In vitro toxicological assessment of modern oral nicotine products

David Smart, Fabio Miazzi, Stela Bozhilova, Lydia Doherty, Elizabeth Richardson, Olivia Chester, Stuart Meredith, Simone Hadley, Damien Breheny

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

CORESTA PSPT 2025
STPOST60

Introduction

Cigarette smoking remains a leading cause of preventable disease, with risks arising primarily from exposure to toxicants generated from the combustion of tobacco rather than nicotine itself. Tobacco Harm Reduction (THR) strategies aim to reduce the negative public health consequences of tobacco use by offering non-combustible nicotine product alternatives to cigarettes. Snus, a traditional Swedish oral tobacco, has strong reduced risk epidemiological evidence as compared to cigarettes. In 2019, certain General snus products were authorised by the U.S. FDA. as Modified-Risk Tobacco Products with approved package claims of reduced risk of certain diseases as compared to cigarettes. Modern Oral Nicotine Pouches (ONPs), which contain nicotine but no tobacco, have emerged as additional and chemically simpler alternatives to cigarettes. However, limited biological data are available across the diverse ONP market. This study compares the *in vitro* toxicological profiles of 13 oral nicotine products, including two snus references and 11 ONPs, to better understand their potential role in THR.

Methodology

Test Articles

The details of the test articles used in this study are displayed in Table 1. Aqueous Extracts (AqEs) were generated in cell culture media by standard method (1) and nicotine content was quantified via LC-MS/MS (2).

Table 1. Smoking/puffing regimes used to generate the TPM from the products used in this study.

No.	Product	Category	Base	Nicotine*	pH	Moisture (%)	Weight (g)	Format
1	1S4 Snus Reference	Benchmark	N/A	7.3	7.96	53.77	0.95	N/A
2	General Classic Blend	Snus Comparator	N/A	7.2	8.7	53.5	0.84	Large
3	Competitor Product 1	ONP Competitor (US)	N/A	8	N/A	N/A	0.26	Mini
4	Competitor Product 2	ONP Competitor (Non-US)	N/A	9	8.4	39	0.77	Slim
5	Velo Tropic Breeze (Hybrid)	BAT ONP	Hybrid	6	8.8	48	0.7	Slim
6	Velo Salt Swedish	BAT ONP	Salt	6	8.8	48	0.7	Slim
7	Velo Tropic Breeze (Low Nic)	BAT ONP	Sweet	6	8.8	48	0.7	Slim
8	Velo Polar Mint (Low Nic)	BAT ONP	Sweet	6	8.8	48	0.7	Slim
9	Velo Urban Vibe	BAT ONP	Hybrid	10.9	8.8	48	0.7	Slim
10	Velo Salt Ice Cool	BAT ONP	Salt	10	8.8	48	0.7	Slim
11	Velo Tropic Breeze (High Nic)	BAT ONP	Sweet	10.9	8.8	48	0.7	Slim
12	Velo Polar Mint (High Nic)	BAT ONP	Sweet	10	8.8	48	0.7	Slim
13	Velo Salt Freeze	BAT ONP	Salt	20	8.8	48	1.28	Large

Contacts
David Smart, David_smart@bat.com

References
1. Bishop E, East N, Bozhilova S, Santopietro S, Smart D, et al. An approach for the extract generation and toxicological assessment of tobacco-free 'modern' oral nicotine pouches. Food Chem Toxicol. 2020;145:111713
2. Jin M, Earla R, Shah A, Earla RL, Gupte R, Mitra AK, et al. A LC-MS/MS method for concurrent determination of nicotine metabolites and role of CYP2A6 in nicotine metabolism in U937 macrophages: implications in oxidative stress in HIV + smokers. J Neuroimmune Pharmacol. 2012;7(1):289-99.

Methodology (continued)

Biological assessments included:
- Cytotoxicity using Real-Time Cell Analysis (RTCA) on human oral fibroblasts.
- Osmolality measurements of AqEs.
- Genotoxicity using the ToxTracker® reporter assay, with and without S9 metabolic activation.
- Benchmark Dose (BMD) analysis for potency ranking.

All assays were performed in triplicate.

Results

In Vitro Cytotoxicity

All products showed some cytotoxicity at 100% extract, but only four (two snus and two ONPs: Salt Freeze, Competitor Product 2) allowed EC50 determination. Nine ONPs were less cytotoxic than the snus reference. When aligning the four most active products by measured nicotine, Salt Freeze demonstrated lowest cytotoxicity per unit nicotine.

