Evaluation of the Genotoxic Potential of a Flavored Oral Nicotine Pouch Product Using Integrated Approaches

Zhang, J., R Morgan, U Doshi, C Anderson, W Kangethe, D Smith, and K. Monica Lee

Altria Client Services LLC, Richmond, VA 23219
Center for Research and Technology

The 1st International Electronic Conference on Toxics
Session B (Drugs Toxicity and Safety) & E (Novel Methods in Toxicology Research)

March 20th 2024

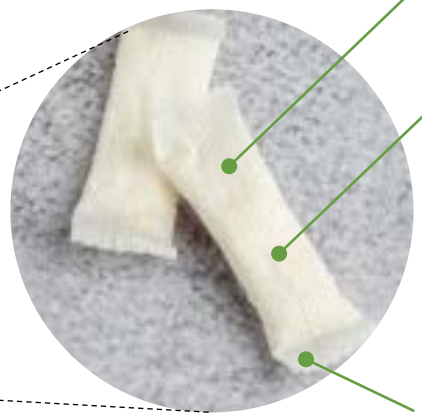
Table of Content

Altria Science

Toxicity Assessment of Oral Nicotine Pouch Products

- Oral nicotine pouch (NP) products may aid adult tobacco consumers to switch from cigarettes to potentially reduced-harm, smoke-free products.
- These products typically consist of tobacco-derived nicotine, flavors and other excipients.
- Flavoring compounds, mostly generally recognized as safe (GRAS), have been thoroughly evaluated for their safety under conditions of the intended use (e.g., food and beverages).
- Although the GRAS status of flavoring compounds are NOT intended for the tobacco products, the toxicological data are relevant, especially for oral products.

