



NEXT GENERATION PRODUCTS

Toxicological assessment of a range of commercially available next generation product aerosols reveals marked reductions in biological activity compared to cigarette smoke

Dr Fiona Chapman

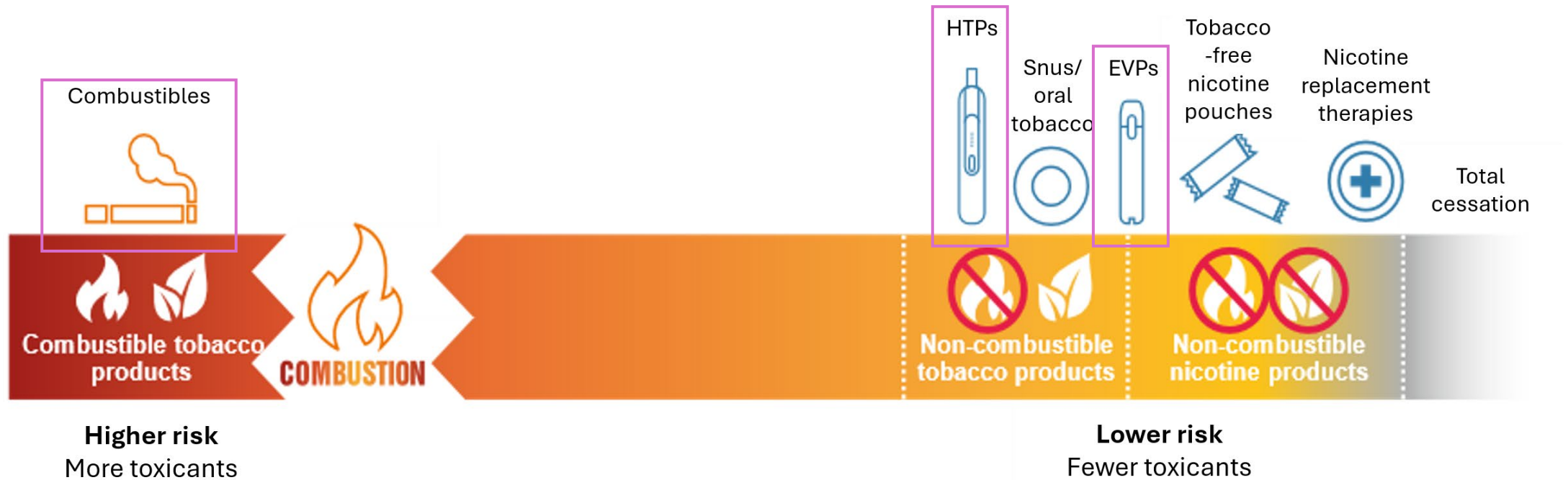
CORESTA Congress 2024

14th October

Background

- Smoking is a cause of serious diseases
 - Attributed to the toxicants present in tobacco smoke
- Next generation products (NGPs) offer nicotine delivery to adult smokers but with reduced numbers/ levels of such toxicants
- Nicotine delivery products are proposed to sit on a relative risk (of exposure to toxicants) scale

