Risk Assessment Strategy of Flavor Ingredients in e-Vapor Products

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PMI SCIENCE
PHILIP MORRIS INTERNATIONAL

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Beyond Science & Decisions…*Flavor Ingredients in e-Vapor Products*

- Building on the ideas of the NAS' Science & Decisions: Advancing Risk Assessment (2009)
- A real-time compendium of practical, problem-driven approaches for "fit for purpose" risk assessments
- Links novel and pragmatic scientific methods and approaches with specific problems faced by risk assessors and risk managers
- Enhanced communication and collaboration across various stakeholders (e.g., regulatory, and industry, academic community)

I. INTRODUCTION

The Importance of Flavor Ingredients in Harm Reduction

Donna Smith

Current Situation

- Cigarette Smoking is still the leading cause of major preventable diseases, morbidity and mortality worldwide.
- The current prevalence of smoking in the US is ~14%[1-2]
- Quit attempts often fail, and long-term cessation is low[3-5]

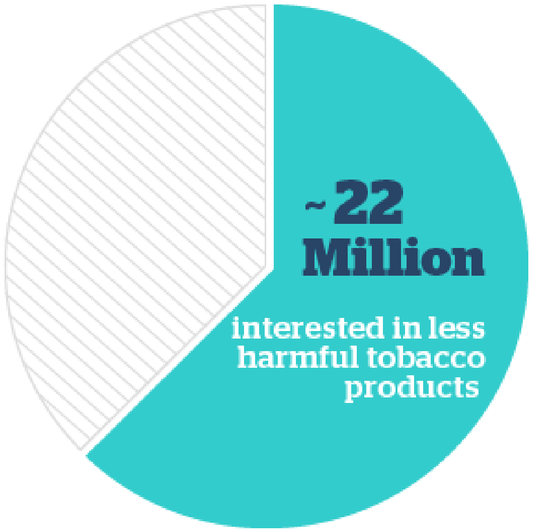
(1) U.S. Department of Health and Human Services. Smoking Cessation: A Report of the Surgeon General— Executive Summary. Atlanta, GA: U.S. Department of Health and Human Services, Centers for Disease Control and Prevention, National Center for Chronic Disease Prevention and Health Promotion, Office on Smoking and Health, 2020.
(2) U.S. Department of Health and Human Services. How Tobacco Smoke Causes Disease: The Biology and Behavioral Basis for Smoking-Attributable Disease: A Report of the Surgeon General. Atlanta, GA: U.S. Department of Health and Human Services, Centers for Disease Control and Prevention, National Center for Chronic Disease Prevention and Health Promotion, Office on Smoking and Health, 2010.
(3) Tobacco Advisory Group of the Royal College of Physicians. Nicotine Without Smoke—Tobacco Harm Reduction. 2016
(4) Hughes JR, *et al.* Shape of the Relapse Curve and Long-Term Abstinence Among Untreated Smokers. *Addiction* 2004;99(1):29-38
(5) Institute of Medicine. Scientific Standards for Studies on Modified Risk Tobacco Products. Washington, DC: The National Academies Press. 2012.

Why Harm Reduction?

- *"A centerpiece of [FDA's] comprehensive regulatory plan is acknowledging that nicotine, while highly addictive, is delivered through products on a continuum of risk. And it's the delivery mechanism – not the nicotine itself – that is truly the issue at-hand."*

  Scott Gottlieb, M.D.
  Former Commissioner of Food and Drugs

- Of those smokers in the US who are unable or unwilling to quit, the majority are interested in "less harmful" tobacco products



According to data from the FDA's PATH study, over half of adult smokers would consider using a tobacco product if it had a reduced harm claim. This equates to about 22 million adult smokers who are interested in less harmful tobacco products, particularly if they receive truthful and accurate risk information.

The Continuum of Risk

- A strong public health consensus has formed that not all tobacco products present the same risk
- These authorities agree that there is a broad continuum of risk among tobacco products, with cigarettes at the highest end of that spectrum
- This continuum recognized that most of the harm caused by tobacco results from the burning of tobacco

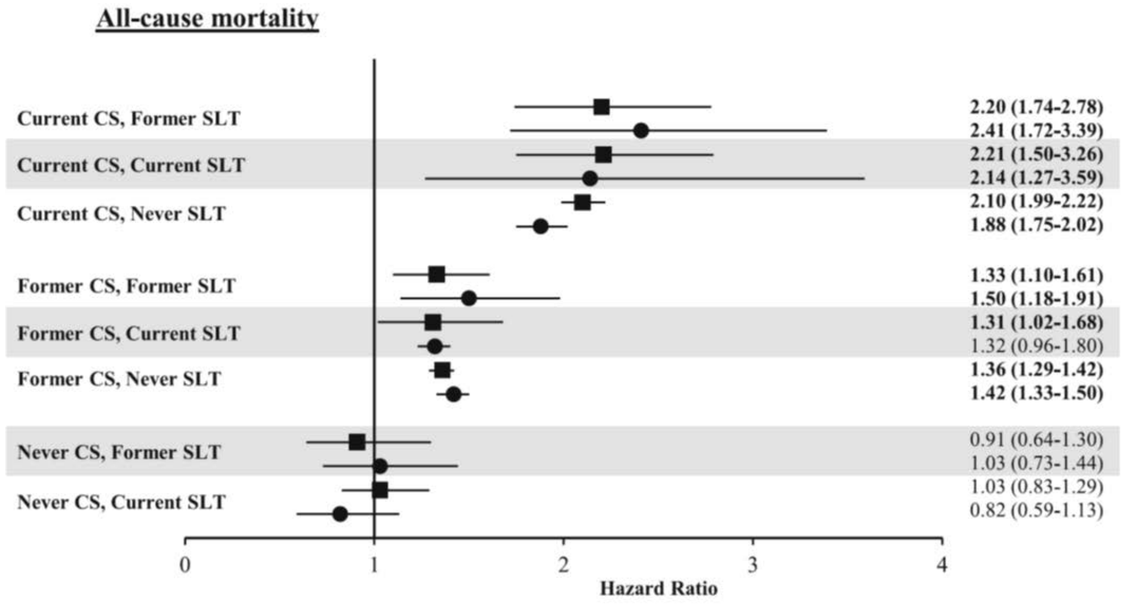
(1) See, e.g., Zeller M, Hatsukami D. The Strategic Dialogue on Tobacco Harm Reduction: a vision and blueprint for action in the US Tobacco Control 2009;18:324-332 & Dorothy K, *et al*. Developing the Science Base for Reducing Tobacco Harm. *Nicotine Tob Res* 2007;9(04):S537–53.

Harm Reduction Equation

- The availability of acceptable combustion-free alternatives to smoking is important
- It is paramount that these alternatives be both:
  - Satisfying
  - Sensorially acceptable

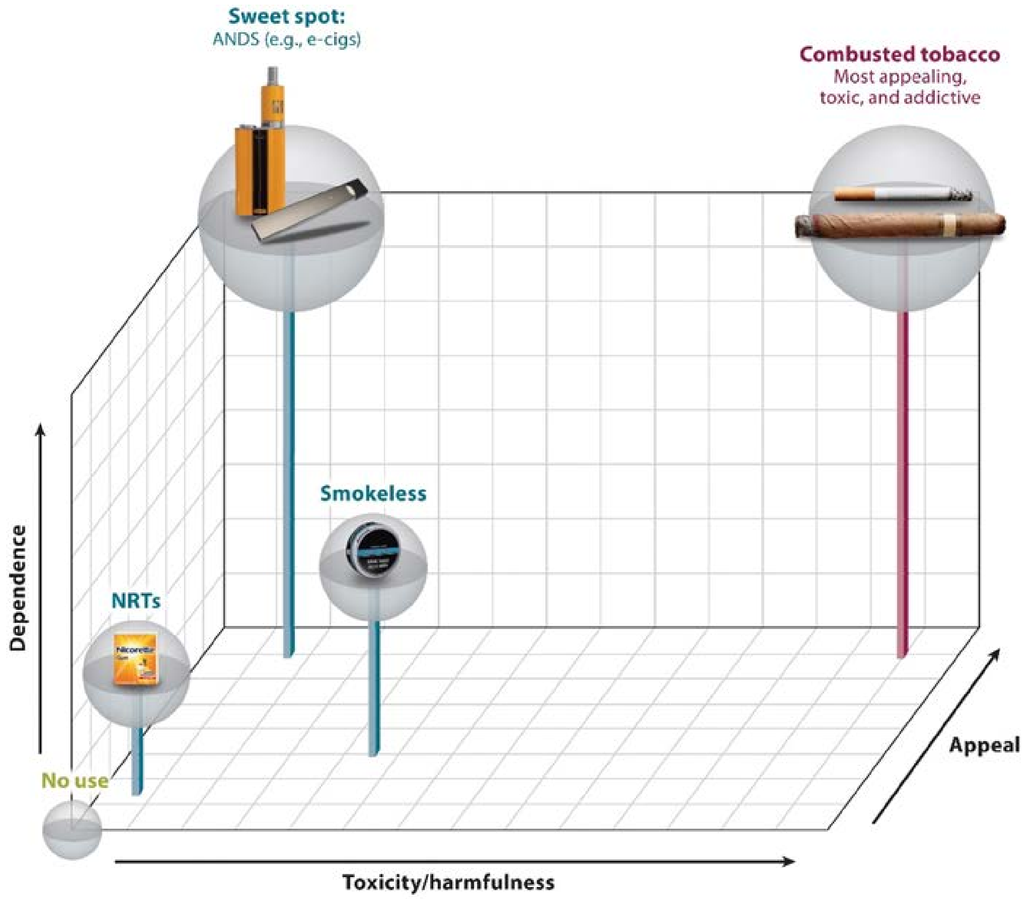
Is the Availability of Reduced Risk Products Enough?

- **Smokeless** tobacco products are widely available in the US, but consumer acceptance on a national level is very low
- Analyses of available epidemiological data show that smokeless tobacco products are significantly less harmful than cigarettes



See, Michael Fisher *et al*. Smokeless Tobacco Mortality Risks: An Analysis of Two Contemporary Nationally Representative Longitudinal Mortality Studies. *Harm Reduction Journal.* 16:27 (2019)

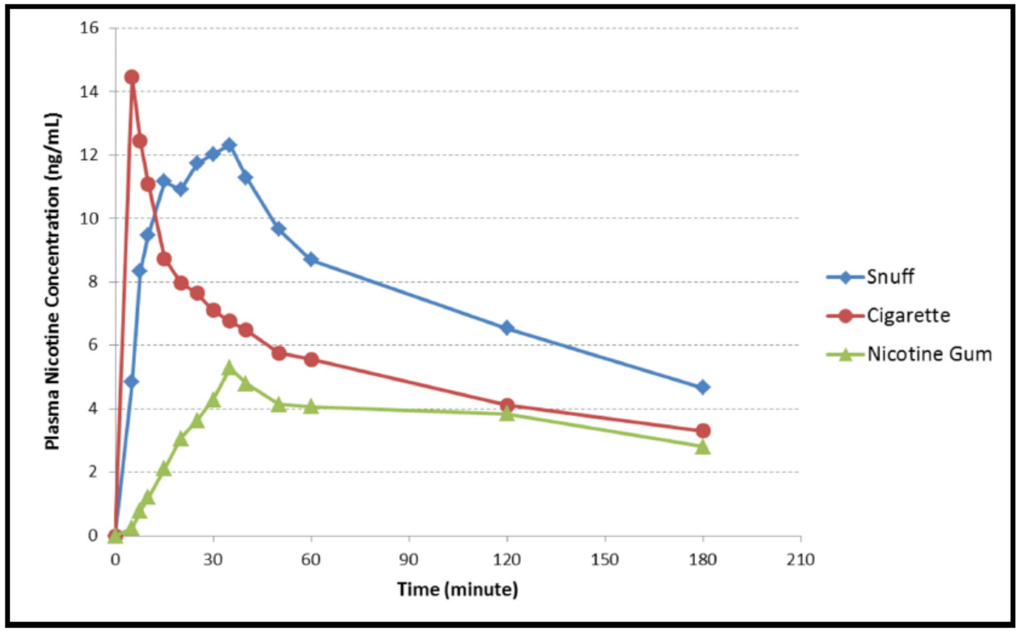
Multidimensional Framework for Nicotine Containing Products



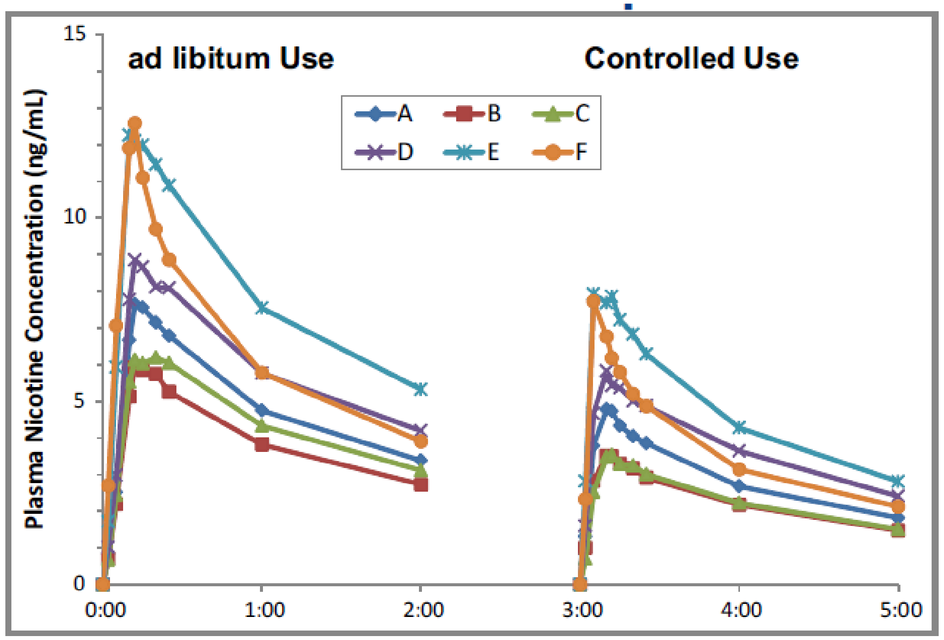
Abrams DB, et al. 2018.
Annu. Rev. Public Health. 39:193–213

Pharmacokinetics

- E-vapor products more closely mimic the PK of cigarettes than smokeless tobacco or NRTs

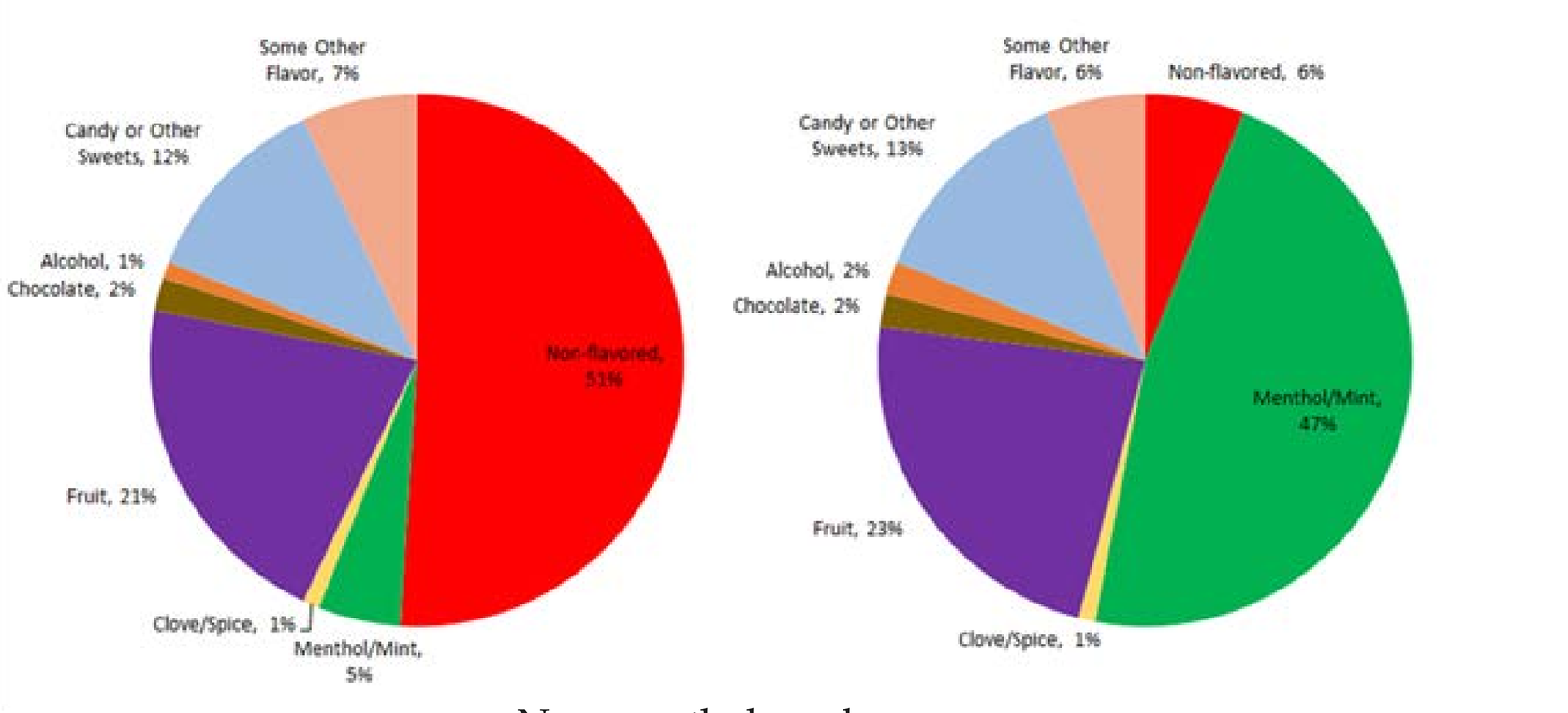


See, Liu *et al*. Assessment of Abuse Potential of a Moist Smokeless Tobacco Product Relative to Cigarette and Nicotine Gum Based on Nicotine Pharmacokinetics and Subjective Effect Measures. Presented at the Global Forum on Nicotine 6/14-6/16, Warsaw, Poland.



See, Liu *et al*. Differences in Plasma Nicotine Pharmacokinetic Profiles for Various E-Vapor Products Used by Adult Smokers Under Ad-Libitum vs. Controlled Use Conditions. Presented at the 71st Tobacco Science Research Conference, 11/28-12/1, 2017, Bonita Springs, Fl.

Flavor Ingredients Selection is Important to Realize the Greatest Harm Reduction on a Population Level



Non-menthol smokers

Data analyzed from the Population Assessment of Tobacco and Health (PATH) at Wave 2 from current adult dual consumers of cigarettes and e-vapor, where this is defined as having used more than 100 cigarettes in their lifetime and now using cigarettes every day or some days, and having ever used e-vapor fairly regularly and now using e-vapor every day or some days.

Flavor Ingredients in E-vapor Products

- Most e-vapor products contain flavor ingredients
- While these flavor ingredients are GRAS for use in food, their inhalation toxicity is generally unknown
- E-vapor products deliver a mixture of flavor ingredients along with carriers such as propylene glycol, glycerine, acids and nicotine
- There are thousands of flavor ingredients that could be used in e-vapor products

U.S. Department of Health and Human Services. Smoking Cessation: A Report of the Surgeon General— Executive Summary. Atlanta, GA: U.S. Department of Health and Human Services, Centers for Disease Control and Prevention, National Center for Chronic Disease Prevention and Health Promotion, Office on Smoking and Health, 2020.

Toxicological Considerations for Flavor Ingredients

- Route of exposure is inhalation
- Complex mixtures
- Stability
- Flavor ingredient transfer from the e-liquid to the aerosol
- Aerosol particle size and resulting deposition
- Extrapolation of data from animal studies to human exposure
- Long-term health effect

II. CASE STUDY – Flavor Ingredients in e-Vapor Products

Flavor Group Representatives (FGRs): Selection Based on Structural Grouping Approach

Davide Sciuscio

Some Considerations….

- Typical flavor mixtures contain 20 flavors
- Food approved flavor ingredients are often used in e-cigarettes
- 2500 flavor ingredients have been approved by EFSA (for food)
- Today >5000+ Flavors are available on the market (growing)

However

- No Inhalation data available for the vast majority of flavor ingredients
- GRAS status for the use of flavor ingredients in food does not mean that GRAS flavor ingredients are safe for use in ENDS
- Lack of standards for flavor testing

Therefore

Imperative to acquire safety data on flavor ingredients used by inhalation in a fast and agile way.

But

Classical approaches for evaluating safety require a series of in vitro and in vivo studies on individual flavors and definition of safe-use levels (not suitable)

Because

- Costly and time consuming (years of animal testing)
- Single Flavor ingredients or Mixtures (numerous flavor combinations possible)
- Additive, synergistic or antagonistic effects?
- Lack of standards: aerosol generation/collection method? *In vitro* tests? *In vivo* tests?

Some Examples to Acquire Safety Data on Chemicals

In recent years, the use of alternative low-testing and/or non-testing methods for the hazard assessment of substances has been promoted by several regulatory frameworks across different sectors and countries, in order to minimize monetary, timing and ethical costs associated with *in vivo* testing

ECHA
EUROPEAN CHEMICALS AGENCY

Read-across is one of the most commonly used alternative approaches for filling data gaps in registrations submitted under REACH. This approach uses relevant information from analogous (‘source') substances to predict the properties of ‘target' substances.

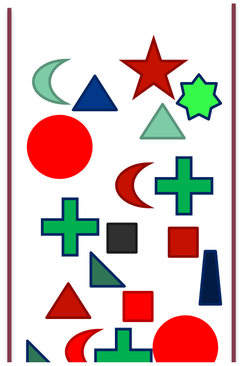
efsa
European Food Safety Authority

EFSA have used a Flavoring Group Evaluation (FGE) approach to assess flavor ingredients in food. The Procedure is a stepwise approach that integrates information on intake from current uses, structure-activity relationships, metabolism and, when needed, toxicity.

