

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

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## Introduction

Tobacco free nicotine pouches (NPs) are a nicotine containing product similar in appearance and concept to Swedish snus. These products aim to further tobacco harm reduction by removing the exposure to tobacco related toxicants. NPs, being a fairly new product category, have limited *in vitro* data. A three-step approach was taken to analyse the biological effects of NPs and snus extracts *in vitro*.

## Methodology

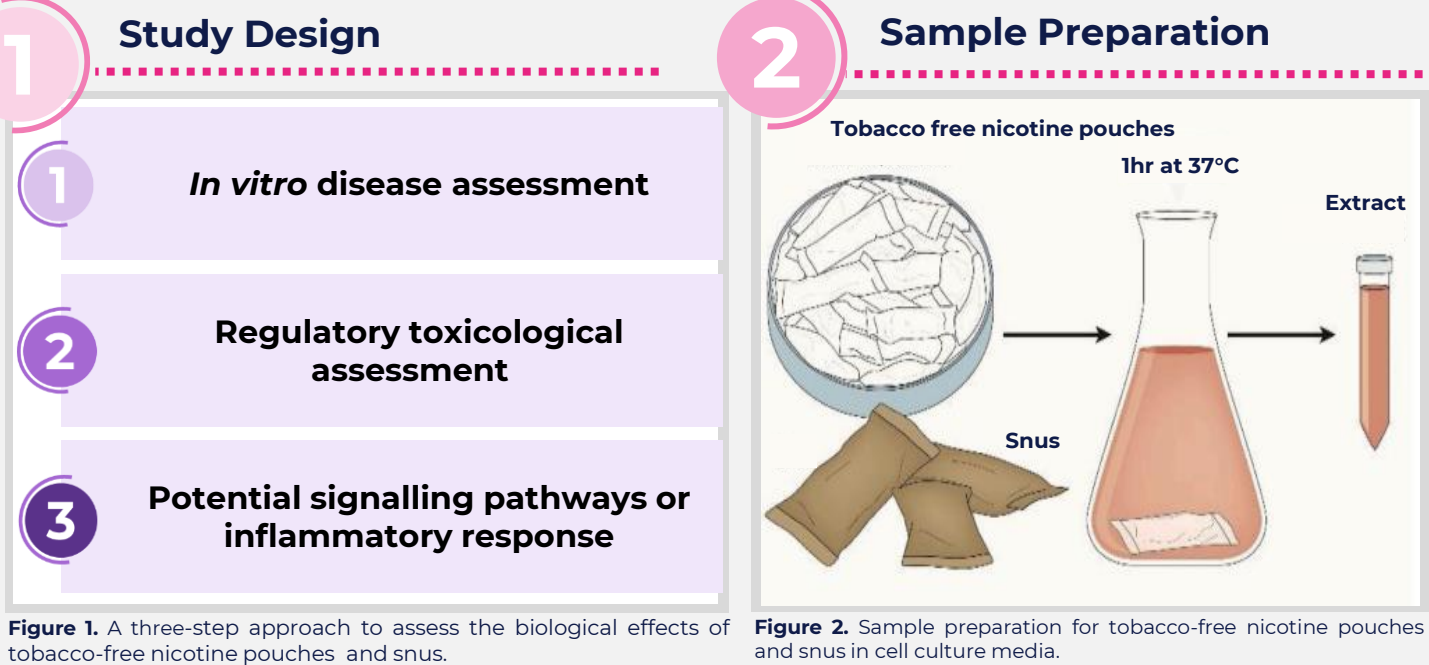


Figure 1. A three-step approach to assess the biological effects of tobacco-free nicotine pouches and snus.

