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NEXT GENERATION PRODUCTS

Reduced in vitro toxicity of nicotine pouches when compared with both snus and cigarettes

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CORESTA Meeting Zimbabwe

29th October 2026

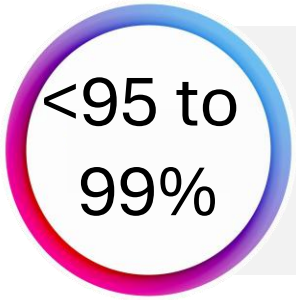
Snus has potential as THR product

- Oral Tobacco-based product
- Many toxicants present in cigarette smoke are not produced
- Nicotine is absorbed through the gums

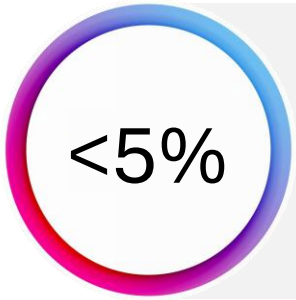


THR= tobacco Harm reduction

The “Swedish experience”

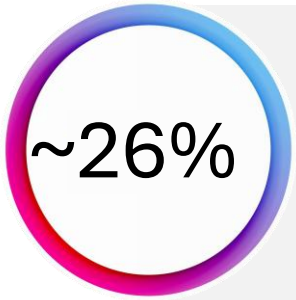


Less harmful than cigarettes[1]



Sweden has the **lowest** smoking prevalence in Europe[2]
(24% in Germany/ 14% Denmark)[3]

Low smoking prevalence in Sweden is widely attributed to availability of snus[4]



Snus use in Sweden

Combined snus, increasingly nicotine pouches and cigarette use is comparable to the average smoking rate in Europe[3]

[1]Gartner CE et al., Assessment of Swedish snus for tobacco harm reduction: An epidemiological modelling study. Lancet 2007;369:2010-4 ; [2]SmokeFreeSweden: https://smokefreesweden.org/2024/11/13/breaking-news-swedes-first-in-world-to-become-smoke-free-its-a-lesson-for-the-world/; [3]Eurobartometer https://europa.eu/eurobarometer/surveys/detail/2995 ; [4]Ramström, et al., (2016) Patterns of smoking and snus use in Sweden: implications for public health. *International journal of environmental research and public health*, *13*(11), p.1110.

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Nicotine Pouches - a new category of oral nicotine products

No tobacco. Plant fibre-based substrate or dry powder with high-purity nicotine

Many toxicants present in cigarette smoke are not produced

Nicotine is absorbed through the gums



As NPs don't contain or burn tobacco, research demonstrates they contain significantly fewer and substantially lower levels of harmful chemicals compared to snus or cigarette smoke.

Confidential

Comparative toxicity in vitro toxicity assessment of Nicotine Pouches (NP); Snus and Cigarettes

Four *in vitro* studies were conducted comparing NP; Snus and 1R6F Reference cigarette

Study 1: NPs, snus and 1R6F reference cigarette using CORESTA battery

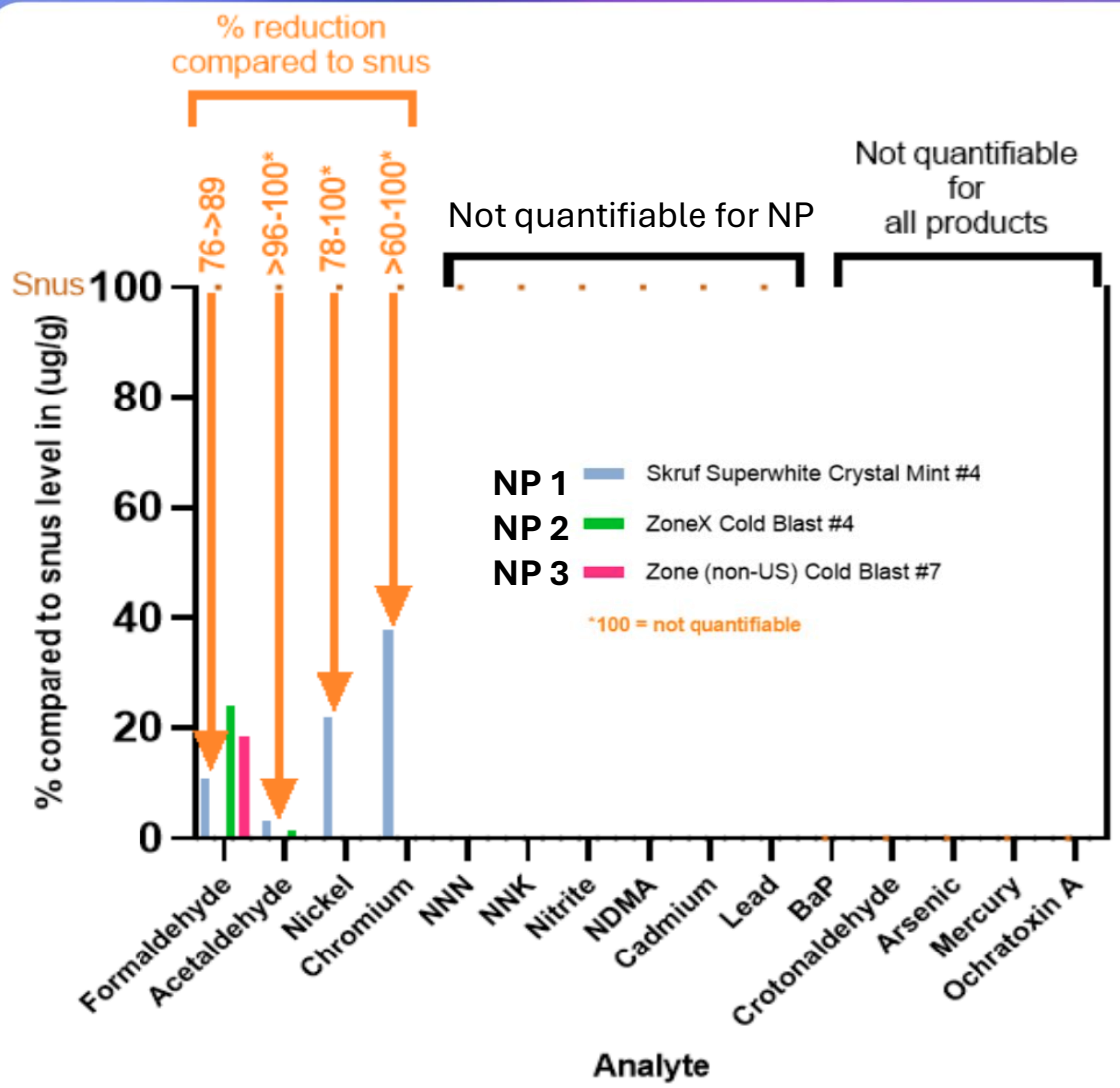
Study 2: Effects of flavours and nicotine on NP biological activity

