Connecting Exposure, Dosimetry and Toxicity Responses in the Preclinical Evaluation of Ingredients: Case Examples of Flavoring Chemicals in Oral Tobacco Products

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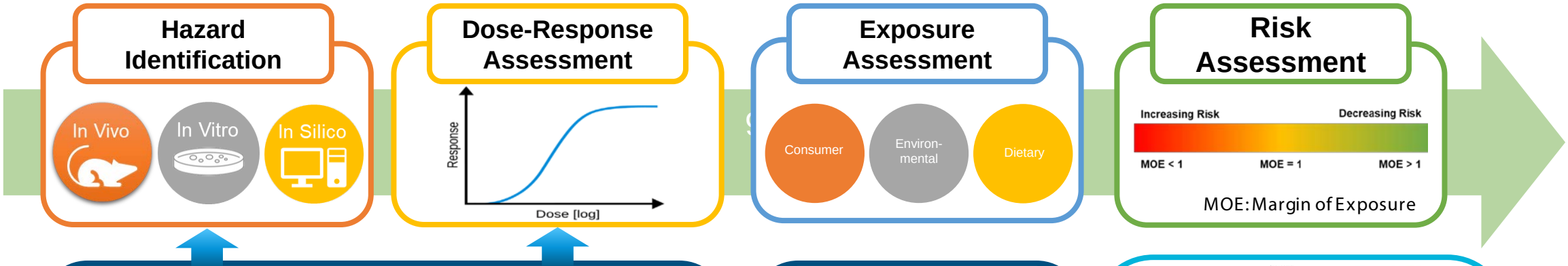
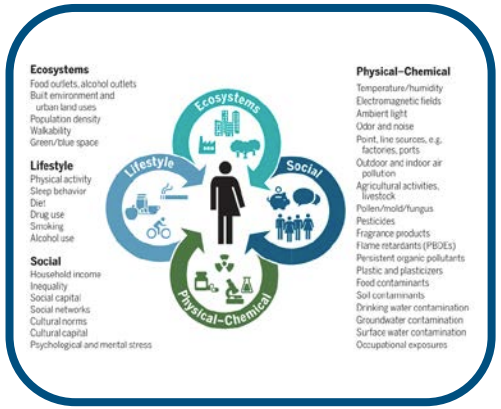
NAMs-Based Toxicity Testing and Risk Assessment

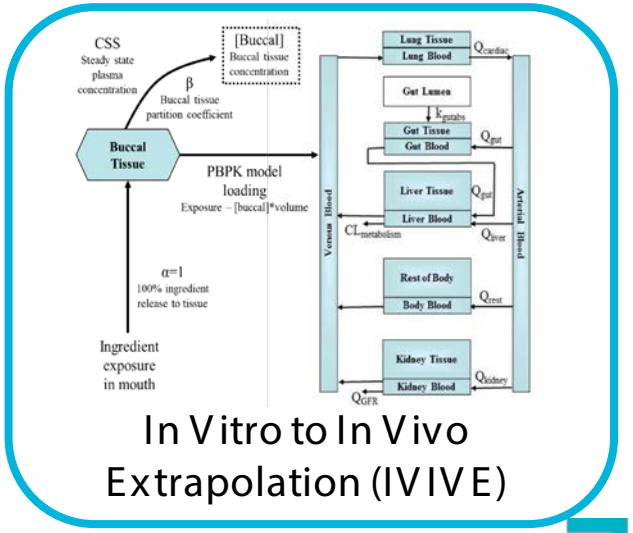
- How to extrapolate from in vitro test results to in vivo human health risk is key for NAMs-based risk assessment.



In Silico and In Vitro Model



Exposure

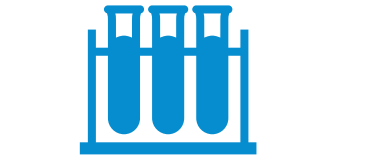


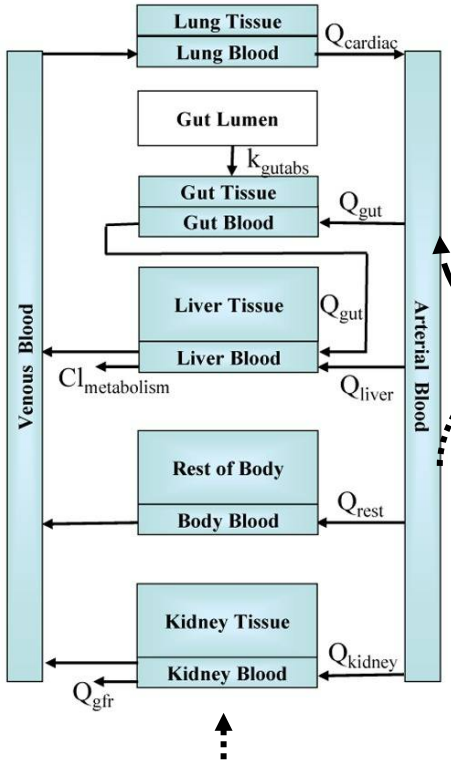
In Vitro to In Vivo Extrapolation (IVIVE)

