Multi-endpoint in vitro toxicological assessment of snus and tobacco-free nicotine pouch extracts

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Multi-endpoint *in vitro* toxicological assessment of snus and tobacco-free nicotine pouch extracts

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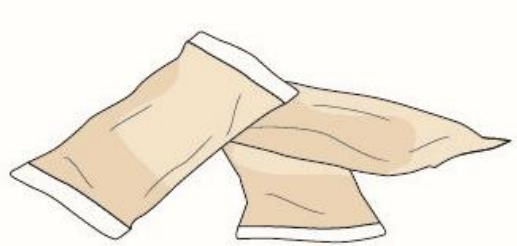
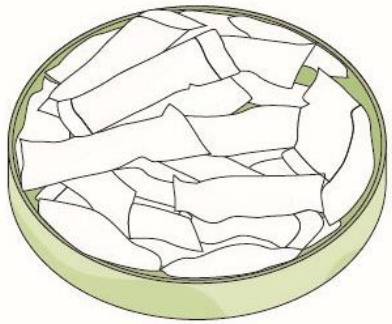
Background

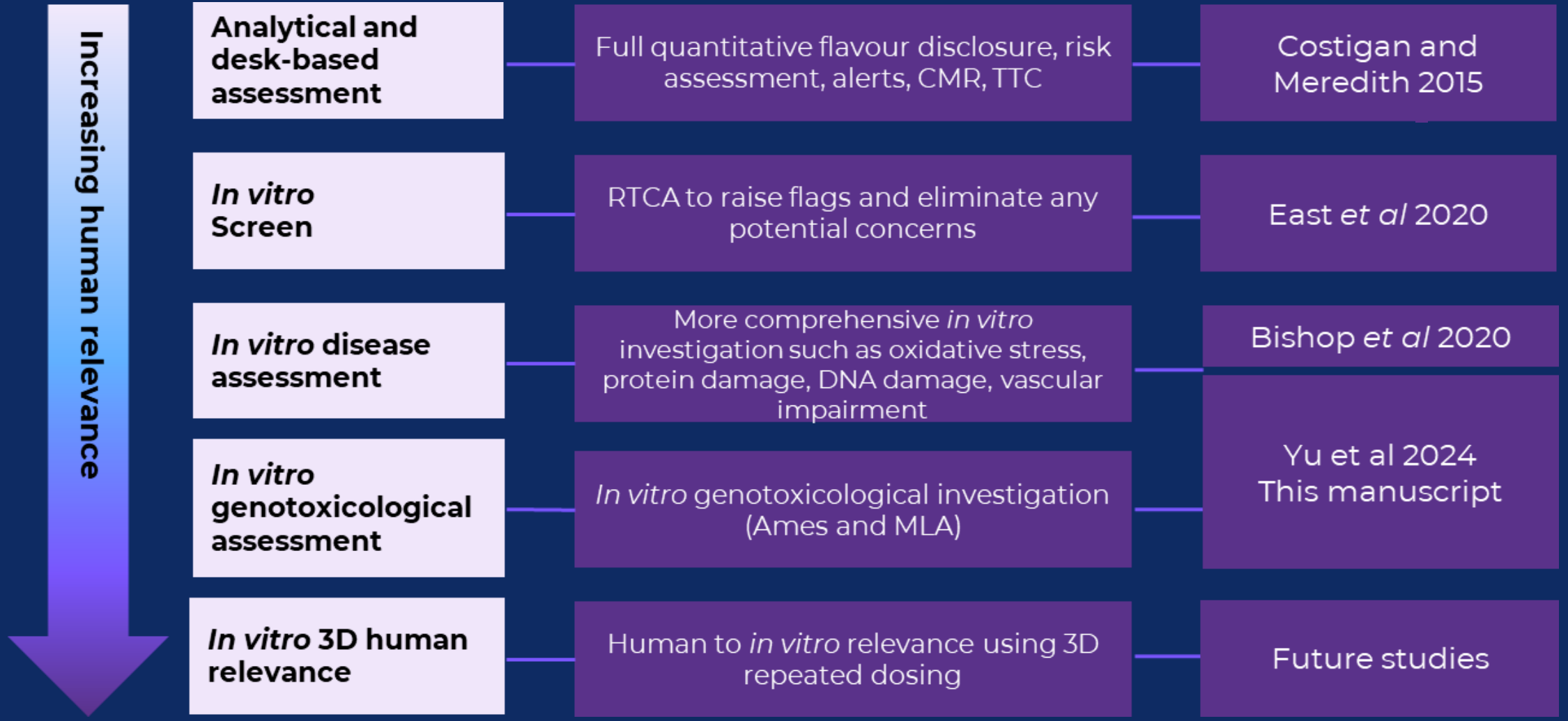
Snus



Tobacco-free nicotine pouches (NPs)



