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

Jingjie Zhang

Altria Client Services LLC, Richmond, VA 23219

Center for Research and Technology

Altria

FAMILY OF COMPANIES

September 11, 2024

Table of Contents

Toxicological Assessment of Oral Nicotine Pouch Products

- Oral Nicotine Pouch Product
- GRAS (Generally Recognized As Safe) Ingredients Used in Tobacco Products

Case Study:
Toxicity Assessment of A Flavored Oral NP Product

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

- In Vitro
- In Vivo
- In Vitro-In Vivo Bridging with Dosimetry

Conclusions

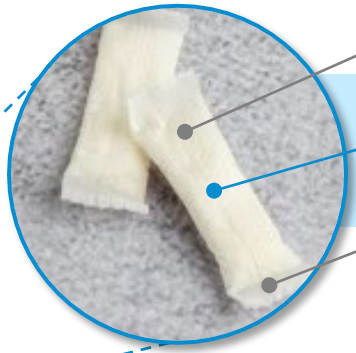
Toxicity Assessment of Oral Nicotine Pouch Products

Oral nicotine pouch (NP) products may be a viable reduced risk alternative for adult smokers who are unable or unwilling to quit all tobacco

These products typically consist of tobacco-derived nicotine, flavors and other excipients

Oral Nicotine Pouches

Image courtesy of iStock.com/Oleksandr Shatyrov



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)

