

A background image showing several pieces of laboratory glassware, including beakers and a test tube, arranged on a surface. The glassware is illuminated with a warm, colorful light, creating a gradient from purple to orange. The text is overlaid on this image.

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

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25 October 2024

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

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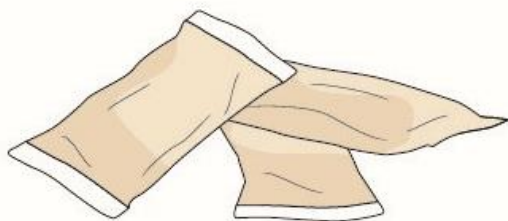
B.A.T. (Investments) Limited, Regents Park Road, Millbrook, Southampton SO15 8TL, UK



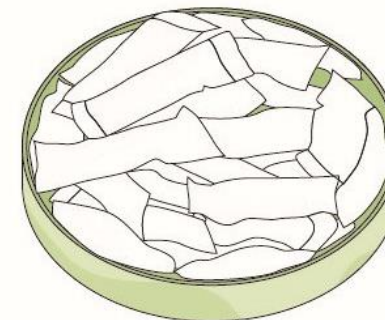
Background



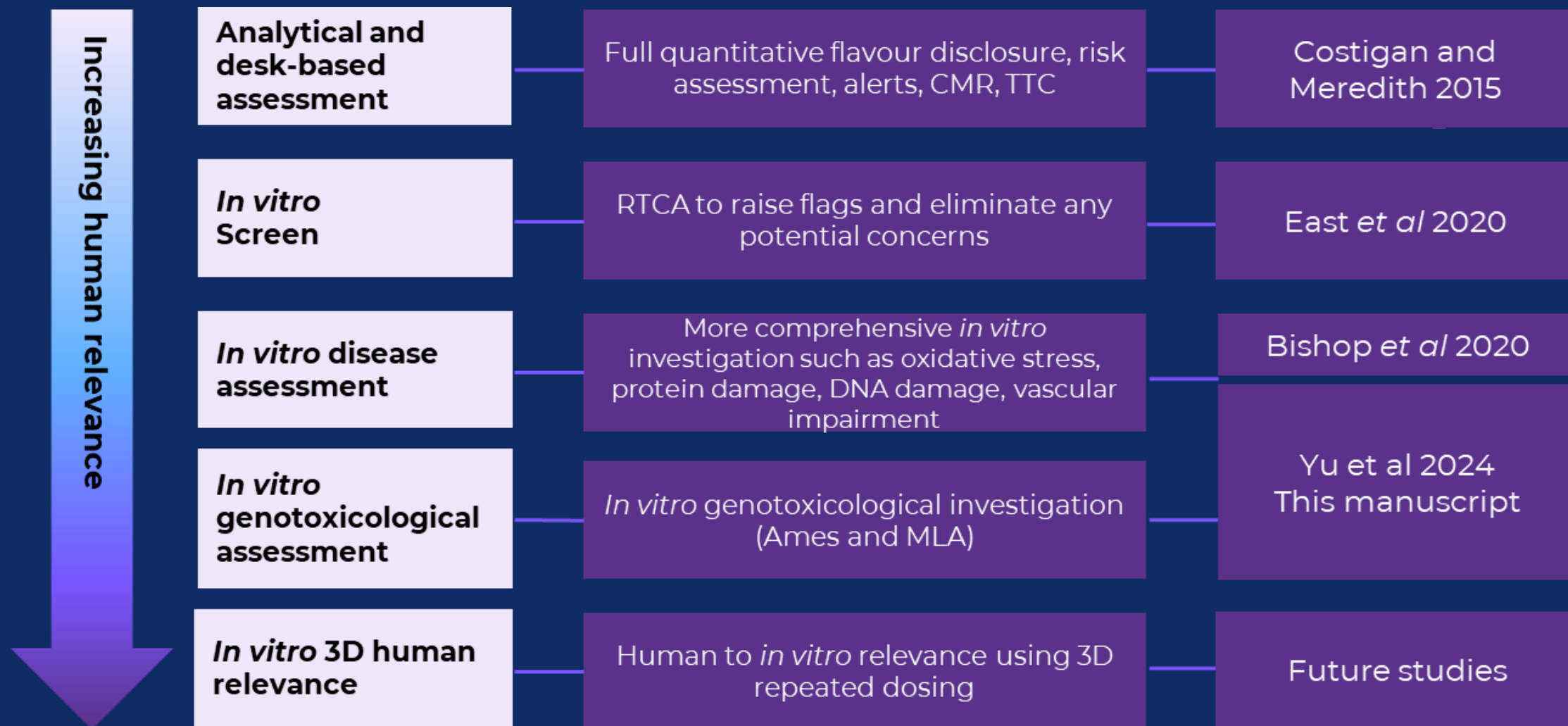
Snus



Tobacco-free nicotine pouches (NPs)