Studies 3 and 4: used High Content Screening (HCS) to compare NP, Snus and 1R6F cigarette using human endothelial cells and an oral cancer cell line



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Lower analytes (GothiaTek®), observed in NP when compared to snus



- In a comparison to Snus, NP had 60-100% lower levels of formaldehyde, acetaldehyde, nickel and chromium
- In the NPs NNN, NNK, nitrite, NDMA, cadmium and lead were not quantifiable
- Both snus and NP did not contain any measurable B(a)P; crotonaldehyde; arsenic, mercury or Ochratoxin A
- Hadley et al., (2026) reported similar decreases for a wider range of compounds

GothiaTek® is a Swedish Match product quality standard; https://www.coresta.org/abstracts/quality-standard-gothiatekr-27960.html
Hadley et al., (2026) BMC Chemistry in press

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Description of test products (Study 1)



Product name	Product type	Coding	Primary substrate	Nicotine content
Skruf Super white Fresh #2	NP	NP#2	Plant fibres	5.8 mg/pouch
Skruf Super white Fresh #3	NP	NP#3	Plant Fibers	10.1 mg/pouch
Skruf slim Fresh white portion	Snus	Snus	Tobacco leaf	10.9 mg/pouch
1R6F Reference cigarette	cigarette	1R6F	Tobacco Leaf	0.7 mg/cigarette

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Extract generation - NP and snus

NP and snus were extracted based on - ISO 10993-12
In brief,

1. 6 g of NP or snus were covered with 20 mL phosphate-buffered saline (PBS) in a 50 mL tube (~ 300 mg/mL)
2. Agitation at 600 rpm for 1 hour.
3. After centrifugation and filtration through 0.45 and 0.2 µm sterile filters
4. Aliquots of 500 µL per extract were frozen at -80°C.

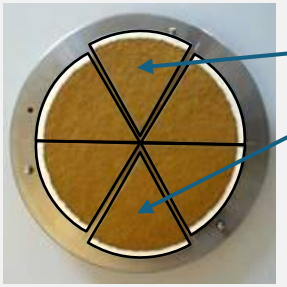
Three independent extracts for each NP test article were generated.
Nicotine was used to evaluate extraction efficiency, (66%–75%) .

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1R6F TPM generation and nicotine concentrations of extracts

1R6F cigarettes were conditioned prior to TPM collection according to the ISO 3308 *(35 ml puff volume/ 2 sec puff duration/ 60 sec interval/ bell shape puff profile, no ventilation block)*

Cigarette Smoke Condensate collected on a Cambridge filter



H_2O and nicotine analysis

Extraction of CSC in DMSO for in vitro tests → TPM

Table 1 Nicotine content of TPM in DMSO, NP, and snus PBS Extracts

Products:	***mg nicotine/mL solvent*** *(±SD; n=3)*
NP #2 PBS *	1.70 ± 0.07 mg/mL PBS
NP #3 PBS *	2.51 ± 0.15 mg/mL PBS
Snus PBS *	2.59 ± 0.10 mg/mL PBS
1R6F TPM	1.35 mg/mL DMSO

* Contained the same flavour

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In vitro testing battery

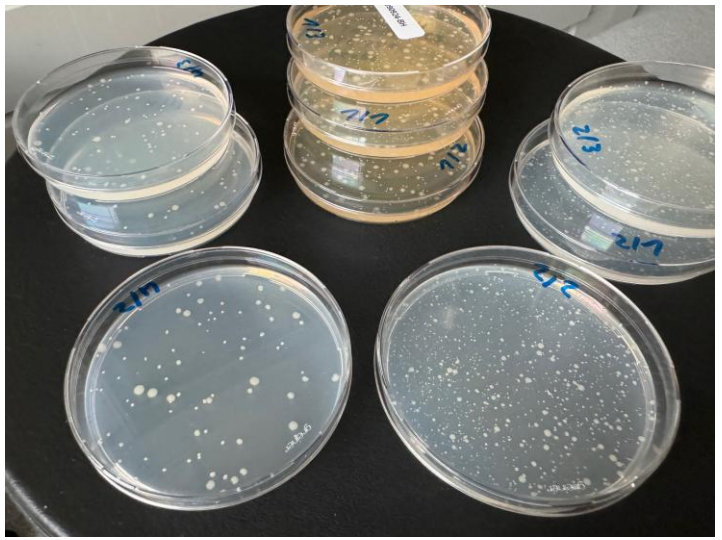
TPM, Snus and NP extracts were tested in three assays (CORESTA battery) in BioToxLab IMB

- **NRU** - neutral red uptake *in 96 multiwell plate in serum free medium over 65h incubation*
  - BEAS-2B (ECACC;Cat.No.:95102433), human bronchial epithelial cell (lung)
  - HepG2 (ATCC,Cat.No.:HB8065), human hepatoma (liver)

- **IVM** - *in vitro* micronucleus *in 24 MWP*
  - V79 (ECACC;Cat.No.:86041102), hamster lung fibroblast

- **Ames** test
  - *Salmonella typhimurium (+/-* S9*)* TA98; TA100; TA102; TA1535; and TA1537



