Substantial reductions in selected harmful and potentially harmful constituents in heated tobacco aerosol, compared to 1R6F reference cigarette smoke, correlated with substantially reduced *in vitro* toxicological outcomes

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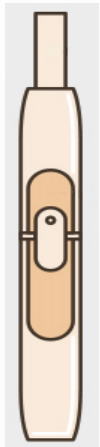
Outline

- Heated tobacco products (HTPs) form a growing category which offer potentially reduced harm nicotine delivery to adult smokers
- This is due to the presence of fewer and lower levels of harmful and potentially harmful constituents (HPHCs) within HTP aerosol compared to combustible cigarette smoke, attributed to the heating, as opposed to burning, of tobacco in HTPs
- There is evidence of this reduced harm potential in the scientific literature, both in adult smokers and *in vitro*

- This study aimed to assess the aerosol chemistry and *in vitro* toxicological effects of a prototype HTP (p-HTP) in comparison to combustible reference cigarette, 1R6F, smoke
- The *in vitro* tests included:
  - Neutral red uptake (NRU) (cytotoxicity) assay
  - Micronucleus (genotoxicity) assay
  - Ames (bacterial reverse mutation) (mutagenicity) test
  - High content screening (HCS) (7 cell stress related endpoints)



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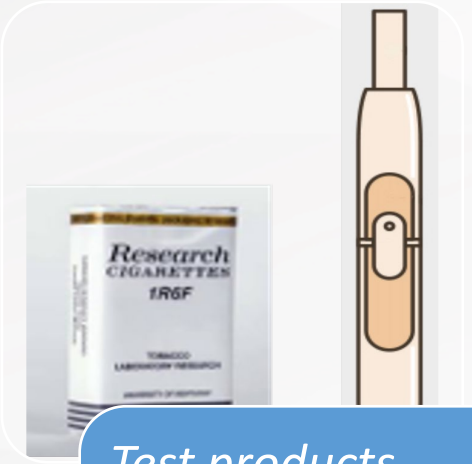
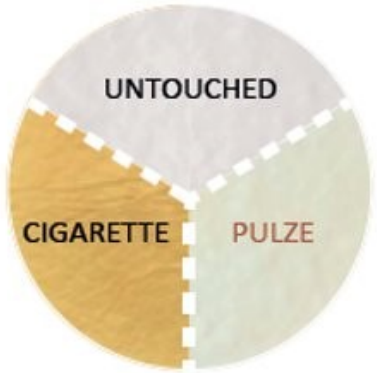
Study design



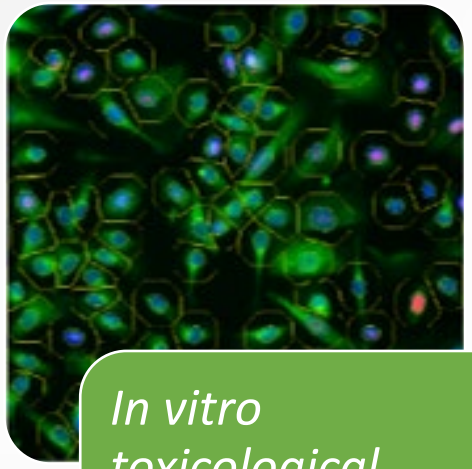
Test products

- **p-HTP** (standard heating mode, modified HCI smoking regime)
- **1R6F reference cigarette** (HCI smoking regime)



Aerosol/ smoke chemistry analysis

- WHO TobReg 9
- Additional selected TSNAs, carbonyls and volatiles
- Nicotine



In vitro toxicological assessment

- NRU (cytotoxicity)
- Micronucleus assay
- Ames test
- HCS with aerosol/ smoke bubbled through PBS