Proposed *in vitro* assessment strategy



Test products



Snus



Nicotine Strength 8mg

Product Code CRP1.1



Tobacco-free nicotine pouches (NPs)



4mg

LYFT_BF04



4mg

LYFT_TB04



10mg

LYFT_IC10

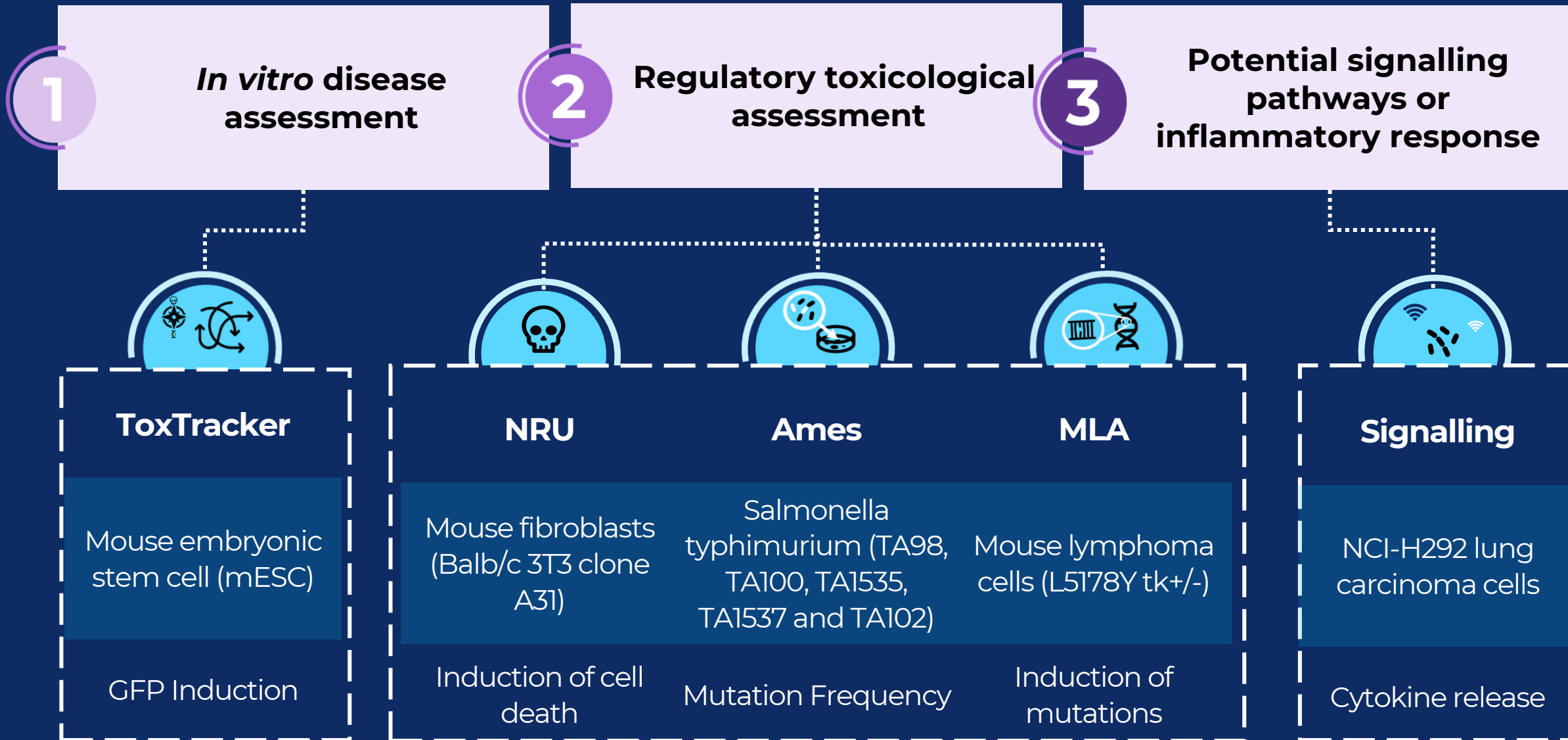


6mg

NDSP_BW06

* VELO previously marketed as LYFT

Testing approach

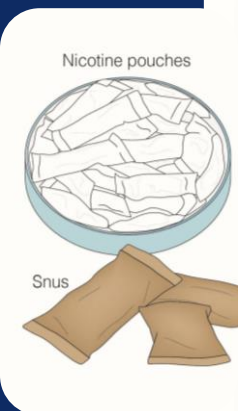