Relative risk (of exposure to toxicants) scale



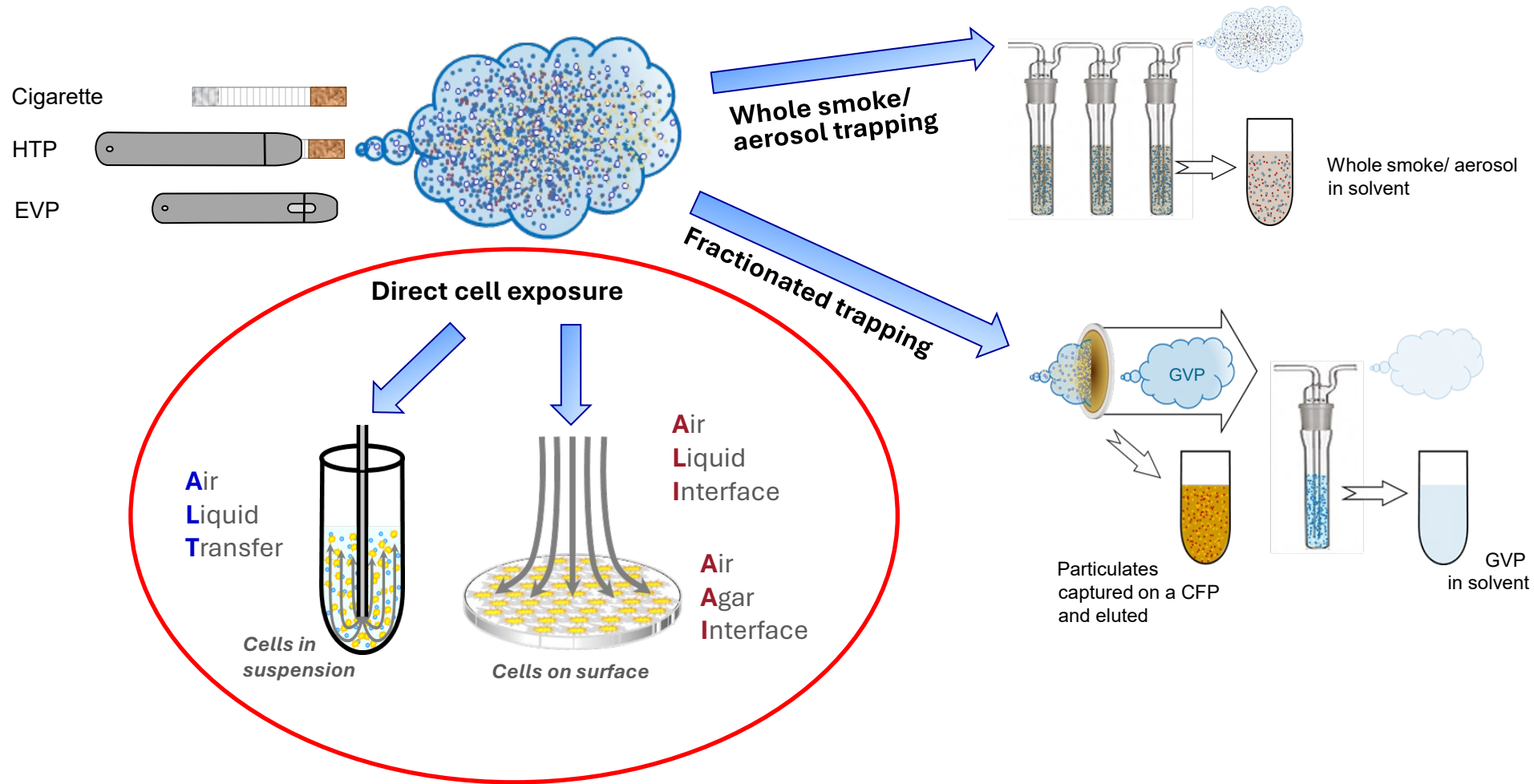
Illustrative representation of the current scientific evidence

Represents products produced by responsible manufacturers

Background

- Novel product iterations require product stewardship assessment
 - Often involving *in vitro* testing
- Range of exposure approaches used for smoke/ aerosol testing *in vitro*
- How do products compare using the same exposure approach?

Smoke/ aerosol exposure *in vitro*



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Study overview

Aim

To assess the effects of a range of inhaled nicotine delivery products using the same whole aerosol exposure approaches in three *in vitro* assays:

- Neutral red uptake (NRU) assay
- Micronucleus (MN) assay
- Reverse bacterial mutation (Ames) test



Study products

- *Cigarettes:*
 - 1R6F Kentucky reference cigarette
 - Very Low Nicotine (VLN) King
- *Heated tobacco products (HTPs):*
 - Pulze 2.0 & iD Rich Bronze
 - IQOS 3 Duo & HEETS Russet
 - IQOS ILUMA & TERA Russet
 - Glo Hyper X2 Air & Neo Dark Tobacco
- *Electronic vapour products (EVPs):*
 - blu Bar Watermelon Ice
 - RELX – Classic Tobacco
 - ELFA – Watermelon
 - ELFBAR 600 – Classic Crème