Proposed *in vitro* assessment strategy



Test products

Snus

Tobacco-free nicotine pouches (NPs)

Nicotine Strength	8mg	4mg	4mg	10mg	6mg
Product Code	CRP1.1	LYFT_BF04	LYFT_TB04	LYFT_IC10	NDSP_BW06

* VELO previously marketed as LYFT

Testing approach

1 ***In vitro* disease assessment**

2 **Regulatory toxicological assessment**

3 **Potential signalling pathways or inflammatory response**

ToxTracker

Mouse embryonic stem cell (mESC)

GFP Induction

NRU	Ames	MLA
Mouse fibroblasts (Balb/c 3T3 clone A31)	Salmonella typhimurium (TA98, TA100, TA1535, TA1537 and TA102)	Mouse lymphoma cells (L5178Y tk+/-)
Induction of cell death	Mutation Frequency	Induction of mutations

Signalling

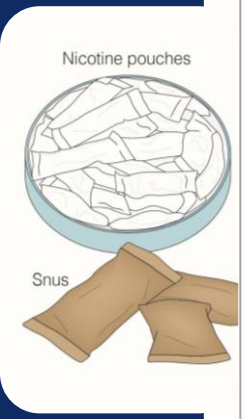
NCI-H292 lung carcinoma cells

Cytokine release

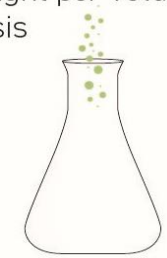
Sample generation

Overview of published extraction procedures for oral tobacco and nicotine products for use *in vitro* assays

1 Prepare material



- Cut open pouch
- Remove pouch contents into a flask
- Addition of fleece material (cut into pieces) (Optional)
- Per pouch basis or weight per volume basis



- 1 pouch per 20 mL of cell culture medium (media specific to each assay).
- The fleece was added to the conical flask.
- Incubated at 37 ◦C and shaken at 150 RPM for 1 h.
- Particulate was removed by centrifugation and supernatant filtered through 5 µm and 0.2 µm filter.