Image courtesy of iStock.com/Oleksandr Shatyrov



Oral Nicotine Pouches

GRAS
GENERALLY RECOGNIZED AS SAFE

Image adapted from Food Business News

GRAS Reference:
Generally Recognized as Safe (GRAS) | FDA

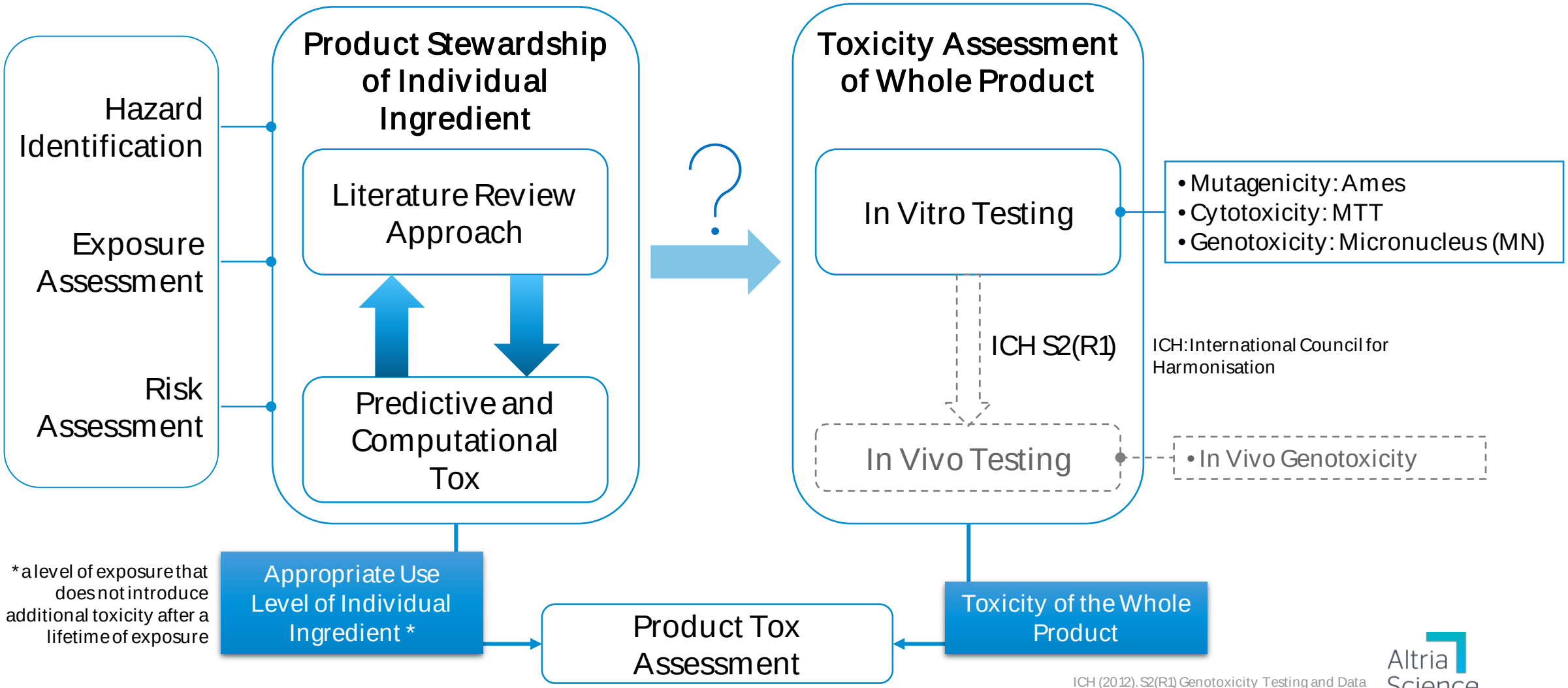
NOT Intended for Tobacco Products

FEMA. (2018) Safety Assessment and Regulatory Authority to Use Flavors – Focus on Electronic Nicotine Delivery Systems and Flavored Tobacco

Altria Science

Concept: Toxicity Assessment of Oral Nicotine Pouch Products

- Can the data from individual ingredients inform the toxicological profile of the whole product?

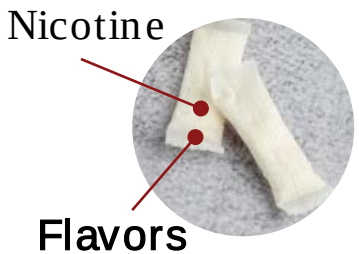


ICH (2012). S2(R1) Genotoxicity Testing and Data Interpretation for Pharmaceuticals Intended for Human Use

Altria Science

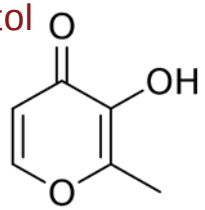
Case Study: Ingredient Assessment of a Prototype Flavored NP Product

Ingredient Assessment: Are there any potential toxicological concerns?

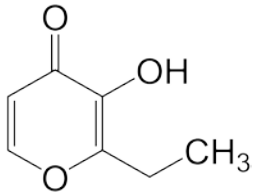


Flavors

- Maltol



- Ethyl Maltol



- Others (proprietary & no concern)

Key takeaways: for both maltol and ethyl maltol ("maltols")

- In Vitro
  - Mammalian in vitro assay: Possibly POSITIVE
  - Ames: Possibly POSITIVE
- In Vivo
  - NEGATIVE

Ingredient Assessment: Maltol and Ethyl Maltol	
In-vitro genotoxicity	• Mixed (equivocal) in mammalian in-vitro studies • With some positive responses observed in Ames assay
In-vivo genotoxicity	• Considered non-genotoxic by JECFA, EFSA, and ECHA (Negative in vivo MN-comet assay) • Negative in vivo MN and comet assay via oral route (EFSA, 2015)

Carcinogenicity	• Not Carcinogenic via oral route
	  • Maltol: in-silico prediction
	  • Ethyl Maltol: chronic toxicity study (Gralla et al. 1969); in-silico prediction

Adapted from: Molly et al. (2024) Hazard Identification and Risk Assessment of Non-Nicotine Ingredients in Oral Nicotine Pouches. SOT 2024. Salt Lake City.
EFSA. (2015) Scientific Opinion on Flavouring Group Evaluation 213, Revision 2 (FGE.213Rev2): Consideration of genotoxic potential for α,β-unsaturated alicyclic ketones and precursors from chemical subgroup 2.7 of FGE.19
Gralla et al. (1969) Toxicity studies with ethyl maltol. Toxicol. Appl Pharmacol. 15(3):604

Altria Science

Case Study: In Vitro Assessment of a Prototype Flavored NP Product

Ingredient Assessment: Are there any potential toxicological concerns?

- YES, maltol and ethyl maltol ("maltols")
- Maltols could lead to POSITIVE in vitro genotoxic response.
- However, maltols are NEGATIVE in vivo via oral route.

