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

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Maintaining Genomic Health in a Changing World.

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

alternatives
TO ANIMAL TESTING

Nicotine delivery products

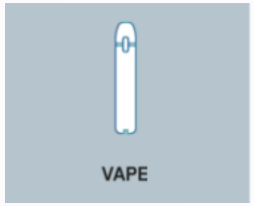
Traditional tobacco products:

- **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:

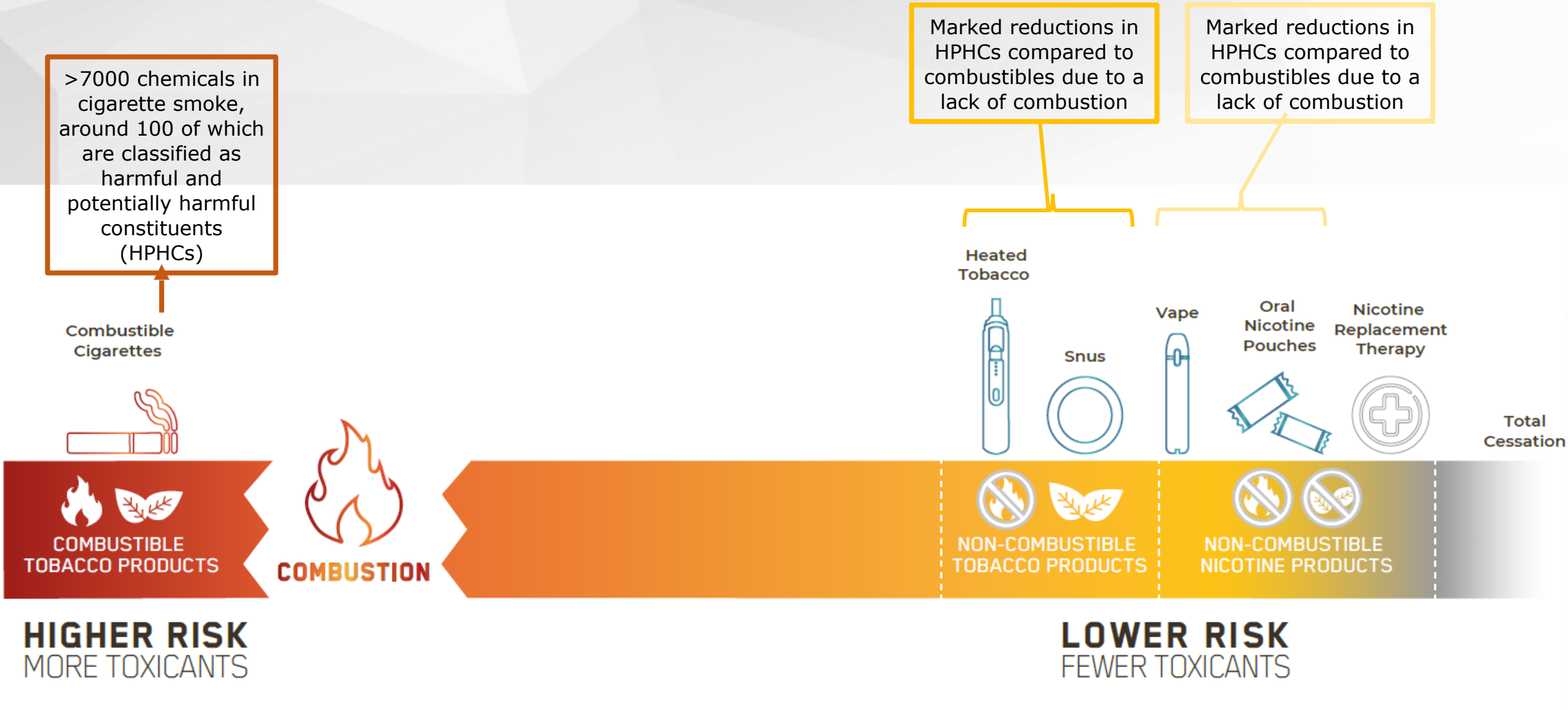
- **Heated tobacco products** (HTPs): reconstituted tobacco stick heated (but not burned) to produce nicotine-containing aerosol
- **Electronic nicotine delivery systems** (ENDS) (vape): e-liquid (base constituents + flavour concentrate ± nicotine) heated to produce an aerosol
- **Oral nicotine pouches**: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