Sample generation

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

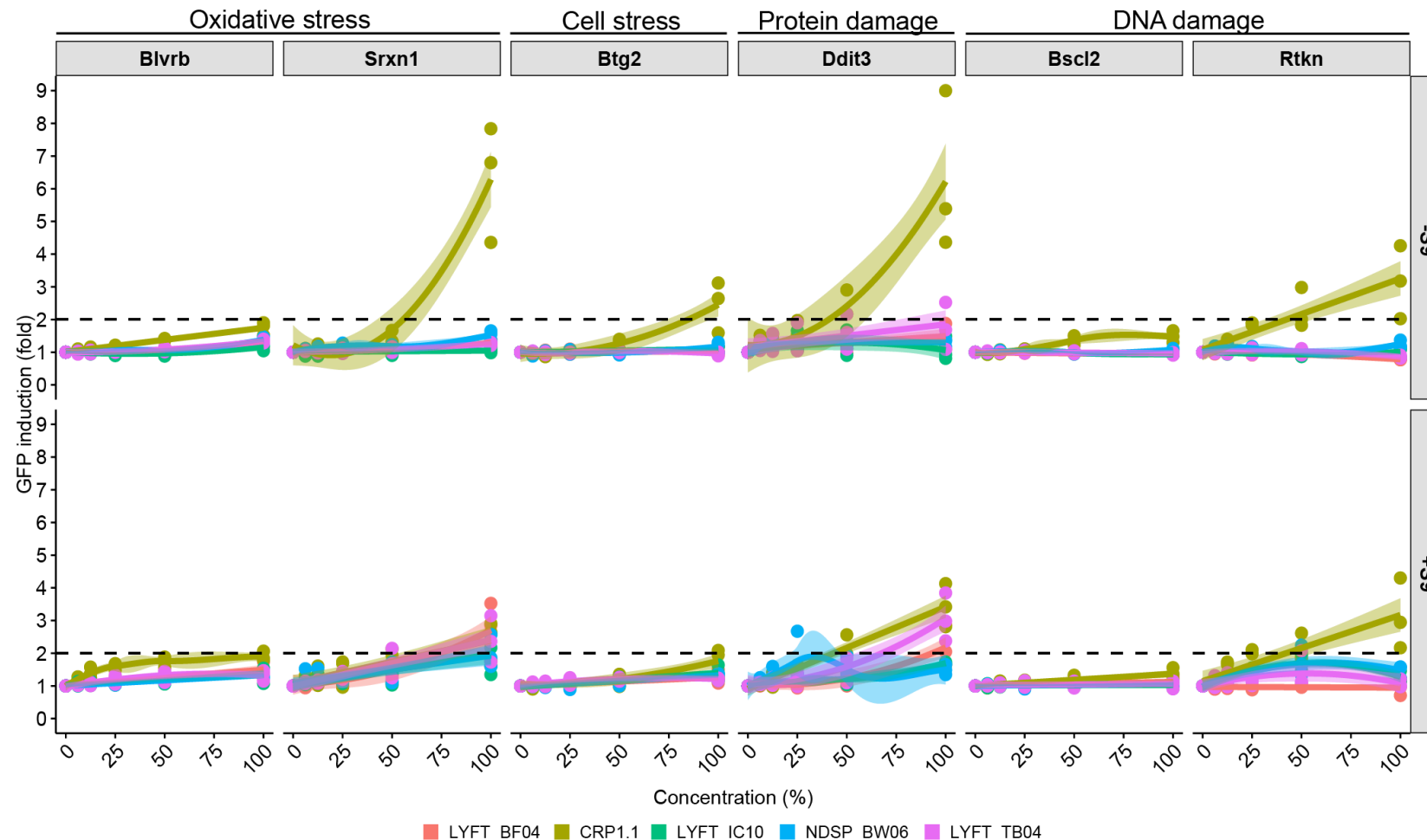
Nicotine quantification

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

1. *In vitro* disease assessment



* Dash line is the 2-fold threshold



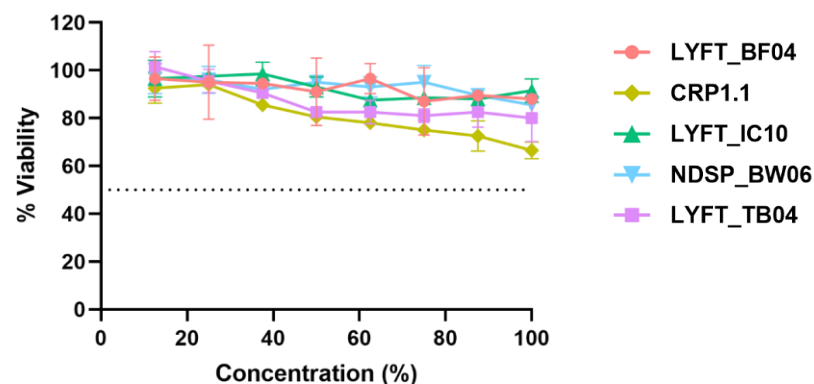