In Vitro: Would the whole Test NP lead to POSITIVE in vitro response?

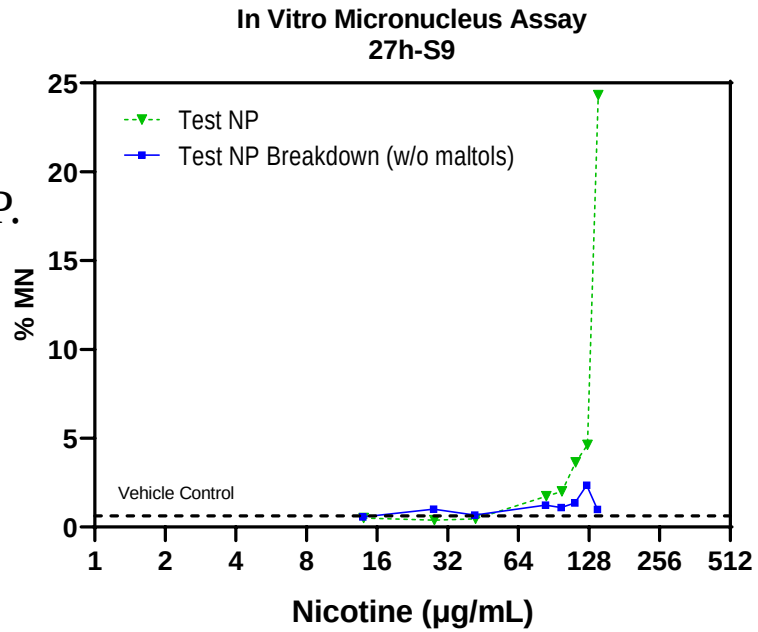
Key takeaways:

- The test NP was tested POSITIVE in vitro in MN assay.
- Maltols drive the in vitro response of test NP.

In Vitro Genotoxicity of Whole Test NP	
Ames Mutagenicity	NEGATIVE
MN Genotoxicity	POSITIVE

Extract in enzyme-free artificial saliva, 10%w/v

Mammalian Cells OR Bacteria



w/ maltols: positive in vitro MN
w/o maltols: substantially reduced response

Adapted from: Farcas et al. (2024). 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. SOT 2024. Salt Lake City.

Altria Science

Case Study: In Vivo Assessment of a Prototype Flavored NP Product

Ingredient Assessment: Are there any potential toxicological concerns?

- YES, maltol and ethyl maltol ("maltols")
- Maltols could lead to POSITIVE in vitro genotoxic response.
- However, maltols are NEGATIVE in vivo via oral route.

In Vitro: Would the whole Test NP lead to POSITIVE in vitro response?

- YES
- The test NP was tested POSITVE in vitro in Ames and MN.
- Maltols drive the in vitro response.

ICH S2 (R1)

In Vivo: Would the whole Test NP lead to NEGATIVE in vivo response?

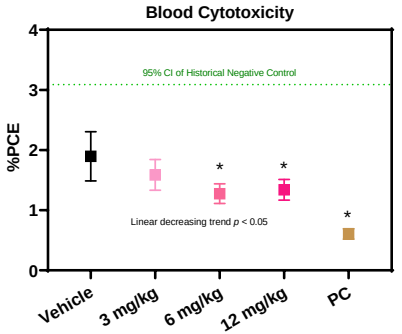
Sprague-Dawley Rats, Oral Gavage

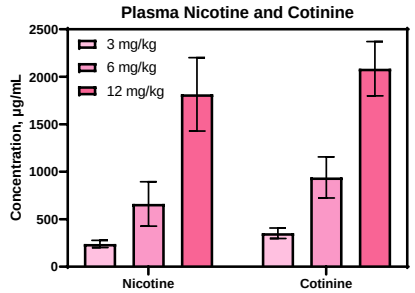
Extract in enzyme-free artificial saliva, 10%w/v

Key takeaways:

- W/ maltols: NOT genotoxic

In Vivo Genotoxicity of Whole Test NP	
MN (Blood, Bone Marrow)	NEGATIVE
Comet (Liver, Stomach)	NEGATIVE





So far, the ingredient assessment has successfully informed the in vitro and in vivo toxicity of the whole product.