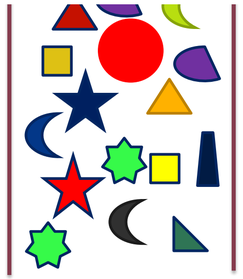
Structurally related compounds are expected to show some metabolic and biological behavior in common

Combinatorial Flavor-Group-Based Approach

Flavor compounds available



2500+ Substances

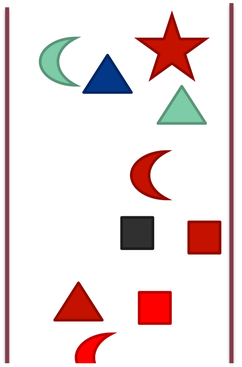


Selection

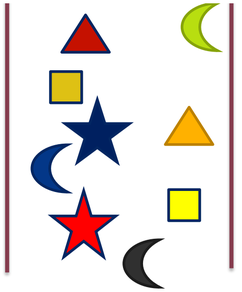
FEMA GRAS

NO
- CMR
- classified respiratory sensitizers
- oils/extracts

Example Flavor Toolbox



245 Substances



Clustering

(EC) No 1565/2000

Refinement on structural similarities

Groups of Relevant Flavor Ingredients

1)

2)

3)

38 Groups

38)

Rank based on potential for inducing toxicity

Experimental data
- Literature (ECHA, ToxPlanet)
- *In vitro* data (RTCA, HCS)

Predicted data
- Toxicological prediction (e.g. with TOPKAT)
- Predicted HCS (Reg. Mod. ToxPi)
- Cramer classes

Flavor Group Representative (FGR)

1) Acetal

2) Linalool

3) Ethyl lactate

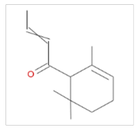
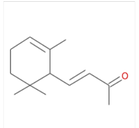
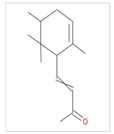
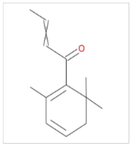
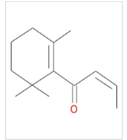
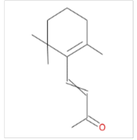
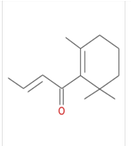
1 Representative for Each Group (38)

38) Furaneol

Example for Selection of an FGR: Group 8

EU definition for group 8:

- Secondary alicyclic saturated and unsaturated alcohols/ketones/ketals/esters with ketals containing alicyclic alcohols or ketones and esters containing secondary alicyclic alcohols. Esters may contain aliphatic acyclic or alicyclic acid component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43052-87-5	127-41-3	79-69-6	23696-85-7	23726-92-3	14901-07-6	23726-91-2
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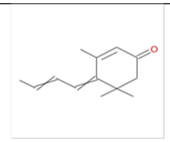
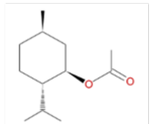
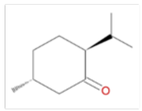
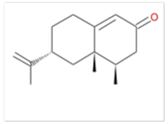
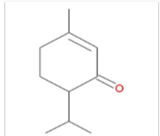
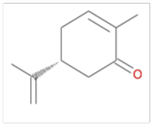
EU Group 8	EU Group 8	EU Group 8	EU Group 8	EU Group 8	EU Group 8	EU Group 8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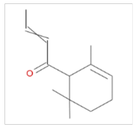
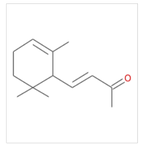
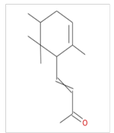
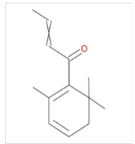
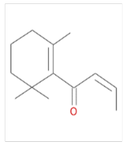
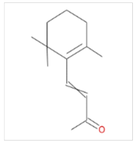
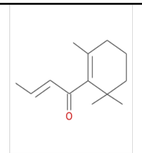
07.173	09.016	07.176	07.089	07.175	07.147
EU Group 8	EU Group 8	EU Group 8	EU Group 8	EU Group 8	EU Group 8

Example for Selection of an FGR: Group 8

EU definition for group 8:

- Secondary alicyclic saturated and unsaturated alcohols/ketones/ketals/esters with ketals containing alicyclic alcohols or ketones and esters containing secondary alicyclic alcohols. Esters may contain aliphatic acyclic or alicyclic acid component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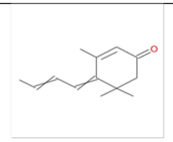
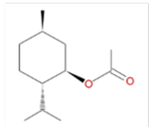
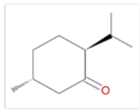
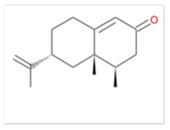
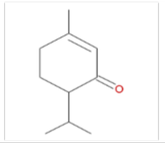
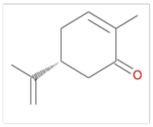
EU Group 8	EU Group 8	EU Group 8	EU Group 8	EU Group 8	EU Group 8	EU Group 8
GROUP 8A						

Ionones and structurally related substances

Carvone and structurally related substances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07.173	09.016	07.176	07.089	07.175	07.147
EU Group 8	EU Group 8	EU Group 8	EU Group 8	EU Group 8	EU Group 8
GROUP 8B					

Example for Selection of an FGR: Group 8a Data Acquisition

- Oral LD_{50}, mutagenicity and genotoxicity data (ECHA or ToxPlanet database)
- *In vitro* cytotoxicity (internal data)
- DNA Damage, Oxidative Stress, Inflammation, etc. (internal data)

Flavoring substance	CAS	EU Chemical group	PMI/ALCS Chemical group	ECHA LD_{50} mg/kg	Toxplan LD_{50} mg/kg	NOAEL Repeated dose toxicity oral	Interpretation Mutagenicity*	Interpretation Genotoxicity*	EC_{50} ratio	ToxPiScore (HCS)
ALPHA-DAMASCONE	43052-87-5	8	8A	.	1670	2,35 mg/kg bw/day	Negative	Equivocal	0.35	0.29
DAMASCENONE, BETA-	23696-85-7	8	8A	.	> 2000	2.35 mg/kg/day	.	.	1.09	.
DAMASCONE, BETA- ISOMER 1	23726-92-3	8	8A	.	2920	2,35 mg/kg bw/day	Negative	Equivocal	0.85	0.33
DAMASCONE, BETA- ISOMER 2	23726-91-2	8	8A	> 2000	2920	.	Negative	.	0.64	.
IONONE, ALPHA-	127-41-3	8	8A	4590	.	.	Negative	Positive	0.86	.
IONONE, BETA-	14901-07-6	8	8A	4590	3290	.	Negative	Negative	0.48	0.23
IRONE, ALPHA-	79-69-6	8	8A	>5000	.	.	Negative	.	0.82	

* Literature data from different studies (Ames, MLA, MN, SCE etc.) have been reviewed and interpreted providing a final recommendation

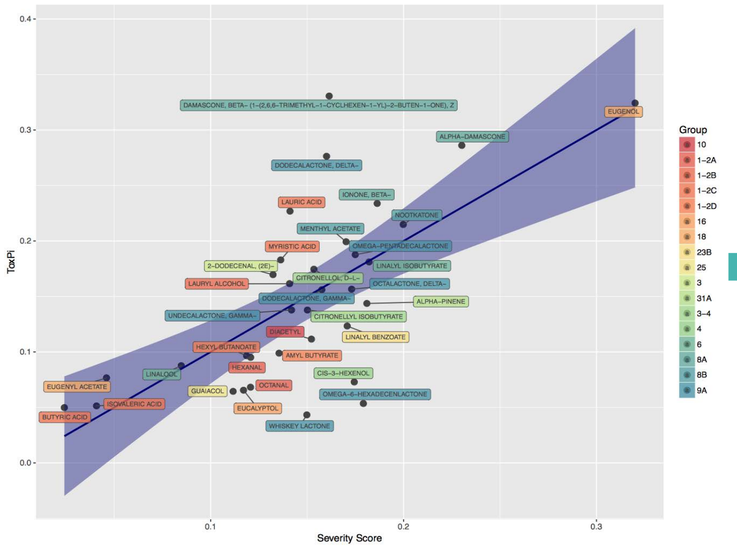
Example for Selection of an FGR: Group 8a
FGRs Data Integration (2)

- Mechanistic data completion using Toxicological Priority Index (ToxPi) developed by EPA and predictive modelling
- A predictive model was developed in order to complement HCS data for all flavor ingredients: pCramer, pIrritancy, pChronicLOAEL, pExpCarcinogenicity and pXCelligence were retained in the final model

Mechanistic Based Screening

Toxicological Priority Index (ToxPi)

Regression model



Predicted ToxPi

Flavoring substance	CAS	PredictedToxPi
ALPHA-DAMASCONE	43052-87-5	0,23
DAMASCENONE, BETA-	23696-85-7	0,08
DAMASCONE, BETA-ISOMER 1	23726-92-3	0,16
DAMASCONE, BETA-ISOMER 2	23726-91-2	0,17
IONONE, ALPHA-	127-41-3	0,19
IONONE, BETA-	14901-07-6	0,19
IRONE, ALPHA-	79-69-6	0,14

HCS data were available for **35 Flavorings**

The model based on the attributes above was the best predictive model (based on CV-RMSE, final model R=0.87) and selected for predicting the ToxPi for all the Flavor ingredients.

Example for Selection of an FGR: Group 8a FGRs Data Integration (1)

- Predictive *in vivo* toxicity modeling (TOPKAT[1])
- Cramer Classes (OECD QSAR Toolbox[2])

Flavoring substance	CAS	EU Chemical group	PMI/ALCS Chemical group	Cramer Class	TOPKAT Ocular Irritancy	TOPKAT Rodent Carcinogenicity	TOPKAT Chronic LOAEL (mg/kg b.w.)	TOPKAT Develop. Toxicity
ALPHA-DAMASCONE	43052-87-5	8	8A	Class I	true	true	10.46	true
DAMASCENONE, BETA-	23696-85-7	8	8A	Class I	true	true	11.71	true
DAMASCONE, BETA- ISOMER 1	23726-92-3	8	8A	Class I	false	true	26.93	false
DAMASCONE, BETA- ISOMER 2	23726-91-2	8	8A	Class I	false	true	26.93	false
IONONE, ALPHA-	127-41-3	8	8A	Class I	false	true	12.57	false
IONONE, BETA-	14901-07-6	8	8A	Class I	false	true	32.56	false
IRONE, ALPHA-	79-69-6	8	8A	Class I	false	true	7.24	true

[1] TOPKAT (TOxicity Prediction by Komputer Assisted Technology) employs robust and cross-validated Quantitative Structure Toxicity Relationship (QSTR) models for assessing various measures of toxicity and utilizing the patented Optimal Predictive Space validation method to assist in interpreting the results.
[2] https://qsartoolbox.org/

Example for Selection of an FGR: Group 8a Ranking and FGR Selection

Flavor within each group was ranked based on :

1. pLD50, pDevToxicity, PredictedToxPi, pChronicLOAEL and pIrritancy scores
2. For each flavor, the average rank is computed which is used to generate the final ranking (FinalGroupRank)

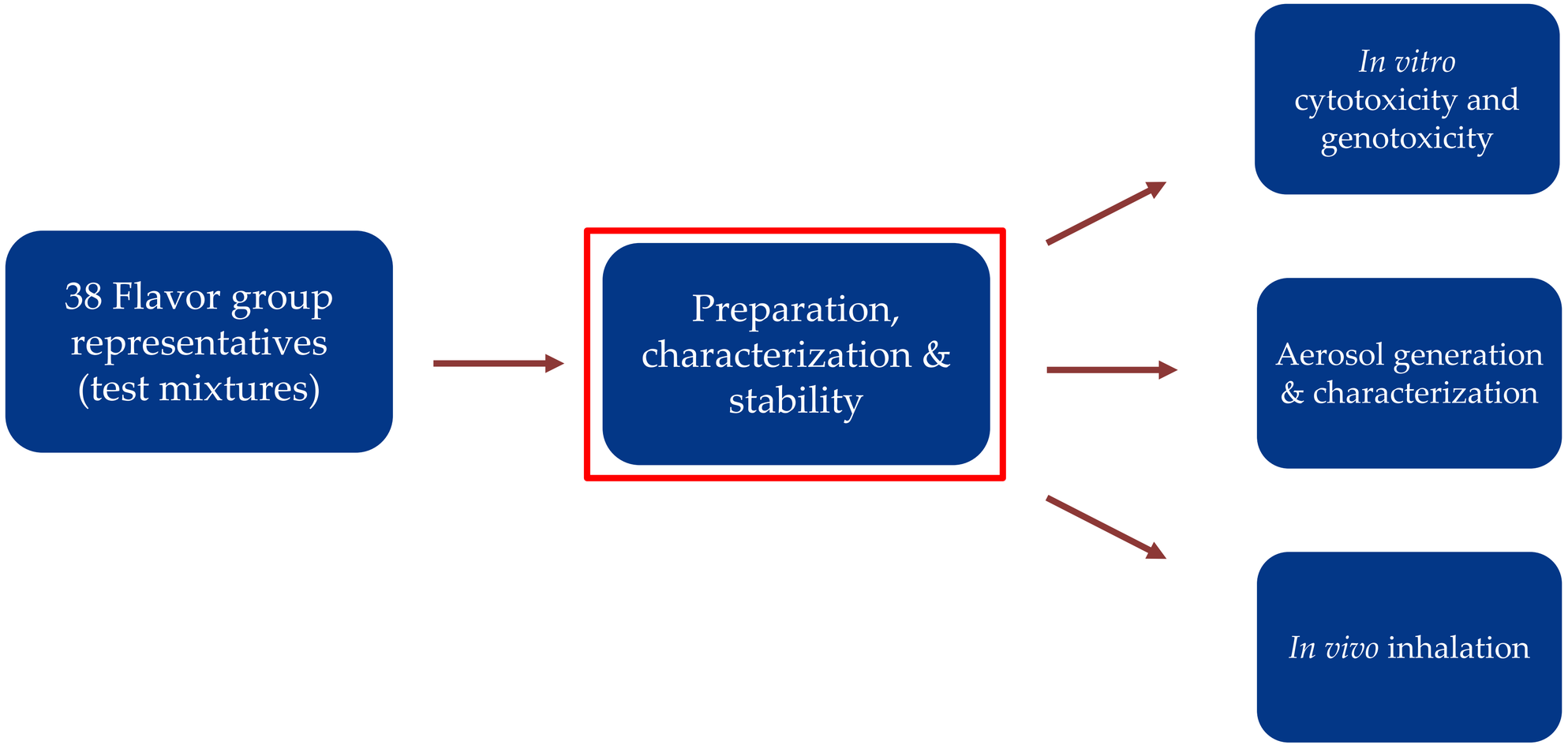
Worst case of the group 8A

Flavoring substance	LD50_GroupRank	pDevToxicty_GroupRank	PredictedToxPi_GroupRank	pChronicLOAEL_GroupRank	pIrritancy_GroupRank	AverageGroupRank	FinalGroupRank
ALPHA-DAMASCONE	**1**	**2**	**1**	**2**	**1,5**	**1,5**	**1**
DAMASCENONE, BETA-	2,5	2	7	3	1,5	3,2	2
DAMASCONE, BETA- ISOMER 1	4	5,5	5	5,5	5	5	6
DAMASCONE, BETA- ISOMER 2	2,5	5,5	4	5,5	5	4,5	5
IONONE, ALPHA-	5,5	5,5	2	4	5	4,4	4
IONONE, BETA-	5,5	5,5	3	7	5	5,2	7
IRONE, ALPHA-	7	2	6	1	5	4,2	3

Flavor Group Representatives – Final Selection

GROUP NUMBER	PMI/ALCS GROUP NAME	FLAVOR GROUP REPRESENTATIVES	GROUP NUMBER	PMI/ALCS GROUP NAME	FLAVOR GROUP REPRESENTATIVES
1	GROUP 1	ACETAL	20	GROUP 13	FURANEOL
2	GROUP 1-2 a	ISOBUTYRALDEHYDE	21	GROUP 15	2-METHYL-4-PHENYL-2-BUTANOL
3	GROUP 1-2 b	ISOAMYL ALCOHOL	22	GROUP 16	AMBROX
4	GROUP 1-2 c	METHYLBUTYRIC ACID, 2-	23	GROUP 18	EUGENYL ACETATE
5	GROUP 1-2 d	ETHYL 2-METHYLBUTYRATE	24	GROUP 20	P-MENTHA-8-THIOL-3-ONE
6	GROUP 3	(E,Z)-2,6-NONADIENAL	25	GROUP 21	ACETANISOLE
7	GROUP 3-4	CITRONELLOL, D-L-	26	GROUP 22	METHYL CINNAMATE
8	GROUP 4	CIS-3-HEXENOL	27	GROUP 23 a	ETHYL VANILLIN
9	GROUP 5 a	ISOPULEGOL	28	GROUP 23 b	BENZYL ALCOHOL
10	GROUP 5 b	1-PENTEN-3-ONE	29	GROUP 24	2,5-DIMETHYLPYRAZINE
11	GROUP 6	LINALOOL	30	GROUP 25	2-METHOXY-4-METHYLPHENOL
12	GROUP 8 a	ALPHA-DAMASCONE	31	GROUP 26	PARA-DIMETHOXYBENZENE
13	GROUP 8 b	PIPERITONE	32	GROUP 27	METHYL ANTHRANILATE
14	GROUP 9 a	DELTA NONALACTONE	33	GROUP 28 a	3-ETHYLPYRIDINE
15	GROUP 9 b	ETHYL LACTATE	34	GROUP 28 b	2-ACETYLPYRROLE
16	GROUP 9 c	TRIETHYL CITRATE	35	GROUP 29	2-ACETYLTHIAZOLE
17	GROUP 10	3-METHYL-2,4-NONANEDIONE	36	GROUP 30	KETOISOPHORONE
18	GROUP 11	DIHYDROACTINIDIOLIDE	37	GROUP 31 a	ALPHA-PINENE
19	GROUP 12	ETHYL MALTOL	38	GROUP 31 b	PARA-CYMENE

Flavor Group Representative Assessment



II. CASE STUDY – Flavor Ingredients in e-Vapor Products

Flavor Group Representatives (FGRs): Preparation and Stability Characterization

Cameron Smith

Definition: Pre-Blends

- Basic concept: concentrated ingredients (flavors) are diluted and combined to make a final mixture or product

- **Pre-blends** used in this study are concentrated (5–20 × more than the test formulation) mixtures containing PG, ethanol, and selected flavor compounds

- Pre-blends can increase shelf life and aid in the repetitive and time-consuming batch characterization necessary in preclinical studies

Study Design

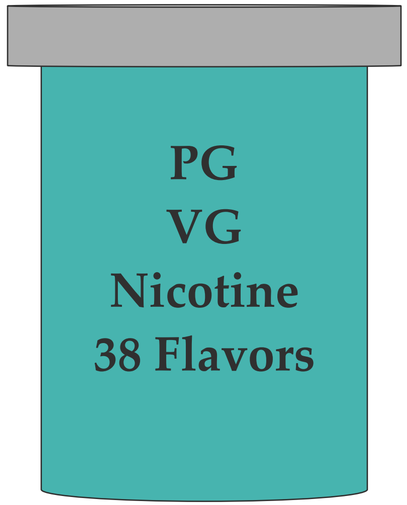
Longer Stability (Weeks)

- Pre-blend IA – 9
- Pre-blend IB – 7
- Pre-blend IC – 6
- Pre-blend II – 7
- Pre-blend III – 2
- Pre-blend IV – 6

Dilute with PG, VG, Water, Nicotine

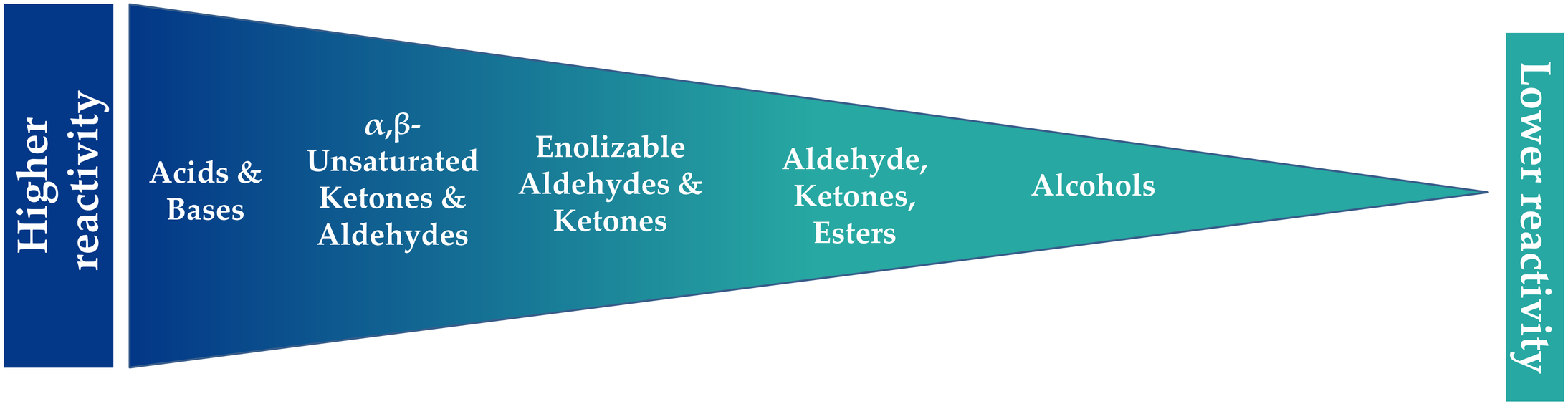
+1 Flavor

Shorter Stability (Days)