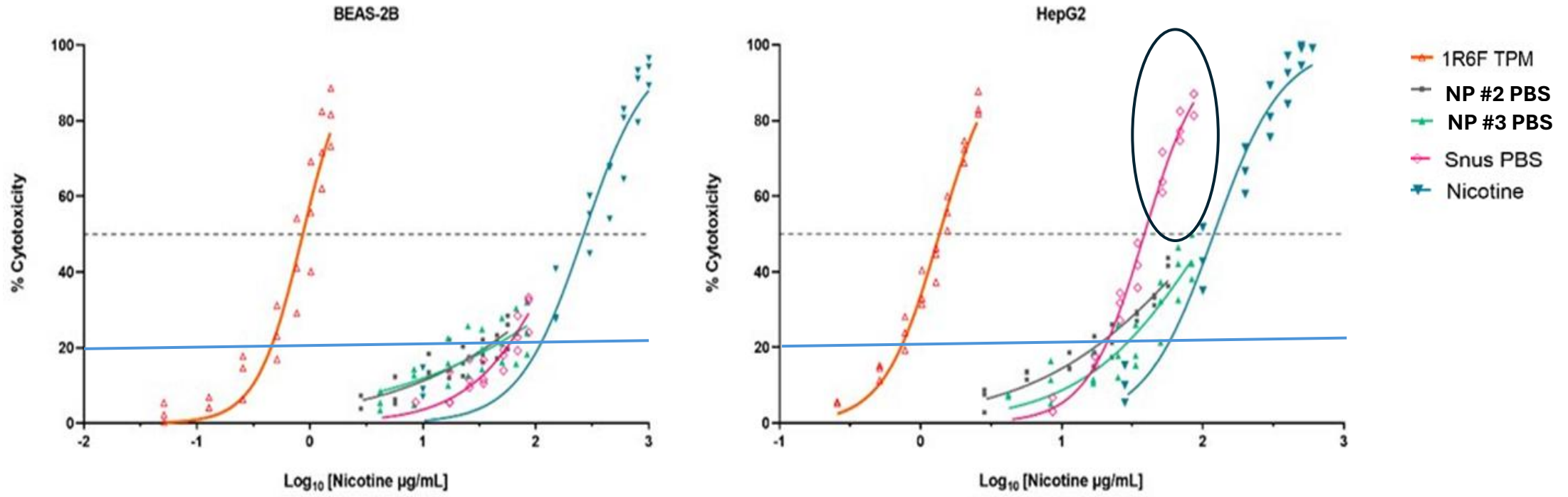


ilac-MRA DAkkS Deutsche Akkreditierungsstelle D-PL-14465-01-00

All methods are ISO17025 accredited

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NRU – cytotoxicity results (µg/ml)



The cytotoxicity with increasing concentrations of NPs and Snus extracts in the NRU assay seems to be related to the nicotine cytotoxicity .

In the EC_{20}, (20% cytotoxicity) 1R6F TPM was >150 times more cytotoxic than NPs, and snus, in both cell lines (Table 1).

- The cytotoxicity with HepG2 cells, suggest that the NP may offer an improved harm reduction profile compared to snus
- The corresponding nicotine concentrations were: 57 (NP #2), 84 (NP #3), and 86 (Snus) µg nicotine/mL, these nicotine concentrations were similar to the nicotine levels found in the saliva of moist snuff users (73 µg nicotine/mL *).

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* Hoffmann D, Adams JD. Carcinogenic tobacco-specific N-nitrosamines in snuff and in the saliva of snuff dippers. Zanetti F, et al. Assessment of a 72-hour repeated exposure to Swedish snus extract

Ames mutagenicity results, negative for snus and NPs (TA98 and TA100 ± S9)

Fig A

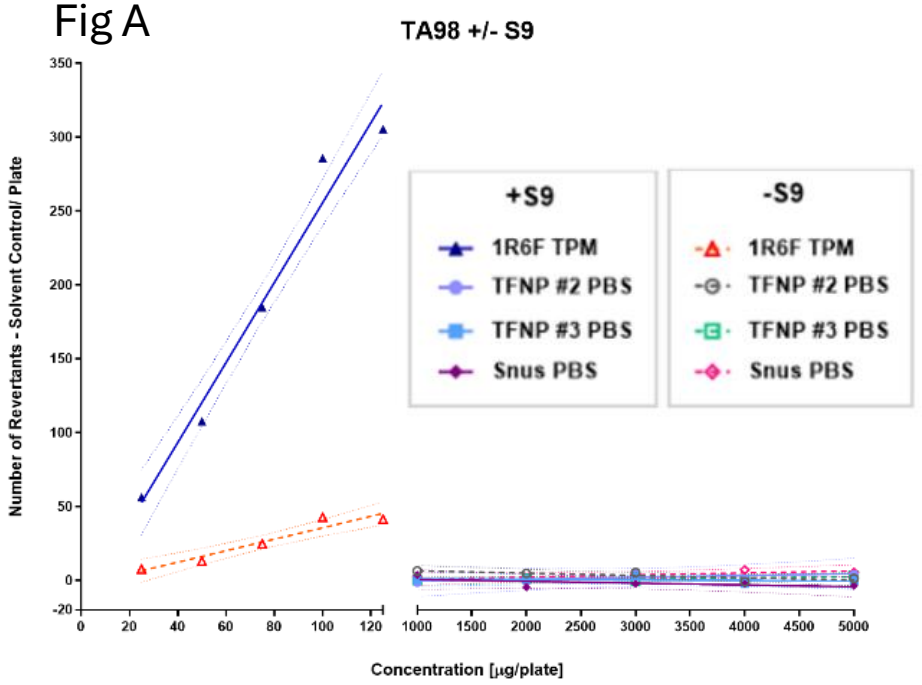
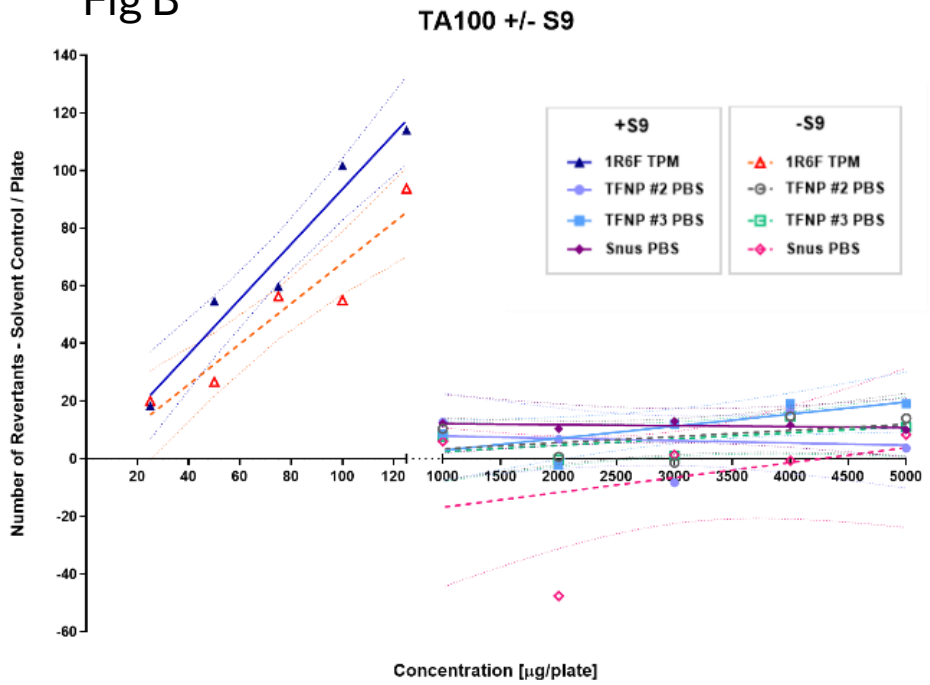