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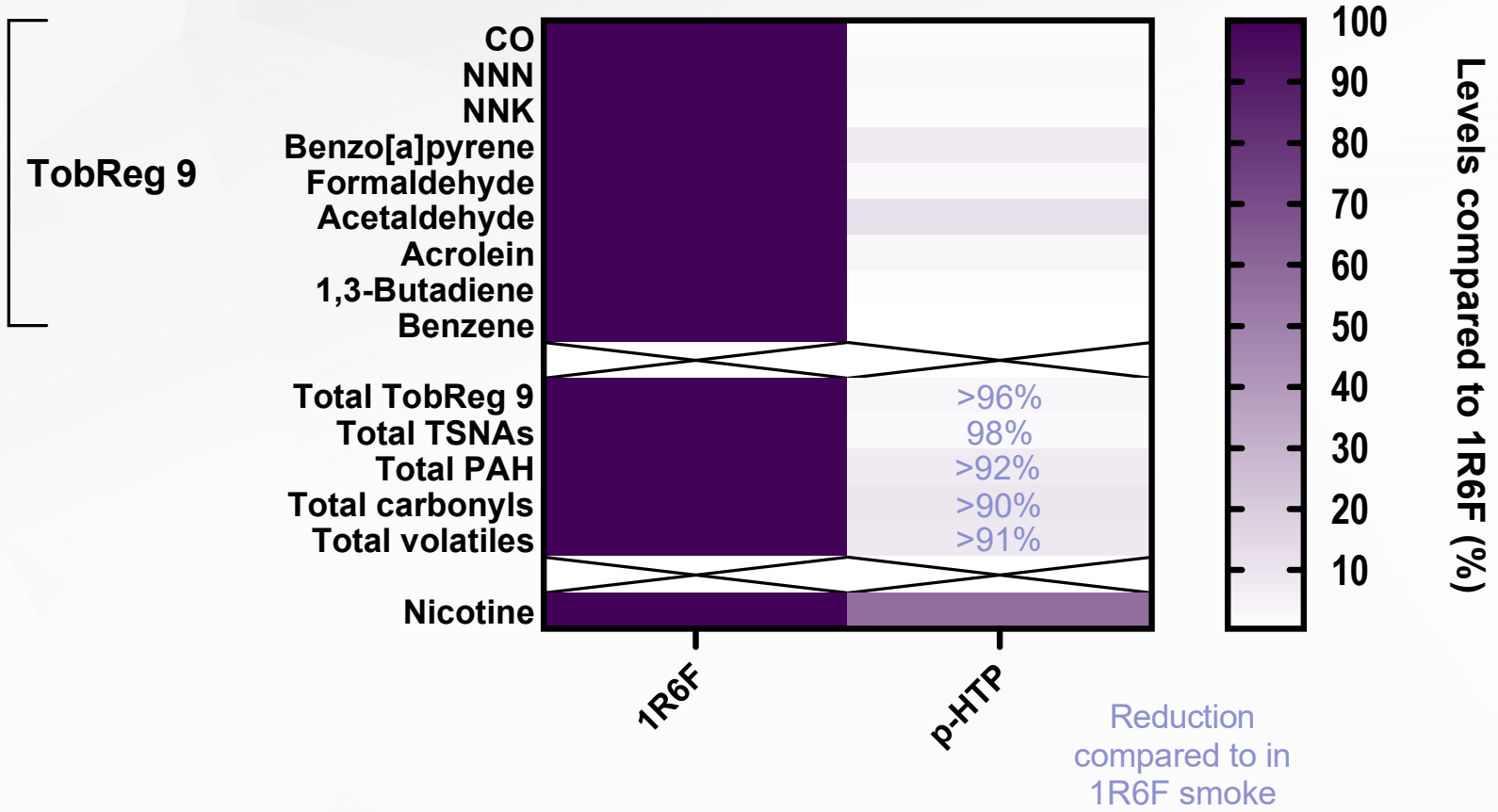
Aerosol chemistry

- On a per puff basis, HPHCs were substantially reduced in the p-HTP aerosol compared to 1R6F smoke, by an average of **>93%**
- In the p-HTP aerosol, the level of nicotine was half that in the 1R6F smoke per puff, however, two puffs of p-HTP aerosol still yield substantially reduced HPHC levels



CO: Carbon monoxide
NNN: N-nitrosonornicotine
NNK: 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone
TSNAs: Tobacco specific nitrosamines
PAH: Polycyclic aromatic hydrocarbon

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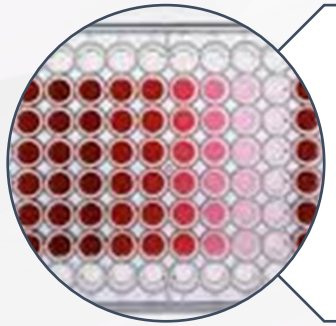
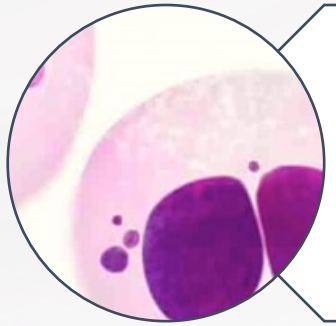
In vitro toxicology: regulatory battery