Test Formulation

Grouping into Stable Pre-Blends



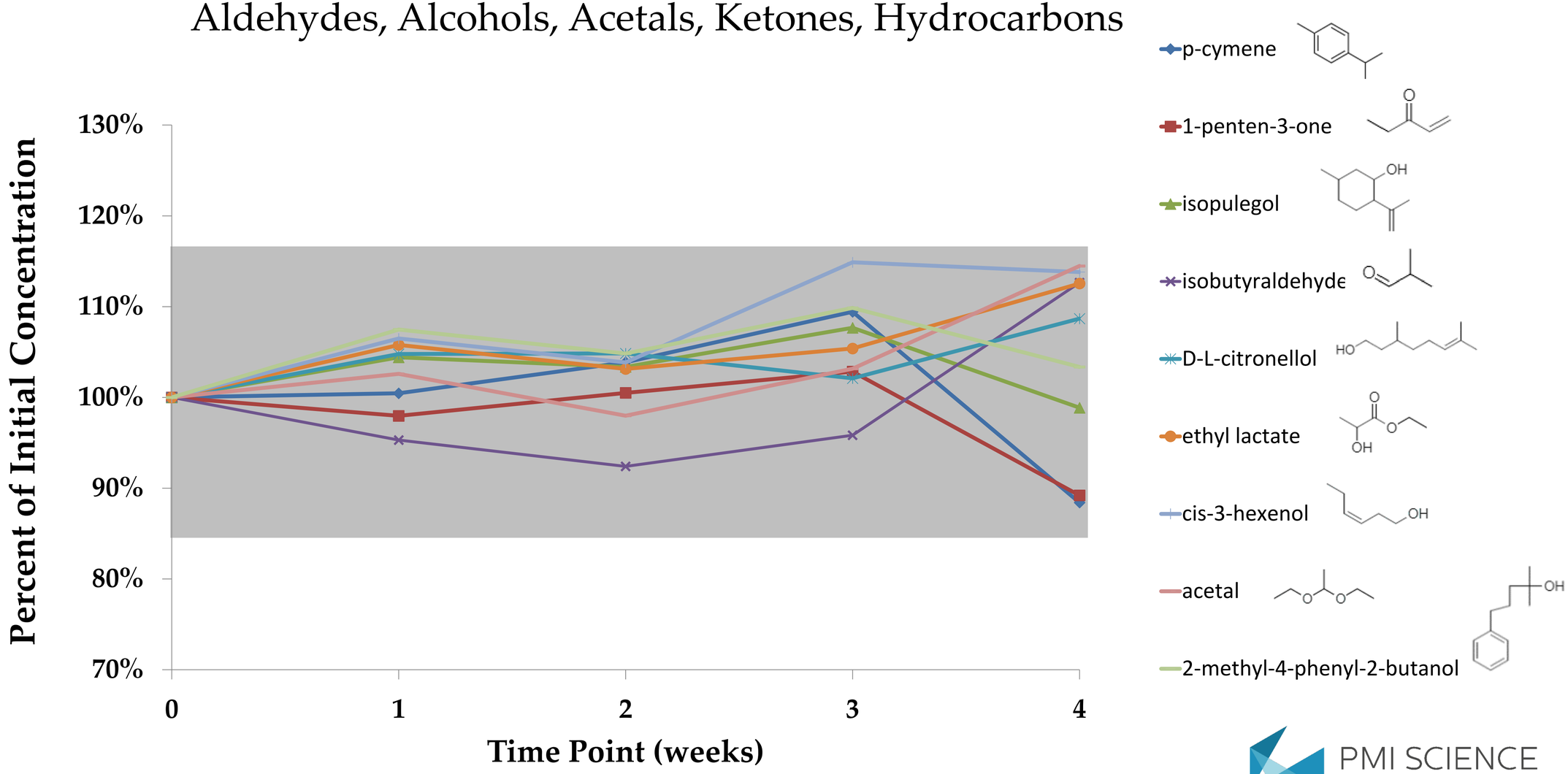
- Evaluated reactivity of compounds based on functional group characteristics
- Define the minimum number of categories as possible
- Ensured compounds within each grouping had limited reactivity

Stability Study Design

- Evaluate using gas chromatography-mass spectrometry (GC-MS)
- 1 Month Stability for Pre-blends
  - Refrigerated and Room Temperature Conditions
- 10 Days Stability for Test Formulations (All 38 FGRs)
  - Refrigerated and Room Temperature Conditions

Example: Pre-blend 1A Stability

Aldehydes, Alcohols, Acetals, Ketones, Hydrocarbons



Test Formulation Without Nicotine

Group #	Flavor Group Representatives	T0	T1 - 1 day	T2 - 7 days (± 1 day)	T3 - 11 days (± 1 day)
1	acetal	100%	102%	107%	95%
2	isobutyraldehyde	100%	106%	102%	86%
3	isoamyl alcohol	100%	98%	99%	98%
4	2-methylbutyric acid	100%	98%	97%	97%
5	ethyl 2-methylbutyrate	100%	100%	104%	105%
6	(E,Z)-2,6-nonadienal	100%	98%	99%	92%
7	citronellol, D-L-	100%	100%	91%	82%
8	cis-3-hexenol	100%	99%	101%	87%
9	isopulegol	100%	103%	104%	88%
10	1-penten-3-one	100%	99%	92%	81%
11	linalool	100%	93%	90%	86%
12	a-damascone (trans)	100%	101%	96%	95%
13	piperitone	100%	97%	102%	97%
14	d-nonalactone	100%	96%	102%	96%
15	ethyl lactate	100%	95%	98%	92%
16	triethyl citrate	100%	102%	114%	106%
17	3-methyl-2,4-nonanedione	100%	100%	105%	101%
18	dihydroactinidiolide	100%	96%	105%	97%
19	ethyl maltol	100%	102%	110%	104%
20	furaneol	100%	97%	101%	96%
21	2-methyl-4-phenyl-2-butanol	100%	99%	99%	88%
22	ambrox (Cetalox©)	100%	99%	96%	95%

Group #	Flavor Group Representatives	T0	T1 - 1 day	T2 - 7 days (± 1 day)	T3 - 11 days (± 1 day)
23	eugenyl acetate	100%	97%	95%	95%
24	p-mentha-8-thiol-3-one	100%	99%	92%	92%
25	acetanisole	100%	95%	90%	89%
26	methyl cinnamate	100%	97%	103%	98%
27	ethyl vanillin	100%	98%	105%	100%
28	benzyl alcohol	100%	97%	101%	97%
29	2,5-dimethylpyrazine	100%	97%	97%	97%
30	2-methoxy-4-methylphenol	100%	98%	103%	98%
31	p-dimethoxybenzene	100%	96%	93%	92%
32	methyl anthranilate	100%	97%	92%	92%
33	3-ethylpyridine	100%	98%	98%	98%
34	2-acetylpyrrole	100%	98%	98%	98%
35	2-acetylthiazole	100%	98%	97%	97%
36	ketoisophorone	100%	97%	101%	97%
37	a-pinene	100%	101%	103%	100%
38	p-cymene	100%	102%	104%	94%

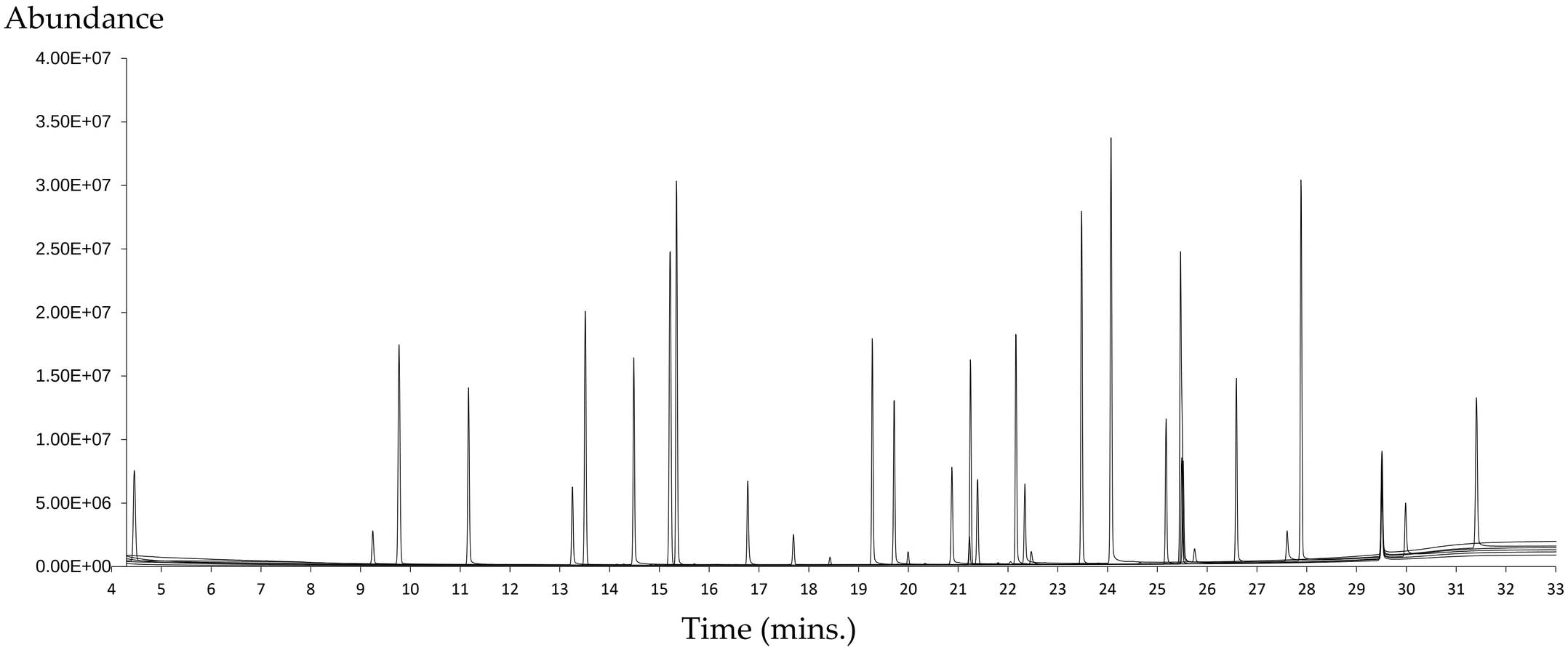
Test Formulation With Nicotine

Group #	Flavor Group Representatives	T0	T1 - 1 day	T2 - 7 days (± 1 day)	T3 - 11 days (± 1 day)
1	acetal	100%	111%	106%	107%
2	isobutyraldehyde	100%	88%	84%	91%
3	isoamyl alcohol	100%	101%	104%	104%
4	2-methylbutyric acid	100%	99%	107%	100%
5	ethyl 2-methylbutyrate	100%	107%	106%	114%
6	(E,Z)-2,6-nonadienal	100%	94%	89%	79%
7	citronellol, D-L-	100%	96%	90%	91%
8	cis-3-hexenol	100%	97%	96%	93%
9	isopulegol	100%	95%	93%	94%
10	1-penten-3-one	100%	93%	56%	45%
11	linalool	100%	90%	83%	81%
12	a-damascone (trans)	100%	96%	90%	89%
13	piperitone	100%	100%	106%	106%
14	d-nonalactone	100%	99%	99%	99%
15	ethyl lactate	100%	96%	90%	94%
16	triethyl citrate	100%	103%	109%	110%
17	3-methyl-2,4-nonanedione	100%	102%	105%	104%
18	dihydroactinidiolide	100%	101%	106%	106%
19	ethyl maltol	100%	100%	111%	106%
20	furaneol	100%	96%	93%	86%
21	2-methyl-4-phenyl-2-butanol	100%	97%	98%	97%
22	ambrox (Cetalox©)	100%	98%	95%	94%

Group #	Flavor Group Representatives	T0	T1 - 1 day	T2 - 7 days (± 1 day)	T3 - 11 days (± 1 day)
23	eugenyl acetate	100%	98%	97%	95%
24	p-mentha-8-thiol-3-one	100%	88%	73%	70%
25	acetanisole	100%	94%	92%	89%
26	methyl cinnamate	100%	101%	107%	106%
27	ethyl vanillin	100%	101%	106%	107%
28	benzyl alcohol	100%	101%	104%	105%
29	2,5-dimethylpyrazine	100%	101%	106%	105%
30	2-methoxy-4-methylphenol	100%	101%	107%	106%
31	p-dimethoxybenzene	100%	96%	96%	94%
32	methyl anthranilate	100%	98%	96%	92%
33	3-ethylpyridine	100%	101%	106%	105%
34	2-acetylpyrrole	100%	102%	106%	106%
35	2-acetylthiazole	100%	101%	108%	105%
36	ketoisophorone	100%	100%	104%	104%
37	a-pinene	100%	103%	109%	105%
38	p-cymene	100%	97%	96%	97%

Addition of nicotine shortens stability period

Analytical Learnings and Optimization



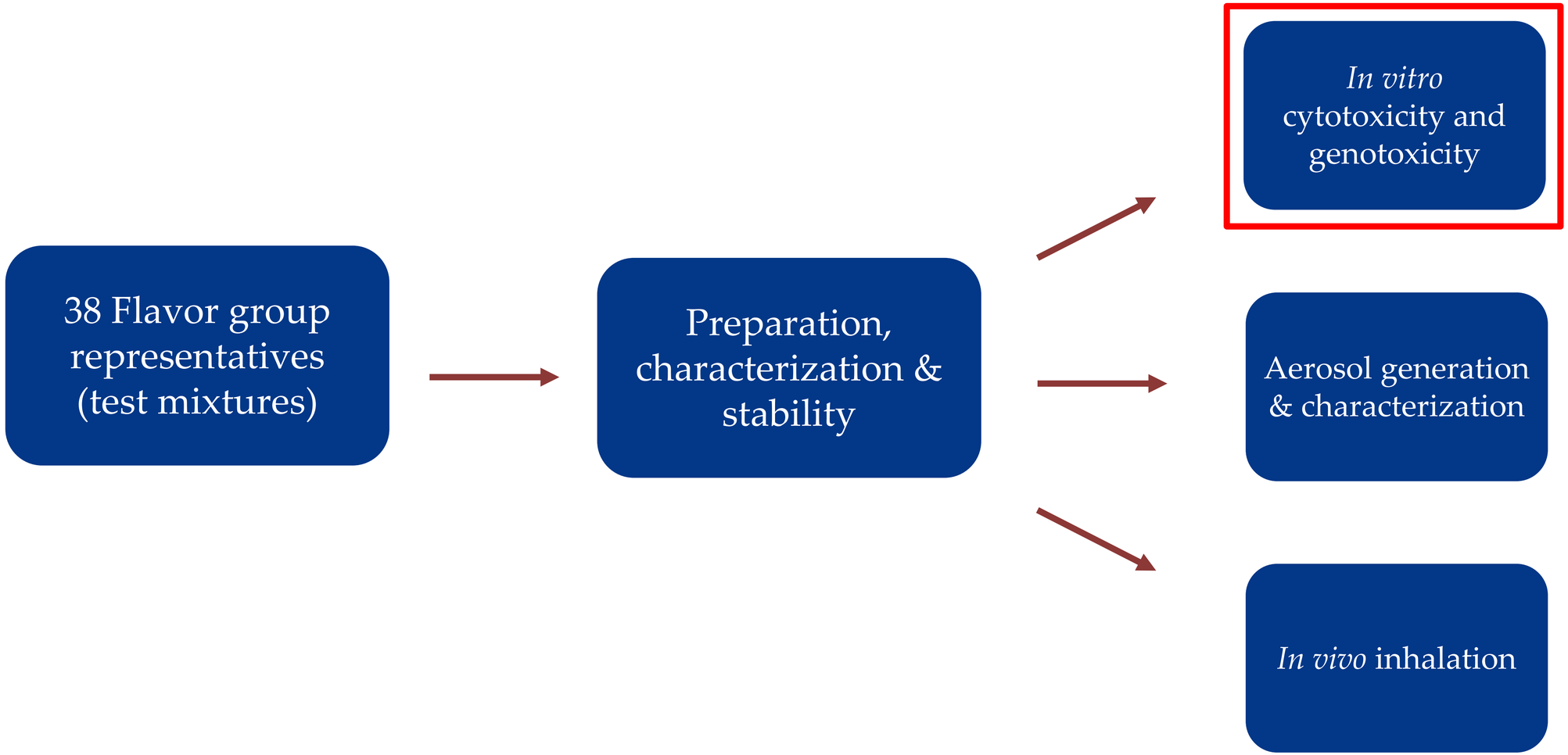
Analytical Learnings and Optimization

- Develop one all encompassing method – All 38 Flavor ingredients
- Develop method using common GC/MS
- Ensure solvent is unreactive
- Full Scan is well suited for identifying impurities
- Selective Ion Monitoring (SIM) useful for co-eluting peaks
- Method is well suited for verifying vendor supplied pre-blend formulations are prepared according to COA and reproducible from batch to batch

Stability Summary

- Depending on the test formulation ingredients, pre-blends are stable for a matter of months in refrigerated conditions
- All test formulation flavor ingredients used in the study were stable for at least 3 days in the presence of nicotine and 10 days without nicotine at refrigerated conditions
- Test formulation was stable for at least 1 day at room temperature
- Based on the stability data, test formulations containing nicotine was prepared fresh every 3 days during pre-clinical testing

Flavor Group Representative Assessment



II. CASE STUDY – Flavor Ingredients in e-Vapor Products

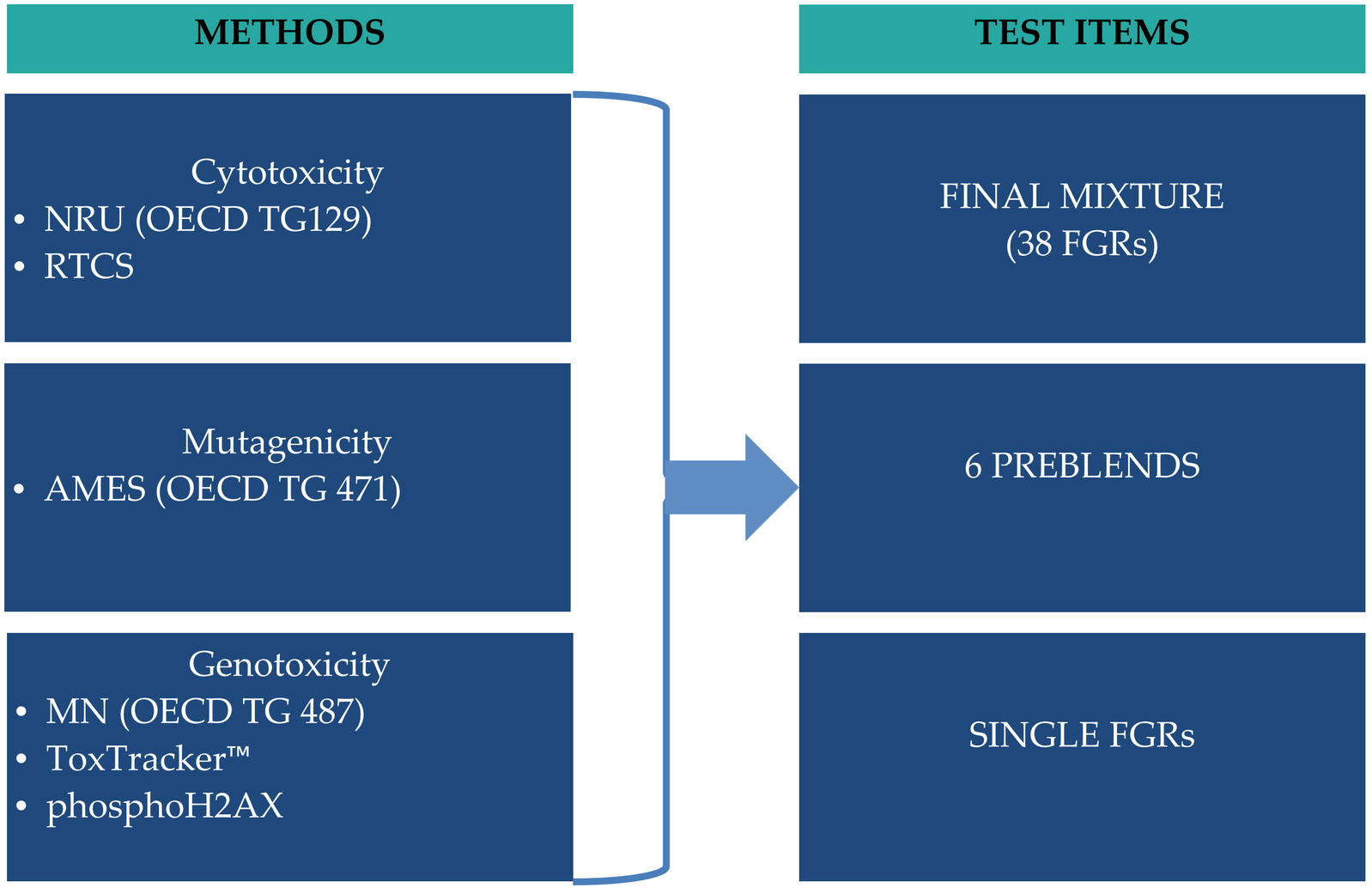
Flavor Group Representatives (FGRs): *In Vitro* Toxicity Screening