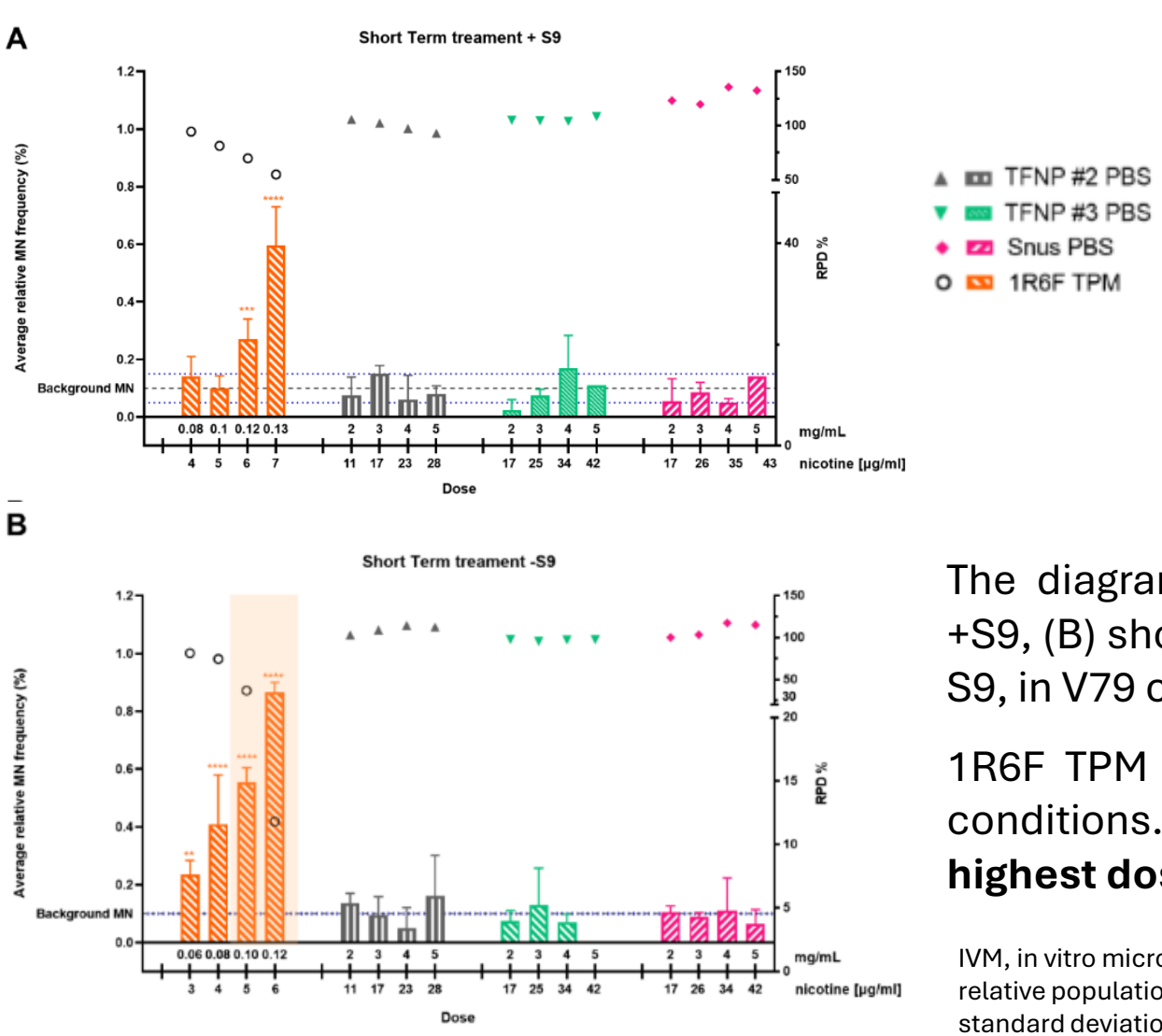
Fig B



- Both +/-metabolic activation after exposure to 1R6F TPM, snus, and two NP extracts in the Ames assay. Dotted lines represent the 95% confidence intervals about the slope
- No dose-dependent or statistically significant increases in revertant frequencies observed for NP and snus extracts in any of the five S. typhimurium strains with or without S9 metabolic activation, when compared with the negative controls.

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IVM Results – genotoxicity negative for Snus and NP



The diagrams show IVM results for (A) short-term treatment +S9, (B) short-term treatment -S9 and (C) long-term treatment -S9, in V79 cells.