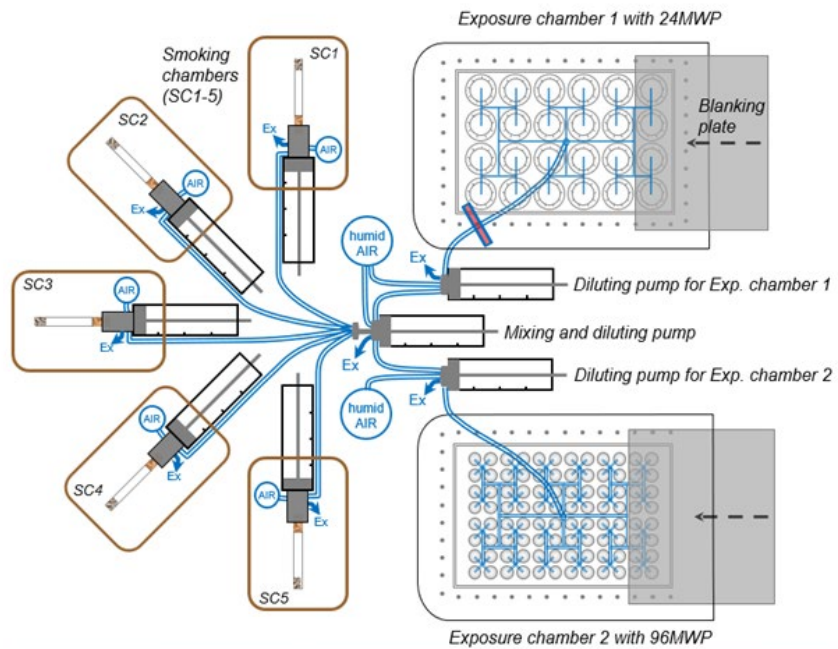


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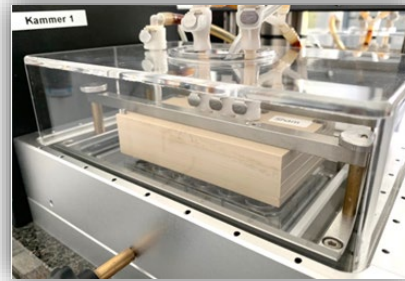
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Methods: Exposure

Smoke Aerosol Exposure *In Vitro* System (SAEIVS): NRU + MN assays



← Cambridge Filter (CF)



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Characterisation of a smoke/ aerosol exposure in vitro system (SAEIVS) for delivery of complex mixtures directly to cells at the air-liquid interface

Roman Wiczorek, Edgar Trelles Sticken, Sarah Jean Pour, Fiona Chapman ✉ Karin Röwer, Sandra Otte, Matthew Stevenson, Liam Simms

First published: 03 February 2023 | <https://doi.org/10.1002/jat.4442>

Vitrocell VC 10 S-Type plus glass impingers: Ames test



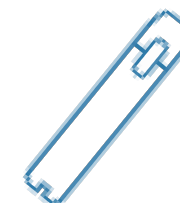
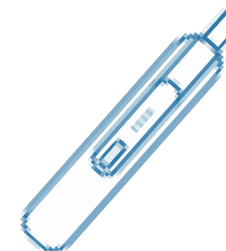
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Methods: Exposure

	Cigarettes	HTPs	EVPs
<i>Regime</i>	ISO 20778	Modified ISO 20778	ISO 20768
<i>Puff volume (ml)</i>	55	55	55
<i>Puff duration (s)</i>	2	2	3
<i>Puff interval (s)</i>	30	30	30
<i>Puff profile</i>	Bell-shaped	Bell-shaped	Square-shaped
<i>Ventilation blocking</i>	Yes	No	-
<i>Conditioning</i>	ISO 3402*	ISO/DIS-5501-1:2023*	Room temperature; dark
<i>Additional details</i>	-	Highest heating setting	-

*at least 48 hours at $22 \pm 1^\circ\text{C}$ and $60 \pm 3\%$ relative humidity



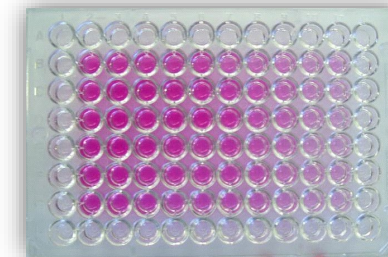
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Methods: Neutral red uptake (NRU) assay

Measure of relative cytotoxicity

- Air-liquid interface exposure using the SAEIVS
- Beas-2B (human bronchial epithelial) cells
- Exposures to increasing puff numbers
- Number of puffs required to induce 20 and 50% cytotoxicity compared to negative control (air) calculated (EC_{20} / EC_{50})



Viable cells take up red dye

More intense colour = more viable cells



Toxicology in Vitro
Volume 86, February 2023, 105510



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

Fiona Chapman ^a, Edgar Trelles Sticken ^b, Roman Wluczczek ^b, Sarah Jean Pour ^b, Ole Dethloff ^b, Jessica Budde ^b, Kathryn Rudd ^a, Elizabeth Mason ^a, Lukasz Czekala ^a, Fan Yu ^a, Liam Simms ^a, Thomas Nahde ^b, Grant O'Connell ^a, Matthew Stevenson ^a



