Nonclinical Toxicity Assessment of Oral Tobacco-Derived Nicotine Products: II. Ingredient Evaluation

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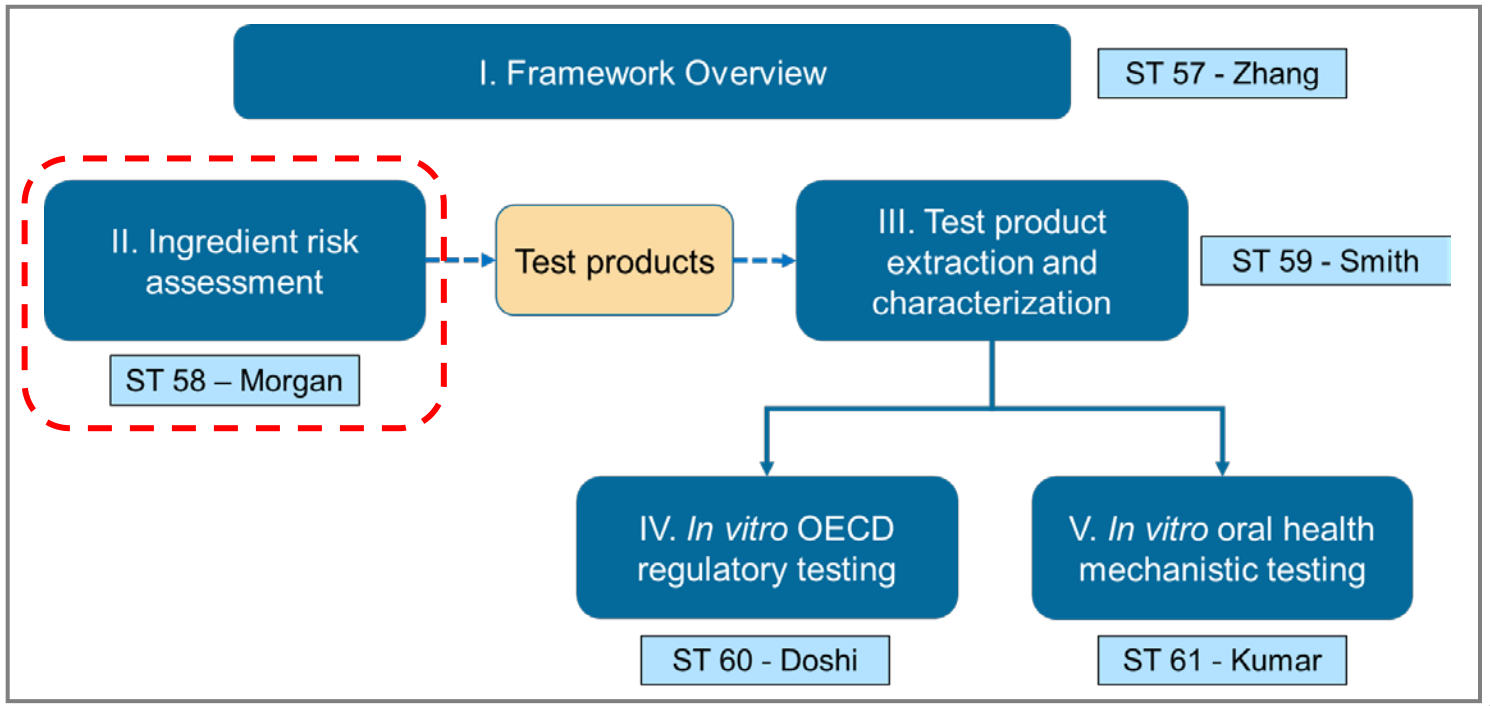
Agenda

- Introduction
- Ingredient Risk Assessment
- *In Silico* Toxicology
- Case Example
- Summary



Tobacco Harm Reduction Framework

Constituent Reduction

The Product

- Manufacturing and quality control
- Physical and chemical characterization
- Product stability
- Hazard assessment

