Preclinical Testing of Flavors in E-vapor Products, Part 1: Selection of Representative Flavor Mixtures for Toxicological Evaluations using a Structural Grouping Approach

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Overview of Session

- **Part 1: Selection of Representative Flavor Mixtures Using a Structural Grouping Approach (Kim Ehman)**
- Part 2: Preparation and Stability Characterization of Representative Flavor Mixtures (Cameron Smith)
- Part 3: In Vitro Cytotoxicity and Genotoxicity of Representative Flavor Mixtures (Utkarsh Doshi)
- Part 4: Flavor Transfer from the Liquid to the Aerosol for Inhalation Exposure (Jingjie Zhang)

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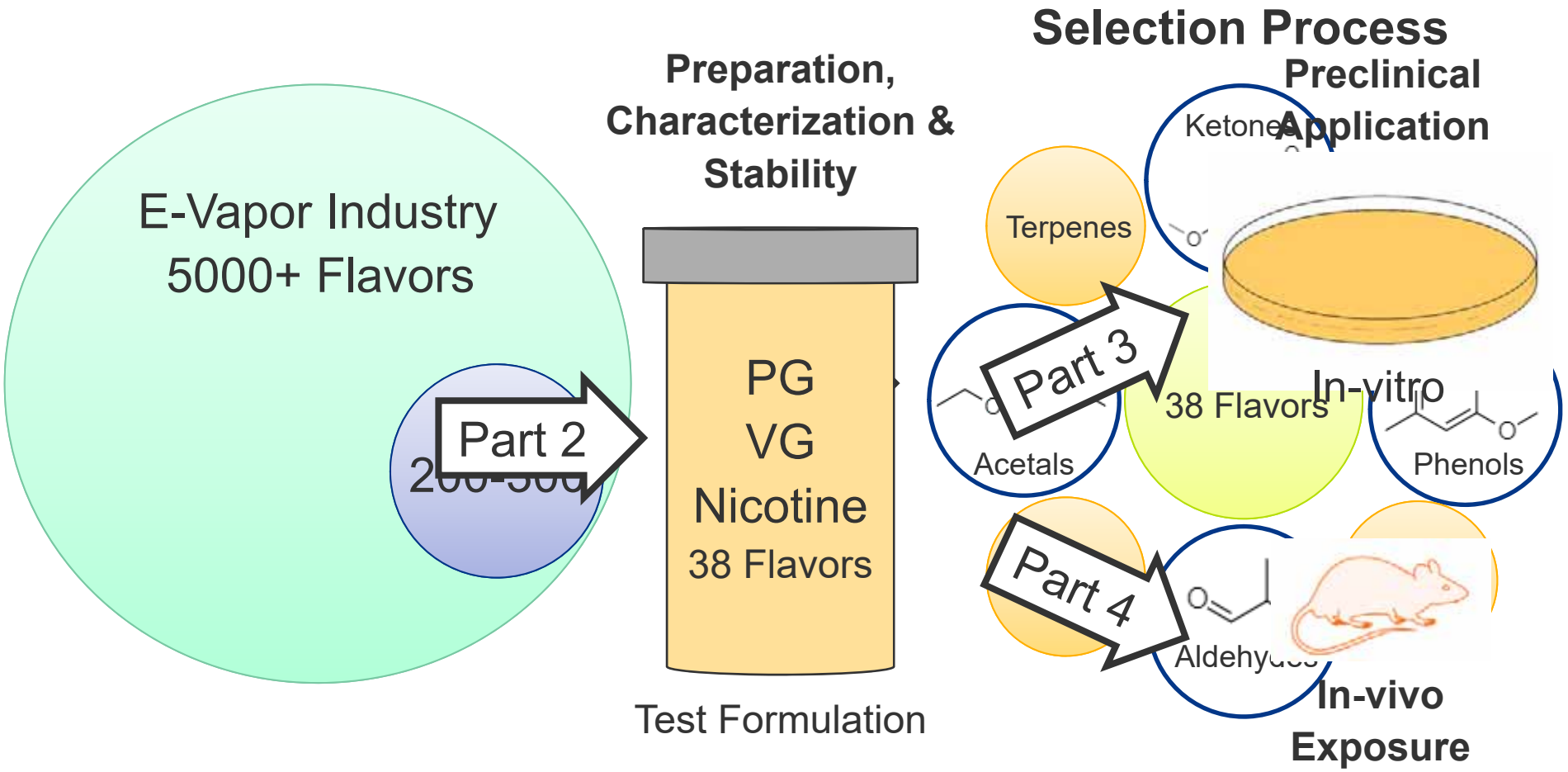
Preclinical Testing of Flavors in E-vapor Products: Overview