Davide Sciuscio

GOALS

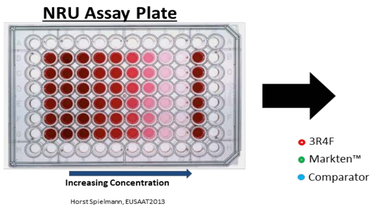
- Define a panel of *in vitro* tests to assess flavor mixtures and enable initial decision making process in product development
- Characterize the biological activity of the test mixture (FGRs)
- Identify the major contributors of the test mixture to biological effects

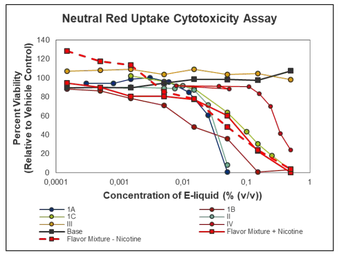
In Vitro Toxicity Screening

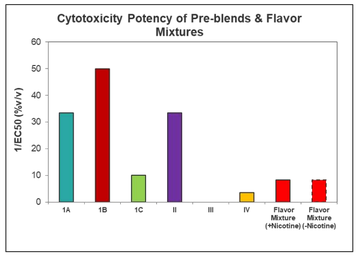


Pre-Blend and FGR Mixtures: *In Vitro* Regulatory Assays

- **Neutral Red Uptake (NRU) Cytotoxicity Assay (OECD TG129)**





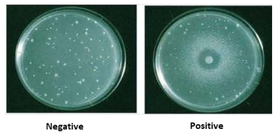


Murine fibroblast cell line

(BALB/c 3T3 cells, clone 31)

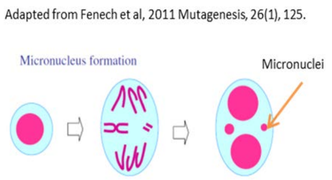
48 hr treatment

- **Ames Mutagenicity Assay (OECD TG 471)**



Test Articles	Mutagenicity
Carrier (PG/G/Nicotine)	Negative
Test Formulation	Negative
Test Formulation + Nicotine	Negative

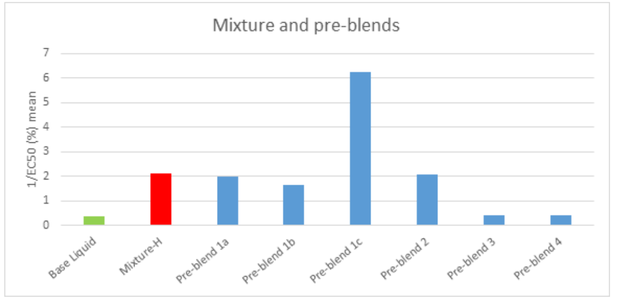
- **Micronucleus (MN) Assay (OECD TG 487)**



Test Articles	Genotoxicity
Carrier (PG/G/Nicotine)	Negative
Test Formulation	Equivocal
Test Formulation + Nicotine	Negative

Pre-Blend and FGR Mixtures: Additional *In Vitro* Assays

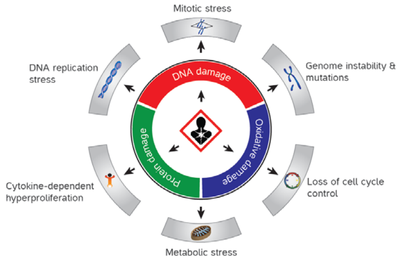
- **Real Time Cell Analyzer (RTCA) Cytotoxicity Assay**

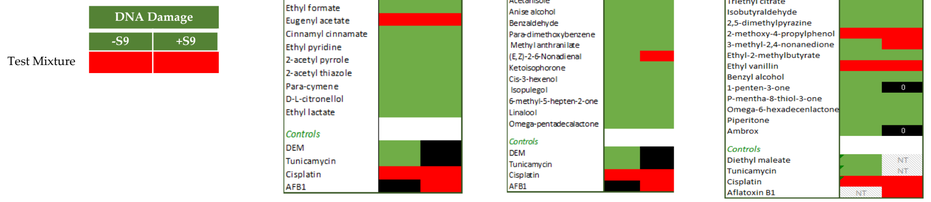


Normal Human Bronchial Epithelial Cells (NHBC)

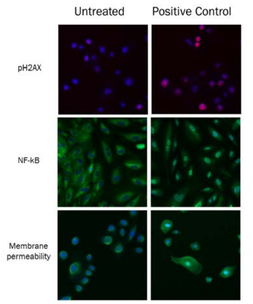
24hr treatment

- **ToxTracker™ Carcinogenicity Assay**





- **High Content Screening γH2Ax**



	Treatment		Literature evidence
	4 hours	24 hours	
(E,Z)2−6 Nonadienal			NA
2−methoxy−4−methylphenol			2-year study available = not carcinogenic
3−methyl−2,4−nonedione			NA
Ethyl Maltol			2-year study available = not carcinogenic
Ethylvanillin			NA
Eugenyl Acetate			NA
Furaneol			2-year study available = not carcinogenic
Matrix			NA
Mixture (18%)			NA

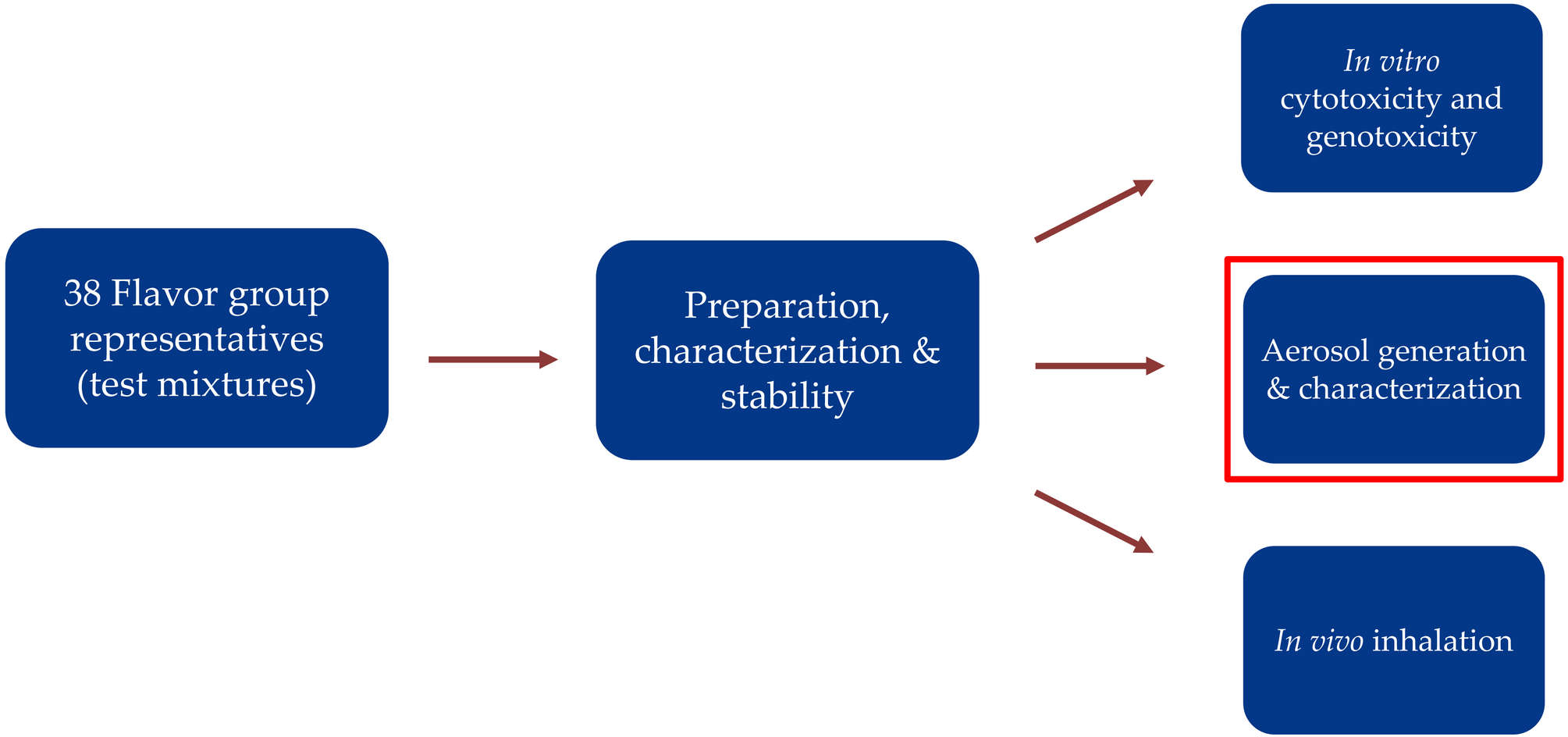
Positive FGRs *In vivo* Findings

FGRs	Carcinogenicity studies	REFERENCE
Ethyl maltol	2-year study available = not carcinogenic	Gralla et al. 1969
Eugenyl acetate	NA	(Miller et al. 1983; Miller et al. 1986; NTP 1983)
Furaneol	2-year study available = not carcinogenic	ECHA
Ethyl vanillin	NA	NA
(E,Z)-2,6-nonadienal	NA	NA
2-methoxy-4-propylpheno	2-year study available = not carcinogenic	ECHA
3-methyl-2,4-nonadieno	NA	NA

General Considerations And Conclusions

- ToxTracker™ and pH2Ax gave a better characterization of the genotoxic effects of test mixture and FGRs
- *The in vitro* panel of tests provided useful information about the hazards associated with the single FGRs, pre-blends and with the test mixture, and might be used to quickly characterize new flavor systems and drive product development
- It is important to highlight that the concentrations tested *in vitro* are often one or more orders of magnitude higher than those achievable *in vivo*, thus the *in vitro* results alone should not be interpreted in isolation to make statements about the safety of flavor ingredients

Flavor Group Representative Assessment

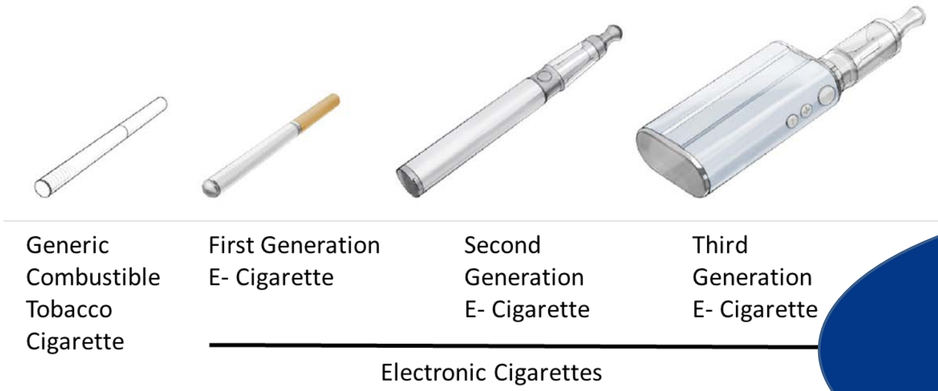


II. CASE STUDY – Flavor Ingredients in e-Vapor Products

Flavor Group Representatives: Aerosol Generation and Characterization

Patrick Vanscheeuwijck

Various Types of E-vapor Generation Systems



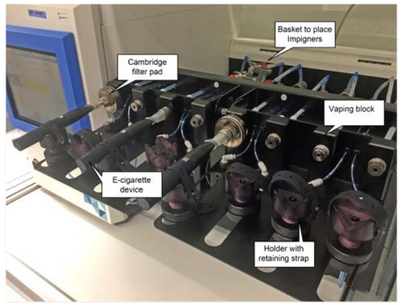
What shall be used?

- Adjustable voltage (3–6 V)
- Varying resistance (1.0–6.5 Ω)

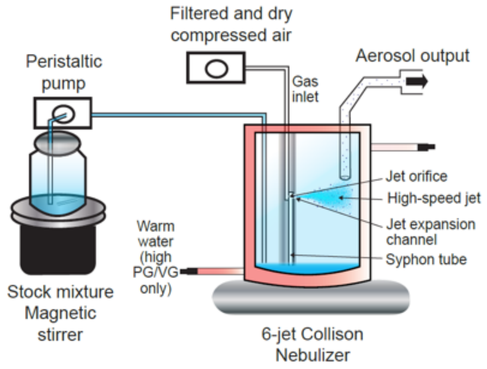
→ Potential for **user-driven changes in delivered power**

- **8000 flavors** available, and numbers are increasing

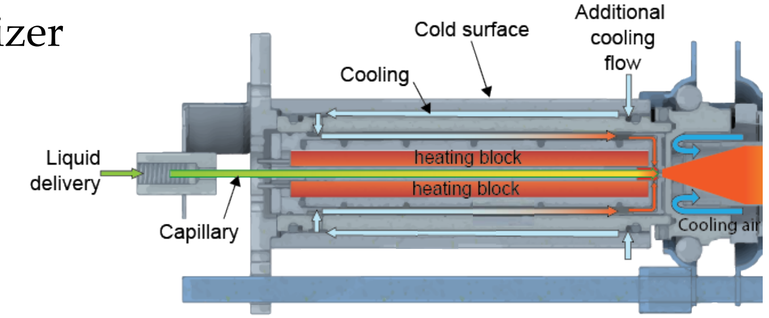
Multichannel e-cigarette vaping machines



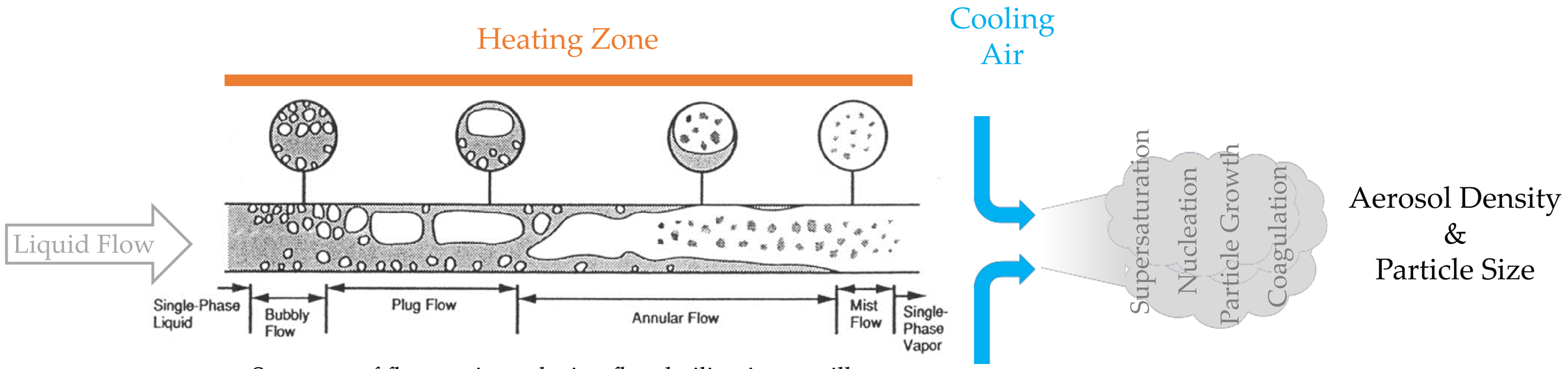
E-liquid nebulization collision nebulizer



Capillary aerosol generator (CAG)



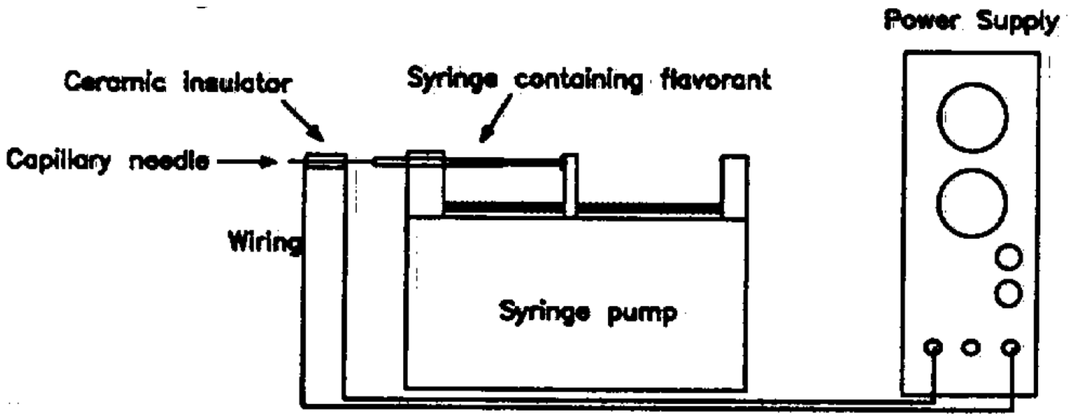
Aerosol Generation Process in CAG



Sequence of flow regimes during flow boiling in a capillary

- The CAG produces a stream of well controlled aerosol by heating and vaporization of a liquid, followed by nucleation and condensation of the vapor
- Liquid is pumped into an electrically heated capillary and hot, saturated vapor exiting from the tip of the capillary is cooled down, leading to homogeneous nucleation of vapors and condensational growth of generated nuclei to form an aerosol

Capillary Aerosol Generator (CAG)



- Invented by Philip Morris, Inc. (Howell and Sweeney, 1998)
- Further developed as a novel aerosol generator for pharmaceutical drug delivery

Benefits of using the CAG for e-vapor inhalation studies:

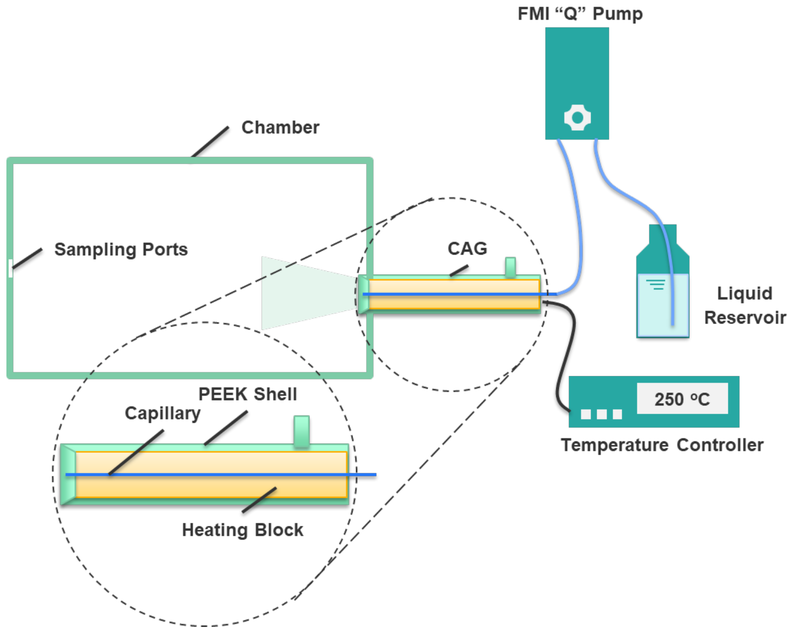
- Ability to assess e-liquid formulations independently of e-cigarette device specificities
- Ability to simulate the operating conditions (temperature) of e-cigarette devices
- Continuous production, over several hours, of a controlled aerosol similar to e-vapor
- Simplified logistics and less labor intensive

Prototype e-Cigarette and the Capillary Aerosol Generator (CAG) Comparison and Qualification for Use in Sub-Chronic Inhalation Exposure Testing

Chemical composition	• Analytical fingerprint chemical analysis: nearly identical number of known and unknown compounds
	• Good correlation of the aerosol levels of formulation constituents. Statistically significant difference in levels of PG will not be seen at the nose-only exposure ports
Particle size measurements	• Similarity in MMAD and GSD
Port-to-port variability	• Differences in exposure port homogeneity below ± 10% and generally not statistically significant
Chemical by-products	• Acetaldehyde below the LOQ for both generators
	• Acrolein levels not statistically significantly different
	• About eight times higher level of formaldehyde from the prototype e-cigarette compared with the CAG

CAG is suitable for use in 28-day, 90-day or longer inhalation studies

Aerosol Generation & Characterization



	Test Formulation w/ Nicotine (n = 4)	Test Formulation w/o Nicotine (n = 4)
MMAD (μm)	0.97 ± 0.07	1.23 ± 0.06
GSD	1.77 ± 0.18	1.82 ± 0.13

	Test Formulation w/ Nicotine (N = 3)			Test Formulation w/o Nicotine (N = 3)		
Analyte	**Liquid**	**Aerosol**	**Transfer[b]**	**Liquid**	**Aerosol**	**Transfer[b]**
Aerosol Mass (mg)	NA	98.1±2.0	NA	NA	108.2±1.8	NA
Ethanol (mg/g)	20.44±0.13	BLOQ	NA	20.19±0.23	BLOQ	NA
Glycerol (mg/g)	144.3±0.3	146.2±2.1[a]	101%	146.1±0.5	147.1±3.1	101%
Nicotine (mg/g)	20.21±0.17	20.61±0.25[a]	102%	ND	ND	NA
PG (mg/g)	580.6±2.14	611.2±14.2[a]	105%	625.3±0.99	656.3±26.5	105%
Water (mg/g)	63.11±0.89	79.90±2.37[a]	127%[c]	55.81±0.71	73.81±0.71	132%[c]

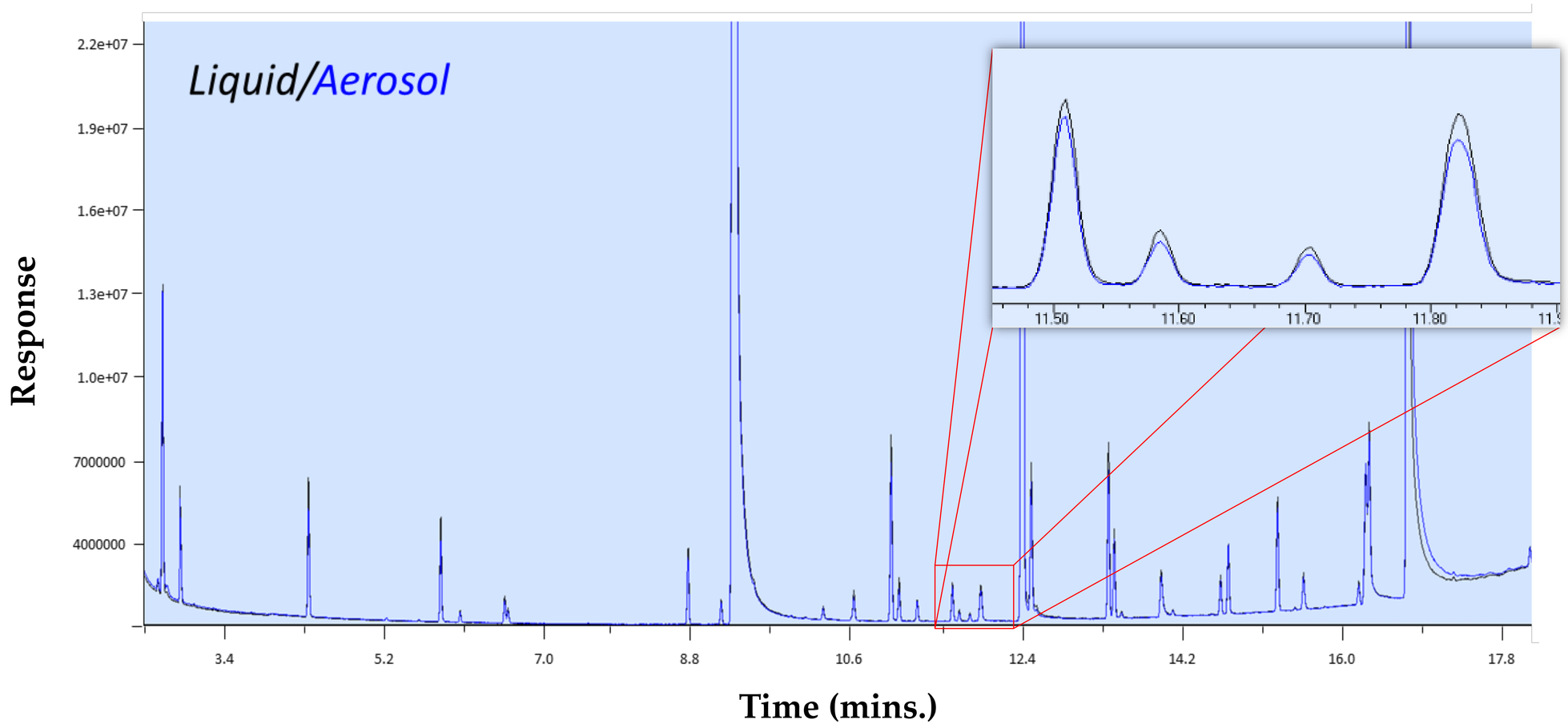
[a] The values were normalized by the collected aerosol mass.