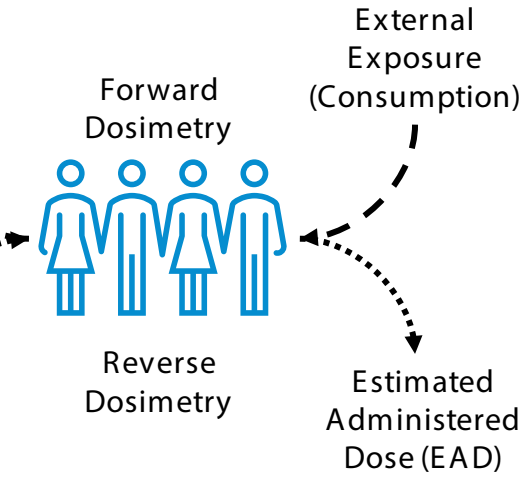
https://theory.labster.com/dose-response/;
https://ntp.niehs.nih.gov/iccvam/meetings/iccvam-forum-2018/07-tox21.pdf ;
Zhang et al. (2022) Use of In Vitro to In Vivo Extrapolation (IVIVE) (altria.com)
The exposure figure from Vermeulen et al. (2020) Science. 367 (6476):392-396 DOI: 10.1126/science.aay3164

In Vitro to In Vivo Extrapolation (IVIVE) – In Vivo Human

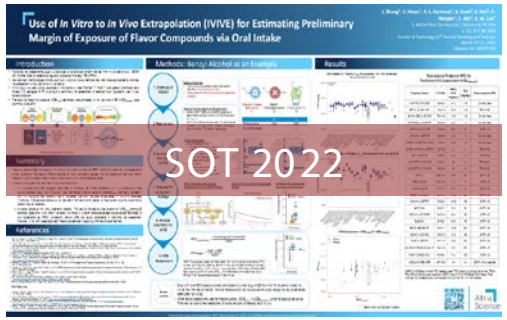
- Pharmacokinetic (PK) modeling has been investigated for its applications in estimating biologically effective dose in target tissues (forward dosimetry) and administered dose in vivo (reverse dosimetry, or IVIVE) for human risk assessment. [1,2]







In Vitro Point of Departure (PoD) ≈ Tissue Concentration
(In Vivo plasma, liver conc., etc.)

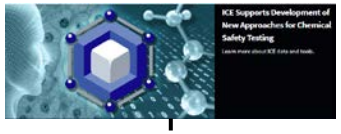


[1] Chang et al. (2023). Toxics. 2022; 10(5):232. https://doi.org/10.3390/toxics10050232;
[2] Hines et al. (2022) Front. Pharmacol., Sec. Predictive Toxicology. https://doi.org/10.3389/fphar.2022.864742
PBPK model adopted from Pearce et al. (2018) J Stat Softw. https://doi.org/10.18637%2Fjss.v079.i04

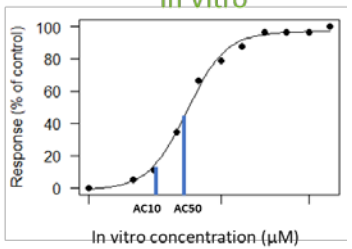
In Vitro to In Vivo Extrapolation (IVIVE) – In Vivo Animal

- This study uses a PK model to connect the in vitro tests and in vivo animal tests, aiming to evaluate the impact of ADME (absorption, distribution, metabolism, excretion) on in vivo test results.

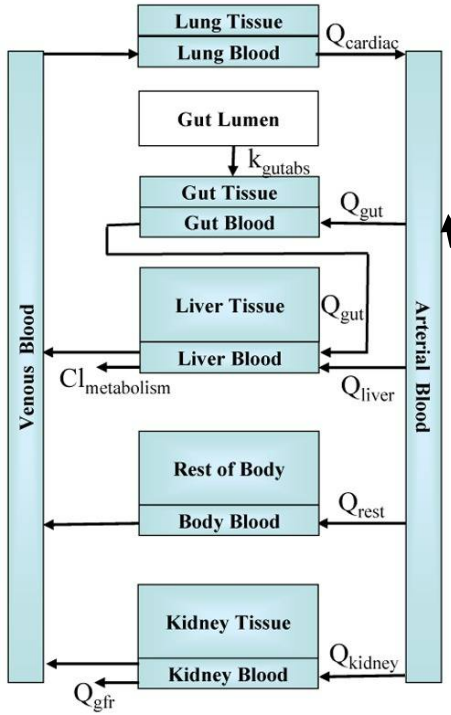
ICE: https://ice.ntp.niehs.nih.gov/ [1]
COSMOS: https://www.ng.cosmosdb.eu/ [2]