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

The relative risk scale for nicotine products

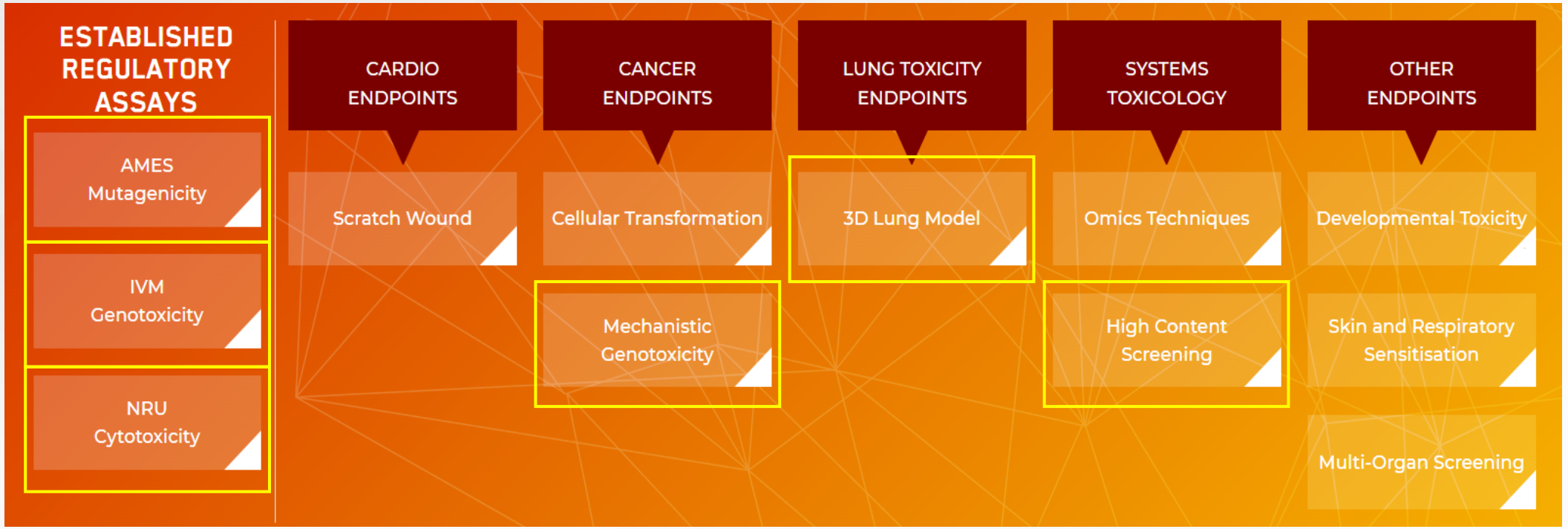
- The current scientific evidence suggests that combustible cigarettes, NGPs, and nicotine replacement therapies can be placed on a relative risk (of exposure to toxicants) scale



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

HTP (Pulze & iD) aerosol

Cytotoxicity: EC_{50} ~38x higher than cigarette
Genotoxicity (micronucleus): EC_{MN3} 8-22x higher than cigarette
Mutagenicity (Ames test; 2 strains): Negative
Chapman *et al.*, under review