2. Regulatory toxicological assessment



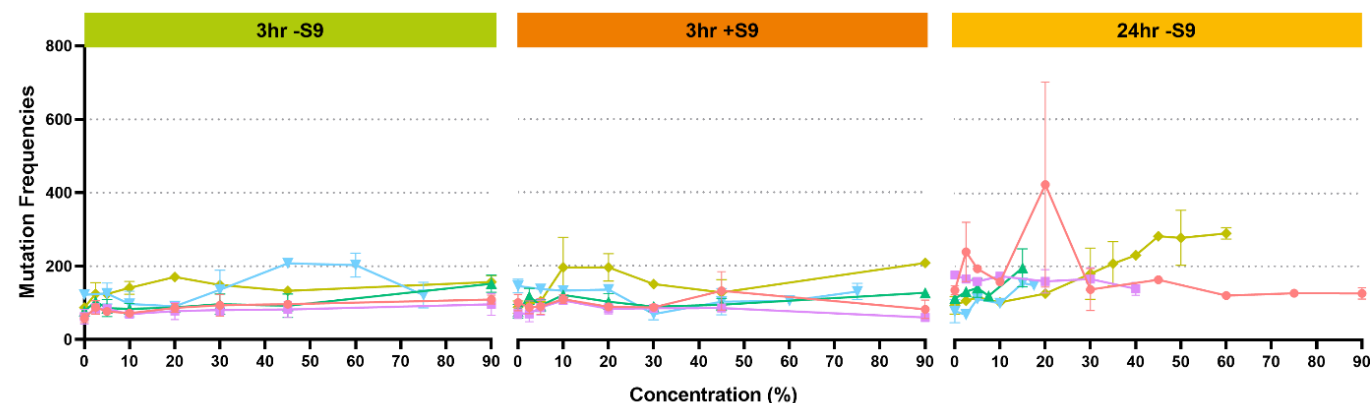
Regulatory Assay		Velo™			Commercial Comparator	CORESTA Reference Product 1.1
		LYFT_BF04	LYFT_TB04	LYFT_IC10	NDSP_BW06	CRP1.1
NRU (Cytotoxicity)		X	X	X	X	X
Ames (Mutagenicity)		X	X	X	X	X
MLA (Genotoxicity)	3 hrs-S9	X	X	X	X	X
	3 hrs +S9	X	X	X	X	X
	24 hrs-S9	?	X	X	X	✓