[b] The transfer was calculated as Transfer (%) $= \frac{Concentration\ in\ Aerosol\ (\frac{mg}{g})}{Concentration\ in\ E-liquid\ (\frac{mg}{g})}\ X\ 100\%$.

[c] Water exceeded 100% by a wide margin due to the hygroscopicity of PG and Glycerin.

NA = not applied; ND = not detected; BLOQ = below the limit of quantification.

Flavor Transfer



Selected Carbonyls in the Aerosol

	Blank (n = 3)	Carrier (PG/VG/Nicotine /Water) (n = 3)	High w/ Nicotine (n = 3)	High w/o Nicotine (n = 3)
Aerosol Mass (mg)	100	107.2 ± 5.4	106.7 ± 1.3	116.1 ± 1.5
Formaldehyde (µg/g) [c]	< LOQ	8.71 ± 0.57	4.98 ± 0.15	5.88 ± 0.24
Acetaldehyde (µg/g) [c]	3.09 ± 0.11	8.34 ± 0.89	Above 1000[b]	Above 1000[b]
Acrolein (µg/g) [c]	< LOD	1.63 ± 0.20	5.36 ± 0.65	2.37 ± 0.13
Crotonaldehyde (µg/g) [c]	< LOD	< LOD	10.57 ± 0.75	8.18 ± 0.17

a. Assumes 100 mg for calculation purposes;

b. Approximations - Above Calibration Curve;

c. Reported values were normalized to the collected aerosol mass.

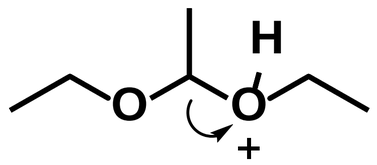
Where did acetaldehyde come from?

1,1-Diethoxyethane is Detected as Acetaldehyde (Artifact of Method)

1,1-diethoxyethane detected as acetaldehyde in the carbonyl analysis due to the sampling limitation

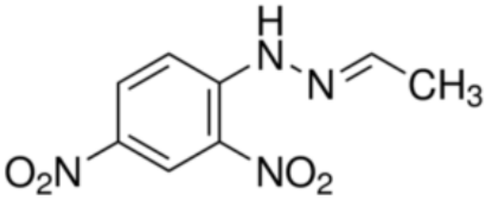
1,1-diethoxyethane as a flavor was transferred to the aerosol around 100% by GC-MS method

Impinger for carbonyl sampling



$[H^+]$

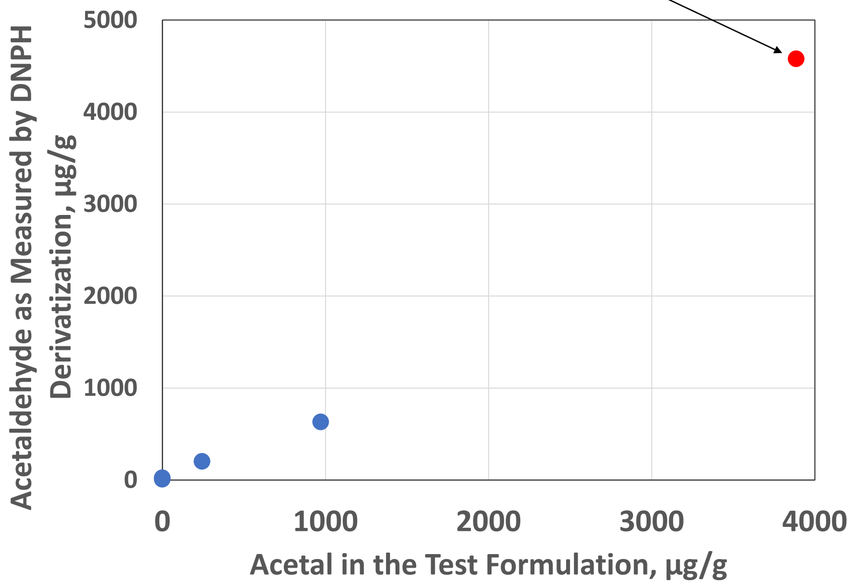
2,4-DNPH



1,1-Diethoxyethane (Acetal; CAS: 105-57-7)

Acetaldehyde - 2, 4-DNPH

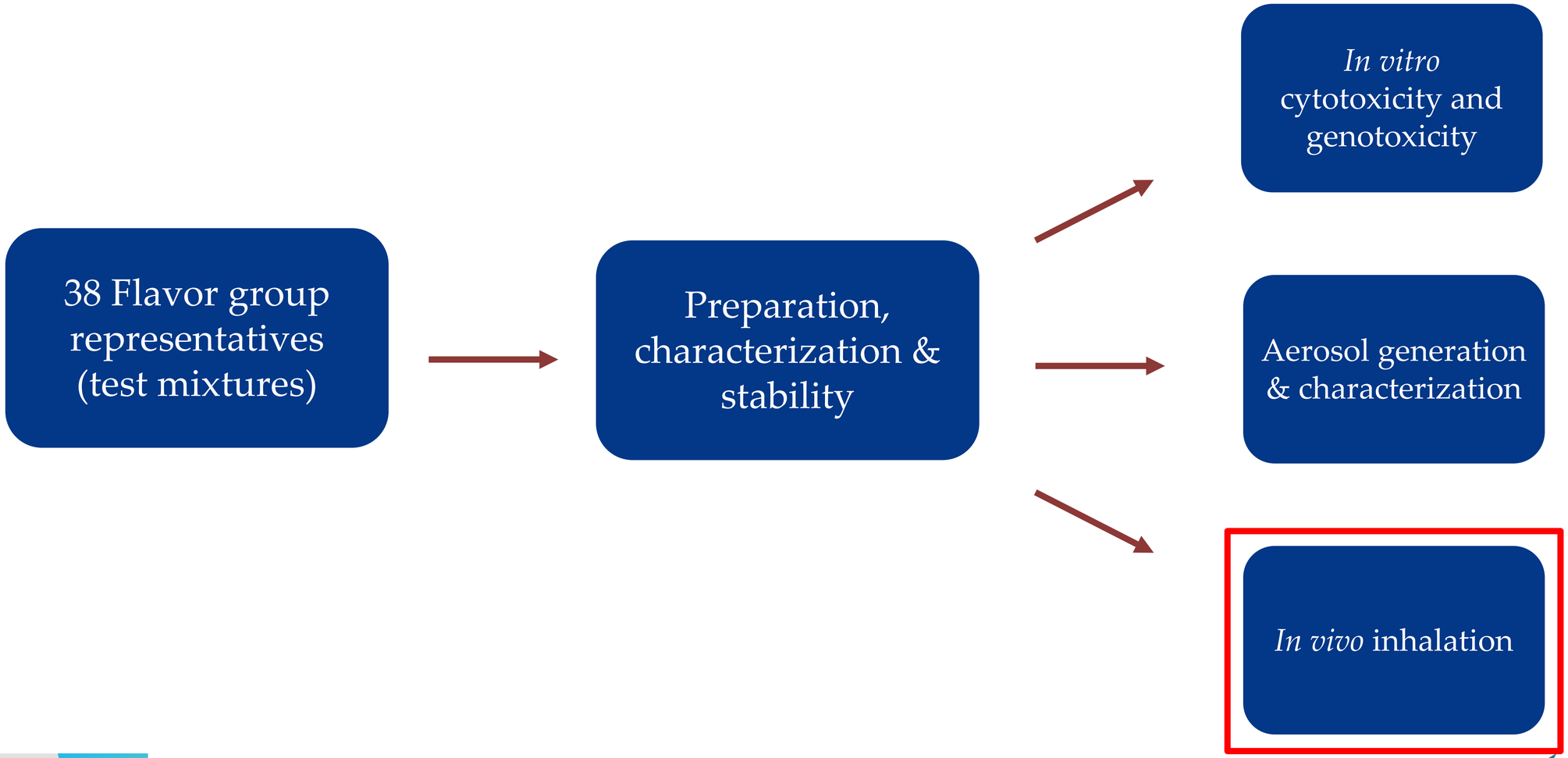
Approximation (above calibration curve)



Summary

- Flavor transfer from liquid formulation into the aerosol was confirmed
- Particle size for both formulations (high with and without nicotine) tested were in the desired range
- Nicotine, PG and glycerol matched in liquid and CAG aerosol for the test formulations
- Selected carbonyls measured in CAG generated aerosols were consistent with previous studies

Flavor Group Representative Assessment

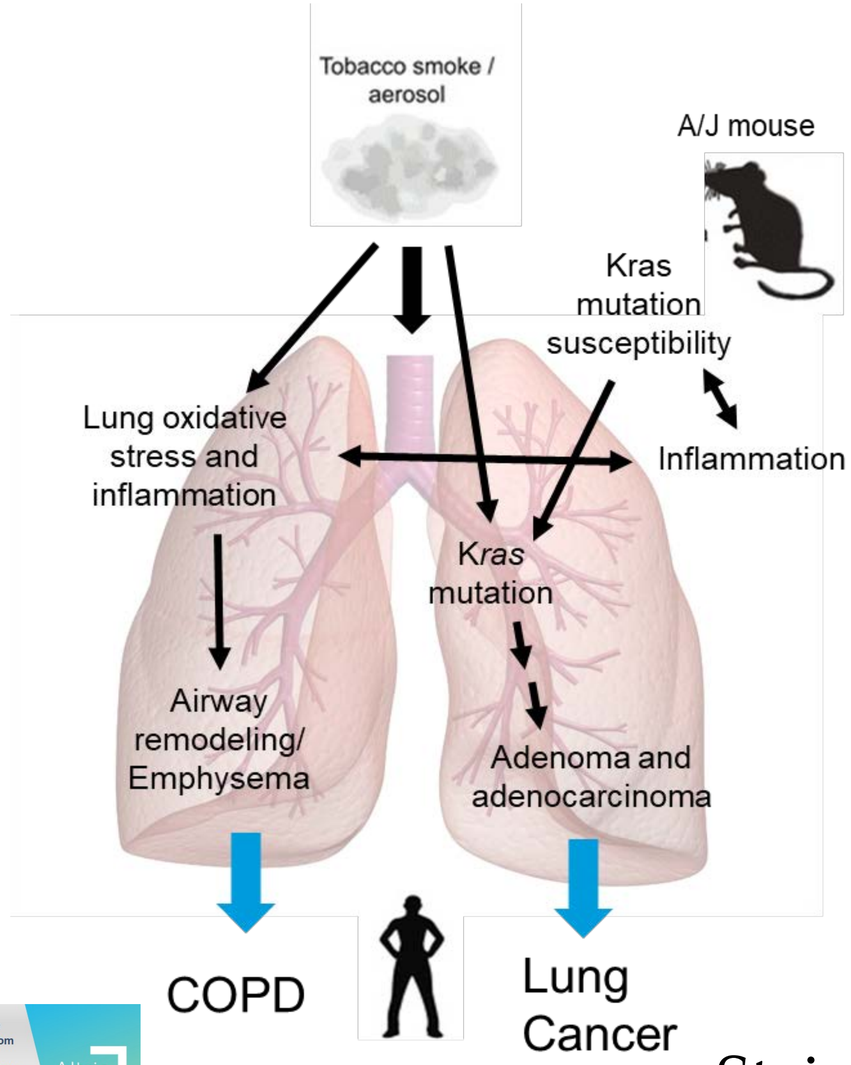


II. CASE STUDY – Flavor Ingredients in e-Vapor Products

Flavor Group Representatives (FGRs): 5-Week Range-Finding Inhalation Study in A/J Mice

Patrick Vanscheeuwijck

Mouse Model of Disease



- Smoke-induced lung cancers in human:
  - Human adenocarcinoma frequently carries *Kras* mutations
- A/J mouse model develops cigarette smoke-induced lung adenocarcinoma, with increased transcription rate of mutated *Kras*
- Suitable to study co-morbitities: inflammation and oxidative stress associated with pathogenesis of lung cancer and COPD

Steinn (2013) – Could not find reference

Dose Selection and Human Relevance

- To derive the test atmosphere concentrations to be used in the A/J study, the following human-relevant approach was used, for the high concentration mixture:
  - Use the 'maximum use level' of the flavoring ingredients, and apply to FGR
  - Assume 4 ml of e-liquid use per day for adults
  - Calculate human dose
  - Calculate corresponding mouse dose *[Alexander formula, CDER conversion factor based on body surface area*[1,2,3]*]*
  - Calculate required test atmosphere concentration to achieve the dose
    - Taking into account : 60% transfer rate, required quantity of aerosol to expose animals in whole body chamber (800L)
- Medium and low concentration mixtures for the A/J mouse study were created by applying a 4-fold serial dilution from the "high mixture"

[1] Alexander et al., 2008, Inhal. Toxicol. 20, 1179-89
[2] Bide et al., 2000, J. Appl. Toxicol. 20, 273-90
[3] CDER, 2005

Objective

- Perform a Dose Range Finding Study on CAG-aerosolized e-liquids with flavor ingredients from the Flavor 'Toolbox' mixture in preparation of a combined chronic toxicity/carcinogenicity study

Study Design and Endpoints

A/J mice (female/male*)

- Exposure: 6 hours/day, 5 days/week for 5 weeks
- Sham (fresh air)
- Control groups: CAG-generated aerosol PG/VG/N, 3R4F cigarette smoke (CS) (Health Canada Intense conditions)
- Test item groups: CAG-generated PG/VG/N/F – Flavor 'toolbox' mixture, Low, Medium, High
- All Nicotine-containing groups: 15.0 µg/L

Endpoints:

- Lung inflammation: free lung cells, cytokines/chemokines in BALF (n=10)
- Histopathology evaluation of respiratory tract (n=11)
- Systems toxicology respiratory tract (n=8)

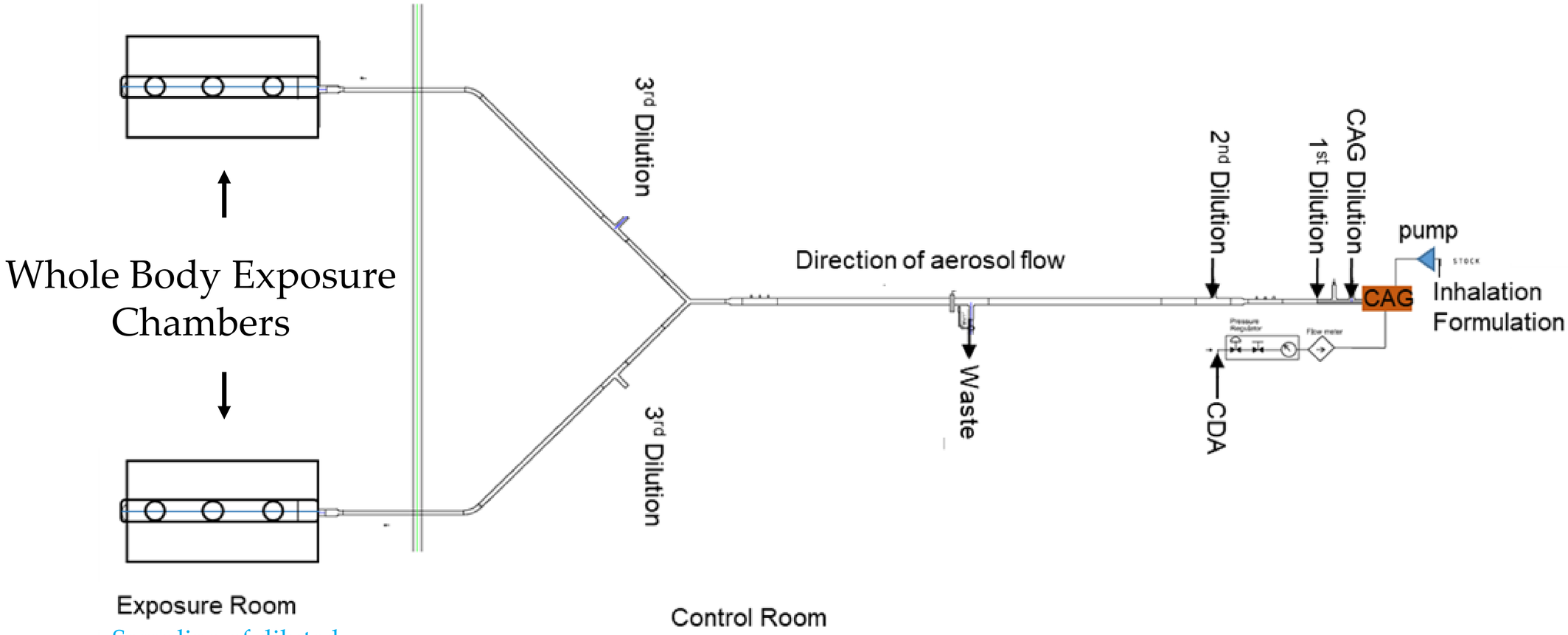
*for male mice: limited study design: Sham, PG/VG/N, and PG/VG/N/F-H groups only

Composition Inhalation Formulations

	Component (g/100g)					
Inhalation formulation	**PG**	**VG**	**Nicotine**	**Water**	**Ethanol**	**Flavor**
PG/VG/N	71.7	17.9	2.0	5.8	2.5	0.0
PG/VG/N/F Low	68.0	17.0	2.0	5.8	2.5	4.6
PG/VG/N/F Med	64.3	16.1	2.0	5.8	2.5	9.3
PG/VG/N/F High	56.9	14.2	2.0	5.8	2.5	18.6