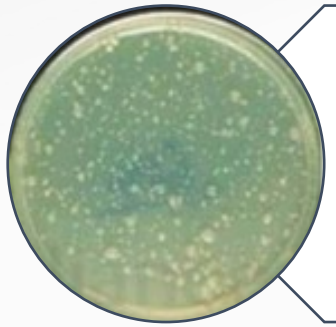
Cytotoxicity testing: NRU cytotoxicity assay

- Beas-2B (human bronchial epithelial) cells cultured at the air-liquid interface (ALI)
- Increasing number of puffs of aerosol or smoke applied using the SAEIVS*



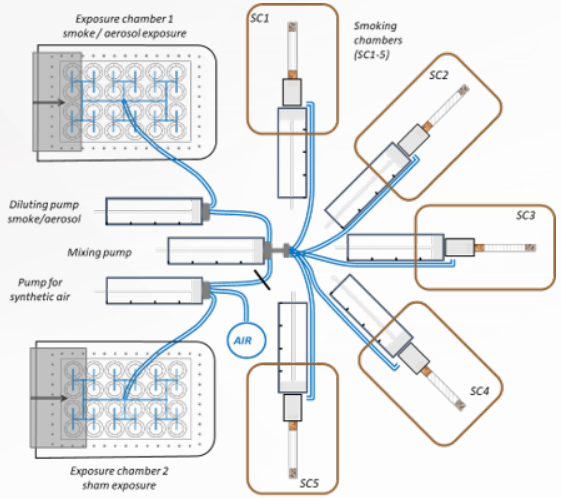
Genotoxicity testing: Micronucleus assay

- V79 (hamster lung fibroblast) cells cultured at the ALI
- Increasing number of puffs of aerosol or smoke applied using the SAEIVS*
- With/ without S9 metabolic activation (post exposure)

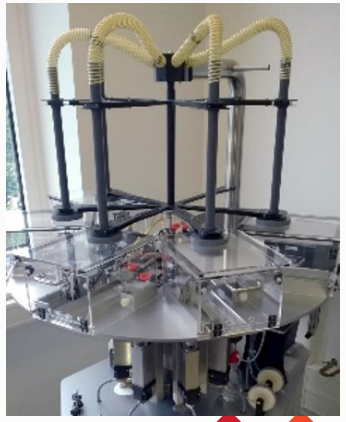


Mutagenicity testing: Ames bacterial reverse mutation test

- TA98 and TA100 *Salmonella typhimurium* strains
- Increasing number of puffs of aerosol or smoke assessed; bubbled directly through bacterial cultures connected to the Vitrocell VC 10 S-Type Smoking Robot
- With/ without S9 metabolic activation (post exposure)