Snus (Skruf) extract

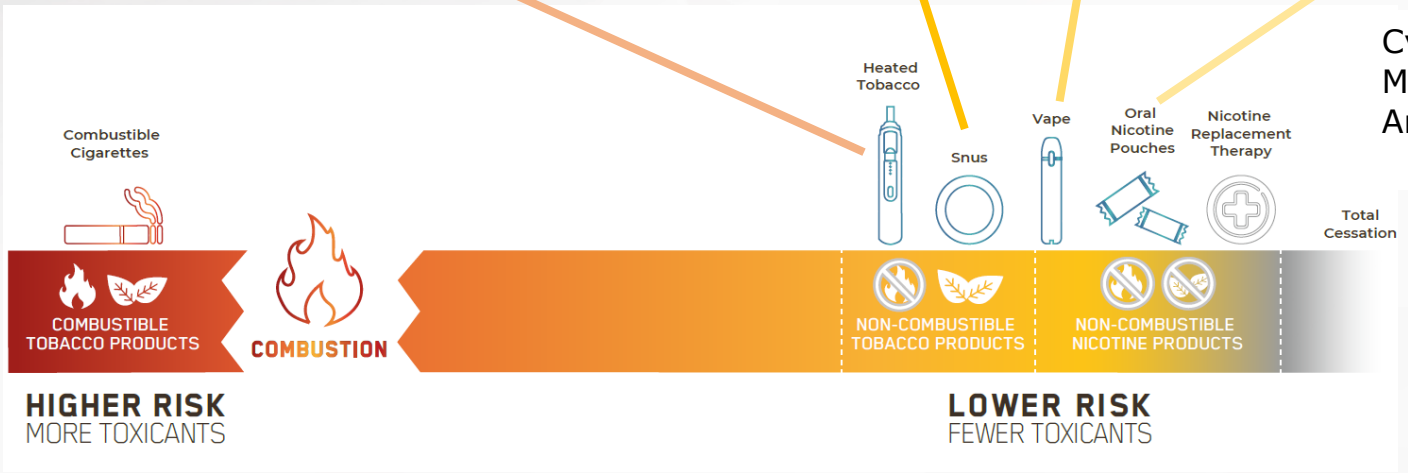
Cytotoxicity*: EC_{50} 172x higher in HepG2 than cigarette, but not reached in Beas-2B
Genotoxicity (micronucleus): Negative
Mutagenicity (Ames test; 5 strains): Negative
Yu *et al.*, 2022

ENDS (myblu) aerosol

Cytotoxicity: EC_{50} 319x higher than cigarette
Genotoxicity (micronucleus): Negative
Mutagenicity (Ames test; 2 strains): Negative
Rudd *et al.*, 2020

OND (Skruf) extract

Cytotoxicity*: EC_{50} not reached (EC_{20} 597-790x higher than cigarette)
Genotoxicity (micronucleus): Negative
Mutagenicity (Ames test; 5 strains): Negative
Yu *et al.*, 2022



Cytotoxicity (neutral red uptake): Beas-2B, *also HepG2
Micronucleus assay: V79
Ames test: **TA98**, **TA100**, TA102, TA1535, TA1537

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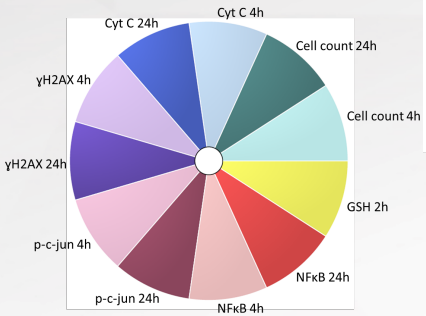
Mechanistic screening supports inhaled products' placement on the risk scale

High content screening

- Normal human bronchial epithelial (NHBE) cells
- 4h and 24h treatments (2h for glutathione depletion (GSH)) with smoke/ aerosol bubbled PBS (bPBS)
- 6 endpoints (below)
- Outcomes plotted as ToxPis (toxpi.org) and sample nicotine levels matched
- Key:



ToxTracker assay

ENDS

Cigarette (1R6F)

