Evaluation of the in vivo genotoxic potential of an oral nicotine pouch product following ICH S2(R1) guidance

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Introduction

In this study, a test oral nicotine pouch (NP) product was evaluated in the in vivo genotoxicity study using two separate endpoints (in vivo micronucleus (MN) and DNA damage). This was an in vivo follow-up investigation according to the ICH S2(R1) guidance (ICH, 2012) for positive in vitro MN responses of the Test NP (Mariana et al., Abstract 3052/P154). The results of toxicity testing was also evaluated in the context of individual ingredient toxicological assessment (Pitegoff et al., Abstract 3636/P137), which identified maltols (maltol and ethyl maltol) were likely the key drivers for in vitro MN genotoxicity, however without leading in vivo genotoxicity or carcinogenicity (Gralla et al., 1969).

3636/P137
- Pitegoff et al.
- Hazard Identification and Risk Assessment of Non-Nicotine Ingredients in Oral Nicotine Pouches

3052/P154
- Farcas et al.
- Comparative Toxicity Assessment of Oral Nicotine Pouches to Combustible Cigarettes, Smokeless Tobacco Products and Market Nicotine Pouches Using Regulatory in vitro Cytotoxicity, Mutagenicity, and Genotoxicity Assays

3108/P212
- Zhang et al.
- Evaluation of the in vivo genotoxic potential of an oral nicotine pouch product following ICH S2(R1) guidance (This Poster)

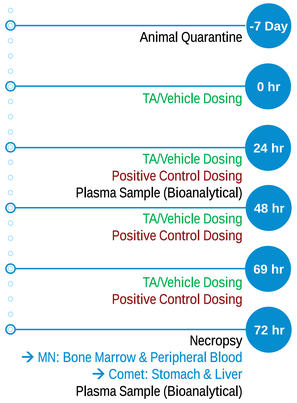
Method

Test Material
- Negative (Vehicle) Control: Enzyme-free artificial saliva
- Test Article (TA): Test Mint NP (6 mg nicotine) extract in the enzyme-free artificial saliva, 10% w/v
- Positive Control: Ethyl Methanesulfonate (EMS)

Main Study (GLP) - Genotoxicity: MN and Comet

Group Number	Sex	Animal Number	Dose Group	Approx. Dose Level (mg nicotine/kg BW)	Gavage Volume (mL/kg BW)
1	M	6	Vehicle Control	0	16
2	M	6	TA-low	3	16
3	M	6	TA-Mid	6	16
4	M	6	TA- High	12 [b]	16
5	M	6	Positive Control [a]	15	10

The study was designed following OECD test Guidelines 474 (OECD, 2016a) and 489 (OECD, 2016b)
[a] EMS, 150 mg/kg bw /day via oral gavage from Day 2 to Day 4. [b] The MTD (max. tolerated dose) as evaluated in a Range Finding study was used as the high dose for test article. The nicotine concentration in the neat test article was ~0.75 mg/mL as measured. Daily gavage volume was 8 mL/kg body weight on Day 1 (half dose) and 16 mL/kg body weight from Day 2 to Day 4.



Conclusions: Based on the totality of evidence (including individual ingredient toxicological evaluation, in vitro and in vivo follow-up results), the Test NP does not pose a meaningful toxicological concern. Overall, these results support the reduced risk potential of the Test NP and its role in tobacco harm reduction.

Results – MTD and Biomarkers of Exposure

Figure 1 Main Study: Body Weight Gain

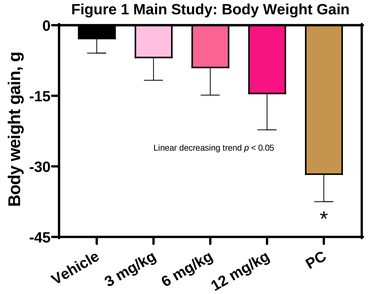
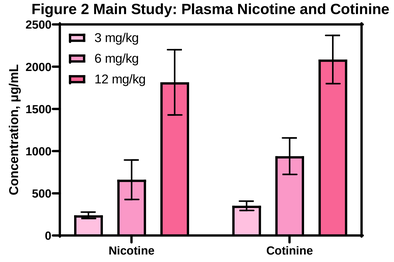


Figure 2 Main Study: Plasma Nicotine and Cotinine



Confirmation of Dose Selection:
The top dose of the main study was the maximum tolerated dose for the test article group, which was confirmed by transient clinical signs of acute toxicity (data not presented) and the dose-related reductions in the body weight gain (Fig. 1).