In Vitro



Bioactive Concentration (or PoD)

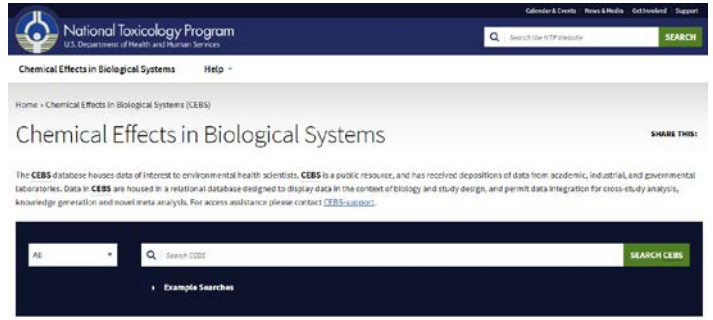


PoD_in vitro v.s. Tissue Concentration ?
(In Vivo plasma, liver conc., etc.)

Forward Dosimetry

External Exposure (Dose Regimen)

In Vivo Study



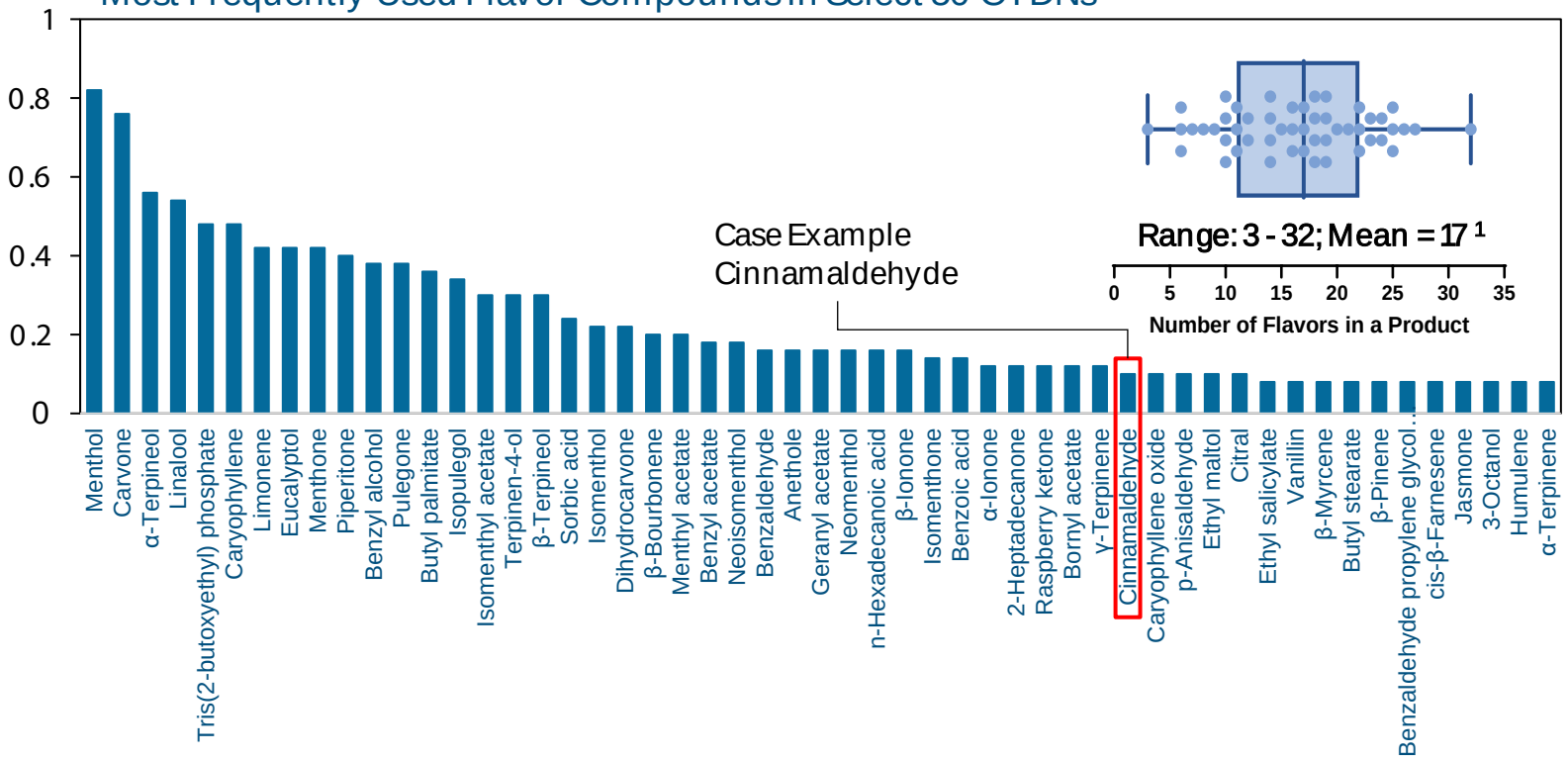
https://cebs.niehs.nih.gov/cebs/

[1] Yang et al. (2021) Computational Toxicology, Volume 19, https://doi.org/10.1016/j.comtox.2021.100175.
[2] Bell et al. (2020) Toxicology in Vitro, Volume 67, https://doi.org/10.1016/j.tiv.2020.104916.