Endpoint	*Marker*
DNA damage	Bscl2 -S9 Bscl2 +S9 Rtkn -S9 Rtkn +S9
p53 activation	Btg2 -S9 Btg2 +S9
Oxidative stress	Srxn1 -S9 Srxn1 +S9 Blvrb -S9 Blvrb +S9
Protein damage	Ddit3 -S9 Ddit3 +S9
— Positive induction	

Czekala *et al.*, 2021

Note, models here applicable to inhaled products, however, further work is needed to develop similar high content assessments of oral nicotine products

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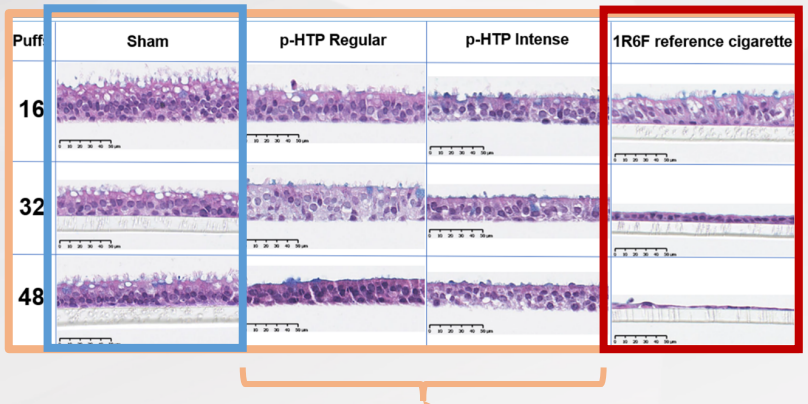
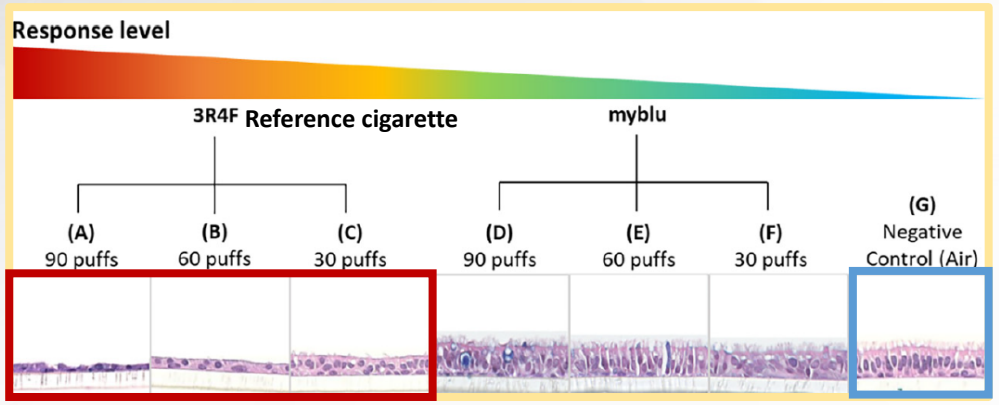
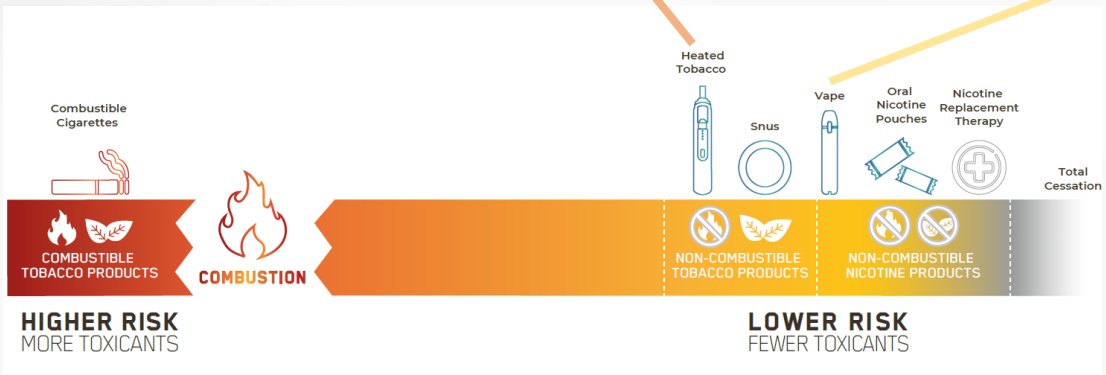
Three-dimensional lung model assessment supports inhaled products' placement on the risk scale