- Typical commercial products (liquid) contain 1g to 3 g flavor/100g

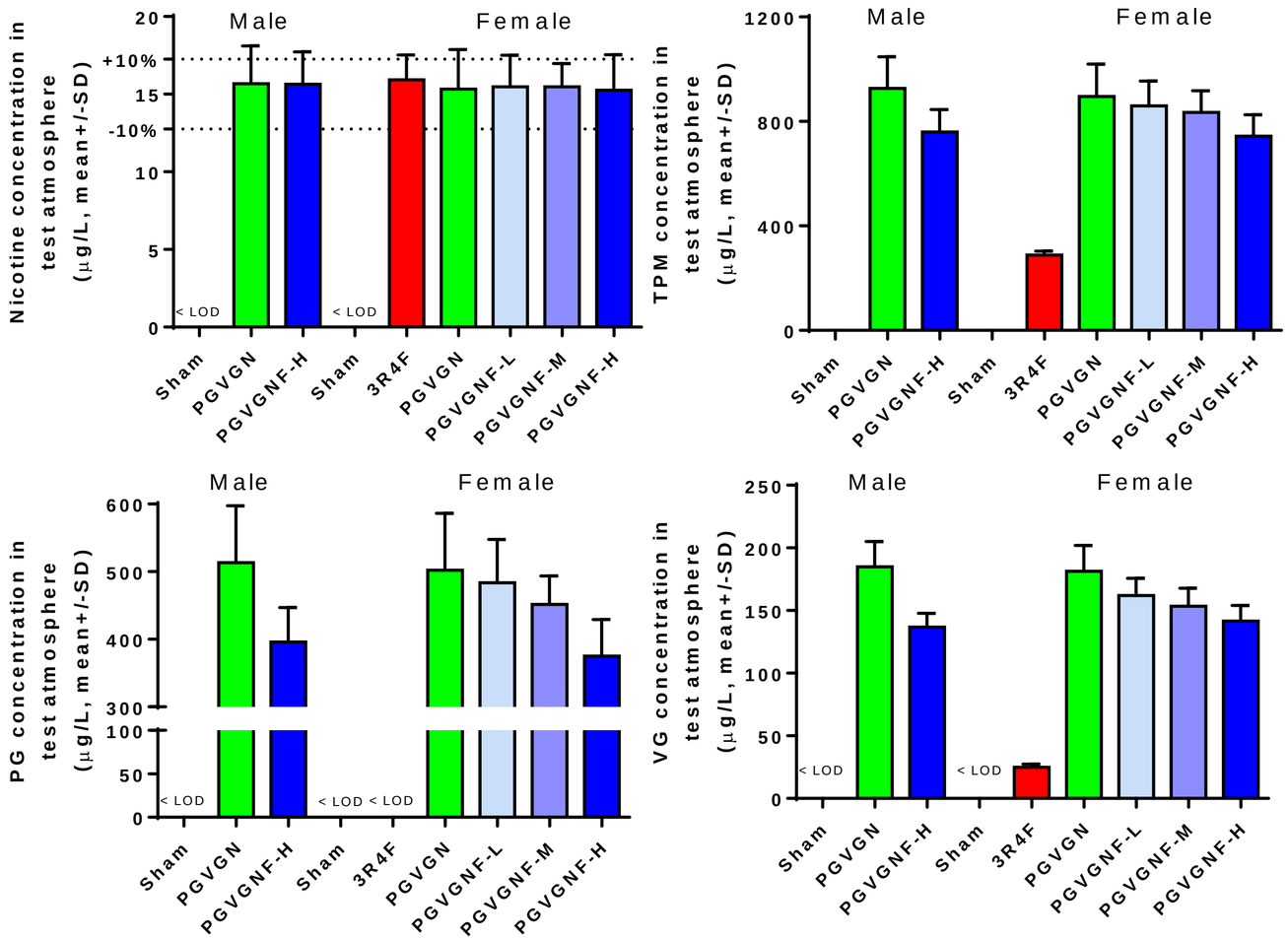
Aerosol Generation and Sampling of Aerosol



Sampling of diluted aerosol in WBEC

Test Atmosphere Characterization

Aerosol composition reflects that of formulation



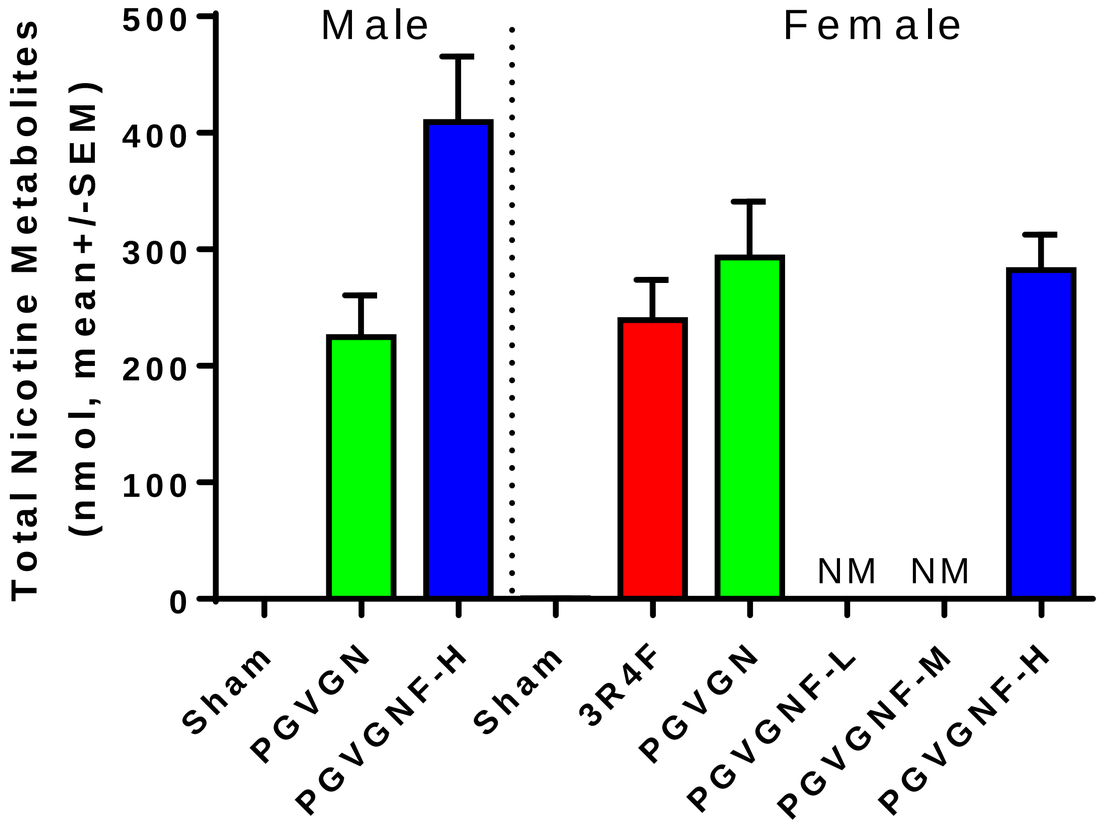
TPM: total particulate matter; LOD, limit of detection

Aerosol Uptake: Urine Nicotine Metabolites

Similar uptake of nicotine by mice exposed to nicotine-containing aerosols, incl. smoke

Higher nicotine metabolites in male PG/VG/N/F-H group because of two outliers.

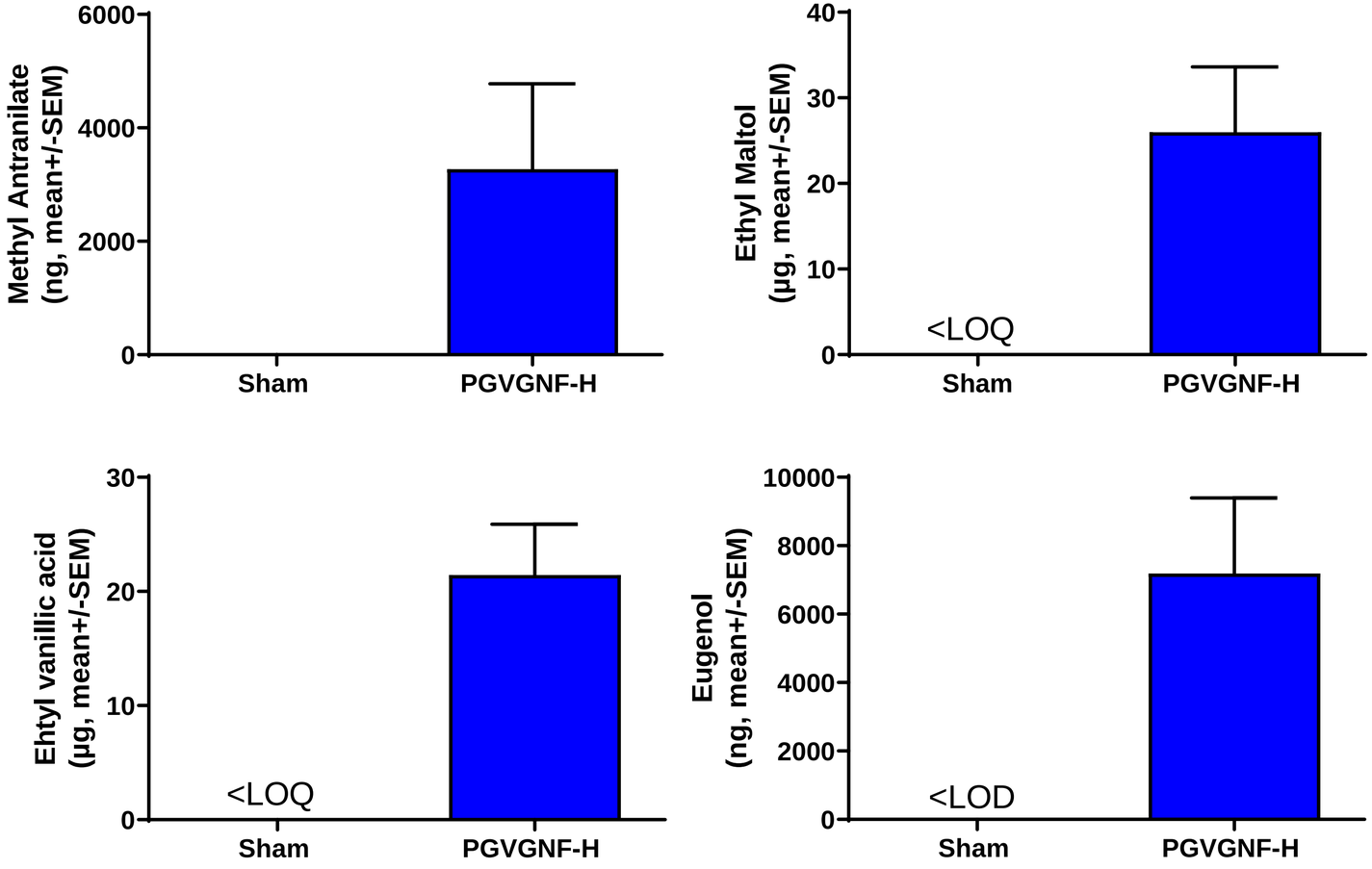
Total Nicotine Metabolites = 6 major nicotine metabolites



NM, not planned for measurement

FGRs Urinary Biomarkers

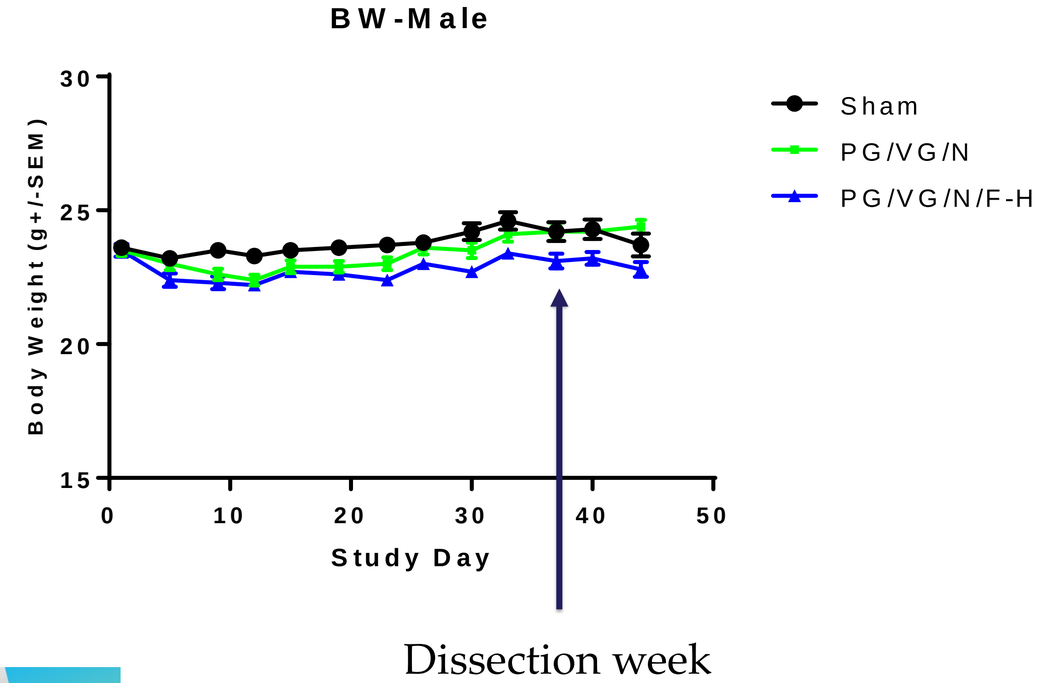
Analysis of 24-h urine samples (n=3) shows good uptake of flavor ingredients



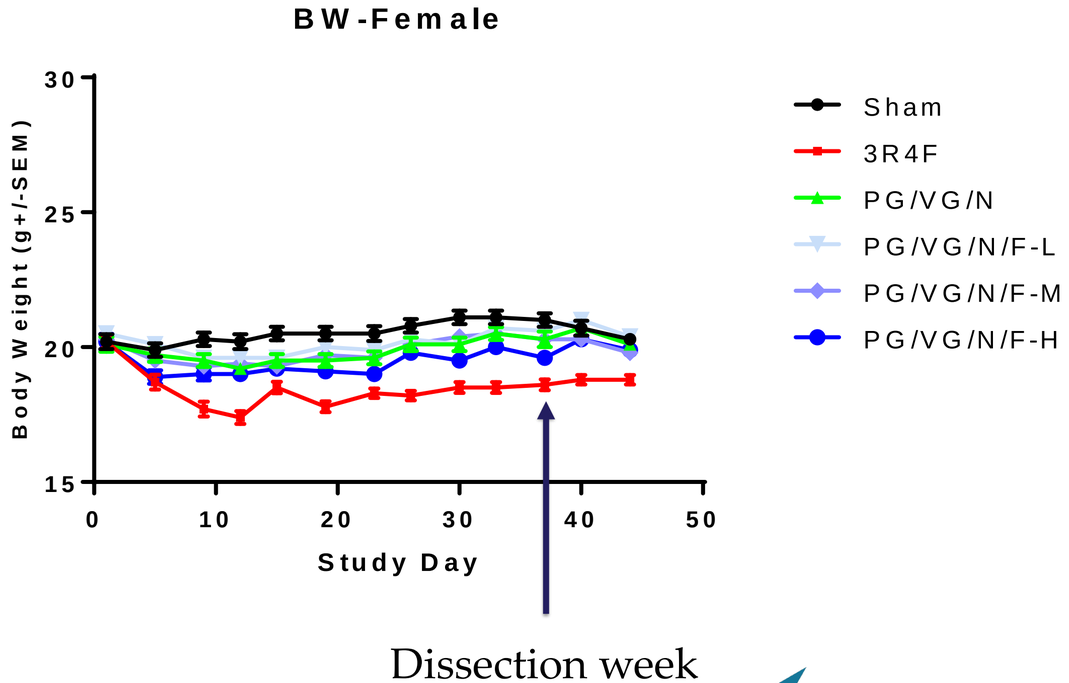
In-Life Body Weight Progression

Transient weight loss was observed during weeks 1-2 and most prominent in 3R4F CS-exposed group.

Body weight measurement were performed twice per week. N=29/group.



Dissection week



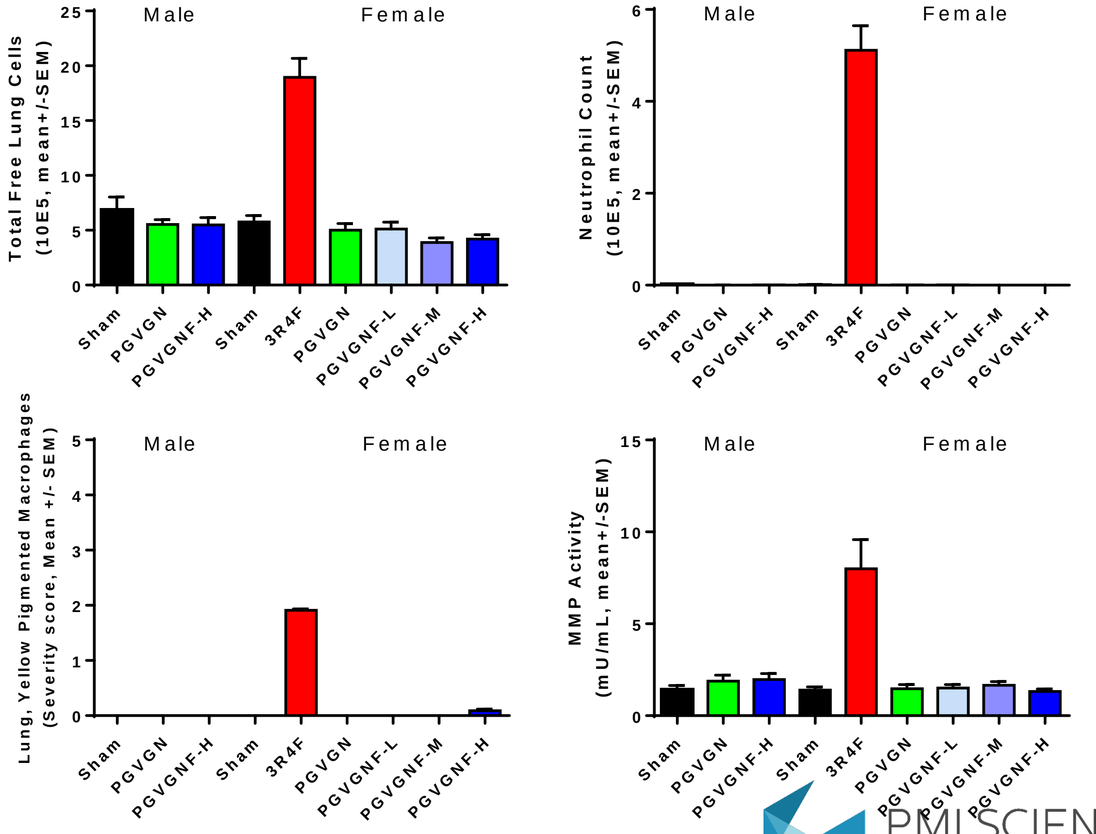
Dissection week

Lung Inflammation Determined in Lavage Fluid

Lung inflammation was prominent in the 3R4F CS-exposed mice but not in the e-vapor exposed groups

Cytokines/chemokines

Free Lung cells (lavage fluid)



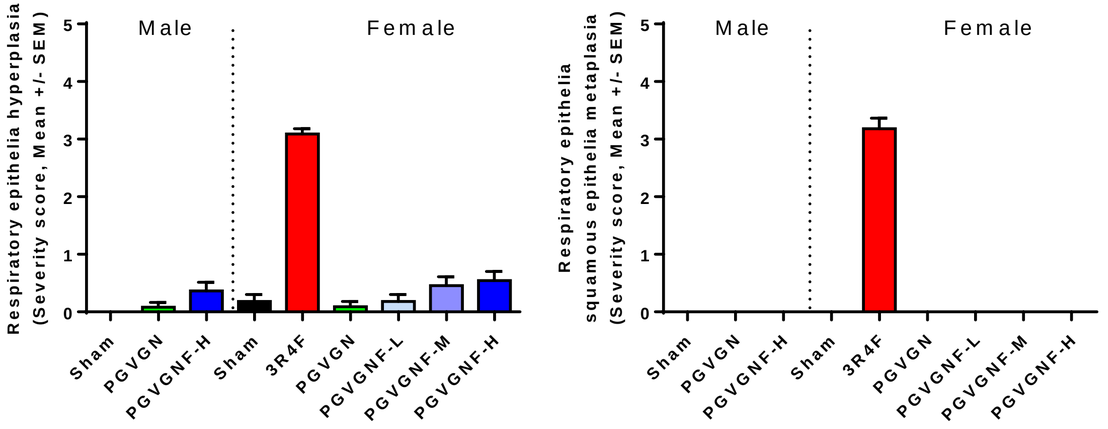
Histopathology Evaluation of the Nose and Larynx

Typical adaptive changes observed in the nasal respiratory epithelium in the 3R4F group – severity higher than in Sham and e-vapor groups.

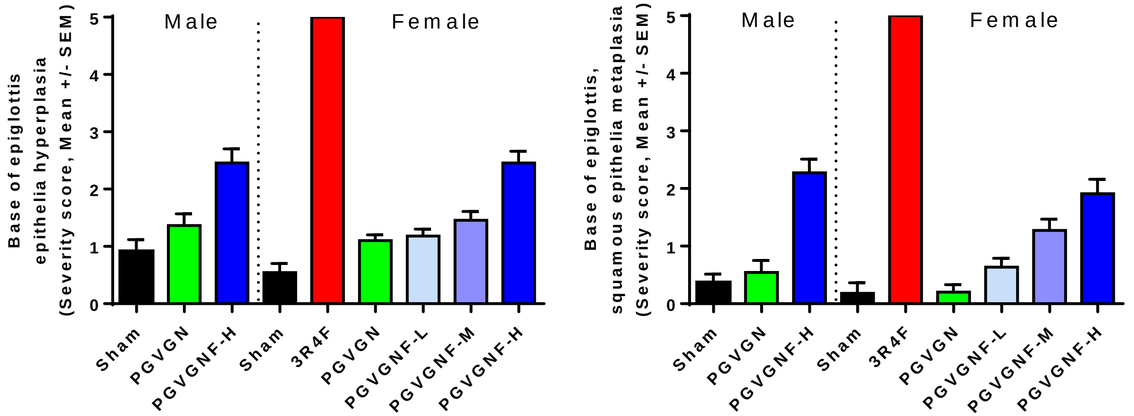
Changes at most sensitive sites of the larynx: Concentration-response in flavor ingredient-exposed groups; much less pronounced than after 3R4F exposure

No other noteworthy epithelial changes in e-vapor exposed groups

Nose level 1



Larynx, base of epiglottis



Conclusions

- 3R4F cigarette smoke causes known adaptive changes in the nasal and laryngeal epithelia, and lung inflammation
- The flavored e-liquid aerosols were well tolerated by the mice, without signs of severe toxicity
- The flavored e-liquid aerosols, even at the highest flavor concentration, did not cause lung inflammation
- Few respiratory tract epithelial changes were observed in mice exposed to aerosols from flavored e-liquids, and when observed, their severity was much lower than in mice exposed to 3R4F cigarette smoke
- The flavor ingredients concentrations used in this dose range finding study are deemed suitable to be used in a chronic toxicity study

Key Takeaways

- Implemented a structural flavor grouping approach to assess flavor ingredients used in e-vapor products
- Flavors and flavor mixtures are well characterized chemically and biologically (in vitro)
- The aerosol dynamics are well characterized
- The results from a 5-week study of the complex flavor mixtures show no effects at human relevant doses

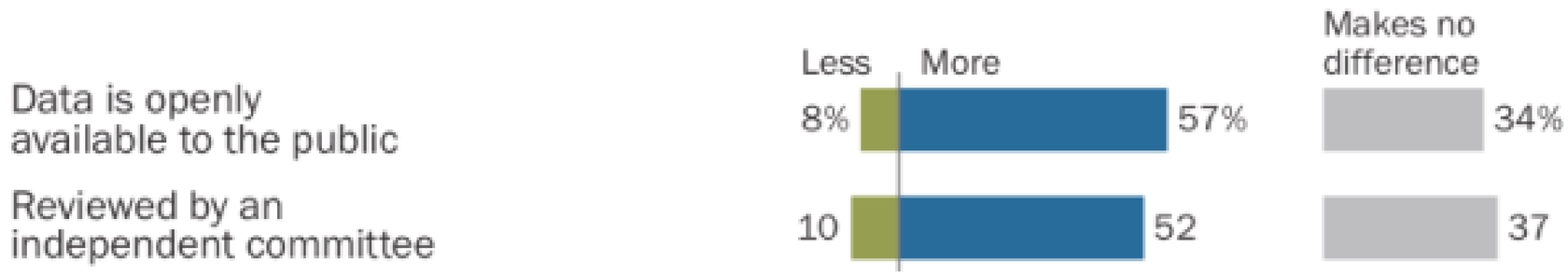
III. BEYOND SCIENCE

Julia Hoeng

Data Transparency Inspires Confidence in Research

Majority of Americans say they are more apt to trust research when the data is openly available

% of U.S. adults who say when they hear each of the following, they trust scientific research findings ...