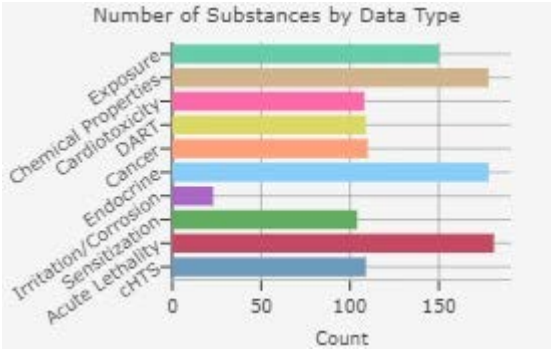
Chemicals of Interest

- Food flavoring compounds are commonly used in potentially reduced-risk (PRR) oral tobacco or nicotine products.[1]
- Flavoring compounds, mostly generally recognized as safe (GRAS), have been thoroughly studied for their safety under conditions of the intended use.

Most Frequently Used Flavor Compounds in Select 50 OTDNs[1]



Tox Data Records in ICE Database



4 out of 186 were not found in ICE database.

23 out of 186 flavoring compounds were evaluated for carcinogenicity in chronic studies, as found in COSMOS.

[1]Based on data from: Mallock-Ohnesorg et al. 2023. Archives of Toxicology. 97:2357–2369;

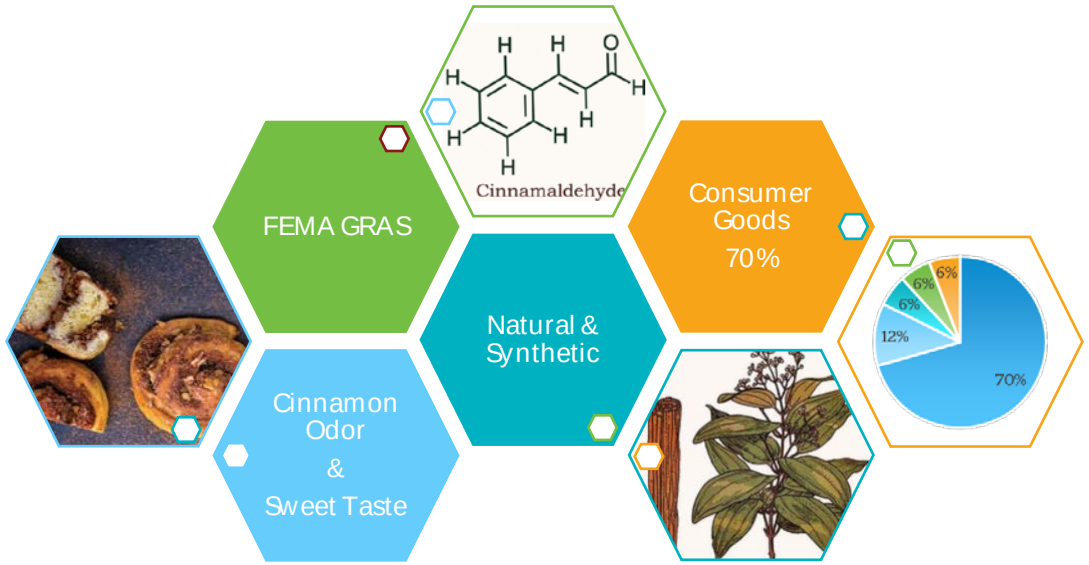
Case Example – Tox Profile of Cinnamaldehyde

- Primary Evidence: NTP 2004 [1]
  - Cinnamaldehyde (CAS: 104-55-2) is not genotoxic or carcinogenic, with in vitro activity in some tests.

	Assay Type	Results	Notes
In Vitro	Ames	Positive in TA 100 (+S9)	(+S9, mouse) TA100 Positive
			Otherwise, non-mutagenic
	Sister chromatid exchange *	Positive	(-S9) Trial 1, 2, & 3: Positive, Negative, Positive
			(+S9) Trial 1& 2: (+S9) Positive
	Chromosomal aberration *	Negative	(-S9) Trial 1: Positive; Trial 2&3: Negative (+S9) Negative