1R6F TPM had a strong genotoxic response in all treatment conditions. **No genotoxicity or cytotoxicity for NP or Snus at highest dose tested (42 µg/ml)**

IVM, in vitro micronucleus; RPD, relative population doubling; SD, standard deviation; **p ≤ 0.01, ***p ≤ 0.005, ****p ≤ 0.0001

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A comparison of 20 NP in the CORESTA Battery (Study 2)



20 Flavoured nicotine pouch extracts were tested in the CORESTA battery and compared to 1R6F

Methods were same as before (Study 1):

- **Pouch Extraction** (ISO 10993-12:2021):
  NRU Assay (BEAS-2B & HepG2):
  NP extracts (0.5–21 mg/mL) vs. 1R6F TPM (0.005–0.05 mg/mL); negative & positive controls.
- **Ames Assay** (OECD 471):
  NP extracts (1–5 mg/plate); controls included.
- **IVM Assay** (OECD 487):
  V79 cells (2–5 mg/mL), 1R6F TPM (0.03–0.14 mg/mL).
- **Statistical Analysis**:
  Chi-Square & Cochran-Armitage trend test for significance

Table 3: Zone flavours tested

Test Article	Nicotine strength (mg nicotine/pouch)	Flavour direction
NP #1	4.08	Menthol
NP #2	6.03	Menthol
NP #3	7.22	Mint/Lime
NP #4	9.63	Red Berry/Vanilla
NP #5	9.63	Watermelon
NP #6	9.63	Orange
NP #7	9.63	Peach
NP #8	9.63	Liquorice
NP #9	10.00	Citrus
NP #10	11.89	Mint
NP #11	12.00	Citrus/Mint
NP #12	12.03	Menthol
NP #13	14.00	Berry/Vanilla
NP #14	14.45	Menthol
NP #15	14.54	Menthol
NP #16	15.05	Berry
NP #17	16.00	Menthol
NP #18	16.00	Sriracha/Lime
NP #19	17.65	Red Berry/Vanilla
NP #20	18.00	Menthol

Bento, J.,et al., In vitro assessment of Tobacco-Free Nicotine Pouches reveals marked reductions in Toxicity when compared to Cigarettes. In *Toxicology Letters* (Vol. 399, pp. S268-S269).

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NRU results : No consistent effect of flavour or nicotine concentration on cytotoxicity (EC20)

Fig A: HepG2

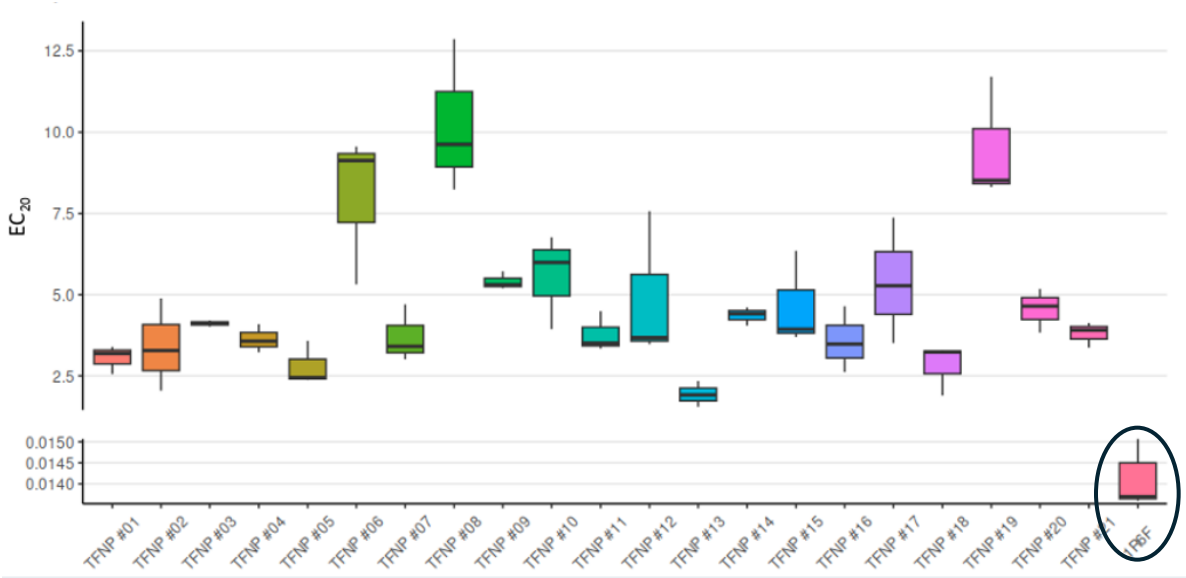
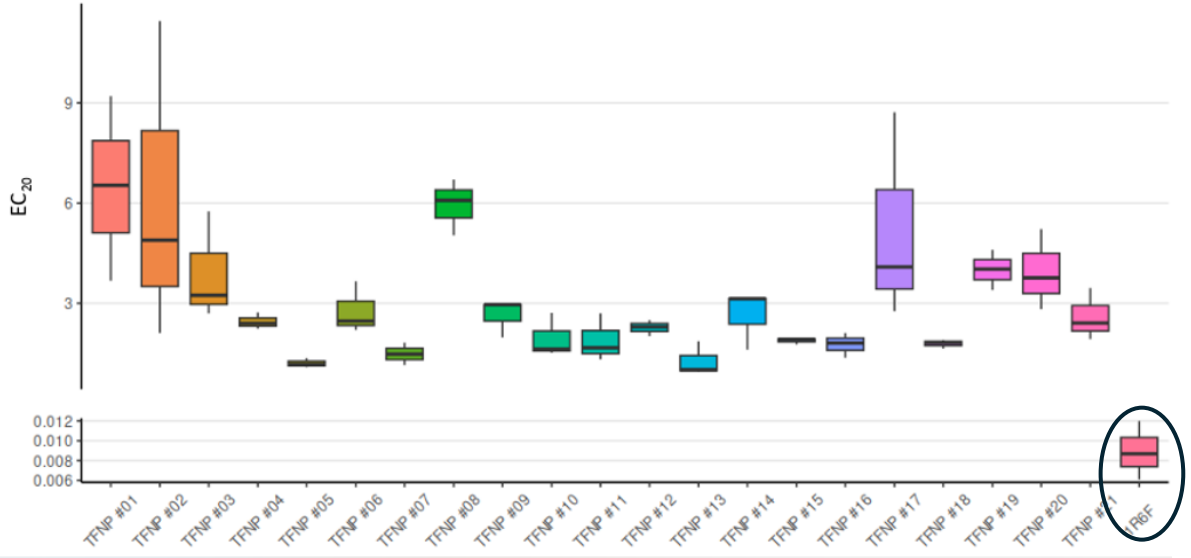


