

IN VITRO TOXICOLOGICAL ASSESSMENT OF NIC NAC ORAL NICOTINE LOZENGE PRODUCTS

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ABSTRACT

The in vitro toxicological assessment of oral nicotine products is an important component of the toxicological evaluation submitted in support of premarket tobacco product applications (PMTAs). The Nic Nac lozenges contain synthetic nicotine blended with xylitol, binders, and flavors. They do not contain cut, ground, powdered, or leaf tobacco and are made by blending all of the ingredients and compressing into a lozenge, using a tablet press. The use of these lozenge products is similar to a typical oral NRT lozenges such as Nicorette.

This toxicological assessment of nine flavored Nic Nac lozenge products with 6mg nicotine load was conducted using a battery of well-established in vitro regulatory assays, including the bacterial reverse mutation Ames assay (OECD TG 471); in vitro micronucleus assay (OECD TG 487); and the neutral red uptake cytotoxicity assay (OECD No. 129). For each product, extracts were prepared in dimethyl sulfoxide (DMSO) and evaluated within the established extract-stability window, as confirmed by nicotine analysis. Under the conditions of the assays and according to the predefined evaluation criteria, the Nic Nac lozenge products demonstrated no evidence of mutagenicity in the Ames assay, no evidence of genotoxicity in the in vitro micronucleus assay, and no evidence of cytotoxicity in the neutral red uptake assay.

These findings support the conclusion that the tested Nic Nac oral nicotine lozenge products do not raise an in vitro toxicological concern for mutagenicity, genotoxicity, or cytotoxicity under the experimental conditions evaluated.

STUDY DESIGN AND METHODS

Nic Nac Lozenge Products

Nic Nac Lozenge products are available in 3 and 6 mg nicotine strengths in nine different flavors. The lozenges are xylitol based. As the nicotine is increased, the amount of xylitol is reduced. All other ingredients except flavors a are the same in the different products. Testing was conducted using the highest nicotine-strength product because it represents the most conservative formulation within the product family with respect to nicotine exposure. The product strengths are manufactured using the same materials, ingredients, manufacturing process, and lozenge configuration. Accordingly, the highest-strength product provides the greatest potential nicotine-related exposure and is considered an appropriate worst-case representative of the lower-strength products. Because the test article was evaluated at a range of concentrations extending to the maximum technically feasible or biologically tolerated concentration, testing the highest-strength formulation also maximized the amount of nicotine and product-derived constituents introduced into the test system.



The tests were conducted at Labstat International, Inc. Kitchener, ON, Canada following OECD and GLP guidelines.

Extract Preparation and Characterization

DMSO was used as extraction solvent for the in vitro toxicological assays and was intended as a conservative product-extract hazard assessment rather than a simulation of physiological oral exposure. The lozenge was ground and sieved through a 4 mm sieve to ensure a particle size of ≤ 4 mm and extracted with DMSO with the ultrasonic homogenizer at 37°C incubator for 21 hours. The extracts were shaken, filtered, and centrifuged to remove any particulate matter. The sterility of each extract was confirmed by the absence of microbial growth. The nicotine in the extracts was determined by a GC-FID method. Appropriate amounts of the filter-sterilized extracts were used for the Ames assay, NRU assay, ivMN assay and chemical analysis. Extract analysis was performed at the initial extraction and at approximately 1 (T1) and 2 months (T2) later.

In Vitro Toxicology Assays

Assay	Method	System
Bacterial Reverse Mutation (Ames) Assay	OECD 471	<i>S. typhimurium</i> strains (TA98, TA100, TA102, TA1535, and TA1537)
Neutral Red Uptake (NRU) Cytotoxicity Assay	OECD 129 HC T-502	Chinese hamster ovary (CHO-WBL) cells
In Vitro Micronucleus (ivMN) Assay	OECD TG 487	Human Lymphoblast TK6 cells

RESULTS

EXTRACT STABILITY

		Peppermint 6 mg			Cool 6 mg			Wintergreen 6 mg			Grapefruit 6 mg			Lemon 6 mg			Tangerine 6 mg			Spearmint 6 mg			Classic 6 mg			Blood Orange 6 mg		
Time Point (month)		T0	T1	T2	T0	T1	T2	T0	T1	T2	T0	T1	T2	T0	T1	T2	T0	T1	T2	T0	T1	T2	T0	T1	T2	T0	T1	T2
Nicotine (mg/mL)	Mean	0.777	0.858	0.802	0.782	0.883	0.879	0.703	0.814	0.841	0.798	0.806	0.85	0.741	0.717	0.799	0.702	0.815	0.758	0.781	0.818	0.834	0.829	0.879	0.83	0.606	0.54	0.576
	SD	0.02	0.038	0.058	0.03	0.067	0.074	0.068	0.061	0.055	0.029	0.045	0.025	0.015	0.037	0.054	0.006	0.045	0.032	0.042	0.007	0.021	0.041	0.15	0.041	0.007	0.005	0.003
% Difference Compared to T0		-	10.4	3.2	-	12.9	12.4	-	15.8	19.6	-	1	6.5	-	3.2	7.8	-	16.1	8	-	4.7	6.8	-	6	0.1	-	10.89	5.56

IN VITRO TOXICITY

NIC NAC PRODUCTS	CYTOTOXICITY	MUTAGENICITY	GENOTOXICITY
Peppermint 6 mg	Not Cytotoxic	Not Mutagenic	Not Genotoxic
Cool 6 mg	Not Cytotoxic	Not Mutagenic	Not Genotoxic
Wintergreen 6 mg	Not Cytotoxic	Not Mutagenic	Not Genotoxic
Grapefruit 6 mg	Not Cytotoxic	Not Mutagenic	Not Genotoxic
Lemon 6 mg	Not Cytotoxic	Not Mutagenic	Not Genotoxic
Tangerine 6 mg	Not Cytotoxic	Not Mutagenic	Not Genotoxic
Spearmint 6 mg	Not Cytotoxic	Not Mutagenic	Not Genotoxic
Classic 6 mg	Not Cytotoxic	Not Mutagenic	Not Genotoxic
Blood Orange 6 mg	Not Cytotoxic	Not Mutagenic	Not Genotoxic

CONCLUSIONS

The in vitro toxicity of the DMSO extracts of Nic Nac nicotine lozenge products were assessed following OECD and GLP guidelines.

- Cytotoxicity (NRU Assay):** Nic Nac nicotine lozenge products were not considered cytotoxic across the tested eight non-zero concentrations in comparison to vehicle control. No concentration-dependent cytotoxicity was observed, and cell viability remained >70% at all tested concentrations, therefore, an EC50 could not be calculated.
- Mutagenicity (Ames Assay):** The Nic Nac nicotine lozenge products were not considered mutagenic in five tester bacterial strains in the absence or presence of metabolic activation across seven non-zero tested concentrations in comparison to vehicle control.
- Genotoxicity (in vitro Micronucleus Assay):** The Nic Nac nicotine lozenge products were not considered genotoxic across five non-zero tested concentrations in comparison to vehicle control under both short-term and long-term conditions of the assay.
- Overall,** the nine flavored Nic Nac nicotine lozenge products do not raise an in vitro toxicological concern for mutagenicity, genotoxicity, or cytotoxicity under the experimental conditions evaluated.
- Comparatively,** published study using OECD-based in vitro assays have reported significant cytotoxicity, mutagenicity and genotoxicity for 1R6F cigarette smoke TPM (Yu et al. 2022).

REFERENCES

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