Risk Reduction Individual

Exposure and Health Risk

- **Literature reviews**
- In vitro studies
- In vivo studies
- **Risk assessments**
- Relative risk comparison across and within tobacco product categories
- Clinical studies

Harm Reduction Population

Impact on the Population

- Secondary analysis of national data
- Risk perception, behavioral intention and product appeal
- Actual use study
- Product instructions comprehension
- Population modeling
- Post-market surveillance

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Ingredient Risk Assessment

Hazard + Exposure = Risk

- Tiered approach to evaluate ingredients
  - Literature review on each ingredient
  - Additional hazard identification using *in silico* software
  - Use assumptions
  - Conduct quantitative risk assessment (QRA)
  - Expert judgement

Ingredient Risk Assessment

- Literature review on each ingredient
  - Identity, occurrence, organoleptic properties, etc.
  - Regulatory information
  - Estimated intake
  - Reference values
  - Metabolism
  - *In vitro* studies (i.e., AMES genotoxicity)
  - *In vivo* studies (i.e., feeding studies, dermal irritation/sensitization)

Ingredient Risk Assessment

- Additional hazard identification using in silico software

*"**CTP** encourages the use of alternative methods for testing toxicity when it is appropriate and has sought to use in vitro and in silico methods synergistically to expand on the results of studies that use research animals in addition to answering unique questions that cannot be addressed using non-human systems. "*

https://www.fda.gov/science-research/about-science-research-fda/advancing-alternative-methods-fda

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Investigating DNA adduct formation by flavor chemicals and tobacco byproducts in electronic nicotine delivery system (ENDS) using in silico approaches

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https://www.sciencedirect.com/science/article/pii/S0041008X20301502

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In Silico Toxicology: CASE Ultra & Derek Nexus

- For initial hazard identification, a statistical rule-based software e.g., CASE Ultra (v.1.8.0.2)[1] is used to assess OTDN ingredients.

- To support the statistical rule-based predictions from CASE Ultra, an expert knowledge-based system (e.g., Derek Nexus v.6.1.0, KB 2020 1.0)[2] was used for further hazard identification.

- Possible Outcomes include:
  - Both approaches provide consistent negative results
  - Results from both approaches are not consistent
    - a tiered approach was adopted with the priority of experimental results >> Derek Nexus > CASE Ultra
  - Both approaches provide consistent positive results, with or without experimental data
    - further investigation is undertaken into the software prediction for positive and known experimental data.

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1 MultiCASE Inc. Beachwood, FL; 2 Lhasa Limited

CASE Ultra

- CASE Ultra is a quantitative structure-activity relationship (QSAR) model (automated) that uses statistical rules to predict biological activities (via structural alerts, fragments) of chemical compounds (known, novel or untested).

- CASE Ultra uses a training dataset of various test models created from databases of biologically active compounds (FDA CDER Archives, 2013; NTP Technical reports; NIH/NCI CCRIS; Cimino database; Various research studies).

- CASE Ultra provides a preview of toxicological properties (hazards) of multiple chemical compounds that could be used to rationalize experimental data or guide experimental studies.

Hazard Endpoints and QSAR Prediction

- CASE Ultra endpoints include Carcinogenicity, Genotoxicity (Mutagenicity and Clastogenicity), Skin Toxicity (Irritation, Corrosion, Sensitization), and Acute Toxicity involving over 35 models.

- Prediction Outcomes Include:

Known Positive
Positive
Known Marginally Positive
Known Negative
Negative
Inconclusive
Out of Domain

Additional Calls:

- Konsolidator (combines model calls for mutagenicity)
- Consensus (combines multiple model calls for other specific endpoints).

Derek Nexus

- Derek Nexus is an expert knowledge-based system for evaluating structure-activity relationships (SAR) in order to make toxicity predictions in mammals and bacteria.