**Smoke aerosol exposure in vitro system (SAEIVS)*



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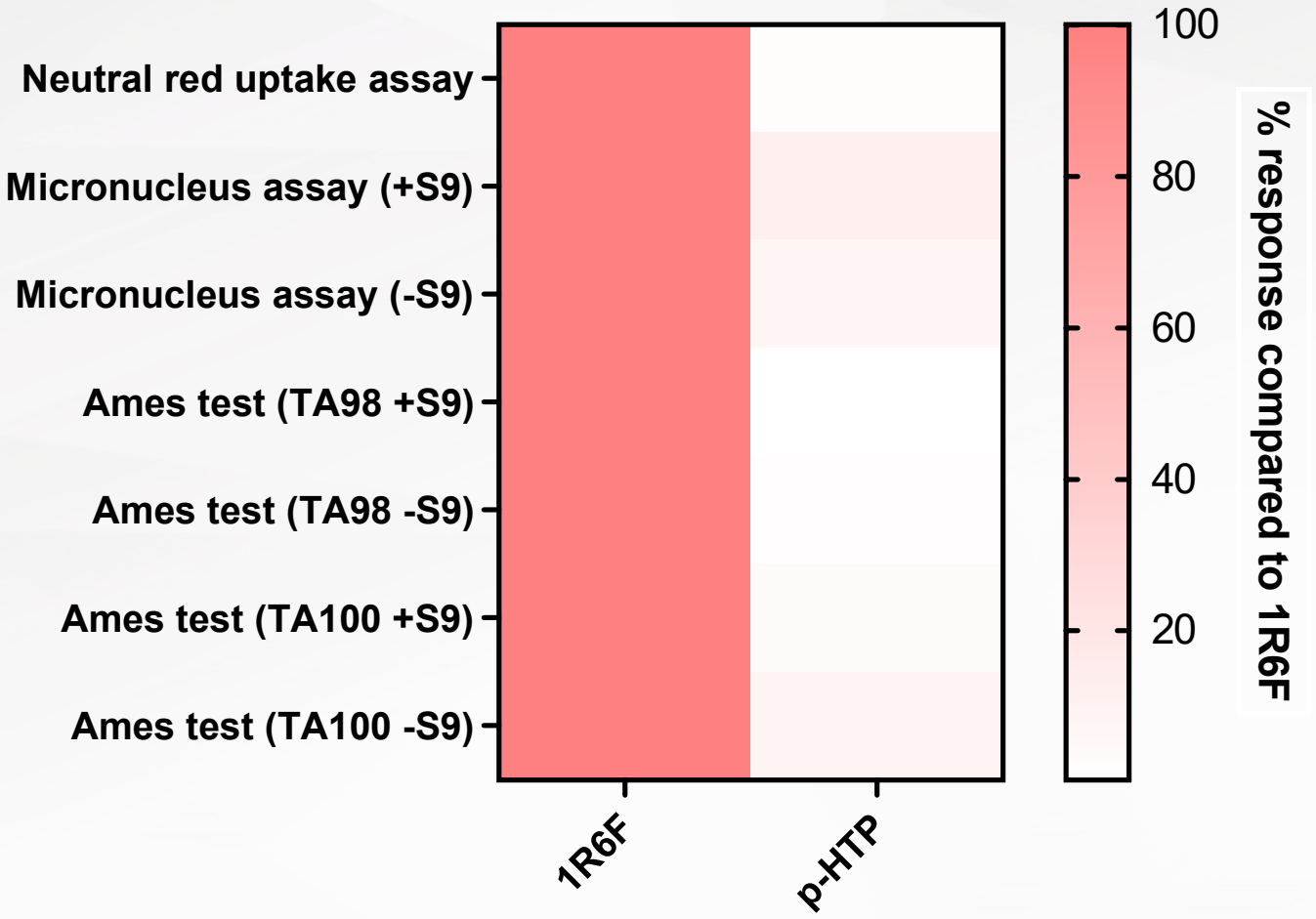
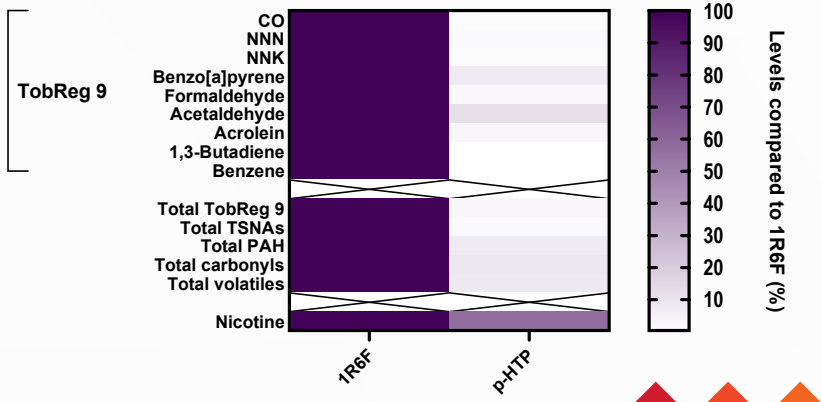
Regulatory toxicity battery results



- Responses to p-HTP aerosol in the NRU, micronucleus and Ames assays were substantially reduced (**87.5 - >99%**) compared to 1R6F smoke
- This reflects the reductions in HPHCs observed in the aerosol/ smoke chemistry



