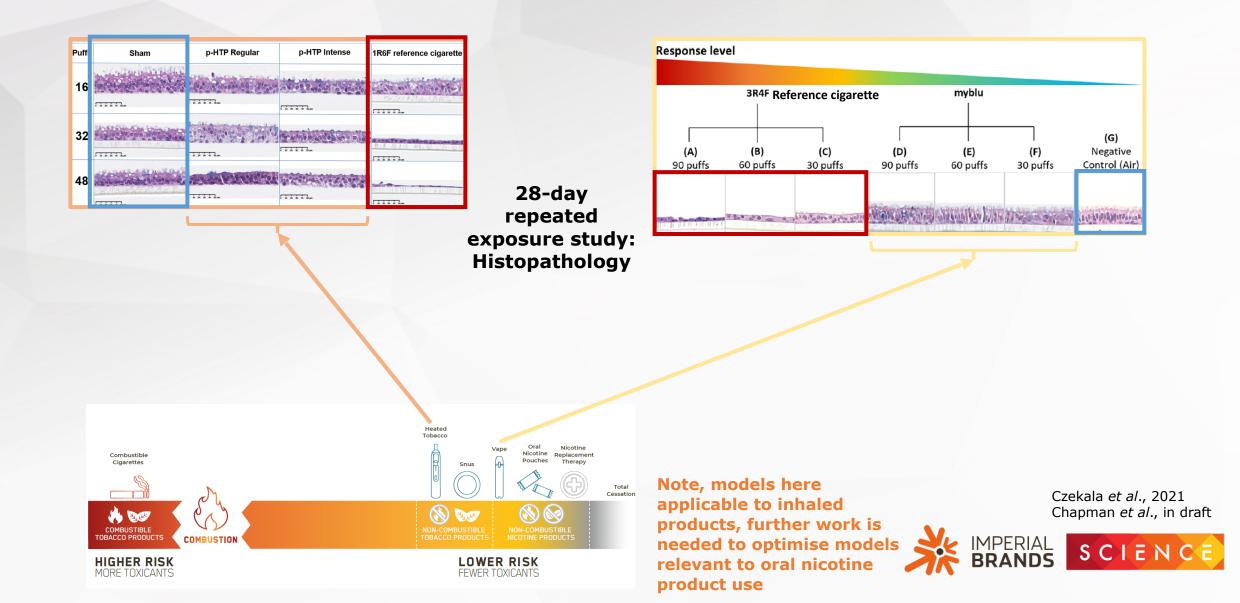
### In vitro toxicological responses reflect chemical composition



## Mechanistic screening supports inhaled products' placement on the risk scale

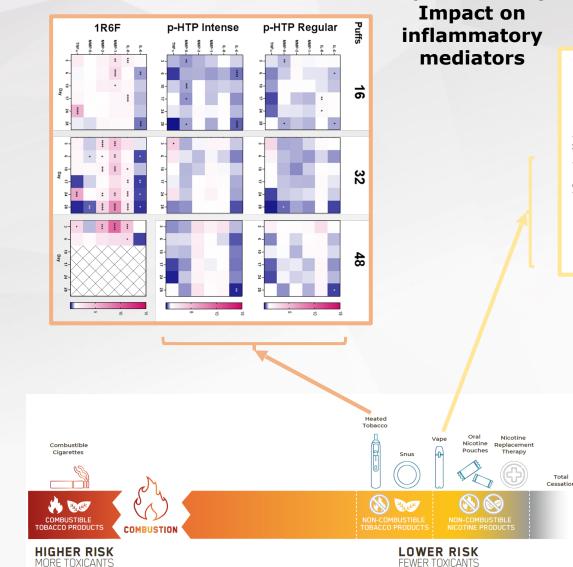


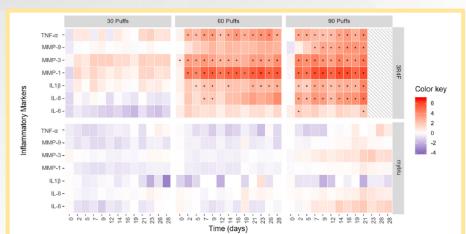
# Three-dimensional lung model assessment supports inhaled products' placement on the risk scale



### Three-dimensional lung model assessment supports inhaled products' placement on the risk scale 28-day repeated

exposure study:





Czekala *et al.*, 2021 Chapman *et al.*, in draft



## Conclusions

The fewer and lower levels of toxicants associated with NGPs is reflected by substantially reduced toxicological outcomes compared to combustible cigarette in a selection of *in vitro* (geno)toxicological assays

Further to this, the outcomes presented support the proposed placement of nicotine products on a relative risk scale