Confirmation of Exposure:

Exposure was confirmed by dose-related increases in the plasma nicotine and cotinine concentrations 1 hour after dosing in the treatment groups. The levels of plasma nicotine and cotinine in the vehicle control group were below the limit of quantitation (5 ng/mL). (Fig. 2)

Results – In Vivo DNA Damage (Comet)

- Test NP is concluded negative in the in vivo DNA damage (comet) genotoxicity in the stomach (first site of contact) and the liver (primary organ of metabolism) tissues.
- The slight (<6%) but statistically significant increases in the liver %Tail DNA were not considered biologically significant, within the assay variability reported (Dertinger et al., 2023).

Figure 3 Main Study Stomach Comet

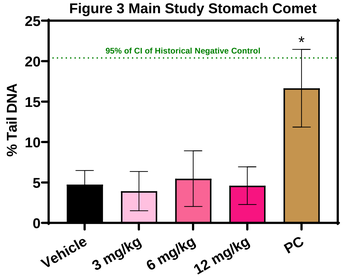
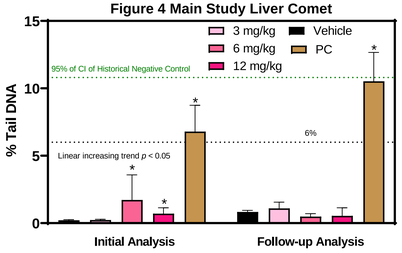


Figure 4 Main Study Liver Comet



Results – In Vivo MN

- Test NP was negative in the in vivo MN genotoxicity in the peripheral blood (Fig. 5) and bone marrow (Fig. 6).
- Significantly lower %PCE in the peripheral blood in the mid- and high dose groups further confirmed exposure to target tissue (Fig. 5a).

Figure 5a Main Study Blood Cytotoxicity

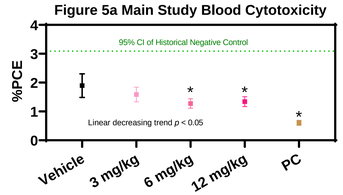


Figure 6a Main Study Bone Marrow Cytotoxicity

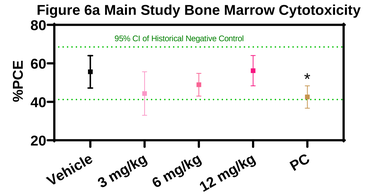


Figure 5b Main Study Blood Genotoxicity

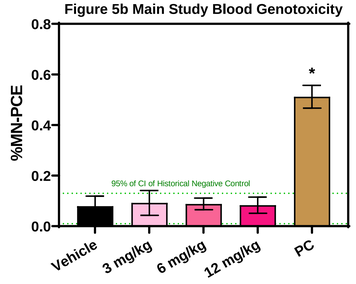
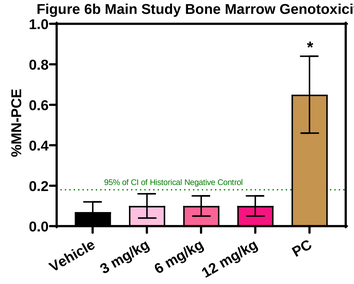


Figure 6b Main Study Bone Marrow Genotoxicity



Strength and Limitation

- **Strength:** The study was conducted according to OECD test guidelines and ICH guidance, meeting all quality criteria. Multiple in vivo genotoxicity endpoints were evaluated in two target tissues: in vivo MN in hematopoietic cells (bone marrow and peripheral blood) and DNA strand breakage in the tissues of the first site of contact (stomach) and the primary organ for metabolism (liver).
- **Limitation:** The route of exposure in this study (oral bolus gavage) is not the same as the main route of exposure in human for NPs (oral mucosa absorption). While the dose range is wide enough to include MTD, the exposures were relative short (4 days), and the long-term carcinogenicity assessment is beyond the scope of testing.

Reference

Dertinger, S.D., et al. (2023). " Assessing the quality and making appropriate use of historical negative control data: A report of the International Workshop on Genotoxicity Testing (IWGT)." Environ Mol Mutagen. https://doi.org/10.1002/em.22541
Gralla, E. J., et al. (1969) "Toxicity studies with ethyl maltol." Toxicology and Applied Pharmacology 15(3): 604
ICH (2012). "International Conference on Harmonisation; S2(R1) Genotoxicity Testing and Data Interpretation for Pharmaceuticals Intended for Human Use."
OECD (2016a). "Test No. 474: Mammalian Erythrocyte Micronucleus Test." OECD Guidelines for the Testing of Chemicals. "
OECD (2016b) "Test No. 489: In Vivo Mammalian Alkaline Comet Assay,: OECD Guidelines for the Testing of Chemicals.

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