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Approach Rationale

- Evaluate structural similarities to develop a representative test formulation for preclinical toxicity testing

- Limitations in toxicological review and testing:
  - Food grade and GRAS (Generally Recognized as Safe) for use in food
  - Ingredient-specific inhalation data
    - Not always available
    - Would require years of animal testing to develop
  - Numerous potential flavor combinations

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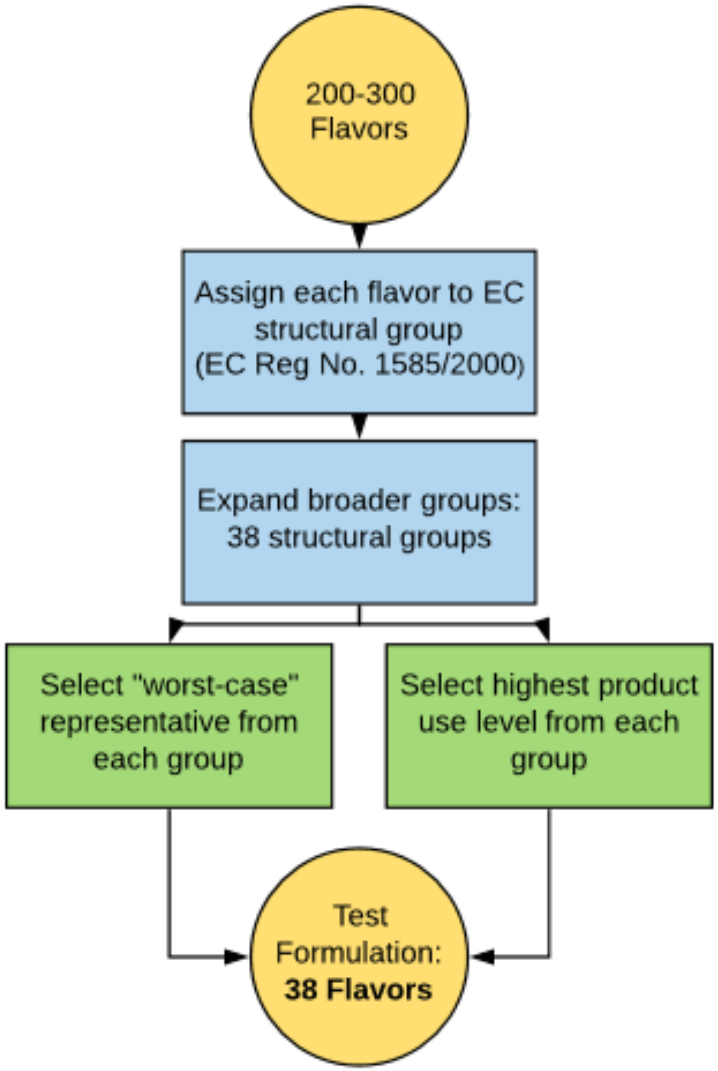
Overview of Flavor Selection Approach