Figure 2. Sample preparation for tobacco-free nicotine pouches and snus in cell culture media.

|                               | Velo*                                                                                                           |               |           | Commercial Comparator                                                                    | CORESTA Reference Product 1.1 |
|-------------------------------|-----------------------------------------------------------------------------------------------------------------|---------------|-----------|------------------------------------------------------------------------------------------|-------------------------------|
| Product Type                  |  Tobacco-Free Nicotine Pouch |               |           |  Snus |                               |
| Source                        | British American Tobacco                                                                                        |               |           | Nordic Spirit                                                                            | CORESTA                       |
| Flavour                       | Berry Frost                                                                                                     | Tropic Breeze | Ice Cool  | Wild berry & Bergamot                                                                    | -                             |
| Nicotine Strength (per pouch) | 4mg                                                                                                             | 4mg           | 10mg      | 6mg                                                                                      | 8mg                           |
| PCode                         | LYFT_BF04                                                                                                       | LYFT_TB04     | LYFT_IC10 | NDSP_BW06                                                                                | CRP1.1                        |

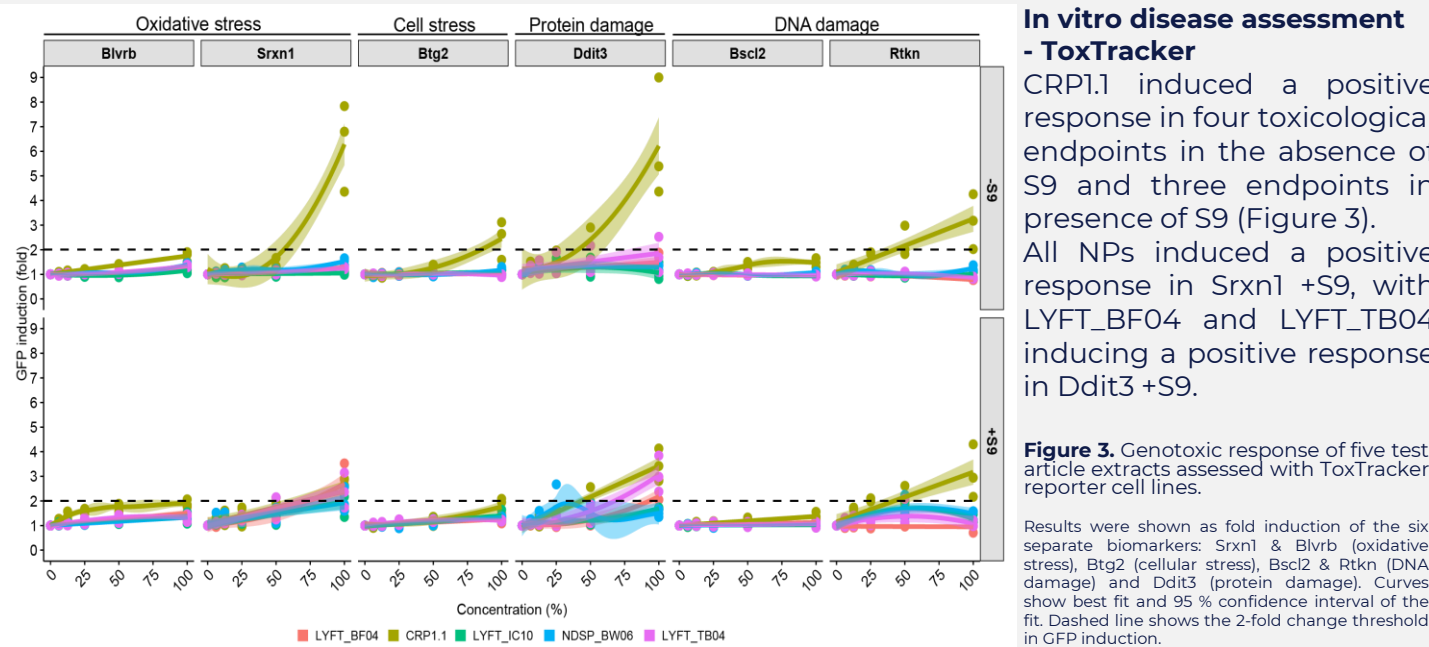
\*VELO previously marketed as LYFT

Table 1. The test products used and assessed

|           | ToxTracker                       | NRU                                      | Ames                                                           | MLA                                              | Signalling                       |
|-----------|----------------------------------|------------------------------------------|----------------------------------------------------------------|--------------------------------------------------|----------------------------------|
| Cell Line | Mouse embryonic stem cell (mESC) | Mouse fibroblasts (Balb/c 3T3 clone A31) | Salmonella typhimurium (TA98, TA100, TA1535, TA1537 and TA102) | Mouse lymphoma cells (LS178Y tk <sup>-/-</sup> ) | NCI-H292 lung carcinoma cells    |
| Readout   | GFP Induction                    | Induction of cell death                  | Mutation Frequency                                             | Induction of mutations                           | Cytokine release                 |
| Citation  | Smart et al 2022 (1)             | OECD Test Guideline No. 129 (2)          | OECD Test Guideline No. 471 (3)                                | OECD Test Guideline NO. 490 (4)                  | Tsolakos et al in submission (5) |

Table 2. Summary of *in vitro* assays used in this study

## Results: *In vitro* disease assessment: ToxTracker



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## Results: 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                             |
|                     | 3 hrs +S9  | X         | X         | X                     | X                             |
|                     | 24 hrs -S9 | ?         | X         | X                     | ✓                             |

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

Table 3. Summary of NRU, Ames and MLA results

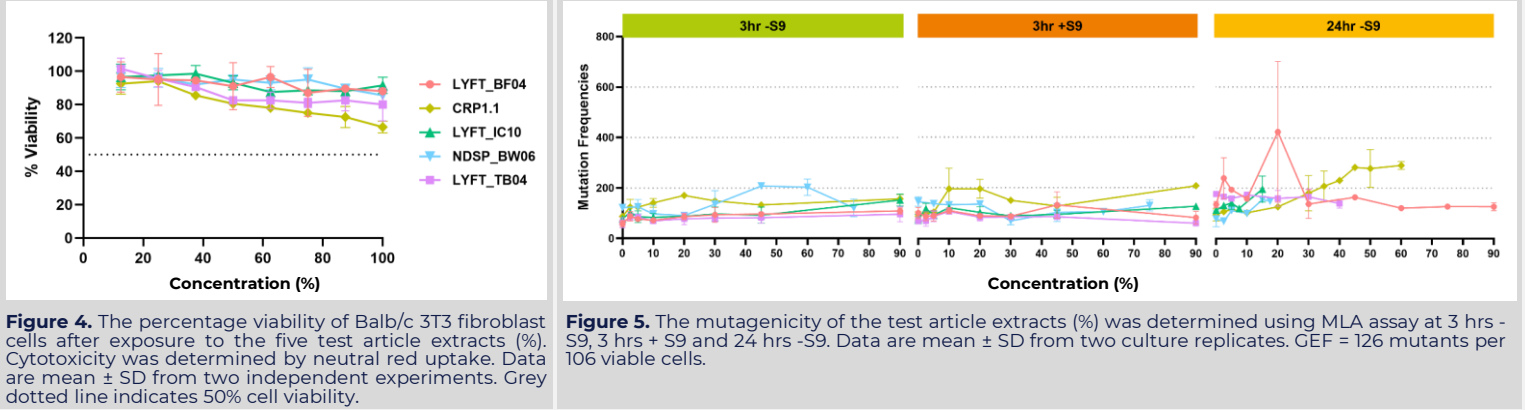
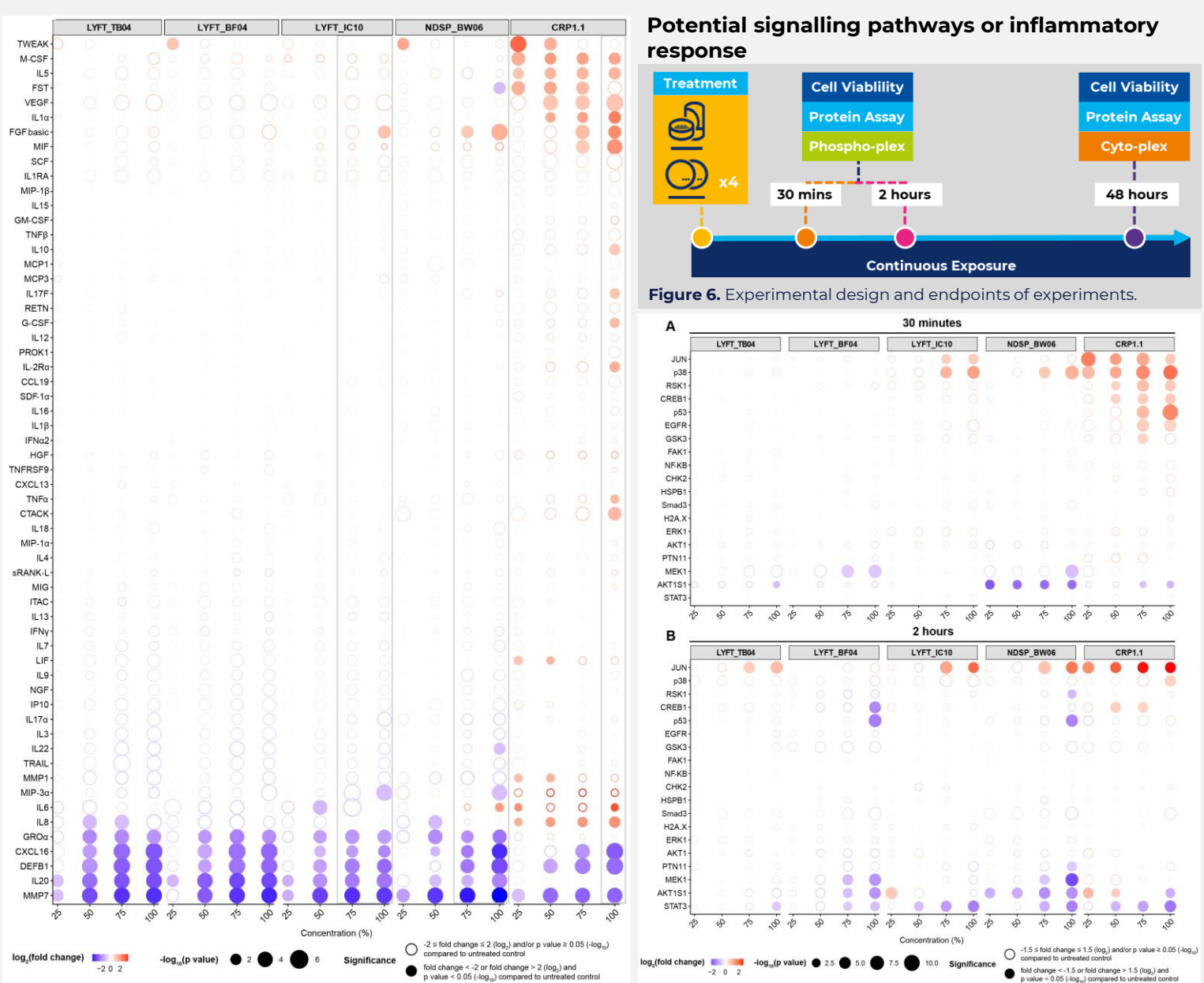