Fig B: BEAS-2B

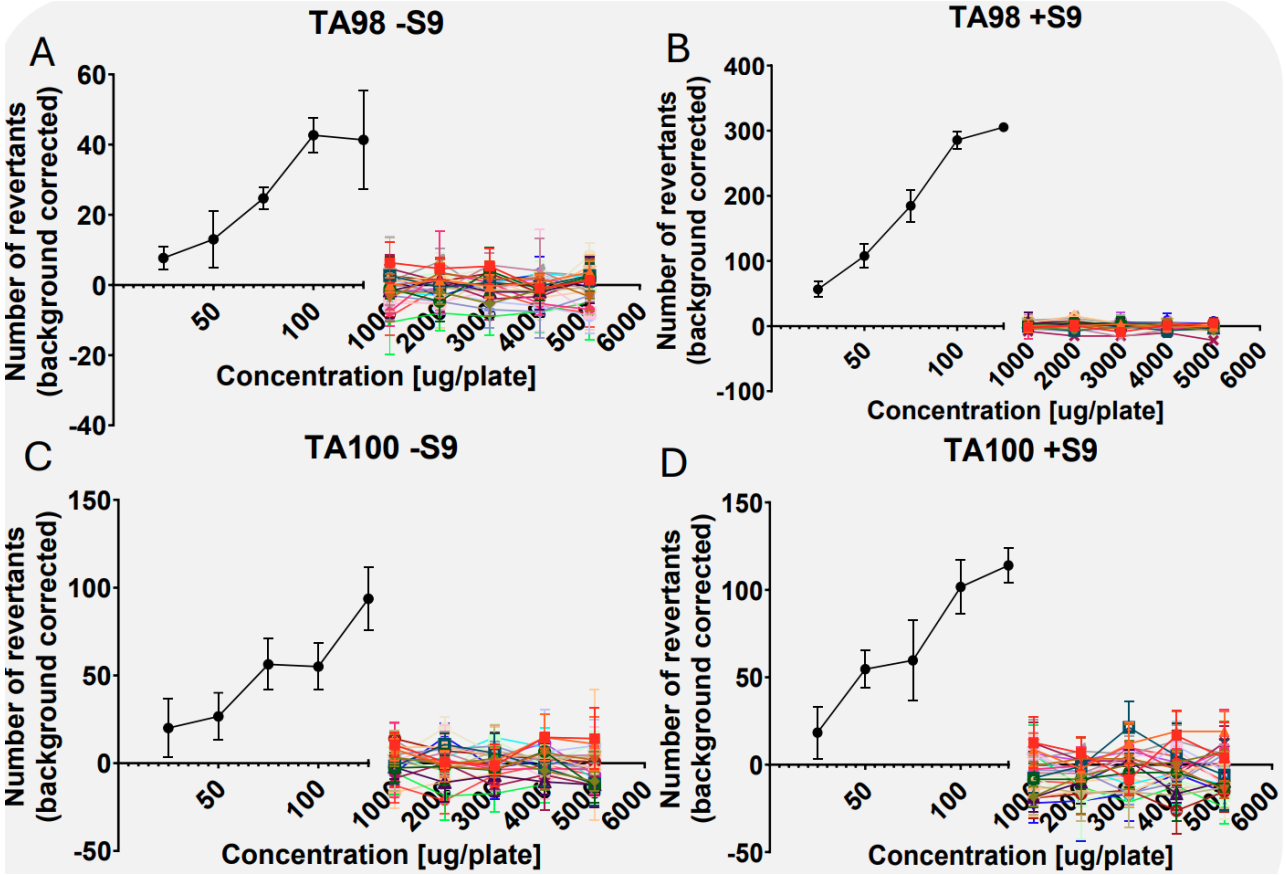


- EC_{20} (Figures A and B) values obtained for the NP extracts ranged between 32 and 618 times less cytotoxic than the 1R6F cigarette TPM when calculated on a per nicotine basis.
- Variation in the EC_{20} values was observed between NP products, but no correlation was observed with nicotine content or flavour. This variance was less pronounced for BEAS-2B

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Negative Ames and IVM, no effects of nicotine or flavours for NPs

1R6F vs NP in TA98 and TA 100 +/- S9



Ames

- **All NP extracts were negative in all of the 5 test strains.**
- 1R6F TPM caused a significant reproducible, dose-dependent increases in the number of revertants in TA98 (+/-S9), TA100 (+/-S9) and TA1537 (+S9).
- The 1R6F TPM was classified as mutagenic under the test conditions.

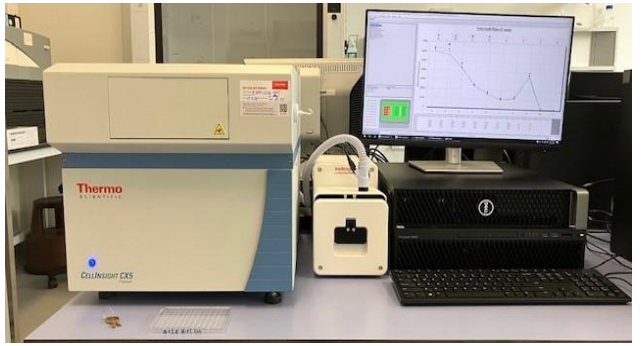
IVM results (Results not shown)

- **No NP extracts increased micronucleus frequencies** (n=3) when compared to the negative controls (short +/- S9; long –S9); not genotoxic in the IVM assay (maximum concentration of 5000 µg/plate).
- 1R6F was genotoxic in the assay

Bento, J.,et al., In vitro assessment of Tobacco-Free Nicotine Pouches reveals marked reductions in Toxicity when compared to Cigarettes. In Toxicology Letters (Vol. 399, pp. S268-S269).