GRAS GENERALLY RECOGNIZED AS SAFE

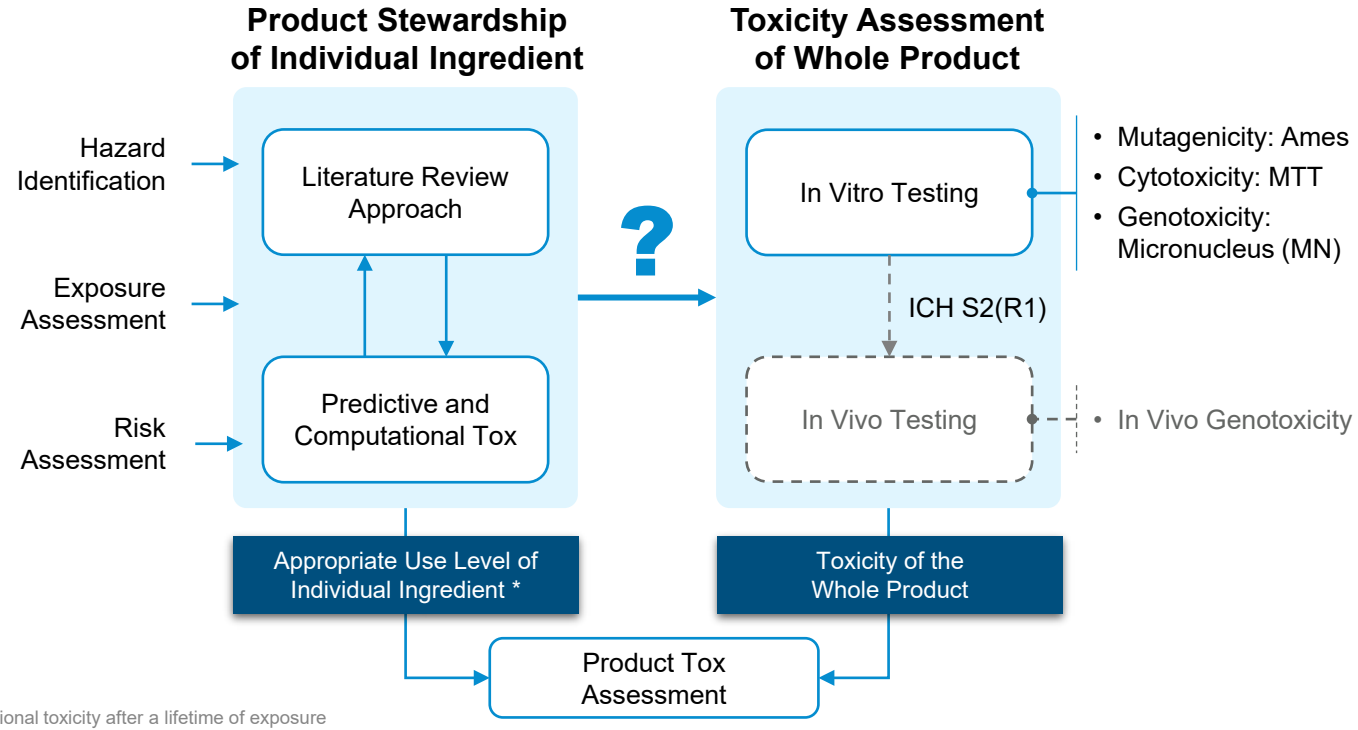
Image adapted from Food Business News

Although the GRAS status of flavoring compounds are **NOT intended for the tobacco products**, the toxicological data are relevant, especially for oral products

GRAS Reference: Generally Recognized as Safe (GRAS) | FDA
FEMA. (2018) Safety Assessment and Regulatory Authority to Use Flavors – Focus on Electronic Nicotine Delivery Systems and Flavored Tobacco

Concept: Toxicity Assessment of Oral Nicotine Pouch Products

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

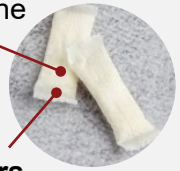


*a level of exposure that does not introduce additional toxicity after a lifetime of exposure
ICH=International Council for Harmonisation
CH (2012). S2(R1) Genotoxicity Testing and Data Interpretation for Pharmaceuticals Intended for Human Use

Case Study: Ingredient Assessment of a Prototype Flavored NP Product

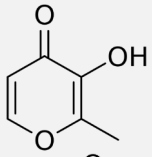
Ingredient Assessment: Are there any potential toxicological concerns?

Nicotine

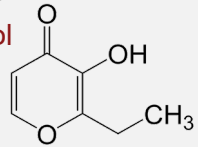


Flavors

- Maltol



- Ethyl Maltol



- Others (proprietary & no concern)

Key Takeaways from Literature:
for both maltol and ethyl maltol ("maltols")

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

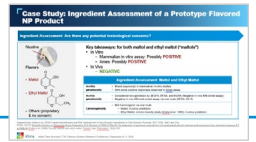
Ingredient Assessment: Maltol and Ethyl Maltol	
In vitro genotoxicity	• Mixed (equivocal) in mammalian in-vitro studies • With some positive responses observed in Ames assay
In vivo genotoxicity	• Considered non-genotoxic by JECFA, EFSA, and ECHA (Negative in vivo MN-comet assay) • Negative in vivo MN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

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?

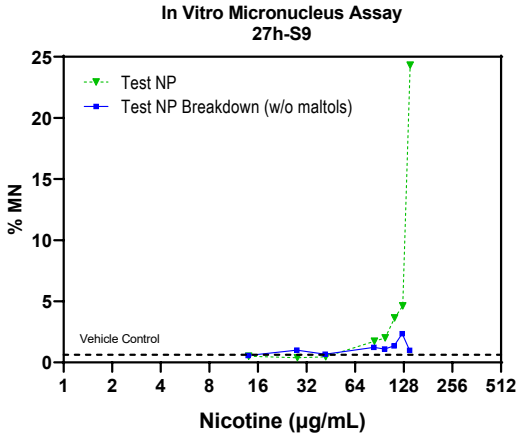
Key takeaways:

- 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



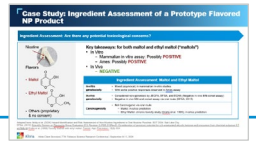
w/ maltols: positive in vitro MN
w/o maltols: substantially reduced response

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.

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

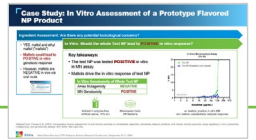
Ingredient Assessment: Are there any potential toxicological concerns?

- 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)

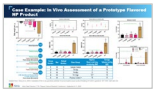
In Vivo: Would the whole Test NP lead to NEGATIVE in vivo response?

