# Comparative Toxicity Assessment of the Harm Reduction Potential of Tobacco-Free Oral Nicotine Products

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2024 SOT Annual Meeting and ToxExpo, Salt Lake City
March 10–14, 2024

**Poster 5140/P241**

## Background and purpose

The growing use of flavored oral nicotine pouches (NPs) has prompted investigation into their potential health impact. Placed between the lip and gum, these products contain nicotine, flavorings, sweeteners, and plant-based fibers; they are considered harm reduction alternatives due to their low levels of toxicants compared to cigarette smoke. The present work assessed the ability of the ToxTracker® reporter assay to characterize the toxicological profiles of five NPs (ZYN®) and four Swedish snus (General®) products on the U.S. market and compare them to those of two reference tobacco products.

## Methods

ToxTracker was used to profile NP products and snus products with different flavours and determine if they could be toxicologically differentiated from a reference American-style loose moist snuff product (CRP 2.1) and combustible reference cigarettes (1R6F). ToxTracker combines six fluorescent reporter cell lines that are specifically activated by different cellular responses associated with DNA damage, p53 activation, oxidative stress, or protein damage. Reporter gene activation was measured by flow cytometry, and cytotoxicity of the tested compounds was simultaneously determined by relative cell count. NPs, snus, and CRP2.1 were extracted in dimethyl sulfoxide (DMSO) or complete artificial saliva (CAS), and total particulate matter (TPM) from 1R6F cigarette smoke produced with the Health Canada Intense regime was extracted in DMSO.

**Table 1.**
Extraction conditions – smokeless products.

| DMSO | Extraction | CAS |
|---|---|---|
| 300 mg product/mL | Amount for extraction (un-cut pouches) | 600 mg product/mL |
| 1 h at 50°C | Condition | 2 h at 37°C |
| 1000 rpm x 10 min | Centrifugation | 3400 rpm x 15 min |
| 0.2-µM PTFE filter | Filtration | 0.2-µM PTFE filter |
| -80°C | Storage | -80°C |

The research described in this poster was supported by Swedish Match, which is a subsidiary of Philip Morris International.

## Results

Our data support the reports of Srxn1 and Ddit3 reporter gene induction in ToxTracker assays by combustible cigarettes and snus reference products (Bishop et al., 2020). Cigarette smoke TPM extract induced cytotoxicity and GFP induction of reporter genes for Srxn1 (oxidative stress), Ddit3 (protein damage), and Btg2 (p53 activation) in both the presence and absence of metabolic activation (+ S9). CRP2.1 extract did not induce cytotoxicity but did increase GFP-Ddit3 expression. The snus extracts in DMSO had similar profiles to the CRP2.1 extract, but GFP-Ddit3 expression was lower or below the positive threshold. However, there was no clear induction of GFP-Ddit3 expression when CAS was used for snus extraction. None of the five NPs extracted in DMSO or CAS induced cytotoxicity or GFP expression, suggesting that the tested ZYN products were the least toxic of the products assessed under these experimental conditions.

**Figure 1.**
Srnx1, Ddit3, and Btg2 GFP reporter gene induction by cigarette smoke TPM extract in DMSO. 1R6F extract induced significant cytotoxicity (black dot line).

**Figure 2.**
Ddit3 and Srxn1 GFP reporter gene induction by CRP2.1 extract in DMSO. CRP2.1 extract did not induce cytotoxicity (black dot line).

**Figure 3.**
Snus extracts in DMSO induced GFP-Ddit3 expression in the absence of S9. There was neither cytotoxicity nor GFP induction observed in the presence of S9 (data not shown). Similar results observed for all flavours.

**Figure 4.**
Snus extracts in CAS did not induce any GFP-reporter gene expression. Neither cytotoxicity nor GFP induction was observed in the presence of S9 (data not shown).