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HCS study with snus; NP and 1R6F in human coronary artery endothelial cells (HCAECs) ± NAC (Study 3)



Methods:

Extract Preparation:

- Snus & NP extracts → EGM-MV2 medium; nicotine quantified; stored at −70 °C
- **TPM**: 1R6F cigarettes → Cambridge pads; extracted with DMSO
- **SbMed**: (1R6F) Smoke bubbled through medium (1R6F only) ; nicotine + 8 carbonyls measured; stored at −70 °C

Cell Culture & Exposure:

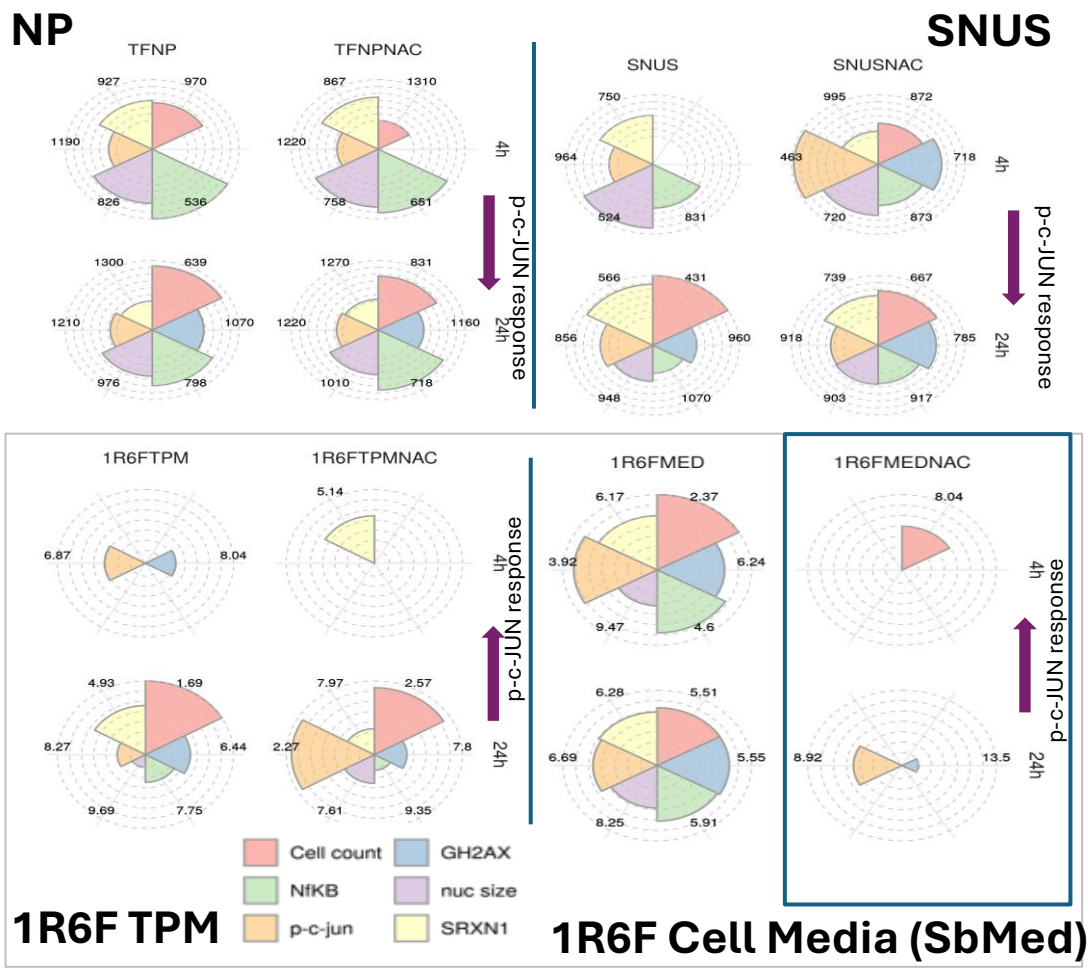
- Primary HCAECs (male 55, non smoker) were seeded in collagen-coated 96-well plates
- Exposed 4 or 24 h ± NAC (final concentration 1mM/well)
- Pure nicotine (20–1500 µg/mL) used to look for nicotine-specific effects (Data not shown)

High content Screening (HCS); NAC n-acetylcysteine an antioxidant

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HCS analysis of HCAECs exposed to NP; snus and1R6F, No effects of NAC for snus and NP

HCAECs: Human coronary artery endothelial cells, lining blood vessels



Results:

- **MECs**: Larger segments = lower MEC (higher potency); lower values. **Expressed as µg/mL of nicotine**
- **p-c-JUN** (orange); key signaling protein for growth, differentiation, cell death.
- **NP/Snus decreased↓ p-c-JUN response** (4 and 24 hours)
- **Smoke/TPM increasing ↑ p-c-JUN response** (4 and 24 hours)

With NAC (antioxidant)

- Minimal impact on Snus/NP
- **Strong mitigation of SbMed effects →** oxidative stress (blue) is the major driver