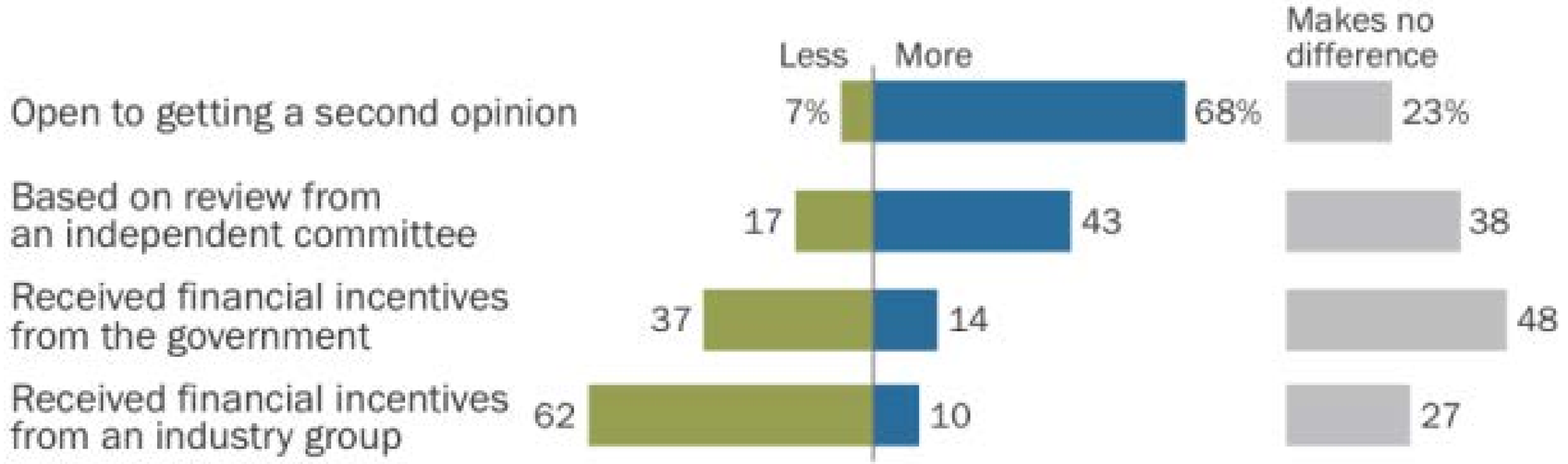
Note: Respondents who did not give an answer are not shown.
Source: Survey conducted Jan. 7-21, 2019.
"Trust and Mistrust in Americans' Views of Scientific Experts"

PEW RESEARCH CENTER

Pew Research Center
Science & Society

Bias Against Industry-Funded Research in Public Opinion

% of U.S. adults who say when they hear each of the following, they trust a science practitioner's recommendation ...

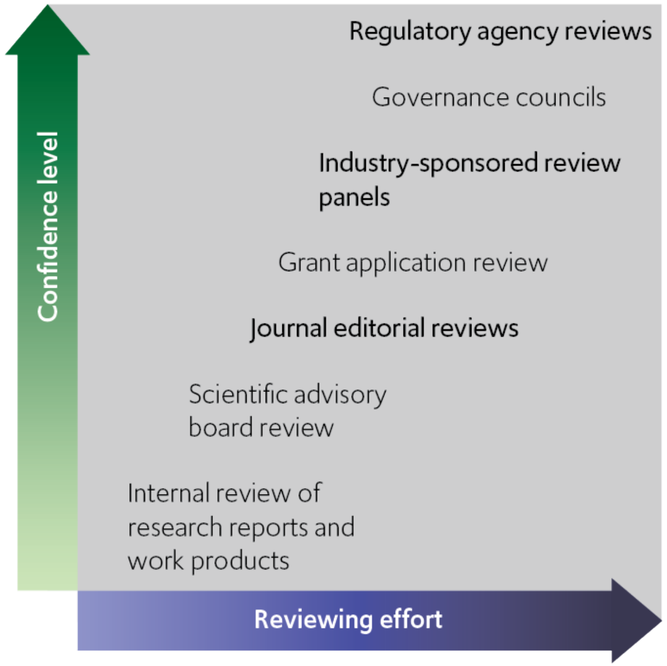


Note: Respondents who did not give an answer are not shown.
Source: Survey conducted Jan. 7-21, 2019.
"Trust and Mistrust in Americans' Views of Scientific Experts"

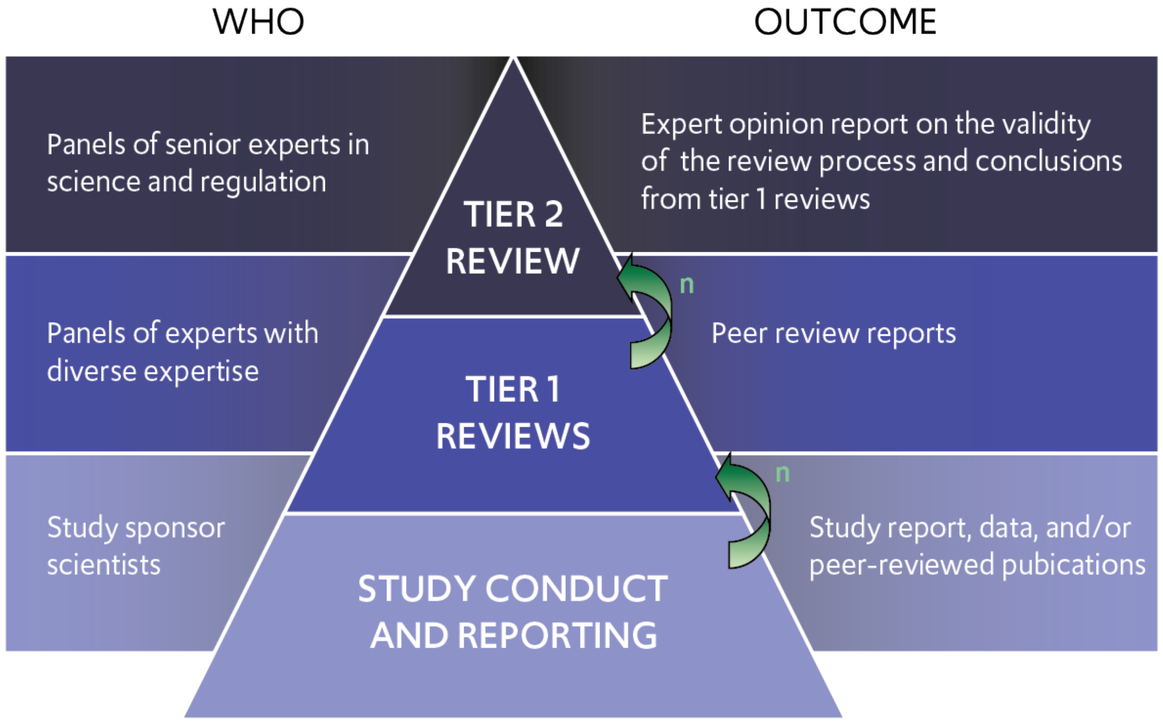
Pew Research Center
Science & Society

Independent Peer Review of the Toxicological Assessment of Tobacco Heating System 2.2

Peer review process

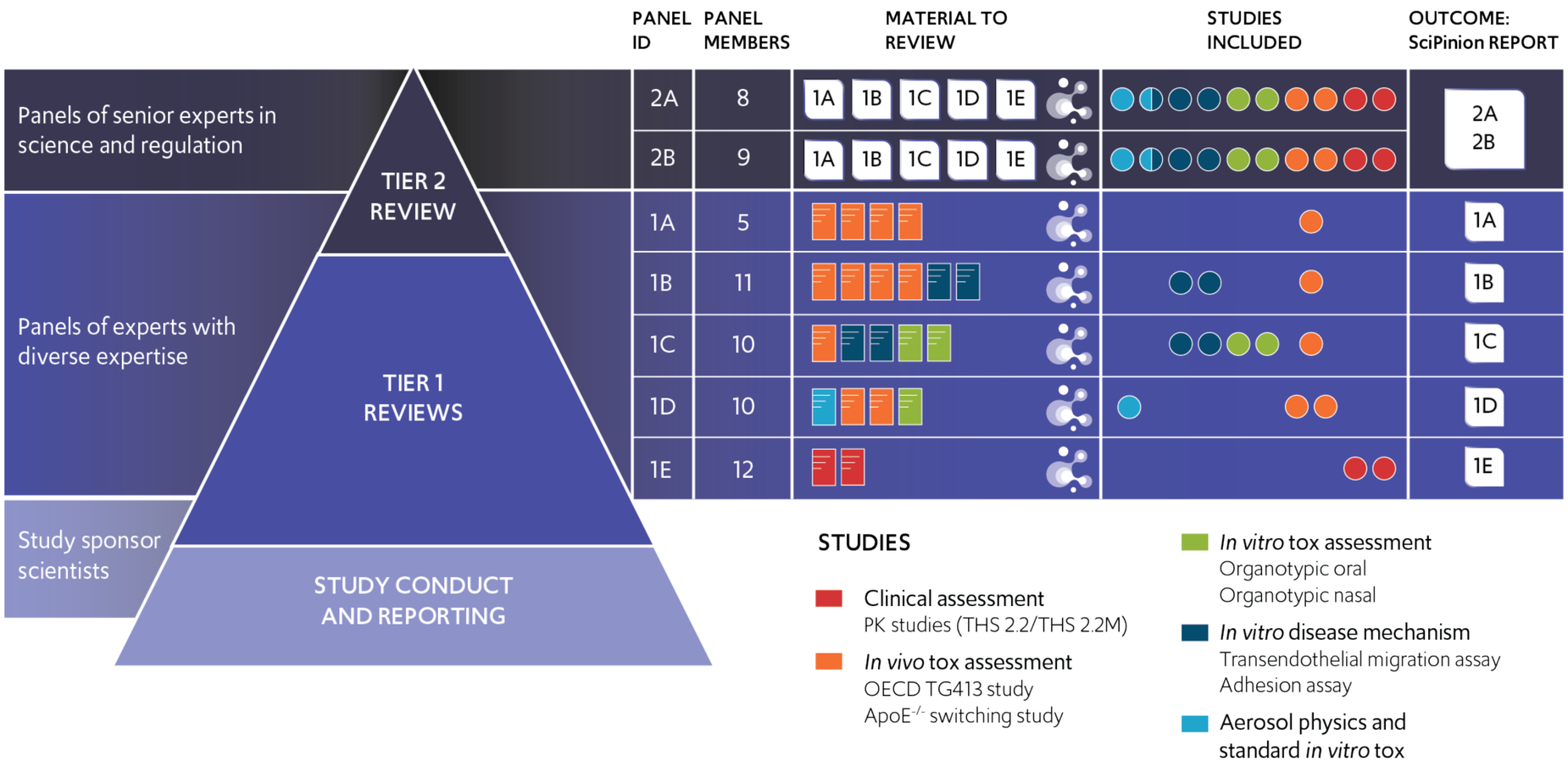


Tiered review of scientific research



Boué S, *et* al. Toxicological assessment of Tobacco Heating System 2.2: Findings from an independent peer review. *Regulatory Toxicology and Pharmacology* 2019;104:115–27. https://doi.org/10.1016/j.yrtph.2019.03.007

Independent Peer Review of the Toxicological Assessment of Tobacco Heating System 2.2 (Continued)



Boué S, *et* al. Toxicological assessment of Tobacco Heating System 2.2: Findings from an independent peer review. *Regulatory Toxicology and Pharmacology* 2019;104:115–27. https://doi.org/10.1016/j.yrtph.2019.03.007

INTERVALS - a Data & Results Sharing Platform, Aimed at Improving Transparency in Industry-Funded Research

https://www.intervals.science/

https://sciences.altria.com/

- Reproducible assessment of alternative products
- Enable evidence-based decisions
- Foster the development of a Smoke Free Future

Considerations for the Development of INTERVALS

"It is not enough to do your best; you must know what to do and then do your best"
W. Edwards Deming

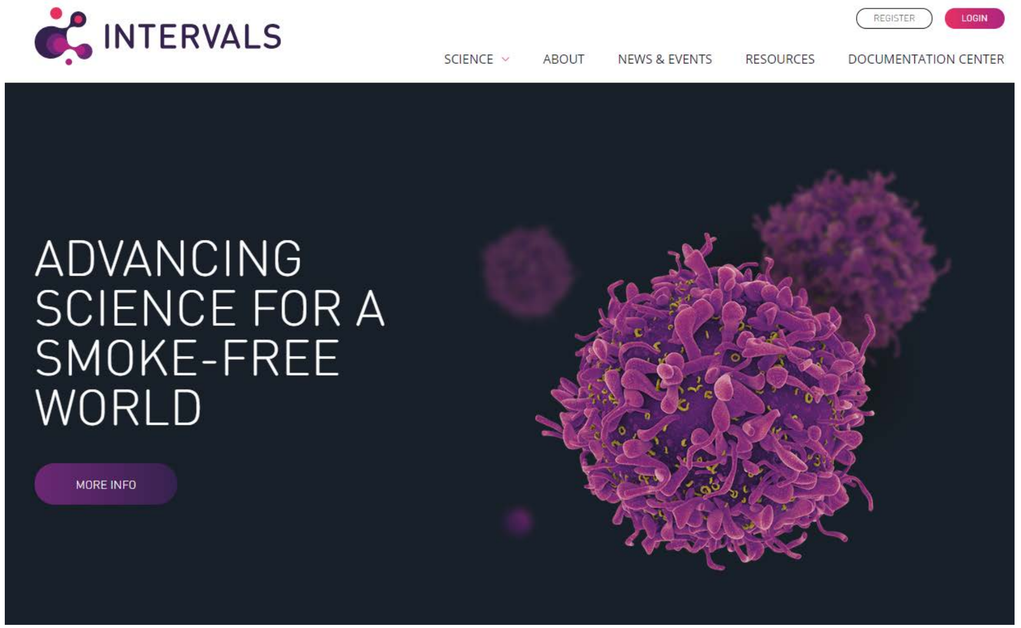
There are many products & flavors to be tested, rapid innovation with many new emerging assay protocols, technologies, and no real data standards

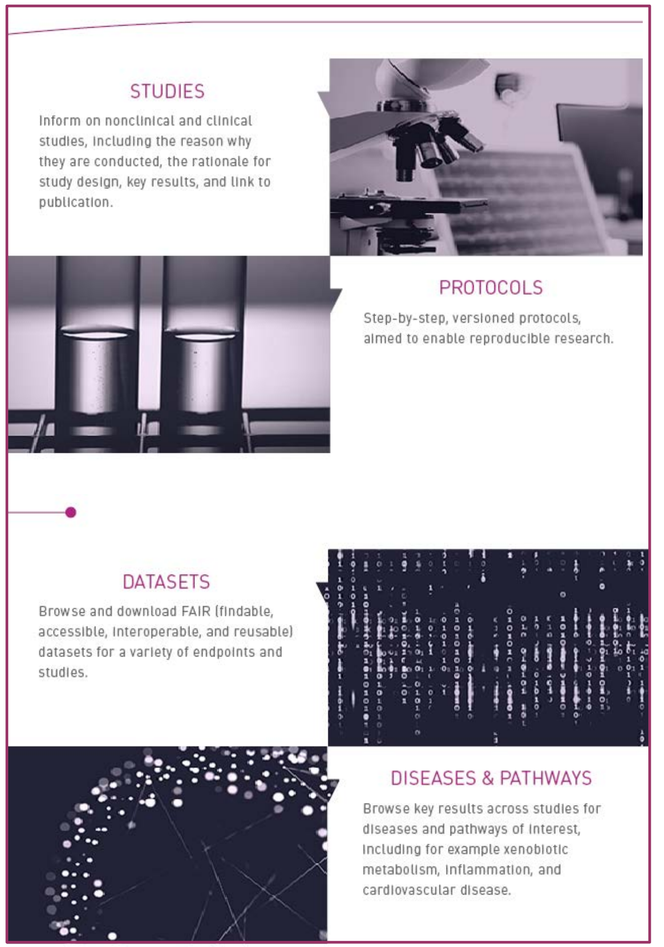
➡ **Need a platform that demonstrates the scientific rigor, thoroughness, precision required in Inhalation Toxicology of candidate reduced risk products to:**

- Ensure quality of the data and that the adequate testing strategies are used
- Enable reuse of data sets (3Rs, generation of new hypotheses)
- Inform the scientific community

INTERVALS: Scientific Data Transparency Applied to Industry

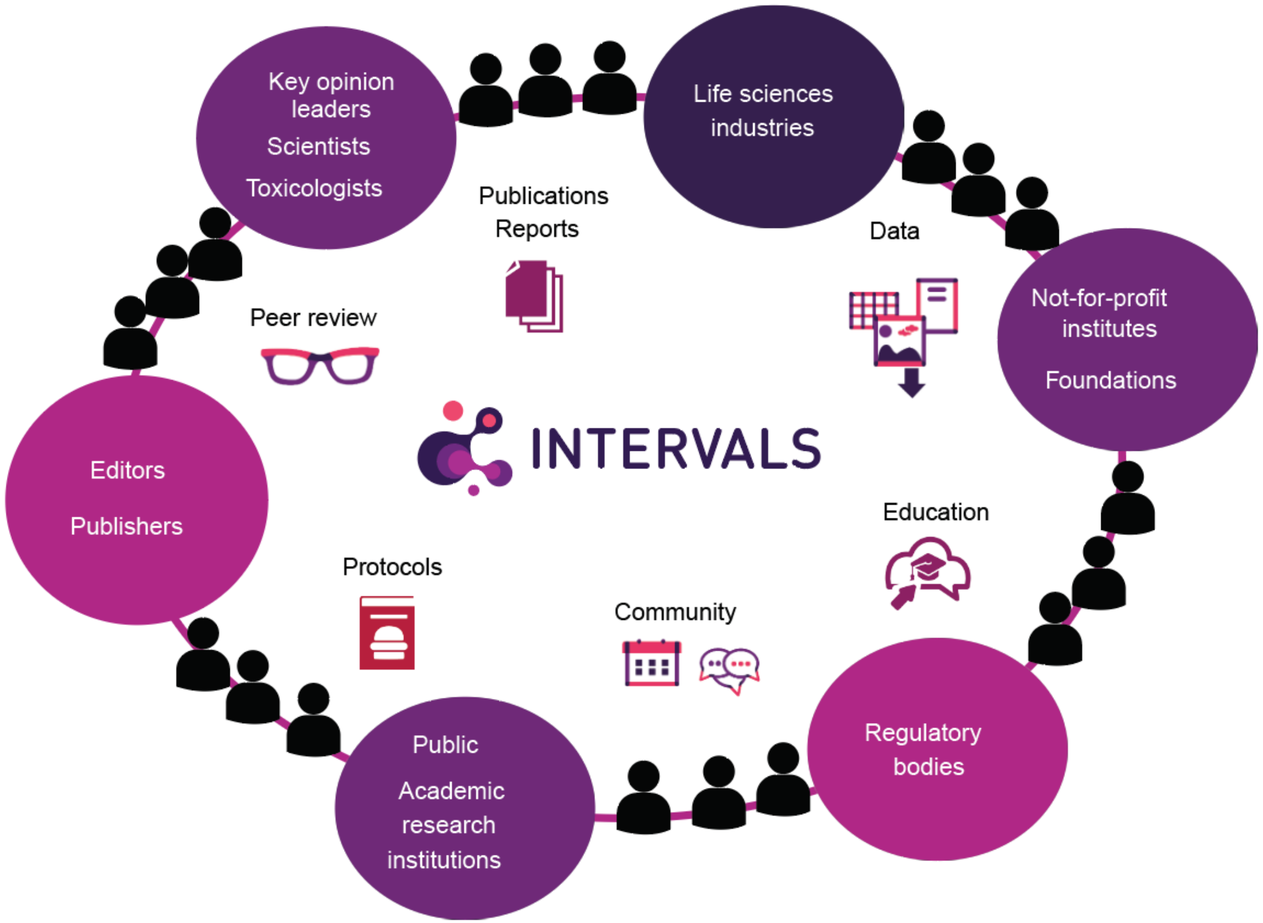
Aim: establish a community and a public **repository** for 21st-century preclinical and clinical (systems) **inhalation toxicology assessment** data and results that supports open data principles