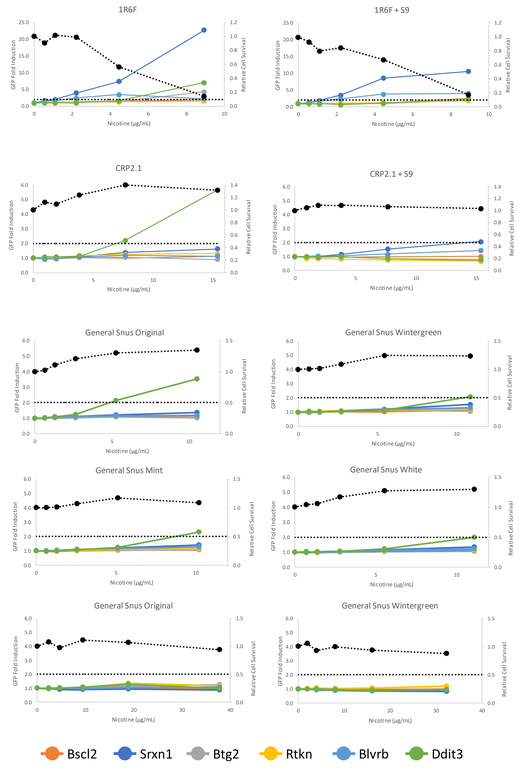


**Figure 5.**
ZYN extracts did not induce cytotoxicity (data not shown) or GFP expression for five different products tested (A-E: DMSO extracts, F-J: CAS extracts).

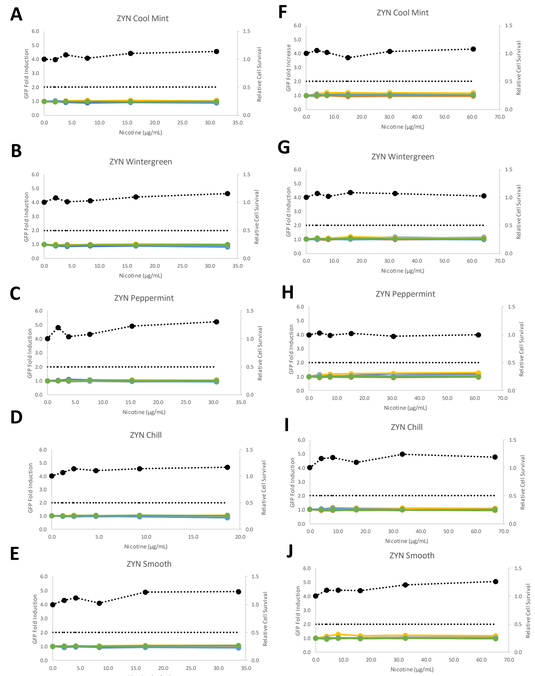


**Table 2.**
Product information.

| Product | Pouch weight (g) | Nicotine (mg/unit) |
|---|---|---|
| **General Snus** Mint[a], White[a], Wintergreen[a], Original[b] | 1.0 | [a]8.0 mg [b]8.5 mg |
| **ZYN** | 0.4 | 6 |
| **CRP2.1** | - | [c]10.4 mg/g |

[c]CORESTA Technical Report 2023

**Table 3.**
Nicotine concentration in extracts.

| DMSO (mg/mL) | Brand name | CAS (mg/mL) |
|---|---|---|
| 0.90 | **C1R6F TPM** | N/A |
| 1.54 | **CRP2.1** | N/A |
| 1.05 | **General Snus Original** | 3.78 |
| 1.09 | **General Snus Wintergreen** | 3.23 |
| 1.02 | **General Snus Mint** | N/A |
| 1.11 | **General Snus White** | N/A |
| 3.32 | **ZYN Wintergreen** | 6.43 |
| 3.12 | **ZYN Cool Mint** | 6.04 |
| 3.06 | **ZYN Peppermint** | 6.14 |
| 1.87 | **ZYN Chill** | 6.70 |
| 3.36 | **ZYN Smooth** | 6.52 |

N/A, not assessed

## References

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## Conclusion

1. This study employed the ToxTracker assay to provide valuable insights into the toxicological profiles of various oral nicotine products to assess their harm reduction potential compared to cigarettes. The results suggest a harm reduction potential of General Snus (tobacco) and ZYN (NP).
2. There was no impact of flavors on cytotoxicity or GFP induction for the different varieties of General Snus or ZYN.
3. Our results indicate that the cruder DMSO extraction induced expression of Ddit3 (CRP2.1 and General Snus Original); however, CAS extracts are more physiologically relevant, and there was no GFP induction for the tested General Snus and ZYN products.
4. These findings contribute to the ongoing research on harm reduction strategies in nicotine consumption, emphasizing the importance of considering specific product types and their safety profiles.