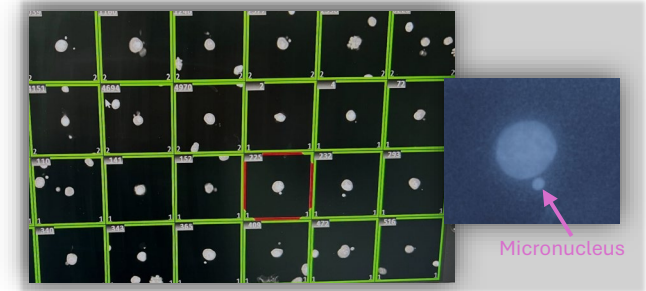
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Methods: Micronucleus (MN) assay

Measure of relative genotoxicity (DNA damage)

- Air-liquid interface exposure using the SAEIVS
- V79 (hamster lung fibroblast) cells (+/-S9)
- Exposures to increasing puff numbers
- Number of puffs required to induce a 3x increase in MN compared to negative control (air) calculated (EC_{MN3})



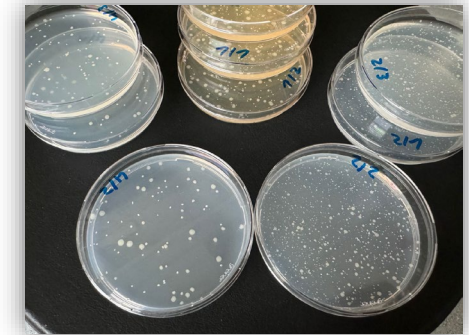
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Methods: Reverse bacterial mutation (Ames) test

Measure of relative DNA mutagenicity

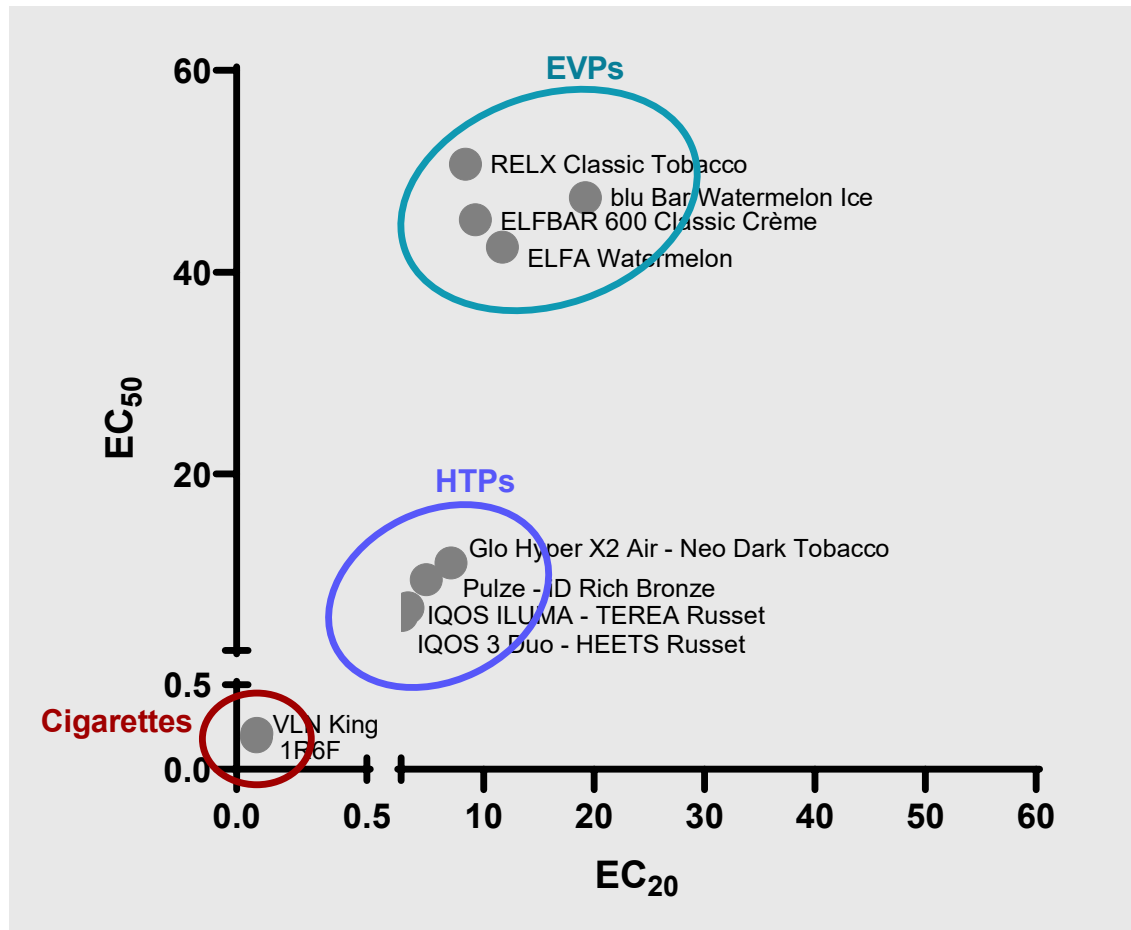
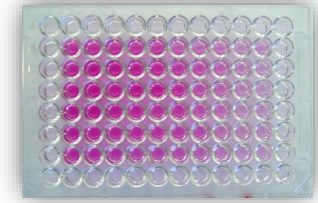
- Whole smoke/ aerosol aqueous bubbling exposure
- *Salmonella typhimurium* strains:
TA98, TA100, TA102, TA1535, TA1537 (+/-S9)
- Exposures to increasing puff numbers
- Test products classified as mutagenic, not mutagenic or equivocal according to specific criteria



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Results: NRU assay



- Responses were distinct between product groups
- Cigarettes were the most potent and EVPs the least
- Outcomes were most variable between EVPs

Results: MN assay

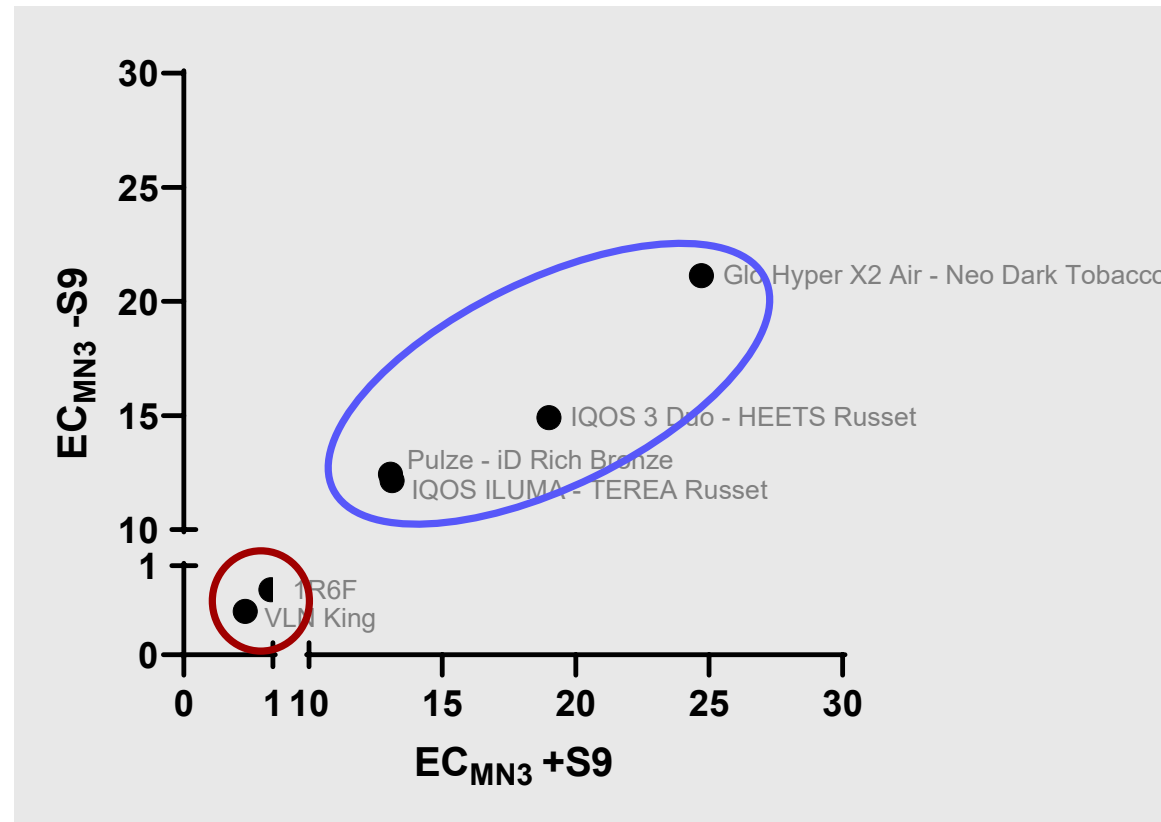
	+S9	-S9
Cigarettes		
1R6F	Genotoxic	Genotoxic
VLN King	Genotoxic	Genotoxic
Pulze - iD Rich Bronze	Genotoxic	Genotoxic
HTPs		
IQOS 3 Duo - HEETS Russet	Genotoxic	Genotoxic
IQOS ILUMA - TEREA Russet	Genotoxic	Genotoxic
Glo Hyper X2 Air - Neo Dark Tobacco	Genotoxic	Genotoxic
blu Bar Watermelon Ice	Not genotoxic	Not genotoxic
RELX Classic Tobacco	Not genotoxic	Equivocal
EVPs		
ELFA Watermelon	Not genotoxic	Not genotoxic
ELFBAR 600 Classic Crème	Not genotoxic	Not genotoxic