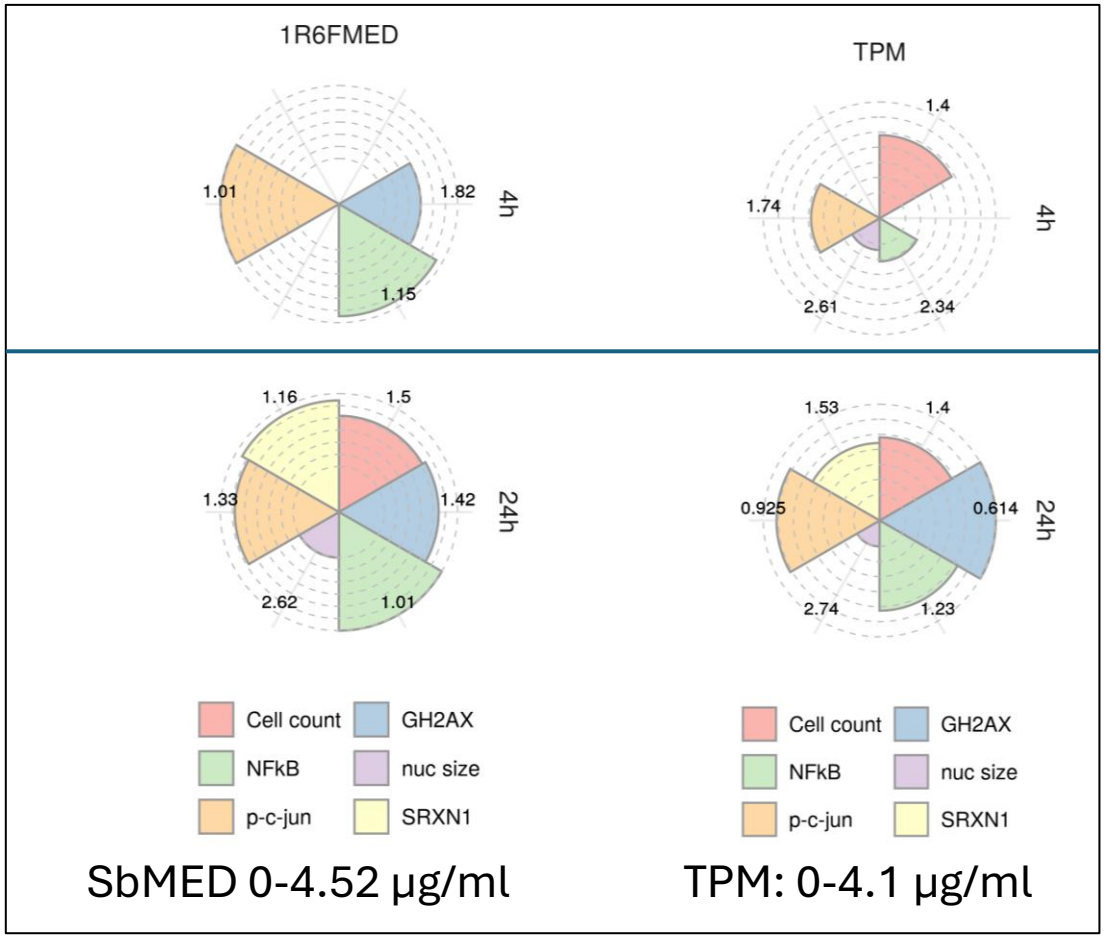
Trelles Sticken et al., (2025) High content screening of tobacco free nicotine pouch extracts shows marked reductions in toxicity when compared to cigarette smoke extracts CORESTA 2025;

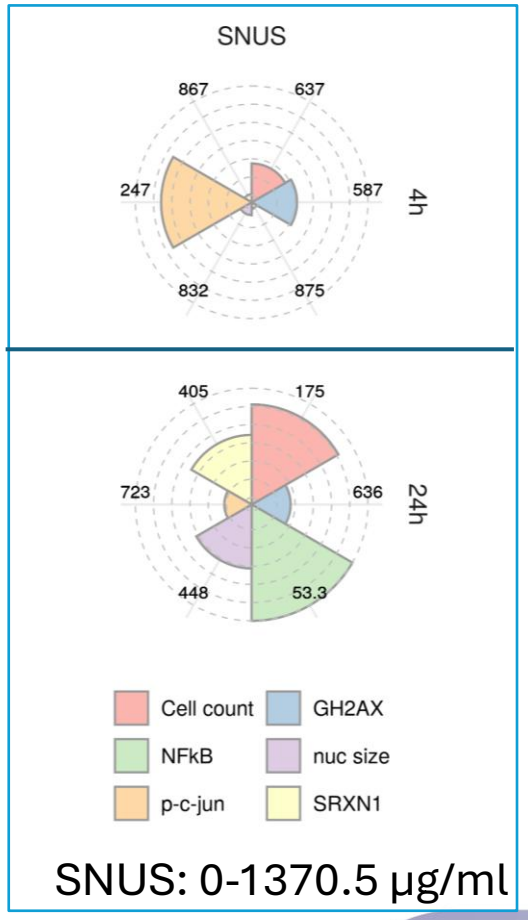
Smoke bubbled medium (SbMed); NAC N-acetyl cysteine; MEC Minimum effective concentration first concentration in excess of control values; TPM total particulate material;

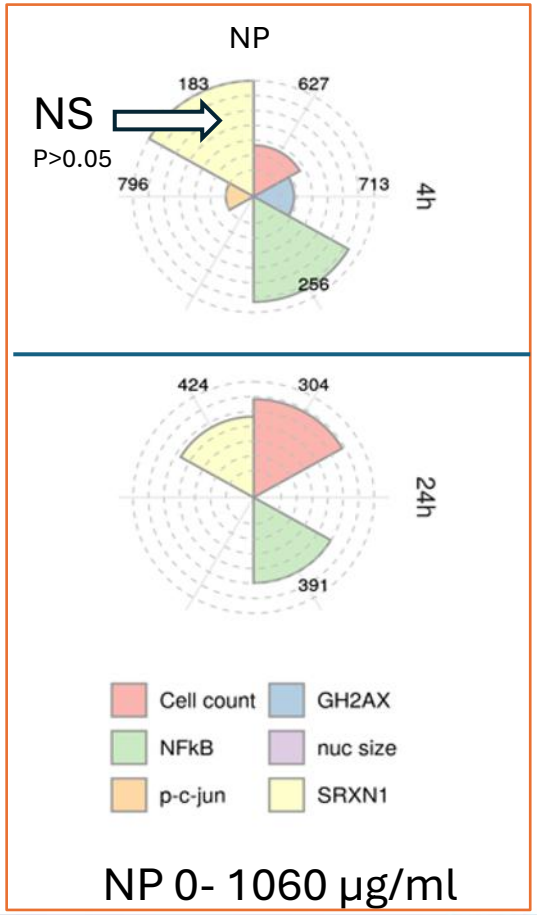
https://github.com/philipmorrisintl/GladiaTOX?tab=readme-ov-file

HCS: Minimal effects seen for snus and NP

Oral cell CA9-22; Dosing on a per nicotine basis; HCS: larger wedge lower MEC more potent (µg/mL)







Cell line CA9-22 is a human gingival squamous cell carcinoma with a TP53 mutation; sbMED smoke bubbled media

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Conclusions

- Snus is widely considered to the be the first tobacco harm reduction product when compared to combusted tobacco; replacement of the tobacco with plant fibers and pharmaceutical grade nicotine may result in greater harm-reduction potential
- This reduction in toxicants may contribute to reduced *in vitro* toxicity data from NP
- HCS also provides evidence of reduced activity of NP when compared to snus this may suggest that tobacco free products may exhibit a more favorable profile than snus under the tested conditions
- These results support a positive role for NP in THR strategies, possibly beyond that of snus; however, further preclinical, clinical, and perception and behavior studies are required for a more complete evaluation

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I would like to thank:

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