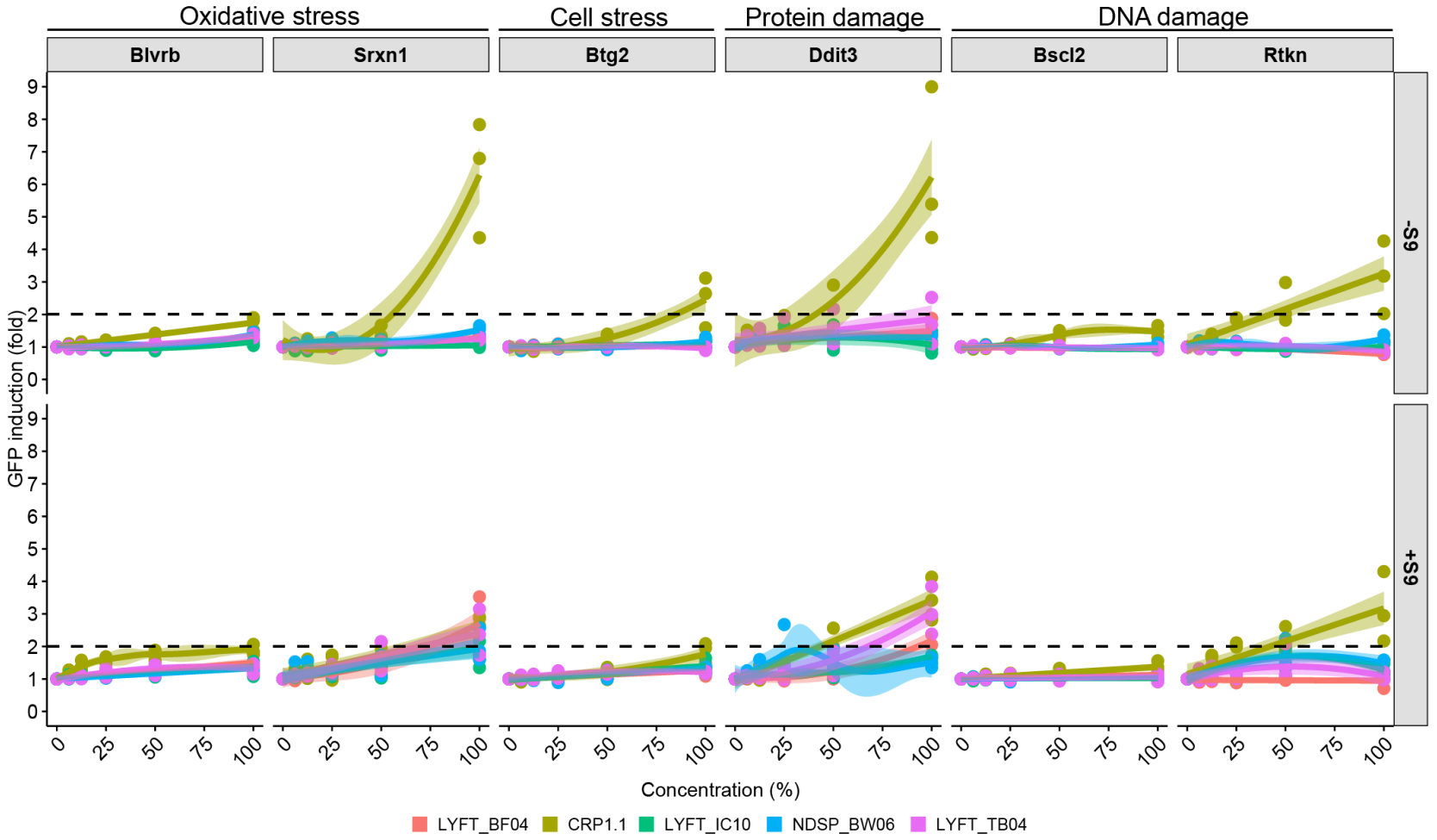
Results

Nicotine quantification

Nicotine quantification of extracts		
Test Article Extract	Nicotine strength (per pouch) (mg)	Average nicotine concentration (µg/mL) ± SD
LYFT_BF04	4	137.2 ±11.1
LYFT_TB04	4	147.1 ± 34.1
LYFT_IC10	10	354.5 ± 36.0
NDSP_BW06	6	207.5 ± 22.9
CRP1.1	8	270 ± 23.3
N=6		

1. *In vitro* disease assessment

* Dash line is the 2-fold threshold

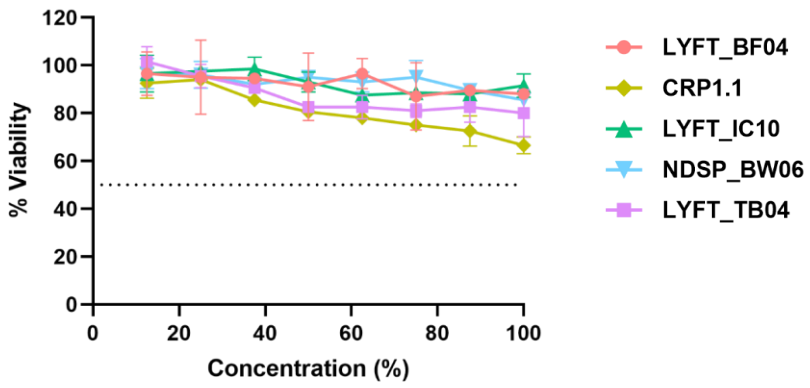


2. Regulatory toxicological assessment

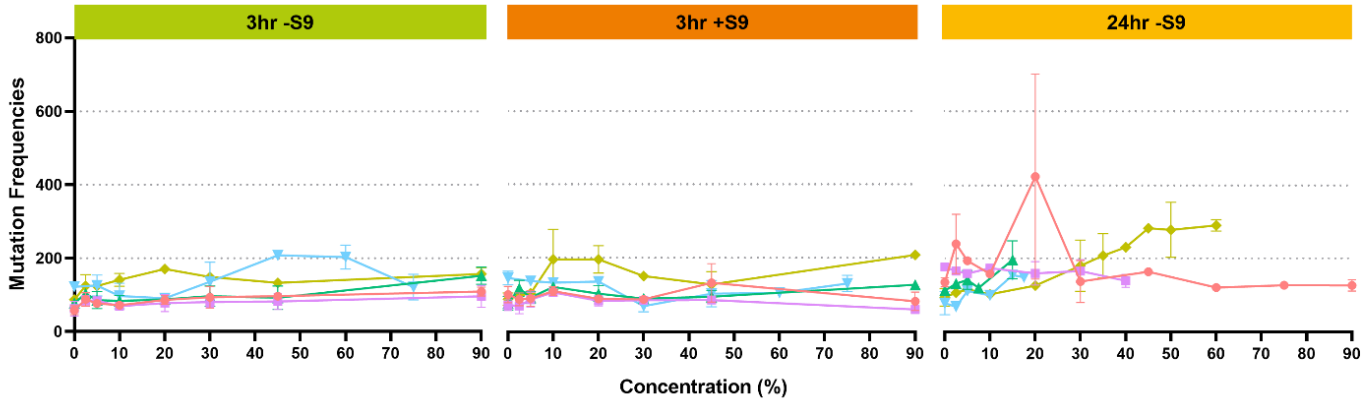
Regulatory Assay		Velo™			Commercial Comparator	CORESTA Refence Product 1.1
		LYFT_BF04	LYFT_TB04	LYFT_IC10	NDSP_BW06	CRP1.1
NRU (Cytotoxicity)		X	X	X	X	X
Ames (Mutagenicity)		X	X	X	X	X
MLA (Genotoxicity)	3 hrs –S9	X	X	X	X	X
	3 hrs +S9	X	X	X	X	X
	24 hrs –S9	?	X	X	X	✓