Key:

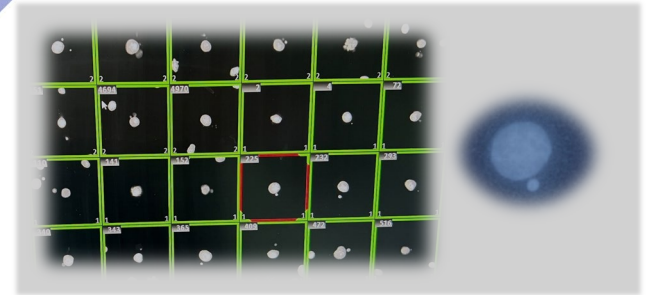
Genotoxic

Not genotoxic

Equivocal



EC_{MN3} = number of puffs required to induce a 3x increase in MN above negative control (air)



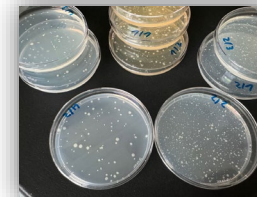
- The cigarettes and HTPs were classed as genotoxic (+/-S9) under the test conditions
 - However, greater numbers of puffs were required to induce equivalent responses for the HTPs compared to the cigarettes
- All the EVPs were classed as either not genotoxic or equivocal



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Results: Ames test



	TA98		TA100		TA102		TA1535		TA1537		Overall classification
	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	
Cigarettes											
1R6F											Mutagenic
VLN King											Mutagenic
HTPs											
Pulze - iD Rich Bronze											Mutagenic
IQOS 3 Duo - HEETS Russet											Mutagenic
IQOS ILUMA - TEREA Russet											Mutagenic
Glo Hyper X2 Air - Neo Dark Tobacco											Mutagenic
EVPS											
blu Bar Watermelon Ice											Not mutagenic
RELX Classic Tobacco											Not mutagenic
ELFA Watermelon											Not mutagenic
ELFBAR 600 Classic Crème											Not mutagenic

- VLN King demonstrated mutagenicity in all strains
 - 1R6F was the next most potent product
- The HTPs were classed at mutagenic (TA100)
 - Responses were induced at higher numbers of puffs for the HTPs compared to the cigarettes
- None of the EVPs demonstrated mutagenic activity

Key:
Mutagenic
Not mutagenic

Conclusions

- Overall, a clear grouping of responses between the different product categories was observed
- The NGP (HTP/ EVP) aerosols demonstrated substantially lower to no *in vitro* toxicity compared to the cigarettes
- VLN King was overall the most potent product tested
- The whole aerosol exposure approach is sensitive in differentiating between the different products/ categories
- **The outcomes support the placement of these products on a relative risk scale and support NGPs' THR potential**

Future directions

- Testing of additional products/ novel NGP categories
- Implementation of more mechanistically insightful techniques, e.g., High Content Screening
- Data to be compared on a nicotine exposure basis (in addition to puffs)

Thank you

Imperial Brands Group Science and Regulatory Affairs (GSRA)

Operational Science

- Roman Wieczorek
- Edgar Trelles Sticken
- Sarah Jean Pour
- Torge Evenburg

Scientific Regulatory Affairs

- Emmanuel Minet
- Annette Dalrymple

Harm Reduction & Engagement

- Matthew Stevenson

Product Safety & Compliance

- Liam Simms
- Kostas Papikinos

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Thank you for listening

Questions