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

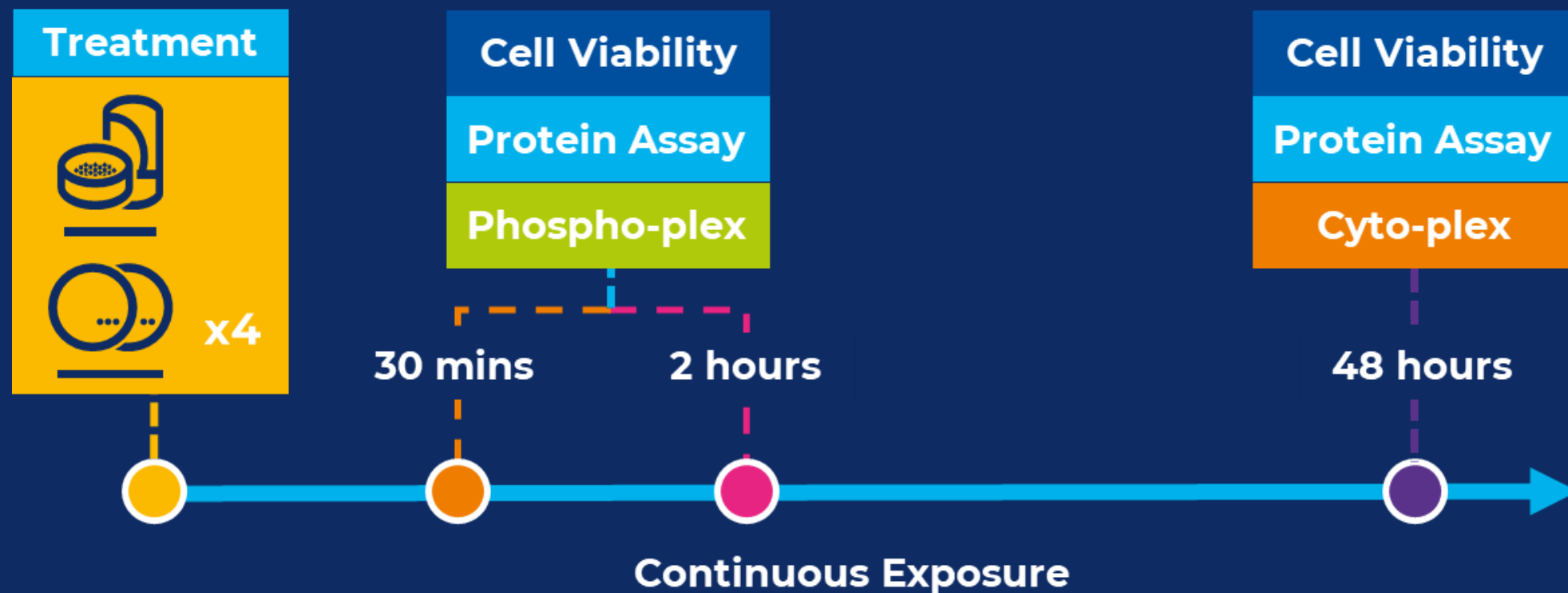
NRU



MLA