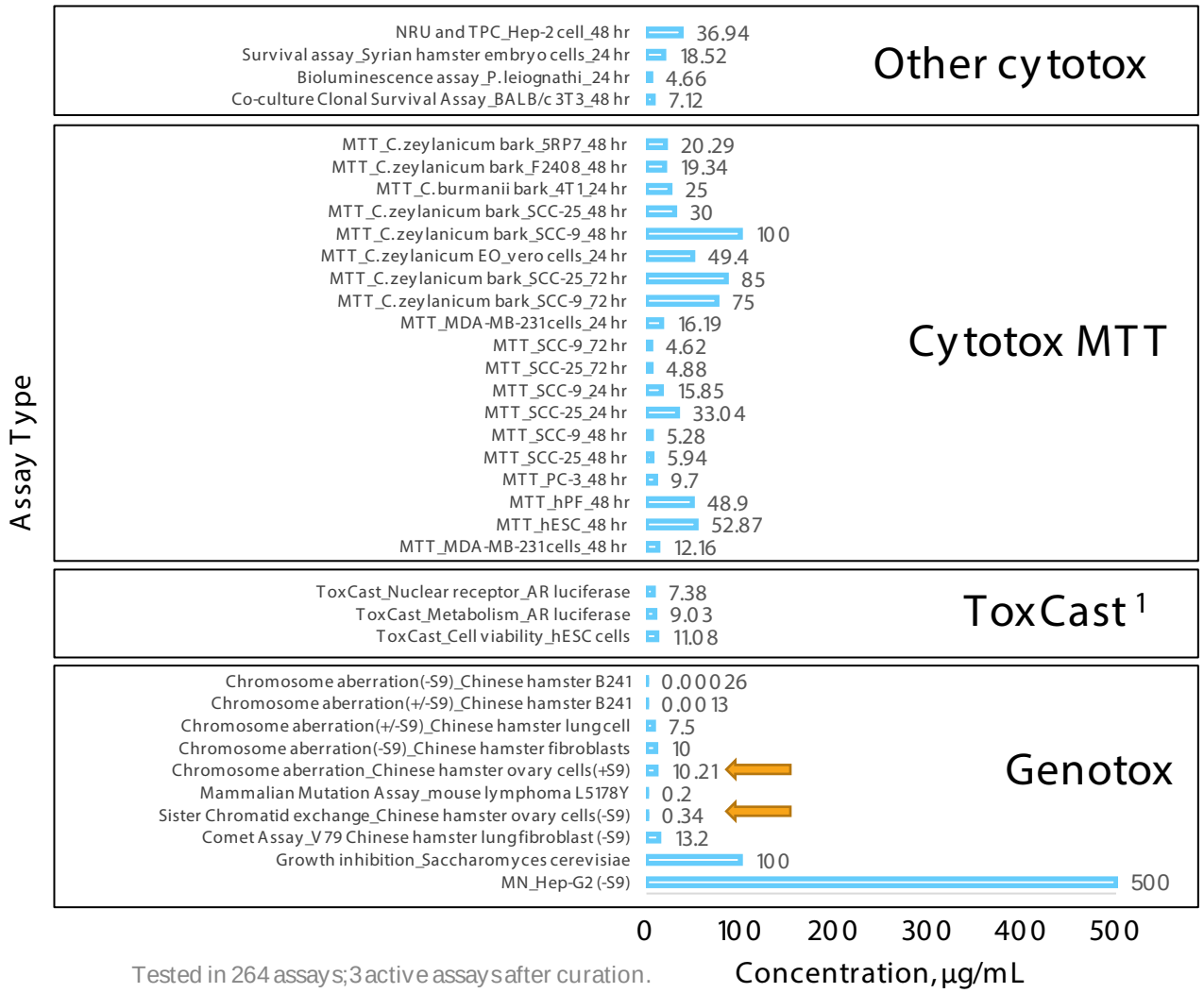
*Chinese hamster ovary (CHO) cells

	Assay Type	Results	Notes
In Vivo	90-day feeding, Mouse MN	Negative	Negative up to 4,000 mg/kg/day
	90-day feeding. Subchronic	Forestomach toxicity	LOAEL 570 mg/kg/day (Rat)
	2-year feeding, Carcinogenicity	No Carcinogenicity	NOAEL 200 mg/kg/day (Rat and Mouse) (Derived ADI 3.2 mg/kg using uncertainty factors of 10 and 6.2 for intra- and inter-species extrapolation.)