Adapted from: Zhang et al. (2024). Evaluation of the in vivo genotoxic potential of an oral nicotine pouch product following ICH S2(R1) guidance. SOT 2024. Salt Lake City.
Image of the rat adapted from Impact of oral gavage technique of drug-containing microcontainers on the gastrointestinal transit and absorption in rats

Altria Science

Case Study: Bridging the In Vitro and In Vivo Outcomes

Ingredient Assessment: Are there any potential toxicological concerns?

- YES, maltol and ethyl maltol ("maltols")
- Maltols could lead to POSITIVE in vitro genotoxic response.
- However, maltols are NEGATIVE in vivo via oral route.

In Vitro: Would the whole Test NP lead to POSITIVE in vitro response?

- YES
- The test NP was tested POSITVE in vitro in MN and Ames assays.

In Vivo: Would the whole Test NP lead to NEGATIVE in vivo response?

- YES
- The test NP was tested NEGATIVE in vivo in MN and comet assay.

Bridging the In Vitro and In Vivo Outcomes

Positive In Vitro → Negative In Vivo

- Fast absorption in the gastrointestinal tract and efficient metabolism in the liver in rats and humans
- Different routes of administration lead to different in vivo MN results (EFSA, 2015)
  - Intraperitoneal injection → Positive
  - Oral gavage → Negative

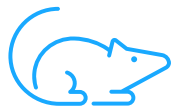
➢ **Dosimetry plays a role in the in vivo genotoxicity of the maltols.**

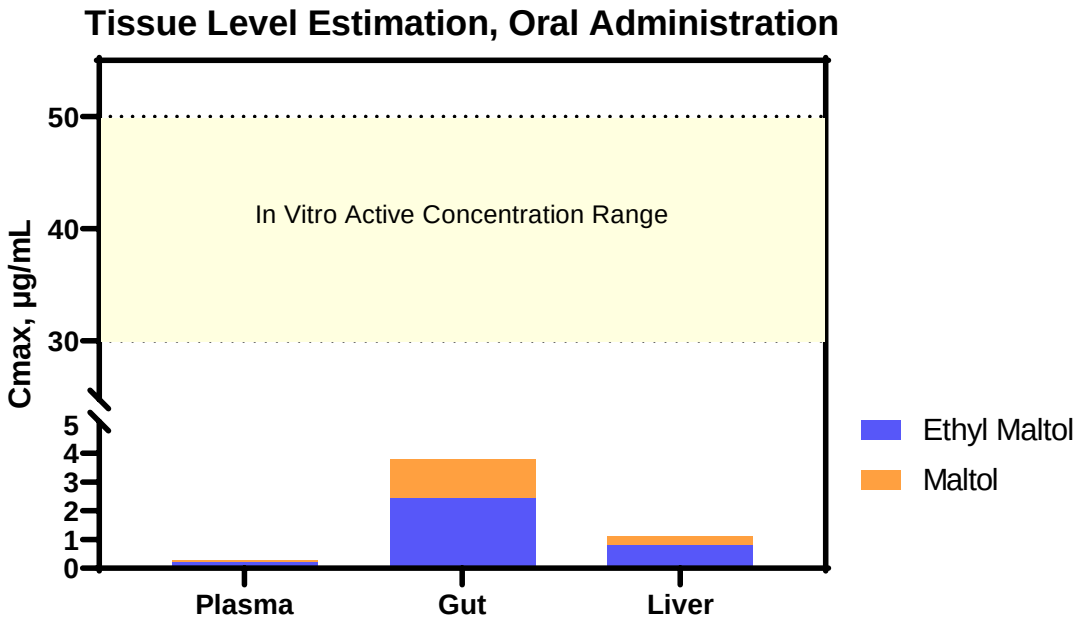
EFSA (2015). Scientific Opinion on Flavouring Group Evaluation 213, Revision 2 (FGE.213Rev2): Consideration of genotoxic potential for α,β-unsaturated alicyclic ketones and precursors from chemical subgroup 2.7 of FGE.19

Altria Science

Case Study: A Dosimetry Hypothesis to Bridge the In Vitro-In Vivo MN Assay

- A dosimetry hypothesis:
  - *The in vivo tissue concentrations of the maltols are substantially lower (negative in vivo outcomes) than the active in vitro concentration (positive in vitro outcomes).*
- Open-source pharmacokinetic model (Oral): EPA httk ICE Tools (nih.gov)
  - Oral; dose once every 24 hours for 4 days
  - ➢ In Vivo Tissue Levels ≪ In Vitro Bioactive Concentration