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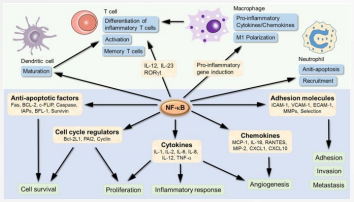
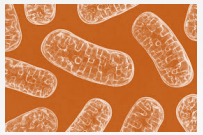
In vitro toxicology: high content screening (HCS)



Cytotoxicity

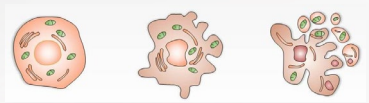
NfκB: pro-inflammatory response

Mitochondrial membrane potential: dysfunction in cellular energetics



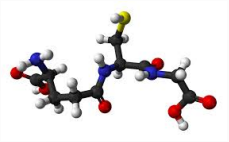
Multiple endpoint assessment

4h and 24h timepoints



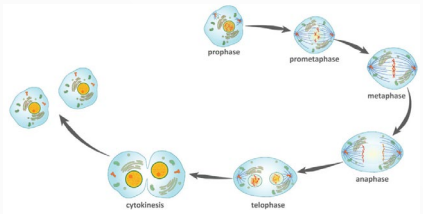
Cytochrome c: initiation of apoptosis

GSH (glutathione): measure of oxidative stress

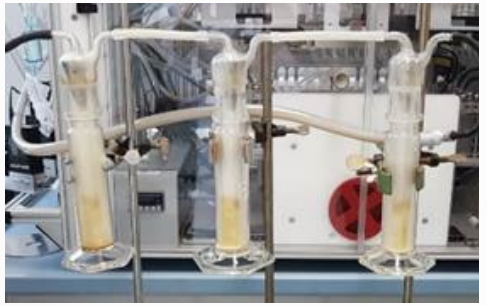


Phospho c-jun: involved in cell stress response (e.g., cell cycle control)

γH2AX: DNA double strand breaks



- Test samples generated by bubbling aerosol/ smoke generated by the Vitrocell VC 10 S Smoking Robot through PBS