Figure 4. The percentage viability of Balb/c 3T3 fibroblast cells after exposure to the five test article extracts (%). Cytotoxicity was determined by neutral red uptake. Data are mean ± SD from two independent experiments. Grey dotted line indicates 50% cell viability.

Figure 5. The mutagenicity of the test article extracts (%) was determined using MLA assay at 3 hrs -S9, 3 hrs +S9 and 24 hrs -S9. Data are mean ± SD from two culture replicates. GEF = 126 mutants per 106 viable cells.

## Results: Potential signalling pathways or inflammatory response



## Conclusion

This study demonstrated that a weight of evidence approach is required to cover a wide range of endpoints to provide sufficient *in vitro* data for the assessment of potential risk of NP and snus.

To this end we have used a three-step approach to analyse the biological effects of NPs and reference snus extracts in the following areas: cytotoxicity, mutagenicity/ genotoxicity, and cell signalling. In summary, NPs extracts were less biologically active in all endpoints tested, compared to snus, relevant to a range of disease processes.

Taken together with previously published data on chemical analysis and clinical studies, the data presented here contribute to the weight of evidence that suggest NPs should be considered as an alternative reduced risk product.

## Acknowledgements

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## References

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- Tsolakos, N. et al. (2024) Comparative toxicological assessment of cigarettes and new category products via an *in vitro* multiplex proteomics platform, *Toxicology Reports* (In Submission)