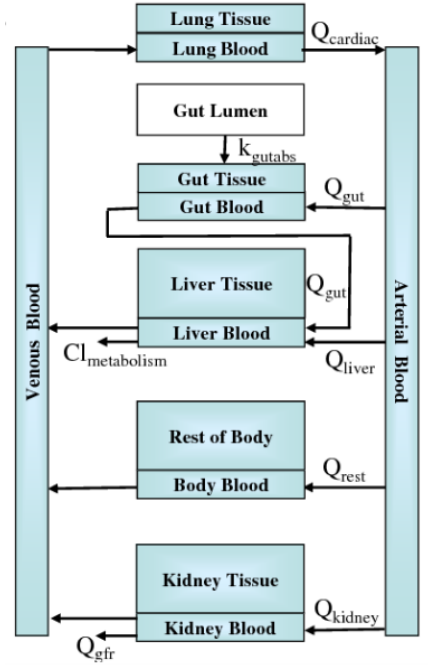


Diagram of the PBTK model in the httk R package (Pearce et al. 2017)

Pearce et al. (2017) httk: R Package for High-Throughput Toxicokinetics

Altria Science

Conclusions

- We conducted a feasibility study and evaluated a flavored oral nicotine pouch product integrating NAMs into standard non-clinical test battery.
- With this specific case example, we demonstrated that
  - Data from individual ingredients (literature and in silico) could inform the in vitro and in vivo toxicological profile of the whole product, as confirmed by experimental results. AND,
  - Dosimetry provides insight into the biological relevance of in vitro testing, which are often sensitive due to lack of complete metabolism or tested in a wide concentration range for hazard identification purposes.
  - Caveat: a simple mixture that allow us to identify the toxicity driver
- We built a case for NAMs-based toxicological assessment without the need for confirmatory in vivo testing.
- New area in new era: Integration of Exposure/Dosimetry and Mechanisms
  - AEP - AOP: Aggregated Exposure Pathway - Adverse Outcome Pathway

Altria Science

Acknowledgement

Please contact me if you have any questions.

Jingjie Zhang, Principal Scientist (Jingjie.Zhang@altria.com)

Altria Client Services, LLC

601 E. Jackson St., Richmond, VA, 23219

Special acknowledgements to our co-authors, Richard Morgan, Utkarsh Doshi, Chastain A. Anderson, Wanyoike Kangethe, Donna Smith, and K. Monica Lee, and those who contributed to the work, Mariana Farcas, Molly Pitegoff, and reviewers, Britt Langston, Sara Hurtado, and Mohamadi Sarkar.

