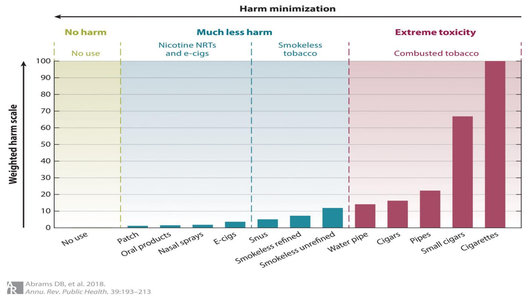
Comparative Analysis of in vitro Testing for Oral Nicotine Products Versus Combustible Cigarettes: Implications for Public Health and Harm Reduction Strategies

[1]Katarina Aleksa and [2]Bonnie Coffa

[1]Labstat International, Kitchener, ON, Canada; [2]ToxPharm LLC

Tobacco harm reduction (THR) refers to strategies designed to decrease the health risks associated with tobacco use, especially for individuals unable or unwilling to quit smoking entirely. THR products provide less harmful alternatives to traditional combustible cigarettes, including heated tobacco products (HTPs), electronic nicotine delivery systems (ENDS), and oral nicotine pouches. The latter category, comprising both oral tobacco-derived nicotine pouches (OTDNs) and oral non-tobacco-derived nicotine pouches (ONPs), is rapidly gaining global popularity, particularly in markets outside traditional smoking cultures. These oral nicotine products, which include smokeless tobacco like chewing tobacco and nicotine pouches that don't contain tobacco leaf, offer a nicotine delivery method without combustion, significantly reducing exposure to harmful chemicals found in tobacco smoke. As in vitro toxicological data on oral pouch products continue to emerge, evidence increasingly supports that these products present a much lower toxicity profile and different health outcomes compared to traditional tobacco products. As the global market for THR products expands, driven by rising demand in the U.S. and Europe as health-conscious users seek safer alternatives to smoking, it is important to understand how these new emerging products affect health outcomes.

Figure 1: Harm minimization by class of nicotine product



Abrams DB, et al. 2018. *Annu. Rev. Public Health.* 39:193–213

Method

This study involved a secondary analysis of existing data, concentrating on oral nicotine products and specifically examining both tobacco-derived and non-tobacco-derived nicotine pouches. A range of flavored products from 17 different brands were examined. In vitro toxicological data were collected following Health Canada methods, including the AMES test (T-501), the Neutral Red Uptake (NRU) assay with CHO cells (T-502), and the in vitro micronucleus (MN) assay with manual scoring (T-503), with modifications based on OECD Test Guidelines. The data was anonymized and evaluated as a product category, allowing for comparison across different brands and flavors.

The same extraction process was utilized for OTDN and ONP. The only difference was the solvent utilized – DMSO or complete artificial saliva (CAS). Samples were diluted with media following the extraction process.

Figure 2: General extraction procedure for OTDN and ONP.

Methods

Nicotine Content of Products Tested:

Seventeen products (12 ONP products and 5 OTNP) with varying flavor profiles and nicotine content were tested. Products were extracted and assayed utilizing Health Canada T-501 (Ames), T-502 (NRU) and T-503 (MN) methods.

Product Type	Nicotine	Flavors	Number of Brands
ONP (Pouches)	6 to 8 mg nicotine	Various flavors	10
OTDN (Snus, MRP, Gum/Lozenge, Pouch)	4 - 12 mg nicotine	Various flavors	17
1R6F	10mg	N/A	1

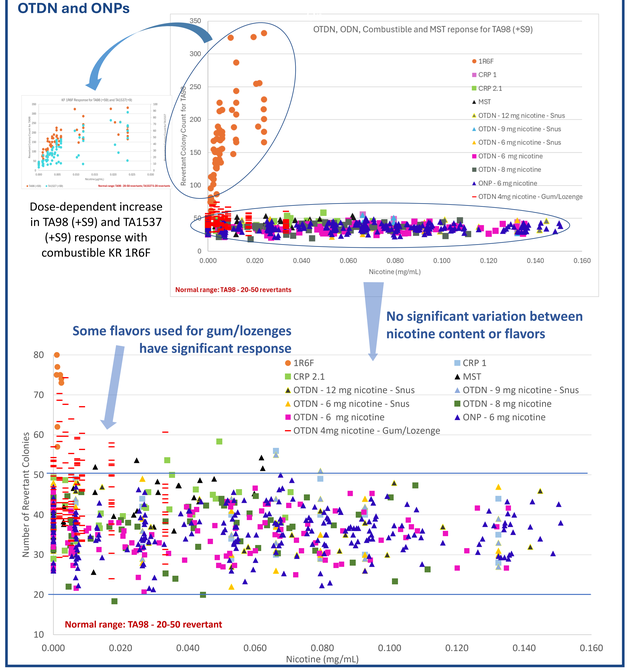
Results

The results indicate that, regardless of flavor, the nicotine pouches tested did not show mutagenicity (AMES test), genotoxicity (MN assay), or cytotoxicity (NRU assay) compared to the solvent control. In contrast, the 1R6F (Kentucky Reference Cigarette) exhibited dose-dependent mutagenicity within the concentration range of 0-24 µg nicotine per plate. The nicotine pouches, tested up to 160 µg/mL nicotine, did not show mutagenicity, cytotoxicity or genotoxicity across the various brands and flavors evaluated. Despite an overall lack of toxicity, some variability in cytotoxicity and mutagenicity assay responses was observed across different flavors, with no clear correlations identified.