- A Derek Nexus prediction includes an overall conclusion (ranging from “certain” to “impossible”) on the likelihood of toxicity resulting from a structure of interest and the detailed reasoning informing that likelihood. The prediction is generated by applying reasoning rules to toxicology data returned from a knowledge base.

- The Derek knowledge base is created and supplied by Lhasa Limited and contains data from both published sources and private data donated by our member organizations. This data is collated, checked and verified by Lhasa scientists before being used to develop new knowledge rules and relationships based on the principles of toxicology.

The Derek Workflow

- Currently, the Derek Knowledge Base is capable of identifying 872 alerts and 74 toxicological endpoints across 9 parent categories.

Endpoints	
Carcinogenicity	Genotoxicity
Irritation	Neurotoxicity
Organ Toxicity	Reproductive Toxicity
Respiratory Sensitization	Skin Sensitization
Misc (Blood in Urine, Cerebral Edema, Mitochondrial Disruption, etc.)	

- The assistance of an expert reasoning system like Derek allows for the rapid processing of large numbers of ingredients against a wide range of relevant endpoints to identify potential hazards. This can help direct future research, compliment experimental data in weight of evidence arguments, and even infer valuable information on compounds with little to no experimental data.

In Silico Toxicology: Recap

- Statistical rule-based software (CASE Ultra) - initial hazard identification
- Expert knowledge-based system (Derek Nexus) - further hazard identification
- *In silico* scenarios:

CASE Ultra	+	-	-	+
Derek Nexus	-	+	-	+

- Integration of *in silico* predictions and experimental data

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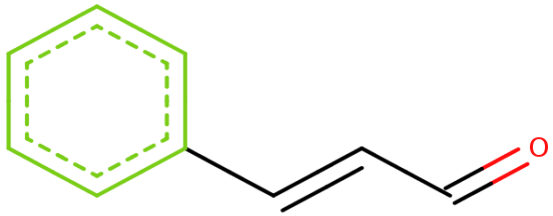
Ingredient Risk Assessment

- Conduct quantitative risk assessment (QRA)
  - Comparison of reference toxicological threshold (e.g., Acceptable Daily Intake [ADI]) identified from literature review
    - Relevant uncertainty factors applied (i.e., inter- and intraspecies extrapolation)
  - Calculate a margin of exposure (MOE)
    - Usage scenarios based on consumer actual use data
      - e.g., 8 or higher OTDN pouches/day, 100% intake assumption
  - MOE ≥ 1 indicates the estimated daily exposure is below a threshold of toxicological concern

Case Example

- Cinnamaldehyde
  - CAS# 104-55-2
  - FEMA GRAS (#2286)
  - Approved flavoring substance pursuant to 21 CFR § 182.60



- Literature Review
  - Unlikely to be genotoxic and no evidence of carcinogenic activity
  - Skin/eye irritation with increasing concentrations and mild to moderate skin sensitizer

Case Example - Cinnamaldehyde

- *In Silico* Software Predictions

Endpoint	Carcinogenicity	Genotoxicity	Skin Toxicity
CASE Ultra	-	+	+
Derek Nexus	-	+	+
Literature Review	-	-	+

- *In silico* predictions and literature review correlate
  - Positive genotoxicity hazard predicted from *in silico*
    - However, MOE ≥ 1 based on
      - Estimated total exposure is less than derived ADI from 2-year carcinogenicity study in rats and mice
      - No clinical findings at maximum average daily (oral) exposure
    - Therefore, based on the overall weight-of-evidence (*in silico*, experimental data, expert judgement), cinnamaldehyde is acceptable for intended use

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Summary

- A tiered approach provides a comprehensive ingredient assessment
- *In silico* toxicology assessment is gaining acceptance by regulatory agencies as it informs hazard identification
- QRA is performed to support the use levels for ingredients
- Experimental testing is an important component of risk assessment outcomes
- Finally, expert judgement is critical and carries significant weight of evidence in the ultimate decision

Questions?

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