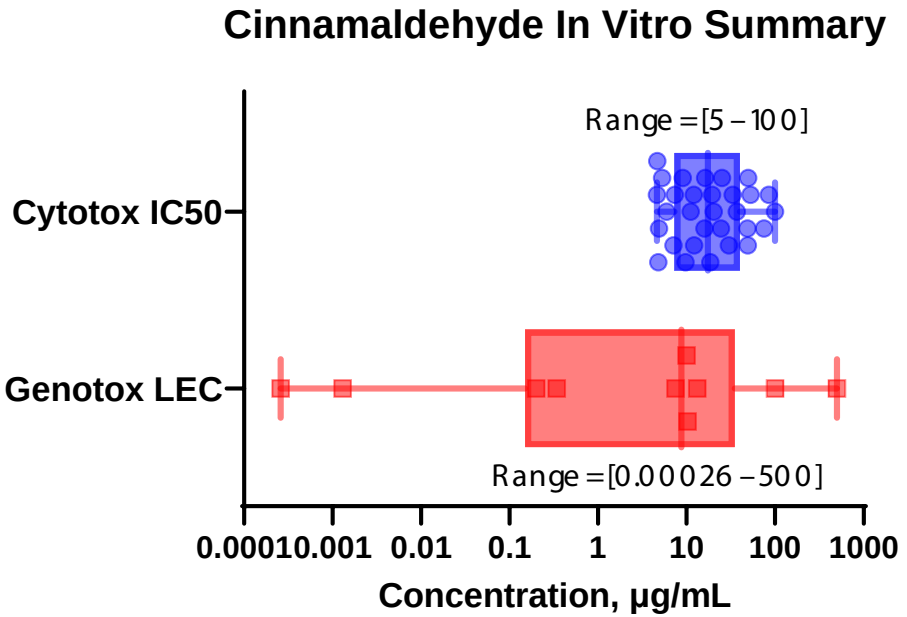
[1] NTP. (2004) https://ntp.niehs.nih.gov/publications/reports/tr/500s/tr514
Curate product category data from https://ice.ntp.niehs.nih.gov/Tools?tool=pbpk
Photos of cinnamon plant adopted from Friedman. (2017) Journal of Agricultural and Food Chemistry. 65 (48), 10406-10423;

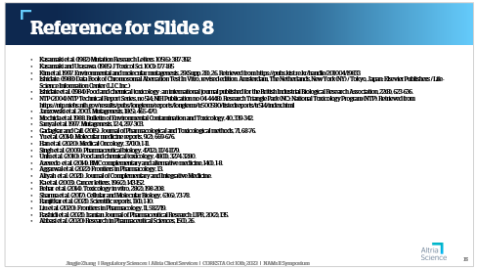
In Vitro Toxicity Profile of Cinnamaldehyde



Tested in 264 assays; 3 active assays after curation.

Other Evidence

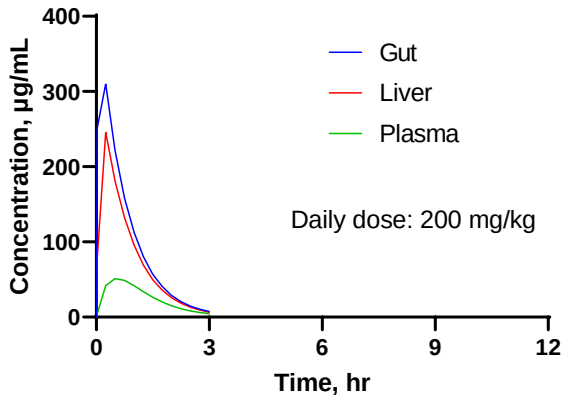




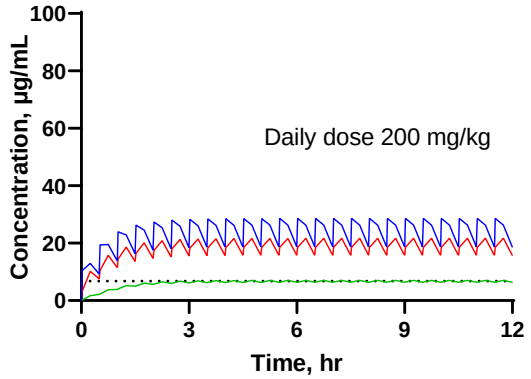
Results: Estimated in vivo dosimetry (target tissue)

- In vivo dose regimen: oral (feed) dose 200 or 570 mg/kg/day for rats [1]
  - Single bolus gavage vs. multiple gavage doses over 12 hours (considering 12-hour light-dark circle)
- Httk [3] modeling to estimate cinnamaldehyde levels in gut, liver, and plasma
  - The generic modeling tool helps visualize the pharmacokinetic profile of various chemicals conveniently, with experimental and predicted (with OPERA) parameters as part of the model.
  - Caveat: linear model, with no chemical-specific metabolic pathway; instead, clearance is adjusted to simulate changes in elimination.
- Scenarios [2]
  - A single (bolus) dose results in a much higher Cmax in all three tissues than multiple (divided) doses.
  - Under the same dosing regimen (30 mins over 12 hours), the Cmax in all three tissues increase linearly to the total daily dose.