AMES ASSAY:

The results for TA100 (+/- S9), TA102 (+/- S9), TA1535 (+/- S9) and TA1537 (+/- S9) were similar to TA98 (+/- S9) and are not shown below.

Figure 3: Comparison of Mutagenicity (AMES) for TA98 (+) S9 between Combustible, OTDN and ONPs



Results

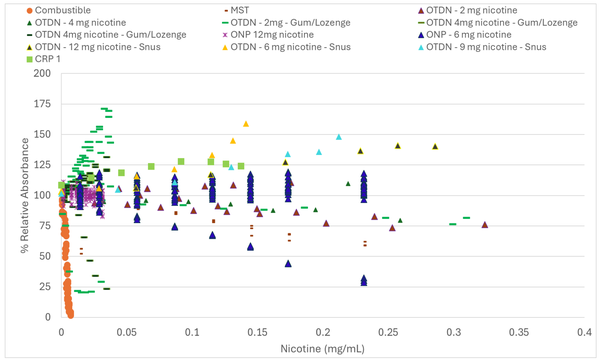
NRU ASSAY:

Cytotoxicity (T-502) was assessed by treating CHO-WBL cells with samples extracted with either CAS or DMSO. No significant differences in cytotoxicity were evident between samples extracted with DMSO or CAS. The data presented is a mix of samples extracted with DMSO or CAS.

The KR1R6F produced a dose-dependent decrease in cytotoxicity, while cytotoxicity was not observed for OTDN or ONP for most flavors tested up to 340ug/mL nicotine.

There was a dose-dependent decreases in cytotoxicity for products containing cinnamon flavoring. This was observed across brands and matrices.

Figure 4: Comparison of Cytotoxicity (MN) in CHO cells between Combustible and Oral Nicotine Products

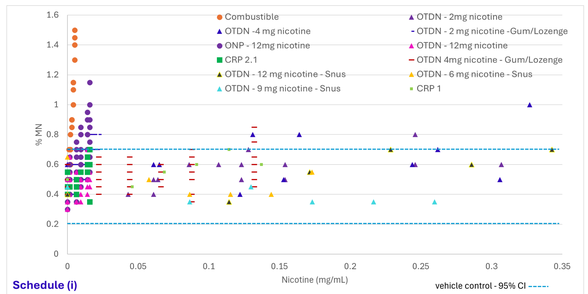


ivMN ASSAY:

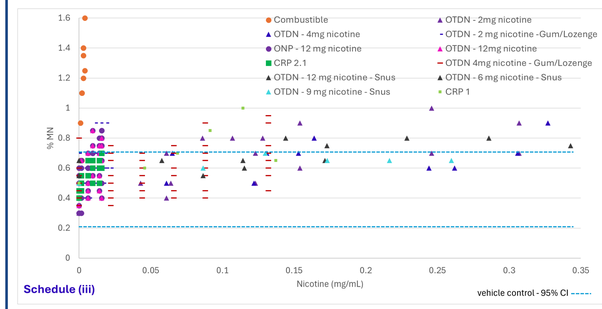
Genotoxicity (T-503) was assessed by treating CHO-WBL cells with samples extracted with either CAS or DMSO. No significant differences in cytotoxicity were evident between samples extracted with DMSO or CAS. The data includes samples extracted using either DMSO or CAS. CHO-WBL cells were treated with CAS or DMSO extracts of KR1R6F, OTDN, or ONP, following the T-503 protocol. Micronucleus was evaluated using the manual slide scoring method.

A dose-dependent increase in %MN was observed for KR1R6F and ONP with 12mg nicotine in schedule (i), (ii) and (iii).

Figure 5: Comparison of % MN in ivMN assay in CHO cells between Combustible, OTDN and ONPs



Results



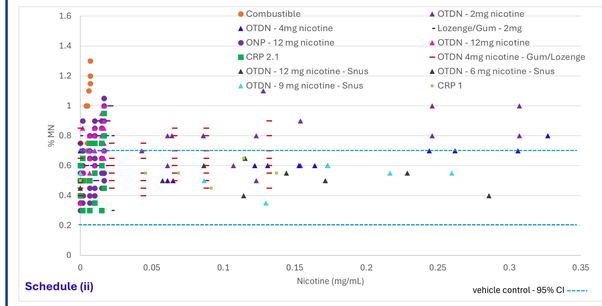
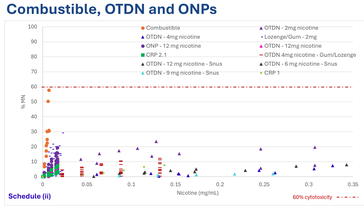


Figure 6: Comparison of % Cytotoxicity in ivMN assay in CHO cells between Combustible, OTDN and ONPs



Cytotoxicity was comparable for all three schedules. The graph (left) is a representation of all three schedules

Conclusion

- Despite the small sample size, data suggests that oral nicotine pouches, whether tobacco-derived or non-tobacco derived (synthetic), do not generally elicit positive responses in mutagenicity, cytotoxicity, or genotoxicity assays.
- In contrast, traditional combustible cigarettes showed positive responses across all three assays.
- OTDN and ONP products with cinnamon flavors exhibited cytotoxicity.
- Further testing is necessary across the nicotine pouch category, particularly for high-nicotine products and other variations, to validate these findings.
- No differences in responses were observed between extractions using DMSO and CAS, suggesting that the extraction vehicle does not influence the assay outcomes.