28-day repeated exposure study: Histopathology





Note, models here applicable to inhaled products, further work is needed to optimise models relevant to oral nicotine product use

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

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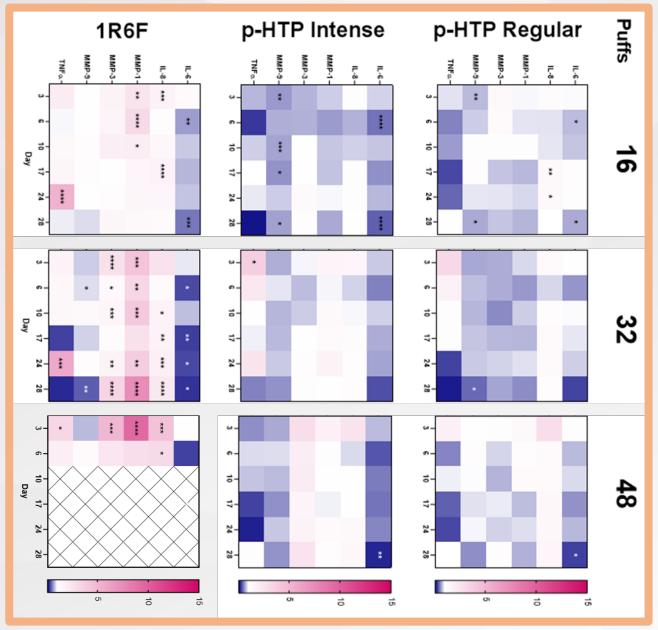
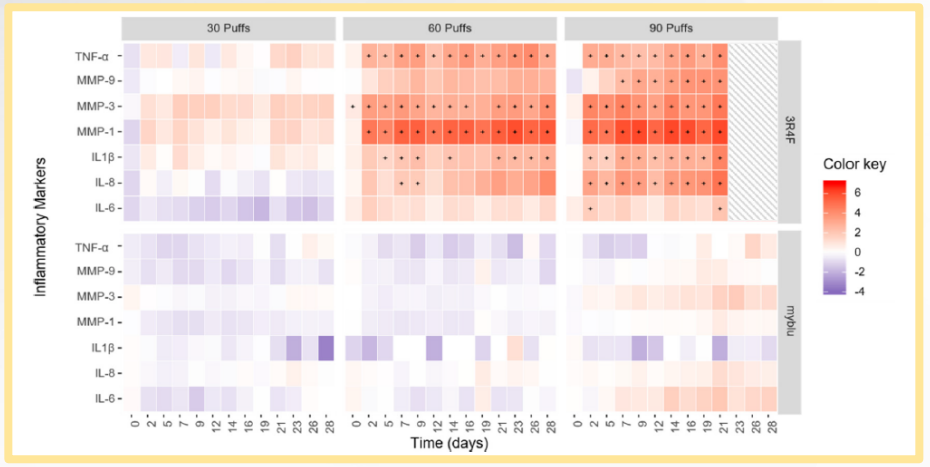
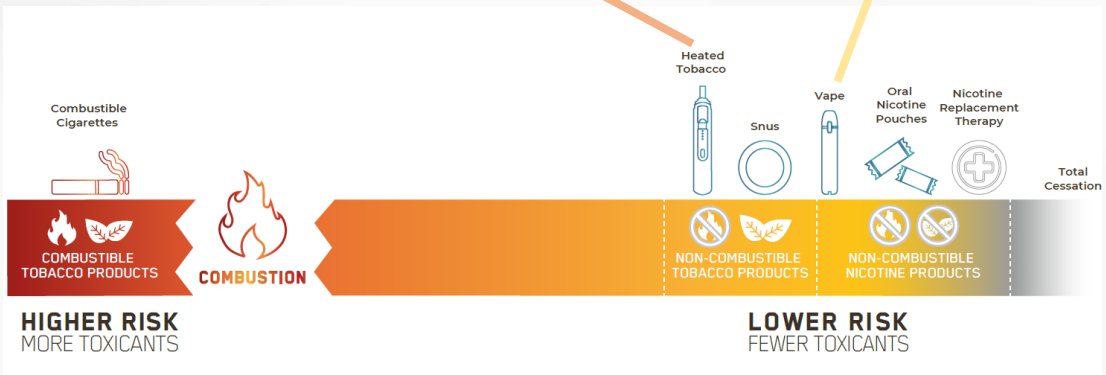
Three-dimensional lung model assessment supports inhaled products' placement on the risk scale