- PBS was then added to Normal Human Bronchial Epithelial (NHBE) cell cultures

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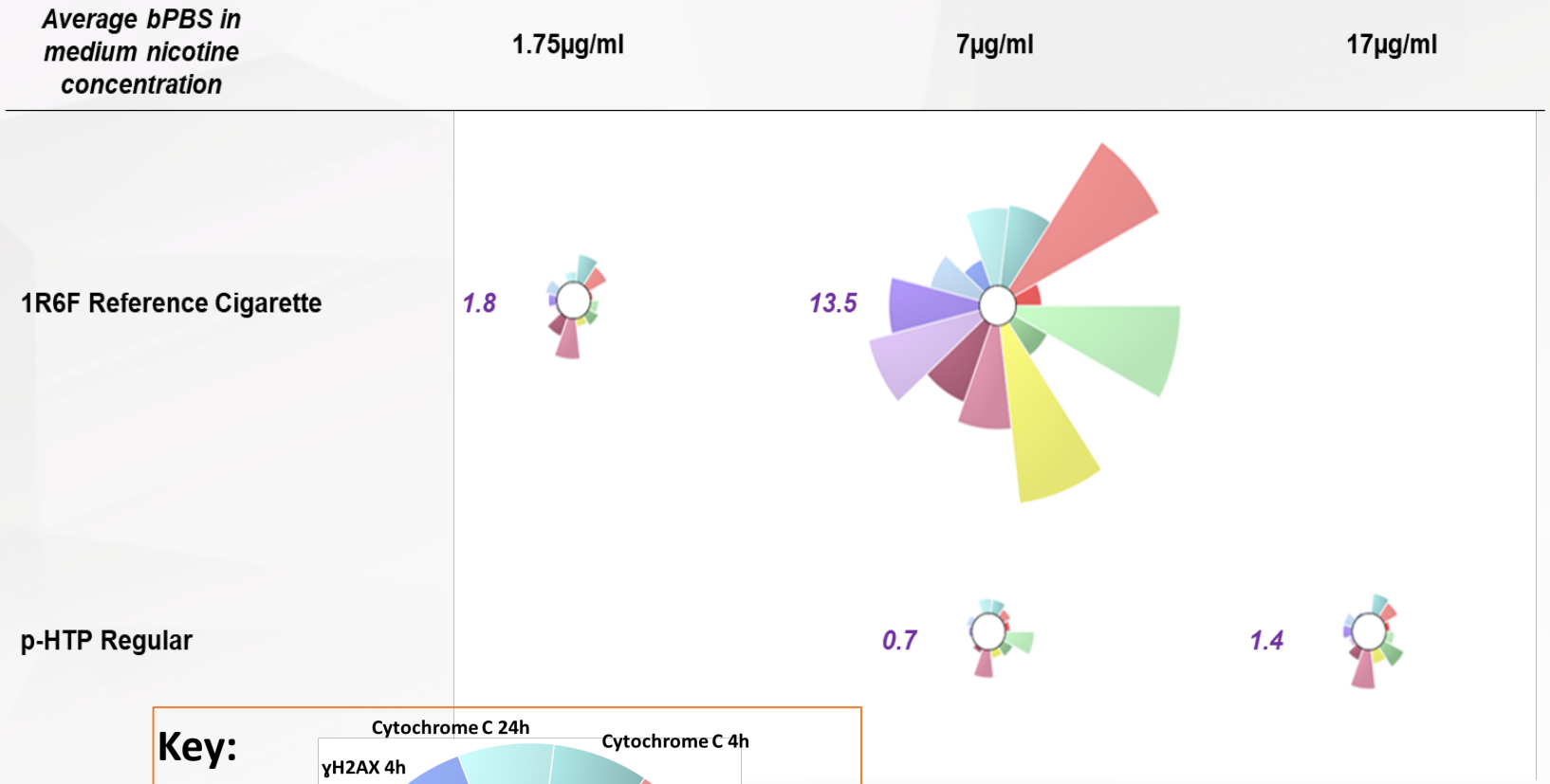
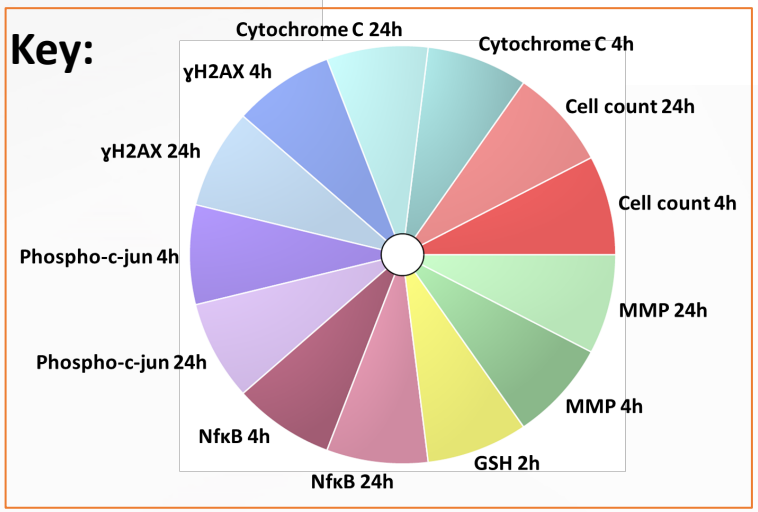
HCS results





- ToxPi plots allow visualisation of relative responses in HCS assessment
- Consistent with the other toxicological outcomes, and aerosol/ smoke chemistry, cellular responses to the p-HTP aerosol were substantially reduced
- This effect for p-HTP was observed at both an equivalent nicotine concentration to 1R6F and high nicotine concentrations

https://toxpi.org/

Conclusions

- Levels of HPHCs were substantially reduced, some even to below the limit of detection, in the p-HTP aerosol compared to 1R6F smoke, on average by **>93%**

- This translated directly to substantially reduced responses in a range of *in vitro* toxicological endpoints
  - The assessment of multiple *in vitro* endpoints provides a more informative view of the toxicological profile of such test articles

- These findings add to the weight of evidence that HTPs have the potential to offer adult smokers a significantly reduced harm mode of nicotine delivery compared to continued combustible cigarette smoking
  - The findings add to the available body of scientific evidence that HTPs do not combust tobacco and therefore demonstrate an emission profile with fewer and substantially lower HPHCs, shown here to directly translate into a potentially less harmful risk profile compared to continued combustible tobacco smoking

- These findings will need to be further substantiated in studies including behavioural and clinical assessments. The pre-clinical data here provides confidence to move into clinical assessment with adult smokers

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Thank you

CORESTA Scientific Commission Reading Committee

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