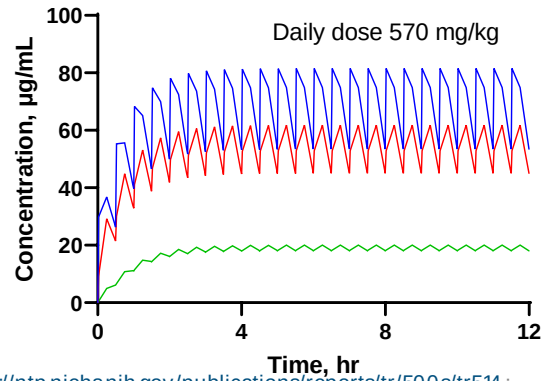
Gavage 200 mg/kg/day, a single dose



Gavage every 30 min (8.33 mg/kg/dose) over 12 hours



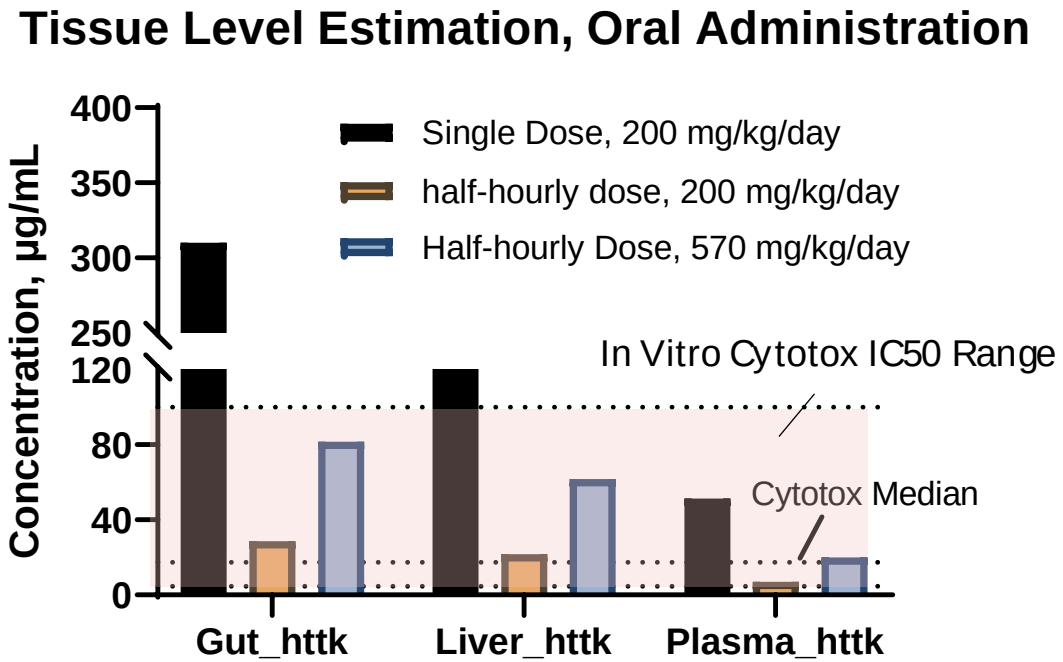
Gavage every 30 min (23.75 mg/kg/dose) over 12 hours



[1] NTP. (2004) https://ntp.niehs.nih.gov/publications/reports/tr/500s/tr514;
[2] Pearce et al. (2017) J Stat Softw. 79(4): 1-26. doi: 10.18637/jss.v079.i04
[3] Assuming a body weight of 0.45 kg for rats

Results: Connect In Vitro Toxicity and In Vivo Dose

- Gut is the entry organ, and mostly similar to in vitro exposure.
  - GI tract - directly exposed to test materials upon oral dosing - similar to in vitro exposure
  - Biological relevance in acute toxicity in vivo
    - Forestomach lesions in subchronic feed studies up to 570 mg/kg/day
    - Not observed in the chronic feed studies up to 200 mg/kg/day
- Blood
  - Plasma level is low as predicted in modeling.
    - Could be even lower based on a rat PK study due to rapid oxidation.[1]
  - The clearance rate in the model is based on OPERA prediction – could be overestimated.
    - Actual clearance in rat plasma is prolonged, possibly due to slow regeneration of cinnamaldehyde from protein conjugates that escape hepatic metabolism.[1]



[1] Yuan et al.(1992) Fd Chem Toxic 30(12): 997 - 1004

Summary

- In this example, we demonstrate that publicly available (and relatively simple) linear TK modeling can help link the dose-response relationship between in vitro and in vivo studies.
- TK modeling tools can provide quantitative relationship and information about the ADME of a chemical.
  - Variation among tissues; Kinetic curves
- Dose-dependent differences in in vivo (and in vitro) results can be partly explained by the TK modeling.
  - Comparison between in vitro and estimated tissue concentration, especially the level in the target organ
- Multiple PBPK models are available with pros and cons – depending on the question:
  - Generic model: offers convenience for the evaluation of a large groups of compounds with less hassle in defining and finding model parameters; often publicly available; easy to use
  - Customized model: offers improved accuracy in prediction with increased knowledge about the compound; commercial software, or expertise in coding
  - The degree of uncertainty depends on the context of use: Sensitivity analysis can help identify which parameters to reduce uncertainty – supplement with experimental data
- Understanding the dosimetry between in vitro and in vivo conditions is critical in ultimate use of the in silico and in vitro-based (NAMs) assay results to quantitative toxicological risk assessment.