Altria Science

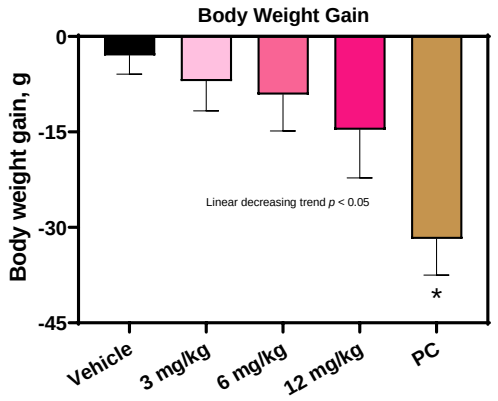
Thanks

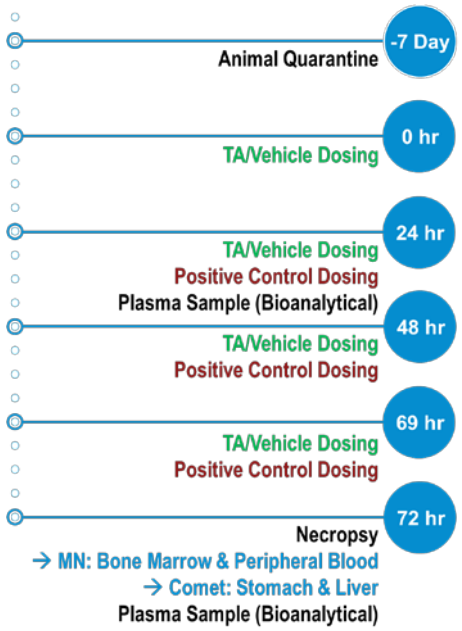
Any Questions

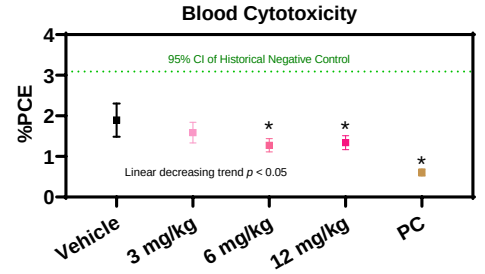
Altria Science

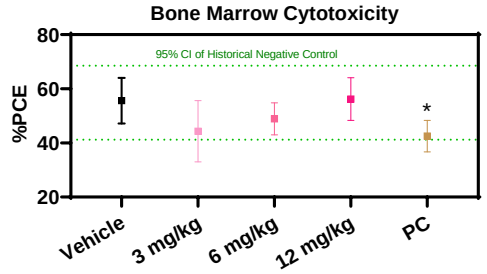
Backup

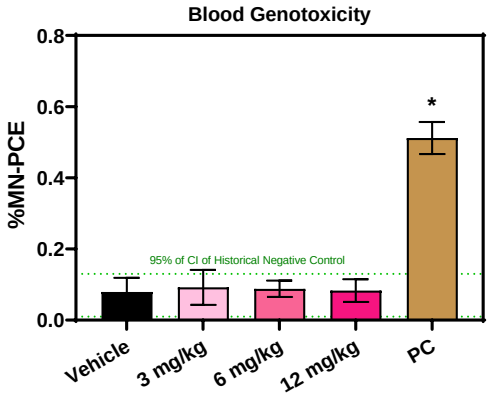
Case Example: In Vivo Assessment of a Prototype Flavored NP Product

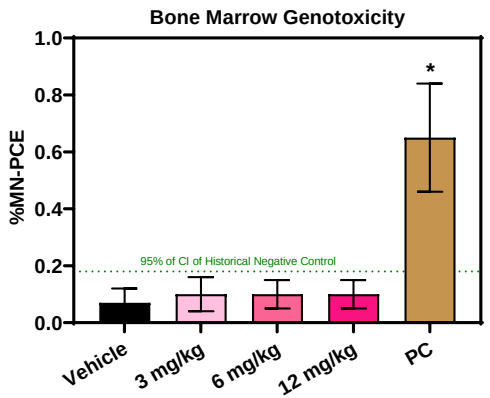


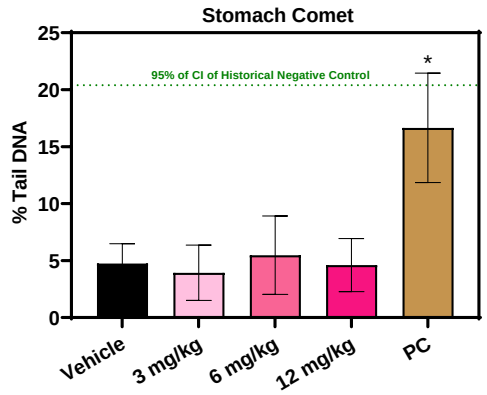


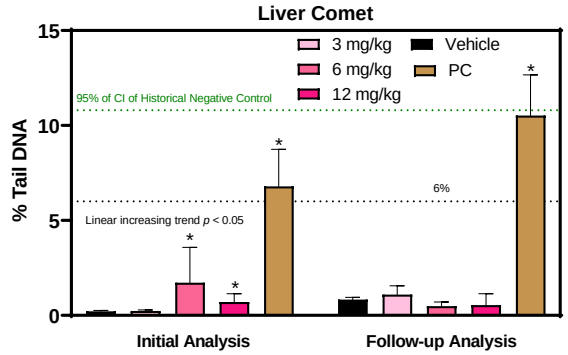












Group Number	Sex	Animal Number	Dose Group	Approx. Dose Level (mg nicotine/kg BW)	Gavage Volume (mL/kg BW)
1	M	6	Vehicle Control	0	16
2	M	6	TA-low	3	16
3	M	6	TA-Mid	6	16
4	M	6	TA- High	12 [b]	16
5	M	6	Positive Control [a]	15	10

Adapted from: Zhang et al. (2024). Evaluation of the in vivo genotoxic potential of an oral nicotine pouch product following ICH S2(R1) guidance. SOT 2024. Salt Lake City.

Altria Science