Sprague-Dawley Rats, Oral Gavage

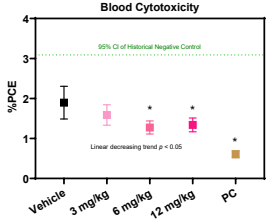
Extract in enzyme-free artificial saliva, 10% w/v

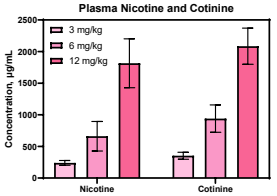
Key takeaways:

- W/ maltols: NOT genotoxic



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





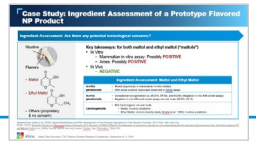
So far, the ingredient assessment has successfully informed the in vitro and in vivo toxicity of the whole product

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

Case Study: Bridging the In Vitro and In Vivo Outcomes

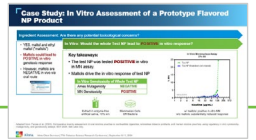
Ingredient Assessment: Are there any potential toxicological concerns?

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



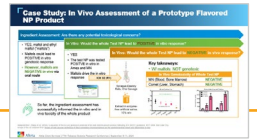
In Vitro: Would the whole Test NP lead to **POSITIVE** in vitro response?

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



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

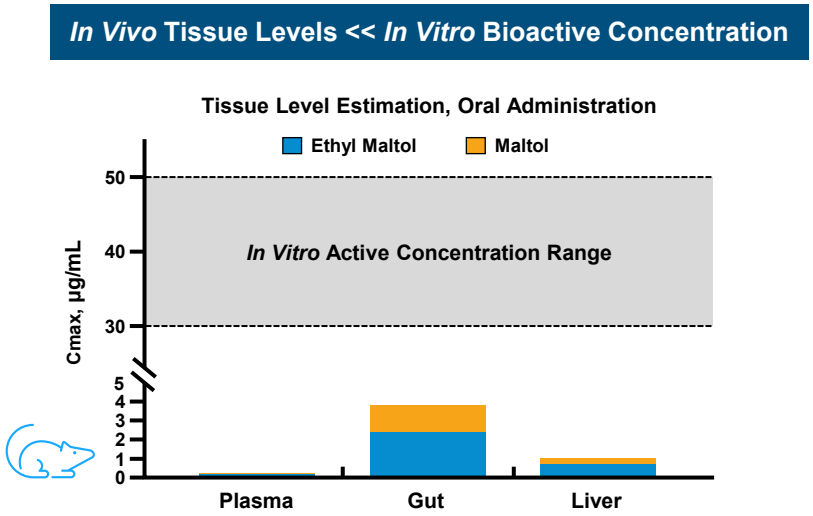
Dosimetry plays a role in the in vivo genotoxicity of the maltols

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

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

Dosimetry hypothesis:

- 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[1]

- Oral; dose once every 24 hours for 4 days

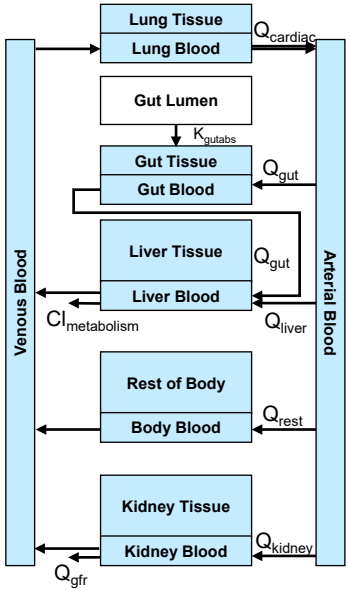


Diagram of the PBTK model in the httk R package[2]

1. ICE Tools (nih.gov); 2. Pearce et al. (2017) httk: R Package for High-Throughput Toxicokinetics

Conclusions

We conducted a feasibility study and evaluated a flavored oral nicotine pouch product integrating New Approach Methodologies (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 allows 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

Acknowledgement

If you have any questions, please contact:

Jingjie Zhang

Principal Scientist

Jingjie.Zhang@altria.com

Altria Client Services, LLC

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

Special acknowledgements those who contributed to the work:

Richard Morgan, Utkarsh Doshi, Wanyoike Kangethe, Donna Smith, K. Monica Lee, Mariana Farcas, Molly Pitegoff, Vanessa Haase, Olushola M. Awoyemi, Chase Anderson and reviewers

Thanks

Any Questions?