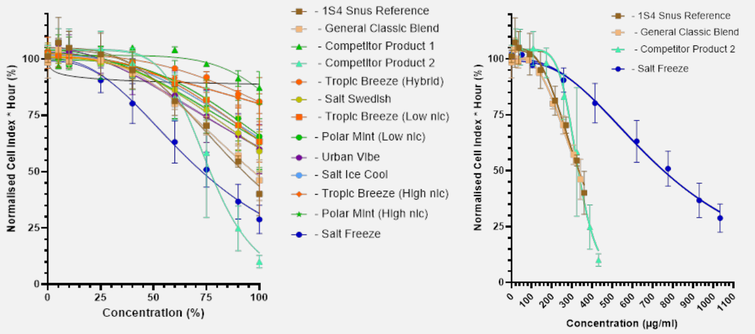

Figure 1. Test article cytotoxicity by RTCA, A) By concentration of AqE product extract, B) Measure extract nicotine concentration.

Osmolality

Cytotoxicity correlated partly with pouch mass and osmolality. Heavier pouches (snus, Salt Freeze, Competitor Product 2) produced the highest osmolality values, suggesting formulation components contribute to observed cytotoxicity.

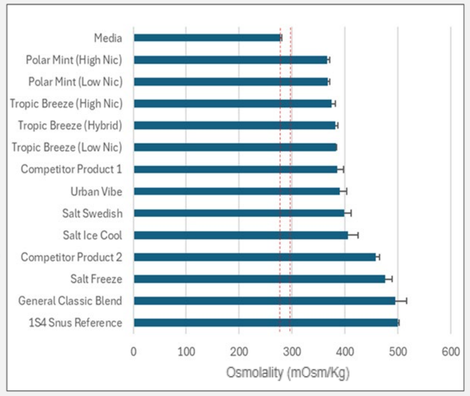

Figure 2. Measured osmolality of test article AqEs, Red dotted lines indicate normal isotonic range (plasma).

Genotoxicity

No product induced DNA damage markers. Snus samples induced oxidative stress and protein/cellular stress responses. Among ONPs, only Salt Swedish (oxidative stress) and Tropical Breeze (protein stress in presence of S9) showed weak positive responses. Overall, ONPs demonstrated equal or lower biological activity compared with snus.

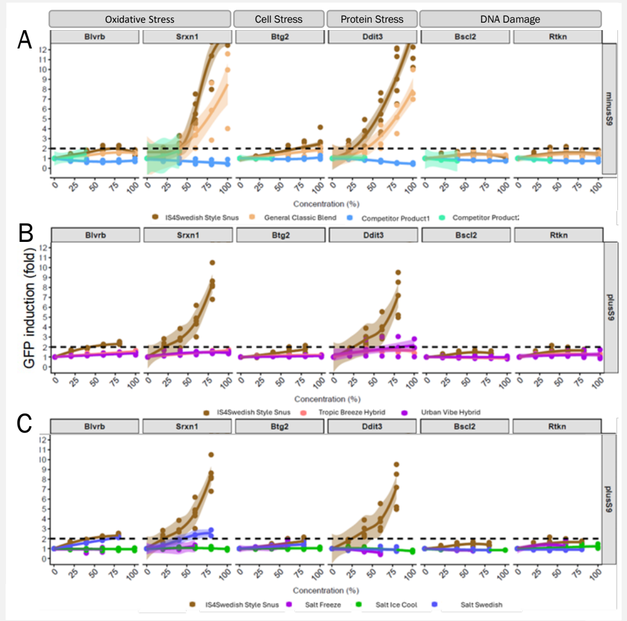

Figure 3. ToxTracker plots for all endpoints +/- S9, A) Commercial competitors B) Hybrid base product C) Salty base products.

Conclusion

The ONPs assessed in this study displayed limited cytotoxic and genotoxic activity *in vitro*, often lower than snus. Cytotoxicity was partly linked to osmolality, though oral mucosa *in vivo* is tolerant of hypertonic solutions.

Importantly, none of the ONPs induced DNA damage markers, and most had weaker responses in stress-related biomarkers compared with the 1S4 snus reference product.

These findings, together with existing epidemiological data on snus, support the potential of ONPs as lower-risk alternatives to cigarettes that may contribute to THR where appropriately regulated.

Point your phone's camera at the QR code to find our library of publications

Follow us:
www.bat-science.com welcometobat @BAT_Sci