X denotes negative; **?** denotes equivocal and ✓ denotes positive response

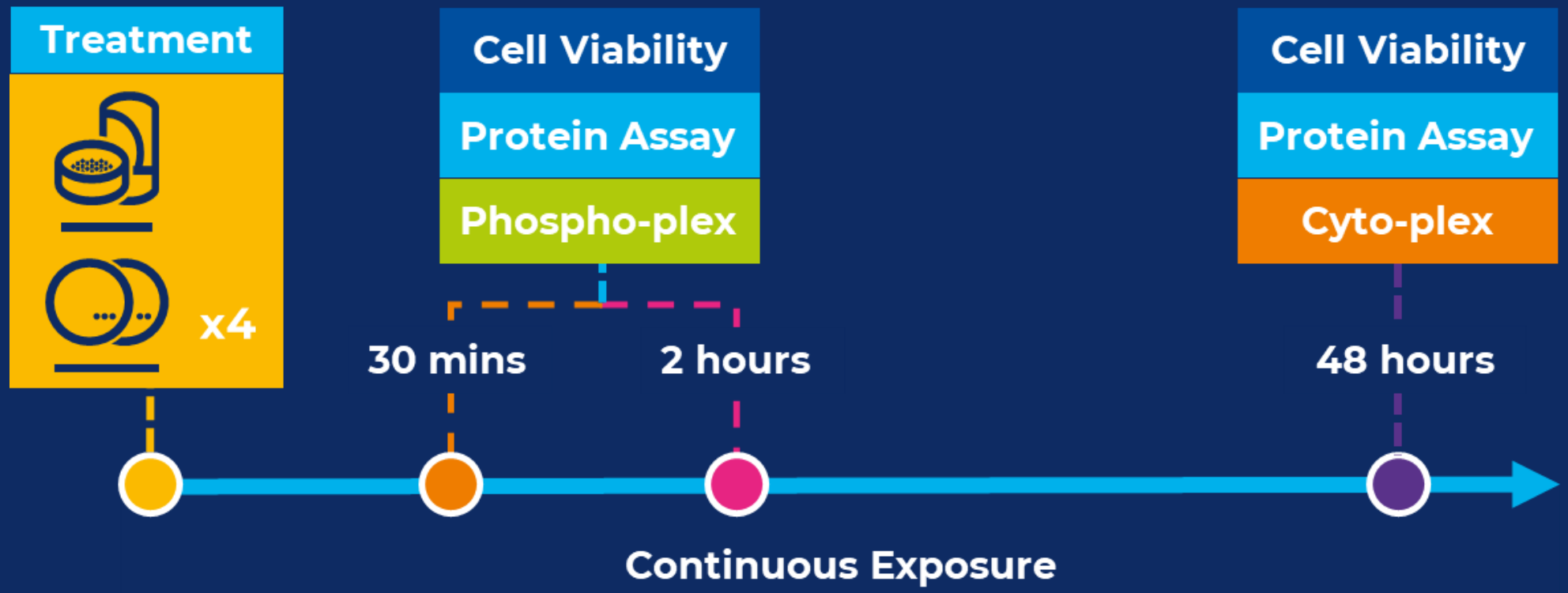
NRU



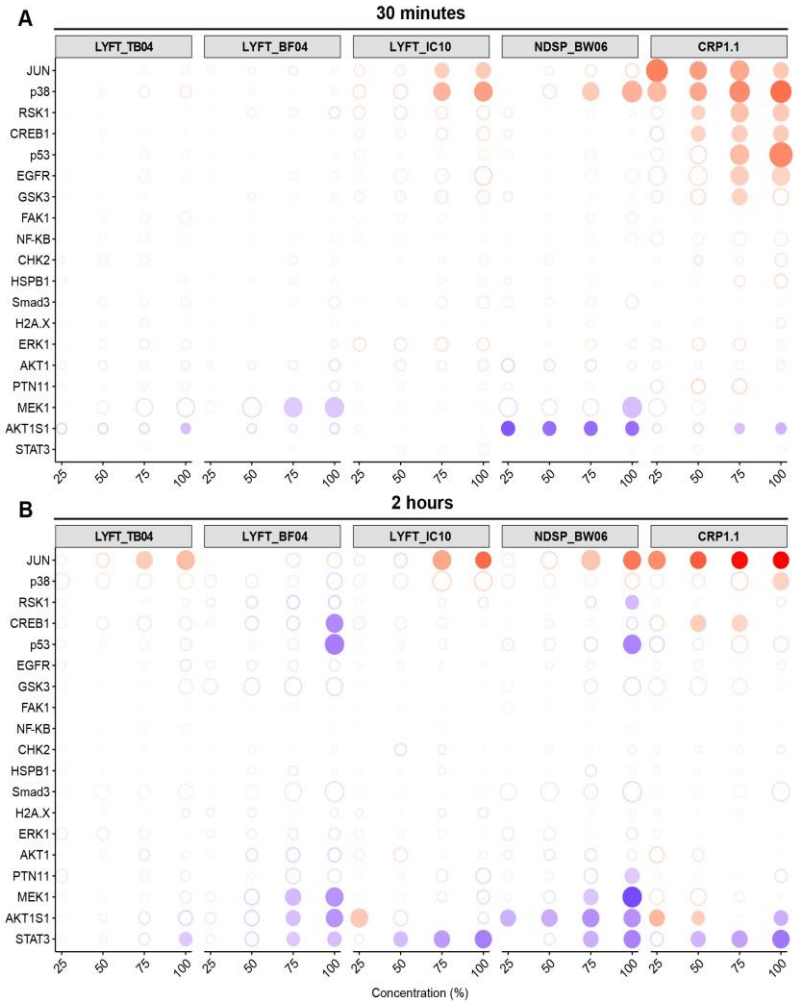
MLA



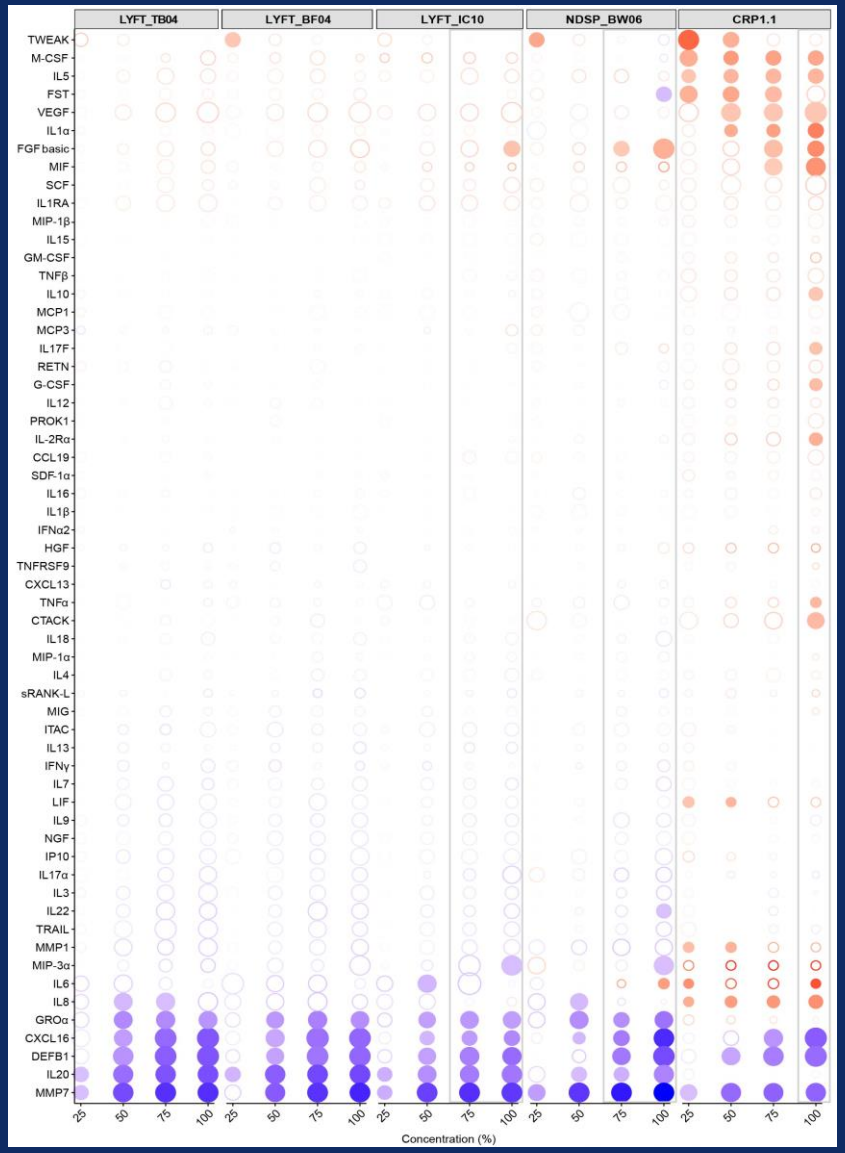
3. Potential signalling pathways or inflammatory response

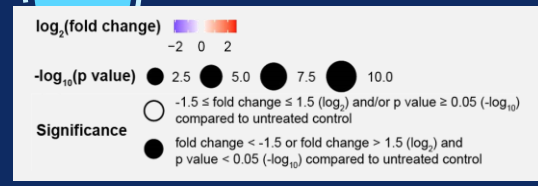


Phosphorylation



Inflammatory markers





- NP extracts in general **did not** induce any significant increase in phosphorylation of protein.
- CRP1.1 extract on the other hand was more active.

- NP extracts in general **did not** induce any significant increase in the secretion of proinflammatory cytokines.
- CRP1.1 extracts induced the production of inflammatory mediators such as IL-1α, IL5, IL6, IL8 after 48hr exposure time.

Conclusions

1. NP extracts were biologically less active compared to the snus reference product in all tested endpoints relevant to a range of disease processes.

2. NP extracts did not induce any cytotoxicity or mutagenic response, genotoxic response was minimal and limited signalling or inflammatory markers were induced.

3. A weight of evidence approach with a wide range of endpoints is required to provide sufficient *in vitro* data for assessment of potential comparative risks of NPs and snus.

4. The data presented here further contribute to the weight of evidence indicating NPs should be considered as an alternative reduced risk profile product in comparison to both combustible tobacco products and snus.

Thank You & Questions?

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