The relative toxicological effects of the products tested is also observed in other *in vitro* endpoints associated with smoking-related disease (e.g., cardiovascular, pulmonary toxicity)

### Future work:

- Expanding on the datasets presented, using the current endpoints to further assess the NGPs (e.g., further testing with oral nicotine pouches)
- Evaluation of transcriptomics data generated across a range of NGP samples vs cigarette samples
- Development and application of further in vitro endpoints



## Thank you

### **Imperial Brands**

Matthew Stevenson Liam Simms Roman Wieczorek Edgar Trelles Sticken Sarah Jean Pour Kathryn Rudd Fan Yu Lukasz Czekala Group Science and Regulatory Affairs



### APPLIED IN VITRO TOXICO Volume 8, Number 1, 2022 Mary Ann Liebert, Inc. DOI: 10.1082/aint.2021.0020



Preclinical Assessment of Tobacco-Free Nicotine Pouches Demonstrates Reduced In Vitro Toxicity Compared with Tobacco Snus and Combustible Cigarette Smoke

Fan Yu<sup>\*</sup>, Kathyn Rudd,<sup>\*</sup> Sarah Jean Pour,<sup>2</sup> Edgar Treiles Sticken<sup>2</sup> Ole Dethioff,<sup>2</sup> Roman Weczorek<sup>2</sup> Thomas Nahde<sup>2</sup>, Lam Simms<sup>1</sup>, Fiona Chapman<sup>1</sup>, Lukasz Czekala,<sup>1</sup> Mattew Steverson,<sup>2</sup> and Grant O'Connel<sup>1</sup>

Introduction: Tobacco harm reduction (THR) represents one of the most promising public health policies with Introduction. Tools of num recursion (THR) represents one of the most promising point material pointers with continued product innovation crucial to making THR a reality. Tools of the most promising point with the product recent THR product innovation available in a growing number of countries as a potentially less harmful alternative to traditional tobacco products.

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the Ames and IVM assays, and demonstrated weak cytotoxicity in the NRU assay compared with TPM, under the the Ames and t-M assays, and a demonstrates weak cytotoxicity in the NRU assay compared with 1PM, under the conditions of test. When using the HerGC cell line in the NRU assay, it was possible to differentiate between TPMP and sums extracts, with only muse extracts resulting in a measurable  $E_{S0}$  response. Conclusion: This initial predictical is write toxicity assessment of TPMPs compared with both tobacco sums and combustible eigarette smoke indicates that the tested TFMPs have a substantially reduced in vitro toxicity activity npared with traditional tobacco products. Given this initial data, both further scientific exploration and public

health discussion on these products are warranted. Keywords: cytotoxicity, genotoxicity, in vitro, tobacco harm reduction, tobacco-free nicotine pouches

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<sup>1</sup>Imperial Brands PLC, Bristol, United Kingdom. <sup>2</sup>Reontsma Cigarettenfabriken GmbH, An Imperial Brands PLC Company, Hamburg, Germany. Part Vu et al., 2022, Published by Mary Ann Liebert, Inc. This Open Access article is distributed under the terms of the Creative ommone Attribution Noncommercial Licence [IC-BY-NC] (http://creative.ormnon.org/licence/by-sci/40) which purprise any non-sumercial use, distributions and reproduction in game products, neuroidable de original publicity and the original publicity and the original publicity and the original publicity. 1



### Multi-endpoint analysis of human 3D airway epithelium following repeated exposure to whole electronic vapor product aerosol or cigarette smoke

Current Research in Toxicology 2 (2021) 99-111

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### ABSTRACT ARTICLE INFO

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### Mategerenis, 2020, XX, 1–14 doi:10.1083/matege/geae033 Original Manuscript or sublication. 18 March 2021

### Original Manuscript

https://imperialbrandsscience.com/

The in vitro ToxTracker and Aneugen Clastogen Evaluation extension assay as a tool in the assessment of relative genotoxic potential of e-liquids and their aerosols

### Lukasz Czekala<sup>1</sup>, Fiona Chapman<sup>1</sup><sup>4</sup>, Liam Simms<sup>1</sup>, Kathryn Rudd<sup>1</sup>,

Edgar Trelles Sticken<sup>2</sup>, Roman Wieczorek<sup>2</sup>, Lisa Maria Bode<sup>2</sup>, Jutta Pani<sup>2</sup>, Nynke Moelijker<sup>3</sup>, Remco Derr<sup>3</sup>, Inger Brandsma<sup>3</sup>, Giel Hendriks<sup>3,6</sup>, Matthew Stevenson<sup>1</sup> and Tanvir Walele<sup>1</sup>