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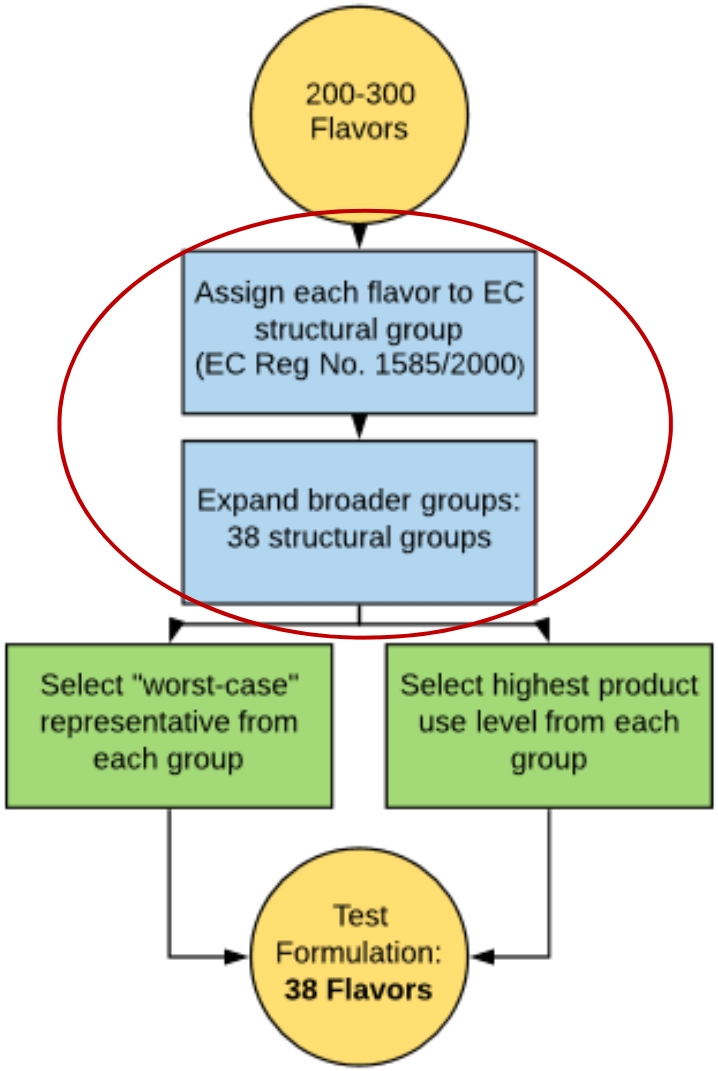

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Structural Groupings (EC Reg No. 1565/2000)

COMMISSION REGULATION (EC) No 1565/2000
of 18 July 2000
laying down the measures necessary for the adoption of an evaluation programme in application of Regulation (EC) No 2232/96 of the European Parliament and of the Council
(Text with EEA relevance)

ANNEX I

CHEMICAL GROUPS FOR FLAVOURING SUBSTANCES (1)

1. Straight-chain primary aliphatic alcohols/aldehydes/acids, acetals and esters with esters containing saturated alcohols and acetals containing saturated aldehydes. No aromatic or heteroaromatic moiety as a component of an ester or acetal.

2. Branched-chain primary aliphatic alcohols/aldehydes/acids, acetal and esters with esters containing branched-chain alcohols and acetals containing branched-chain aldehydes. No aromatic or heteroaromatic moiety as a component of an ester or acetal.

Our approach:

- Instead of 1 representative for Group 1 and 1 representative for Group 2, the groups were combined and 5 representatives were selected to better represent the broad category

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Example of Structural Groupings

Group	Representative Flavor	EC Groups: Group 1 (straight-chain) and Group 2 (branched-chain)
1	Acetal	Acetals
1-2a	Isobutyraldehyde	Aldehydes
1-2b	Isoamyl alcohol	Alcohols
1-2c	2-Methylbutyric acid	Acids
1-2d	Ethyl 2-methylbutyrate	Esters

Flavors within a given chemical group are "*expected to show some metabolic and biological behavior in common*" (EC No. 1565/2000)