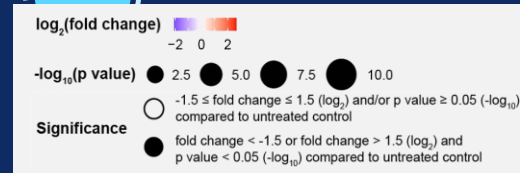
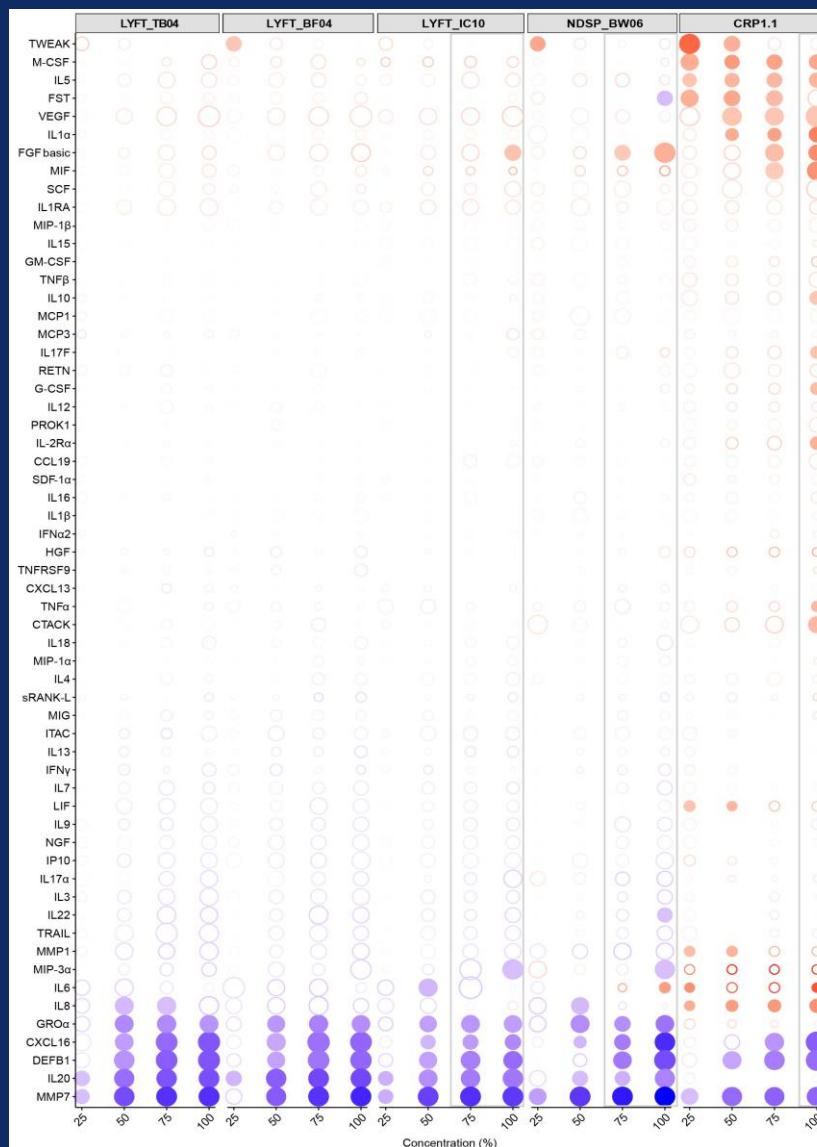
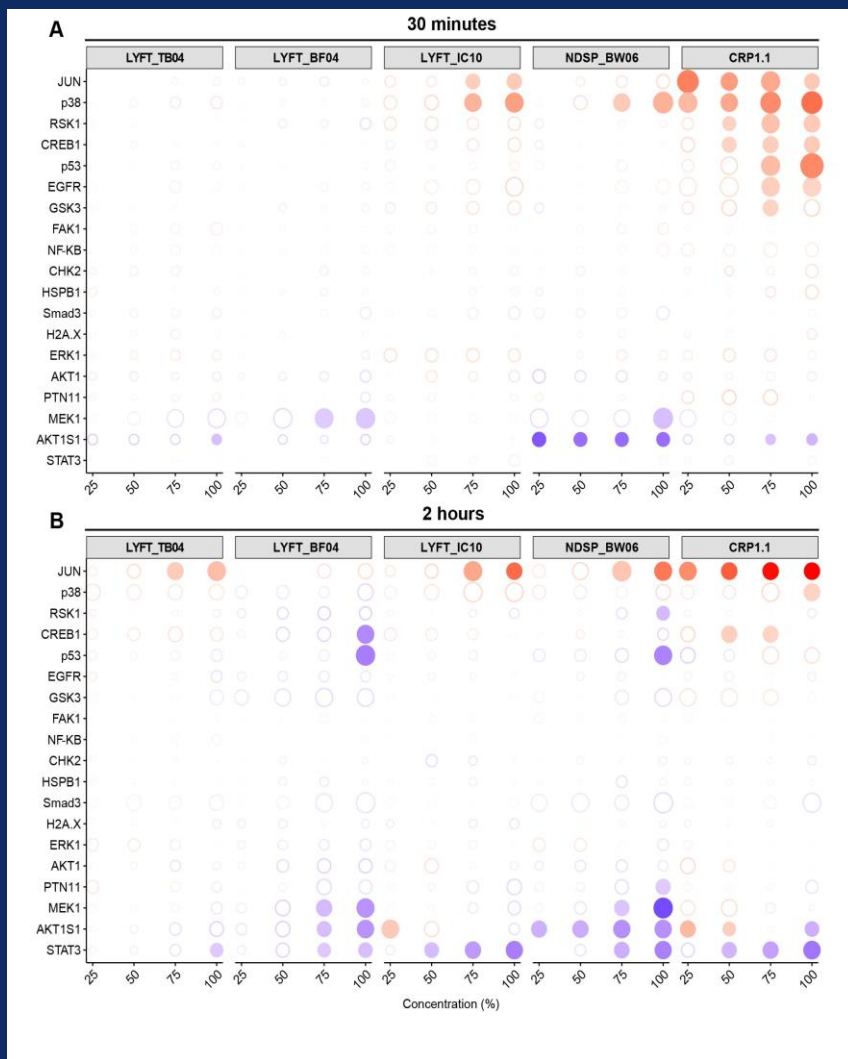