28-day repeated exposure study: Impact on inflammatory mediators







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

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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

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Thank you

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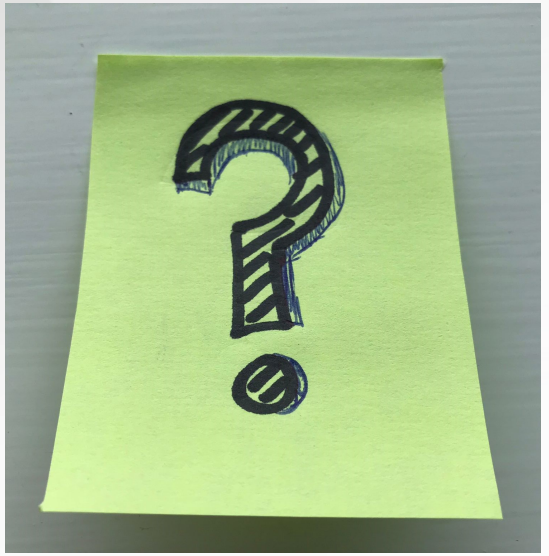

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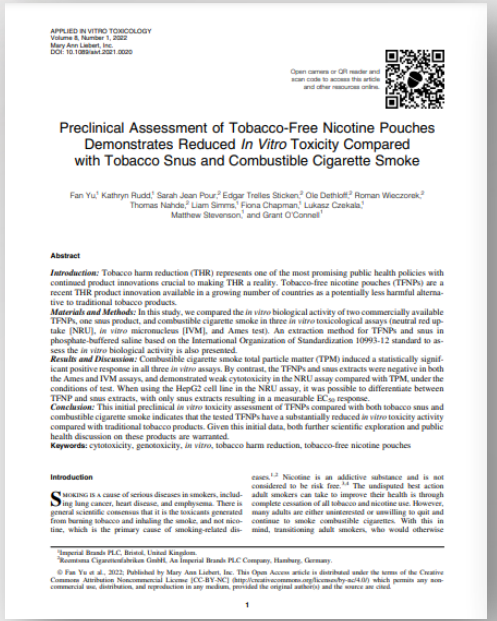


APPLIED IN VITRO TOXICOLOGY
Volume 8, Number 1, 2022
Mary Ann Liebert, Inc.
DOI: 10.1089/aivt.2021.0020

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

Abstract

Current Research in Toxicology 2 (2021) 99–115

Current Research in Toxicology

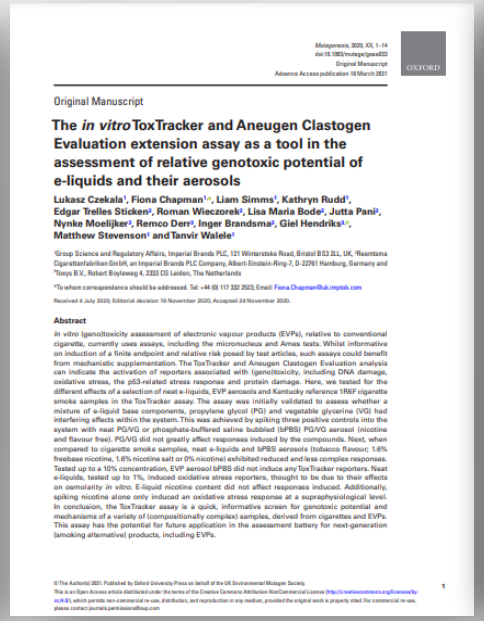
journal homepage: www.elsevier.com/locate/crtox