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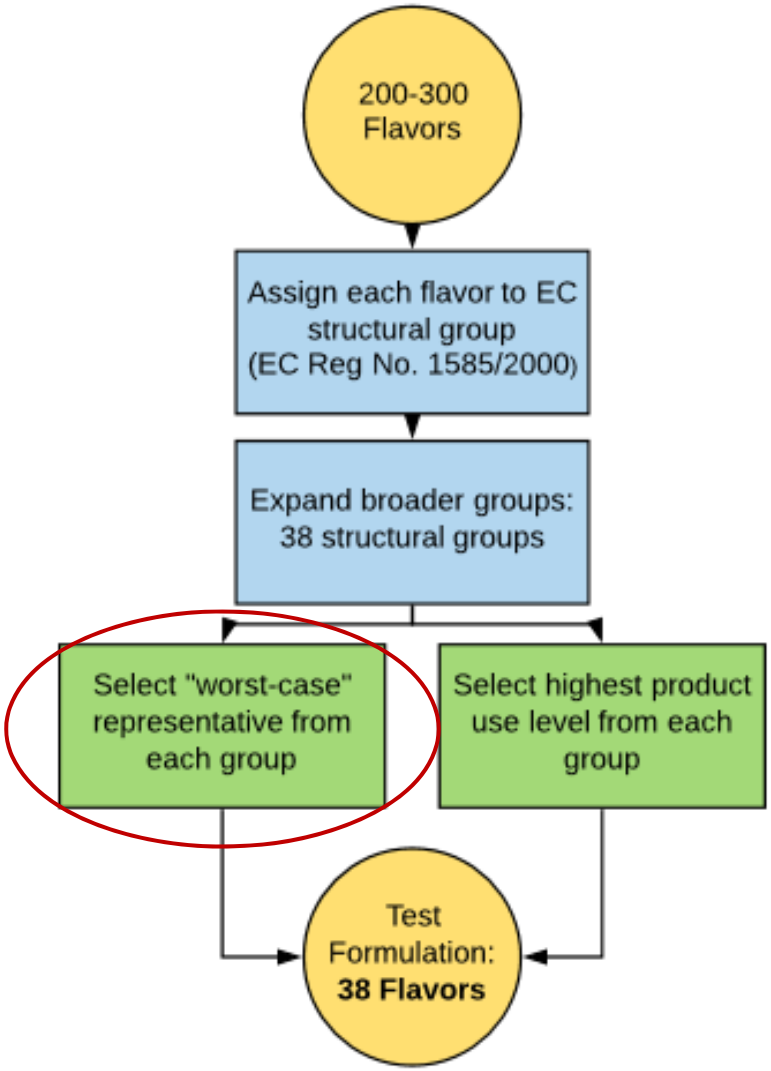

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Toxicological Review for Each Flavor

- Conducted comprehensive literature search for each flavor
  - Selected reliable experimental studies, for example:
    - Acute toxicity
    - Repeated dose toxicity
    - *In vitro* and *in vivo* genotoxicity
    - Developmental/reproductive toxicity
    - Irritation/sensitization
    - Carcinogenicity

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- Applied *in silico* predictions to fill in data gaps
  - Cramer Classification
  - TOPKAT (predictive software)
    - Predicted: acute inhalation toxicity and repeated dose toxicity (including chronic), irritation, carcinogenicity, developmental toxicity

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Predictive data allowed for comparisons within a group

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Selection of Flavor Group Representative

- Considered both experimental and predicted data
  - Gaps in experimental data created difficultly for comparison among compounds within a group
  - Predicted data provided a consistent comparison
    - "Worst-case" could be approximate
- Endpoints were assigned a numerical code or converted to rank data
- Applied objective computational procedures to rank flavors within the assigned groups
  - Included positive controls to test scoring/ranking approach

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Attributes for Selection of Flavor Group Representative

Example: Aliphatic and Aromatic Hydrocarbons

Name	LD50 rank	DevTox rank	ToxPi™ rank[a]	Chronic LOAEL rank	Irritation rank	Avg. group rank	Final group rank
	Experimental	Predicted	Predicted	Predicted	Predicted		
Alpha-pinene	1	2.5	4	1	2	2.1	1
Beta-caryophyllene	5	2.5	3	3	6	3.9	2
Cis-ocimene	5	2.5	7	4	2	4.1	3
D-limonene	2	6.5	1	6	6	4.3	4
Alpha-phellandrene	7	6.5	6	2	2	4.7	5.5
Beta-pinene	5	2.5	5	5	6	4.7	5.5
Terpinolene	3	6.5	2	7	6	4.9	7
1,3,5-Undecatriene	8	6.5	8	8	6	7.3	8

[a]Toxicological Priority Index: Numerical index developed by EPA that can be used to rank multiple domains of information (Reif et al., 2010, 2013)

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Summary

- Approach creates a representative mixture for preclinical testing to support >200 flavors
  - Reduces time needed to generate data on a large number of individual flavors
  - Reduces animal testing
  - Supports read-across strategies for inclusion of future flavors

- Limitations of approach
  - Use of predicted data may represent an approximate “worst-case” flavor representative
  - Mixture toxicity could be driven by most toxic compounds
  - Solubility and stability

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