3. Potential signalling pathways or inflammatory response



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

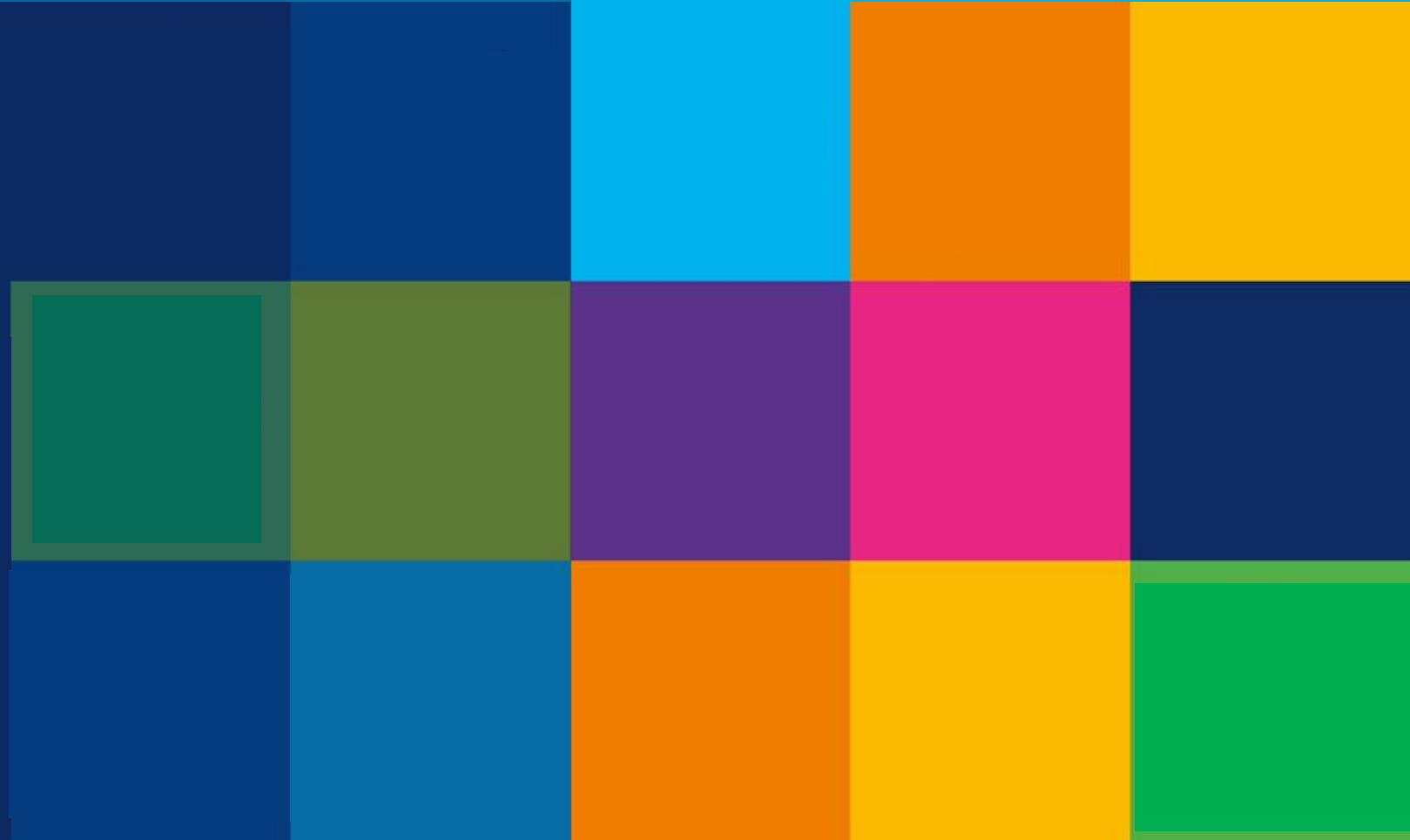
NP extracts did not induce any cytotoxicity or mutagenic response, genotoxic response was minimal and limited signalling or inflammatory markers were induced.

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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