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Acknowledgement

Please contact me if you have any questions.

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Can we connect Exposure, Dosimetry and Toxicity Responses in the Preclinical Evaluation of Ingredients

?

Case Examples of Flavoring Chemicals in Oral Tobacco Products

October 10th, 2023

Jingjie Zhang | Regulatory Sciences | Altria Client Services | CORESTA Oct 10th, 2023 | NAMs II Symposium

Abstract

Flavoring ingredients that are GRAS ("generally recognized as safe") in food are commonly used in oral tobacco derived nicotine (OTDN) products. While the GRAS status is not de facto approval for the use in oral tobacco products, the accompanying toxicological information is relevant and useful in the safety evaluation of OTDN products, considering the similarity of how the products are consumed. In addition to available regulatory limits, a weight of evidence of all available nonclinical and clinical information is used to assess the suitability of using flavoring chemicals in OTDNs and the potential health effects of these products. For some GRAS ingredients, specific in vitro and in vivo toxicity outcomes sometimes present apparently different responses – for instance, cinnamaldehyde, a common flavor in oral consumer products is known to induce positive in vitro genotoxicity; however, these in vitro hazard findings do not lead to in vivo sequelae based on negative long-term carcinogenicity outcomes. In this talk, we have investigated the dosimetry basis for these apparently different in vitro versus in vivo genotoxicity and carcinogenicity outcomes using cinnamaldehyde as an example flavor in OTDN products. PBPK modelling and in vitro to in vivo extrapolation (IVIVE) approaches are used to estimate the equivalent human daily exposures (EADs) and to evaluate in vitro toxicity findings in the in vivo context. Using open-source PBPK models, we estimated the Cmax in the target organ (e.g., plasma and liver) of cinnamaldehyde under in vivo (rodent and human) exposure conditions and compared the estimated doses to the in vitro exposure ranges for cytotoxicity and genotoxicity findings. We also compared the estimated EADs from nonclinical testing to the likely use levels in human use and discuss the estimated margin of exposure in the context of known toxicological profiles of the ingredients. Using the case example, we demonstrate the relevance and opportunity of incorporating target tissue dosimetry as a consideration as part of nonclinical toxicity evaluation and risk assessment.

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Reference for Slide 8

- Kasamaki et al. (1982) Mutation Research Letters. 105(6): 387-392
- Kasamaki and Urasawa. (1985) J Toxicol Sci. 10 (3): 177-185
- Kim et al. 1997. Environmental and molecular mutagenesis, 29(Supp. 28), 26. Retrieved from https://pubs.kist.re.kr/handle/201004/19833
- Ishidate. (1988) Data Book of Chromosomal Aberration Test In Vitro, revised edition. Amsterdam, The Netherlands, New York (NY) / Tokyo, Japan: Elsevier Publishers / Life-Science Information Center (L.I.C. Inc.)
- Ishidate et al. (1984) Food and chemical toxicology : an international journal published for the British Industrial Biological Research Association, 22(8), 623-636.
- NTP (2004) NTP Technical Report Series, no 514; NIH Publication no 04-4448). Research Triangle Park (NC): National Toxicology Program (NTP). Retrieved from https://ntp.niehs.nih.gov/results/pubs/longterm/reports/longterm/tr500580/listedreports/tr514/index.html
- Janzowski et al. 2003. Mutagenesis, 18(5), 465-470.
- Mochida et al. 1988. Bulletin of Environmental Contamination and Toxicology, 40, 339-342.
- Sanyal et al. 1997. Mutagenesis, 12 4, 297-303.
- Gadagkar and Call. (2015). Journal of Pharmacological and Toxicological methods, 71, 68-76.
- Yu et al. (2014). Molecular medicine reports, 9(2), 669-676.
- Han et al. (2020). Medical Oncology, 37(10), 1-11.
- Singh et al. (2009). Pharmaceutical biology, 47(12), 1174-1179.
- Unlu et al. (2010). Food and chemical toxicology, 48(11), 3274-3280.
- Azeredo et al. (2014). BMC complementary and alternative medicine, 14(1), 1-8.
- Aggarwal et al. (2022) Frontiers in Pharmacology, 13.
- Aliyah et al. (2021). Journal of Complementary and Integrative Medicine.
- Ka et al. (2003). Cancer letters, 196(2), 143-152.
- Behar et al. (2014). Toxicology in vitro, 28(2), 198-208.
- Sharma et al. (2017). Cellular and Molecular Biology, 63(6), 73-78.
- Ranjitkar et al. (2021). Scientific reports, 11(1), 1-10.
- Liu et al. (2020). Frontiers in Pharmacology, 11, 582719.
- Rashidi et al. (2021). Iranian Journal of Pharmaceutical Research: IJPR, 20 (2), 135.
- Abbasi et al. (2020) Research in Pharmaceutical Sciences, 15(1), 26.