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

ARTICLE INFO

ABSTRACT

1. Introduction

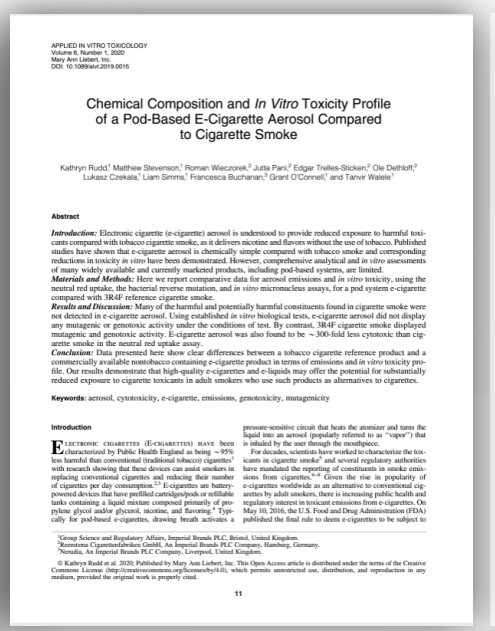


Mutagenesis, 2020, XX, 1–14
doi:10.1093/mutage/gexa033
Original Manuscript
Advance Access publication 19 March 2021

Original Manuscript

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

Abstract



APPLIED IN VITRO TOXICOLOGY
Volume 6, Number 1, 2020
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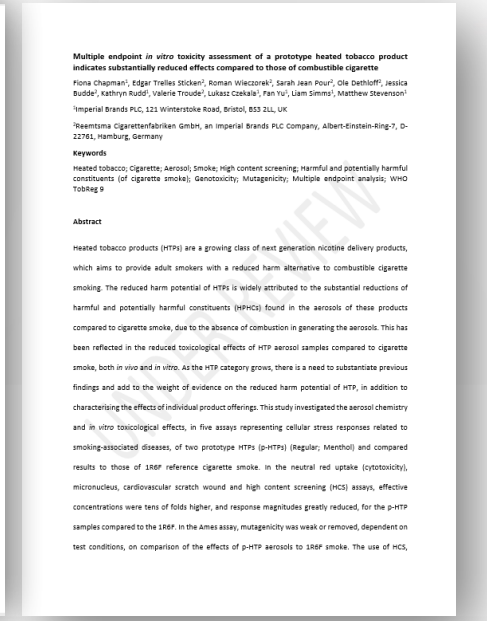
DOI: 10.1089/aivt.2019.0015

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

Abstract

Introduction



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

Abstract

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 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 1R6F smoke. The use of HCS,

References

Chapman, et al. (under review). Multiple endpoint in vitro toxicity assessment of a prototype heated tobacco product indicates substantially reduced effects compared to those of combustible cigarette. Under peer review.

Czekala, L., et al. (2020). 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. *Mutagenesis*, doi:10.1093/mutage/geaa033.

Czekala, L., et al. (2019) High Content Screening in NHBE cells shows significantly reduced biological activity of flavoured e-liquids, when compared to cigarette smoke condensate. *Toxicology In Vitro*, 58, 86-96.

Rudd, K., et al. (2020). Chemical composition and *in vitro* toxicity profile of a pod-based e-cigarette aerosol compared to cigarette smoke*. Applied In Vitro Toxicology*, 6(1), 11-41. DOI: 10.1089/aivt.2019.0015.

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.

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