Group Science and Regulatory Affairs, Imperial Brands PLC, 121 Winterstoke Road, Bristol BS3 2LL, UK, 'Reemtama Cigarettenfabriken GinbH, an Imperial Brands PLC Company, Albert-Einstein-Ring-7, D-22761 Hamburg, Germany and Toxys B.V., Robert Boyloweg 4, 2333 CG Leiden, The Netherlands

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### Abstract

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### APPLIED IN VITRO TOXICOLO Volume 6, Number 1, 2020 Mary Ann Liebert, Inc. DOI: 10.1086/abs.co.0015

### Chemical Composition and In Vitro Toxicity Profile of a Pod-Based E-Cigarette Aerosol Compared to Cigarette Smoke

Kathryn Rudd,<sup>1</sup> Matthew Stevenson,<sup>1</sup> Roman Wieczorek,<sup>2</sup> Jutta Pani,<sup>2</sup> Edgar Trelles-Sticken,<sup>2</sup> Ole Dethloff,<sup>2</sup> Lukasz Czekala,<sup>1</sup> Liam Simms,<sup>1</sup> Francesca Buchanan,<sup>3</sup> Grant O'Connell,<sup>1</sup> and Tarwir Walele<sup>1</sup>

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Keywords: aerosol, cvtotoxicity, e-cigarette, emissions, genotoxicity, mutagenicity

Introduction	pressure-sensitive circuit that heats the atomizer and turns the liquid into an aerosol (popularly referred to as "vapor") that
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cally for pod-based e-cigarettes, drawing breath activates a	published the final rule to deem e-cigarettes to be subject to

Group Science and Regulatory Affairs, Imperial Brands PLC, Bristol, United Kingdom. Reentsma Cigaretterfabriken GmbH, An Imperial Brands PLC Company, Hamburg, Germany Nerudia, An Imperial Brands PLC Company, Liverpool, United Kingdom.

yrs Rudd et al. 2020; Published by Mary Ann Liebert, Inc. This Open Access article is distributed under the terms of the Creative License (http://creativecommons.org/licenses/byl4.0), which permits unrestricted use, distribution, and reproduction in any

### Multiple endpoint in vitro toxicity assessment of a prototype heated tobacco product indicates substantially reduced effects compared to those of combustible cigarette

Fiona Chapman<sup>1</sup>, Edgar Trelles Sticken<sup>2</sup>, Roman Wieczorek<sup>2</sup>, Sarah Jean Pour<sup>2</sup>, Ole Dethloff<sup>2</sup>, Jessica Budde<sup>2</sup>, Kathryn Rudd<sup>1</sup>, Valerie Troude<sup>2</sup>, Lukasz Czekala<sup>1</sup>, Fan Yu<sup>1</sup>, Liam Simms<sup>1</sup>, Matthew Stevenson<sup>1</sup> <sup>1</sup>Imperial Brands PLC, 121 Winterstoke Road, Bristol, 853 2LL, UK

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Keywords Heated tobacco; Cigarette; Aerosol; Smoke; High content screening; Harmful and potentially harmful constituents (of cigarette smoke); Genotoxicity; Mutagenicity; Multiple endpoint analysis; WHO

Heated tobacco products (HTPs) are a growing class of next generation nicotine delivery products. which aims to provide adult smokers with a reduced harm alternative to combustible cigarette smoking. The reduced harm potential of HTPs is widely attributed to the substantial reductions of harmful and potentially harmful constituents (HPHCs) found in the aerosols of these products compared to cigarette smoke, due to the absence of combustion in generating the aerosols. This has

Abstract

been reflected in the reduced toxicological effects of HTP aerosol samples compared to cigarette smoke, both in vivo and in vitro. As the HTP category grows, there is a need to substantiate previous findings and add to the weight of evidence on the reduced harm potential of HTP, in addition to characterising the effects of individual product offerings. This study investigated the aerosol chemistry and in vitro toxicological effects, in five assays representing cellular stress responses related to smoking-associated diseases, of two prototype HTPs (p-HTPs) (Regular; Menthol) and compared results to those of 1R6F reference cigarette smoke. In the neutral red uptake (cytotoxicity), micronucleus, cardiovascular scratch wound and high content screening (HCS) assays, effective concentrations were tens of folds higher, and response magnitudes greatly reduced, for the p-HTP samples compared to the 1R6F. In the Ames assay, mutagenicity was weak or removed, dependent on test conditions, on comparison of the effects of p-HTP aerosols to 186F smoke. The use of HCS.

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Yu, F., et al. (2022). Pre-clinical assessment of tobacco-free nicotine pouches demonstrates reduced in vitro toxicity compared to tobacco snus and combustible cigarette smoke. Applied In Vitro Toxicology, https://doi.org/10.1089/aivt.2021.0020.