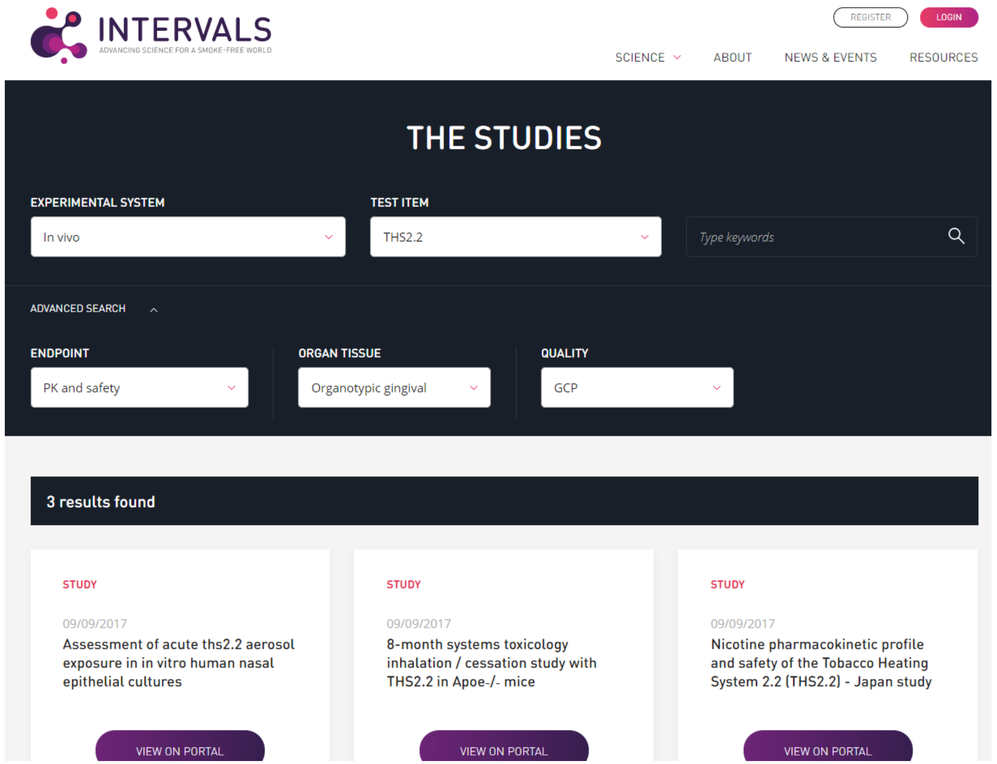
Boué S, *et al*. Supporting evidence-based analysis for modified risk tobacco products through a toxicology data-sharing infrastructure [version 2; referees: 2 approved] F1000Research 2017, 6:12 (doi: 10.12688/f1000research.10493.2)

The INTERVALS Community/Ecosytem



Boue S, et al. Embracing Transparency Through Data Sharing. International journal of toxicology 1091581818803880. https://doi.org/10.1177/1091581818803880

Overview of the Platform



- Faceted search enables quick retrieval of resource of interest
- Detailed protocols
- Clear contact detail
- Community features (news/commenting/events)

Detailed Study Results and Direct Link to Data

Micro-CT at month 7

The additional quantitative micro-CT investigation of the aortic arch plaque formation *in situ* at the 7-month time-point **confirmed the morphometric results from the plaque surface assessment**: for 3R4F-exposed mice, all 3 parameters (plaque volume, plaque area, and aortic occlusion) were significantly higher compared with sham-exposed mice, but the THS2.2, cessation, and switching groups were not different from sham (see Figure 2 and videos below). The aorta plaque surface area (the micro-CT parameter most closely resembling the morphometric plaque area) was 78% higher for the 3R4F group versus sham, while manual quantification of plaque area in the isolated aortas showed a 39% higher value following 3R4F CS exposure.

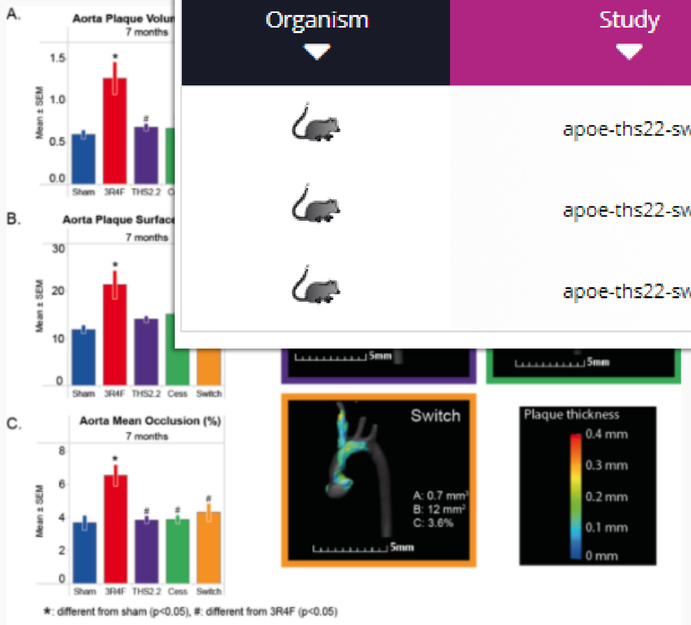


Figure 2 - Micro computed tomography (micro-CT)-based aortic arch (*in situ*) plaque measurements.

A, Plaque volume. B, Plaque surface area. C, Aortic occlusion (mean 6 SEM). D, Representative micro-CT images.

Organism	Study	Tissue	Endpoint (Species/tissue - Test item)	Data Download: PROCESSED
	apoe-ths22-sw	Aorta	Plaque size (Mm - THS2.2)	588.9 kB
	apoe-ths22-sw	Aorta	Aorta transcriptomics (Mm - THS2.2)	26.6 MB
	apoe-ths22-sw	Aorta	Aorta lipidomics (Mm - THS2.2)	switch data type to access data

Method: Plaque size measurements - planimetry and microCT

Planimetry

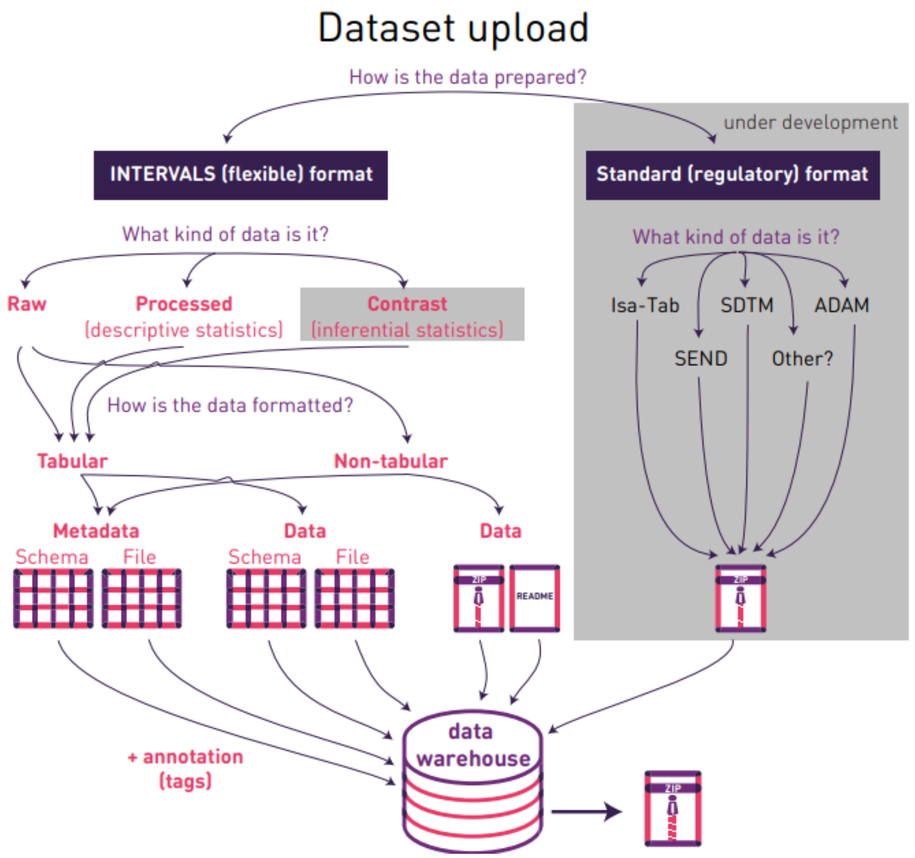
After removal of the aortic arch, the aortic wall was opened longitudinally, stained with Oil Red O, and the intimal area covered by plaques normalized to the whole area was determined from digital images. The intimal area covered by plaques was determined by planimetry and

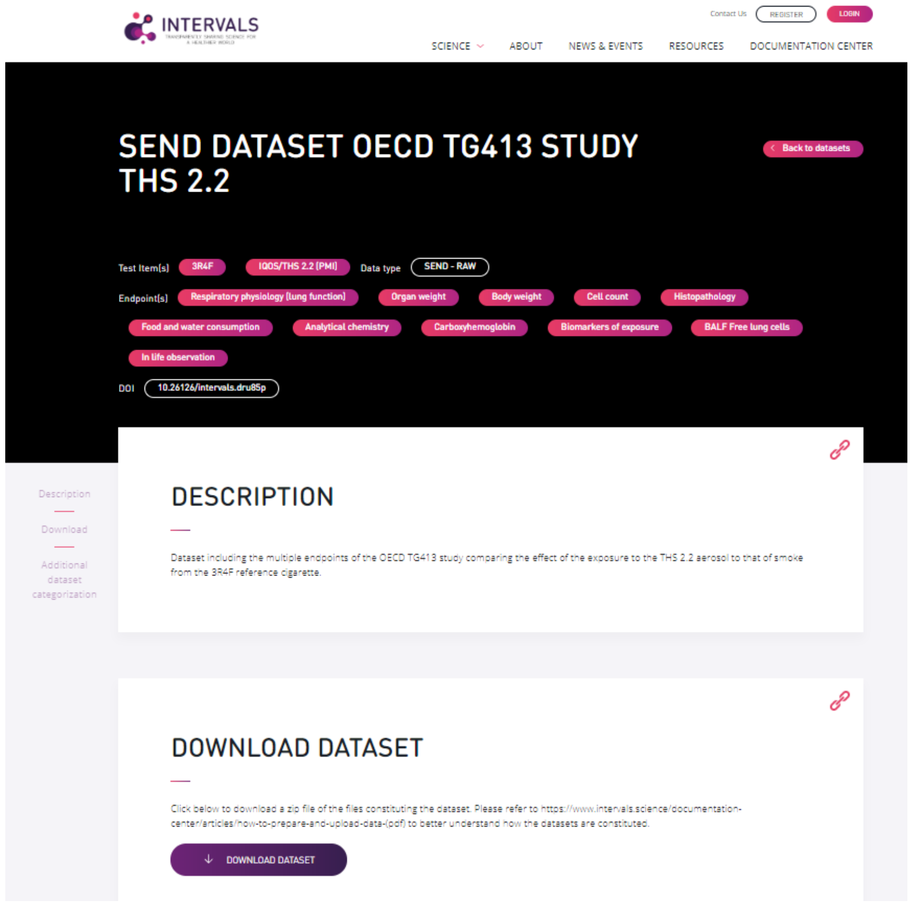
gray, the plaque in dark yellow. A centerline embedded in the aorta is pseudo-colored to indicate the crosssectional area of plaque at each point along the aorta. At the bottom of this frame, the slice distance and plaque cross-sectional area are reported, as well as total measurements (average occlusion, total plaque volume, total plaque surface area) for each of the regions (sinus, aortic arch, thoracic aorta, brachiocephalic trunk).

- **Linear Distance Measurements (top-right)** – for each slice along the curved centerline, the average aorta radius, maximum plaque thickness, and average plaque thickness are plotted. As the animation proceeds, a black time-bar indicates the current slice distance along the graph.
- **Percent Measurements (middle-right)** – For each slice along the curved centerline, the percent coverage (percent of the vessel wall that has plaque attached) and percent occlusion (percent of the vessel cross-section that is occluded with plaque) are plotted. As the animation proceeds, a black time-bar indicates the current slice distance along the graph.
- **Two planar slices (bottom-right)** – the grayscale slices cut through the aorta in an orientation centered around and perpendicular to the centerline. The right side is displayed with segmented aortic plaque overlaid in red, and segmented brachiocephalic trunk plaque overlaid in blue.

All metrics and 3D movies were created for the aortas using SCIRun (Scientific Computing and Imaging Institute, University of Utah). All samples were scanned and analyzed blind to treatment assignment.

A Mine of Data





Studies Published on INTERVALS

		Aerosol	Environment..	In situ	In vitro	In vivo	Clinical	PBA	Epidemiology	Grand Total
Cigarette	1R4F	1			1					1
	2R4F	1			1					1
	3R4F	13		1	20	5	1	1	1	32
	Commercial cigarette	3	2		1		6	1	2	10
E-cigarettes	Base (Blu PLUS, Fontem Ventures)				1					1
	Base (MarkTen, Altria)	1			1	1				2
	Base (MESH, PMI)	2			2					2
	Blueberry flavor (Blu PLUS, Fontem Ventures)				1					1
	Carrier (MarkTen, Altria)	1			1	1				2
	Carrier (MESH, PMI)	2			2					2
	Classic tobacco (MESH, PMI)	2			3					3
	Puritane™ EVP (Fontem Ventures)						1			1
	TestMix (MarkTen, Altria)	1			1	1				2
HNB	CHTP 1.2 (PMI)	1			2	1	1	1	1	4
	Glo/THP 1.0 (BAT)	2			1		1	1	1	2
	IQOS/THS (PMI)	12	2	1	15	5	7	1	1	31
Hybrid tobacco product	iFuse (BAT)	2			1		1	1	1	2
	Pax by Ploom	1					1	1	1	1
	Ploom Tech/PNTV by JTI	2	1		1		2	1	1	3
Mixture	Mixture of flavors				1					1
NRT	Nicotine gum	1					3	1	1	3
Single compound	Aflatoxin B1 (AFB1)				1					1
	Glycerol			1						1
	Propylene glycol (PG)			1						1
	Single flavoring agent/flavor				1					1
Grand Total		18	3	2	26	7	9	1	2	48

The numbers indicate the number of published studies for each test item/type of study

Published Study Titles

- Comparative assessment of HPHC yields in THS 2.2 and commercial cigarettes
- 3D vasculature-on-a-chip model to assess the effect of THS 2.2 exposure on monocyte-to-endothelium adhesion *in vitro*
- 6-month Systems Toxicology Inhalation/Cessation Study with CHTP 1.2 and THS 2.2 in Apoe$^{-/-}$ Mice
- 8-month Systems Toxicology Inhalation/Cessation Study with THS 2.2 in Apoe$^{-/-}$ Mice
- 90-day OECD Rat Inhalation Study with THS 2.2 (TG413 Guideline)
- A 2-year clinical study evaluating the safety profile of an electronic vapor product
- A Cross-sectional Study of the Socio-demographic and Other Determinants of Chronic Obstructive Pulmonary Disease (COPD) Among Those Who Smoke, Quit Smoking and Never-smoking Cigarettes
- A lung/liver-on-a-chip platform for acute and chronic toxicity studies
- A system toxicology approach to investigate the impact of an acute exposure to cigarette smoke and electronic cigarette on human lung and oral *in vitro*
- Acute exposure of human organotypic buccal epithelium cultures to e-liquid aerosols – Comparison with cigarette smoke by using a systems toxicology approach
- Assessment of acute CHTP 1.2 aerosol exposure in *in vitro* human buccal epithelial cultures
- Assessment of Acute THS 2.2 Aerosol Exposure in *in vitro* Human Bronchial Epithelial Cultures
- Assessment of Acute THS 2.2 Aerosol Exposure in *in vitro* Human Buccal Epithelial Cultures
- Assessment of Acute THS 2.2 Aerosol Exposure in *in vitro* Human Nasal Epithelial Cultures
- Assessment of Repeated CHTP 1.2 Aerosol Exposure in *in vitro* Human Gingival Epithelial Cultures
- Assessment of repeated THS 2.2 aerosol exposure in *in vitro* human gingival epithelial cultures
- Atherogenesis Study *in vitro* – Transendothelial Migration Assay with THS 2.2

Published Study Titles (Continued)

- Cigarette smoke reduces colitis severity in mice
- Cigarette smoke vs. e-cigarette aerosol: toxicological comparison with a 3D *in vitro* human respiratory model
- Clinical reduced exposure study with 5 days in a confinement setting (REX-C) – EU
- Clinical reduced exposure study with 5 days in a confinement setting (REX-C) – Japan
- Determination of eight carbonyls in aerosols trapped in PBS for *in vitro* assessment
- Effect of 3R4F smoke and THS 2.2 aerosol on the color stability of teeth.
- Effects of 3R4F smoke and THS 2.2 aerosol on the properties of dental resin composites
- Effects of cigarette smoke and electronic cigarette aerosol on the coloration of dental hard tissues and composite resin restorations
- Evaluation of a Novel Tobacco Vapor (NTV) product impact on the indoor air quality (IAQ)
- Heat-not-burn tobacco products: a systematic literature review (up to Nov 2017)
- IIS.PMI.2017.16 Research on the Effects of Exhaled Pollutant from Tobacco Heating System (THS) on Indoor Air Quality
- Impact of E-vapor aerosols on the cardiovascular and respiratory systems in $ApoE^{-/-}$ mice
- Impact of THS 2.2-generated environmental aerosol on indoor air quality in comparison with smoke from a commercial cigarette.
- *In vitro* biological effects of selected individual smoke constituents and mixtures of smoke constituents
- *In vitro* systems toxicology assessment of selected flavoring substances in e-liquid formulations (flavor toolbox)
- *In vitro* toxicological and biological responses of aerosols from a novel hybrid tobacco product as compared with two tobacco heating products and a reference cigarette
- Investigation of Solid Particles in the Mainstream Aerosol of THS 2.2 and 3R4F
- Long-term exposure to THS 2.2 of human bronchial epithelial cells
- Nicotine pharmacokinetic profile and safety of the THS 2.2 Menthol - ZRHM-PK-05-JP
- Nicotine pharmacokinetic profile and safety of the Tobacco Heating System (THS) 2.2 - ZRHR-PK-02-JP
- Novel Tobacco Vapor product aerosol: chemistry analysis and *in vitro* toxicological evaluation in comparison with 3R4F cigarette smoke

Published Study Titles (Continued)

- Physico-chemical studies of direct interactions between components of electronic cigarette liquid mixtures and lung surfactants
- Systems toxicology assessment of the biological effects of an e-liquid and its corresponding aerosol using 2D and 3D airway epithelial cultures
- Systems Toxicology Meta-Analysis: Biological Impact of a Candidate MRTP Aerosol on Human Organotypic Cultures of the Aerodigestive Tract
- THS 2.2 Menthol: Aerosol *in vitro* toxicology (Neutral Red Uptake, Ames assay and Mouse Lymphoma Assay), in comparison with 3R4F.
- THS 2.2 Menthol: Chemical composition of aerosol in comparison with the mainstream smoke constituents of 3R4F.
- THS 2.2 regular: Aerosol *in vitro* toxicology (Neutral Red Uptake, Ames assay and Mouse Lymphoma Assay), in comparison with 3R4F.
- THS 2.2 regular: Chemical composition and physical properties of the aerosol in comparison with the mainstream smoke of 3R4F.
- THS 2.2 regular: influence of tobacco blends on aerosol composition
- Tier I peer review of toxicological assessment of the Tobacco Heating System 2.2.
- Tier II peer-review of toxicological assessment of the Tobacco Heating System 2.2

Acknowledgements

Altria
Altria Client Services

PMI R&D

BACK UP SLIDES

Questions

Questions

- Describe the dose-response relationship in the dose range relevant to human exposure?
- Address human variability and sensitive populations?
- Incorporate existing biological understanding of the likely mode of action?

Flavor Group Representatives (FGRs) Selection Based on Structural Grouping Approach

- Question 1:

  Is the clustering approach appropriate? What would you add to strengthen the approach?

- Question 2:

  Is the FGR selection appropriate?

- Question 3:

  What would you do differently?

- Question 4:

  Are you familiar with similar approaches for the assessment of complex mixtures?

- Question 5:

  We consider the approach is applicable to other flavoring ingredients with further supporting *in vitro* work to establish specificity and sensitivity beyond the 246 flavoring ingredients evaluated in this study

Representative Flavor Mixtures (RFMs): *In Vitro* Toxicity Screening

- Question 1:

  Do you consider the *in vitro* methods used appropriate for the flavor ingredient hazard characterization?

- Question 2:

  Do you consider a battery of *in vitro* tests (informed with *in vivo* data) appropriate to drive flavor system development?

- Question 3:

  What would you do differently?

Representative Flavor Mixtures (RFMs): Aerosol Generation and Characterization

- Question 1:

  Do you consider the aerosol generation by CAG appropriate for animal testing?

- Question 2:

  Do you consider the aerosol characterization in this project sufficient?

- Question 3:

  What would you do differently?

Representative Flavor Mixtures (RFMs): 5-Week Range-Finding Inhalation Study in A/J Mice

- Question 1:
- Question 2:
- Question 3:
- Question 4: