



# The *in vitro* assessment and testing of electronic cigarettes and liquids

Presenter David Smart

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# The role for e-cigarettes in public health

## Key points

- > Vaping exposes vapers to a far narrower range of toxins than does smoking cigarettes, and levels of toxins absorbed from vaping are generally low. It is therefore likely that vaping poses only a small fraction of the risk of smoking.
- > Vaping nicotine is not associated with a high frequency of adverse health effects after accounting for past smoking history.

© Royal College of Physicians 2024

E-cigarettes and harm reduction: An evidence review. UK Royal College of Physicians 2024 at p. 67.

# Role of flavours in adult consumer transition from cigarettes

## Factor 1

### UK smoking rates declining (UK Adult Population 2023)

Cigarettes : 11.6%<sup>(1)</sup>

E-cigarettes : 9.1%<sup>(2)</sup>

## Factor 2

### Consumer satisfaction

For adult consumers to transition from traditional cigarettes, e-cigarettes must be perceived as a satisfying alternative

## Factor 3

### Most popular flavours are not tobacco (12%)

Fruit (47%) and mint / menthol (17%)<sup>(2)</sup>

Data taken from:

<sup>1</sup>Smoking profile for England: statistical commentary,, Office for Health Improvement & Disparities, UK Gov, October 2024 update.

<sup>2</sup>Use of e-cigarettes (vapes) among adults in Great Britain, Action on Smoking Health (ASH), 2023.

# The different e-cigarette types



## Closed Rechargeable System

Replaceable manufacturer specific prefilled cartridge, liquid, wick & power  
Consumer cannot refill; cartridge (liquid, coil wick) must be replaced once empty.



## Closed Disposable System

Prefilled device, liquid, wick & power supply,  
Consumable cannot be refilled. All discarded once empty.



## Open Rechargeable System

Totally open system,; consumer can customise:  
coils, coil resistance ( $\Omega$ ), wicks and materials, liquid flavours, PG/VG ratio, reservoir size and device power settings.

# What is an e-liquid

## Contents

Pharmaceutical / food grade

- Propylene Glycol (PG) (typical 20-60%)
- Vegetable Glycerol (VG) (typical 80-40%)
- Nicotine (0 – 60mg/ml, 20 mg/ml European Limit)
- Flavourings

PG provides solvent for Nicotine & Flavour

VG customises sensory aspects (sweetness/rich and Cloud formation)

PG/VG ratio impact of cloud formation, harshness and device performance

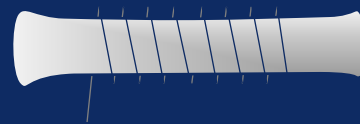
Viscosity            VG > PG

Boiling Point        VG (287 °C) > PG (188°C)



# The e-cigarette atomiser

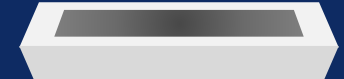
Microporous substrate or wick draws the e-liquid into heating atomiser



Coil & Wick



Mesh & Wick

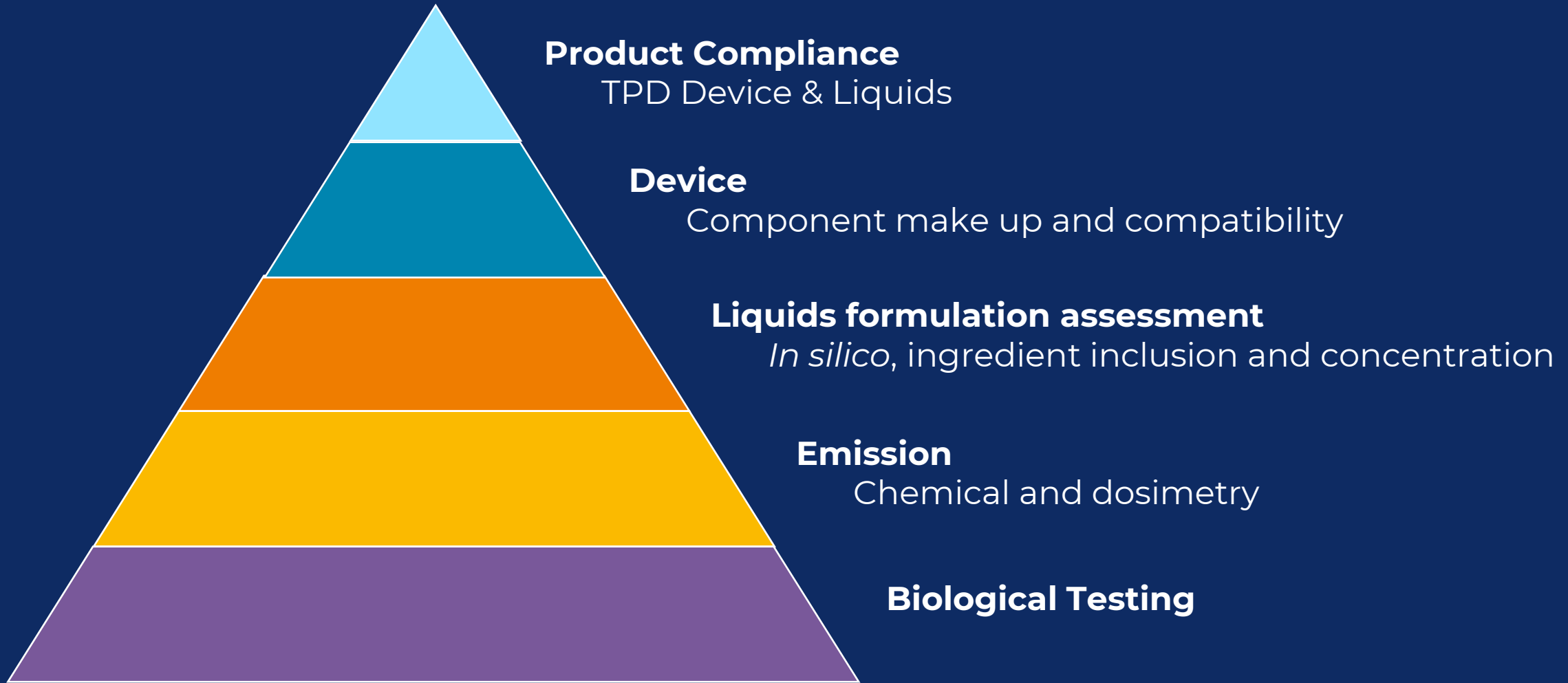


Ceramic

Combination of heating and puffing associated pressure drop leads to Vapour formation.



# Product Assessment



# Direct testing of e-liquids



# Direct dosing

## Pros

- Time-efficient, easy to implement
- Good for comparing individual changes in flavours and formulations
- Versatile application to range of *in vitro mechanistic* assays

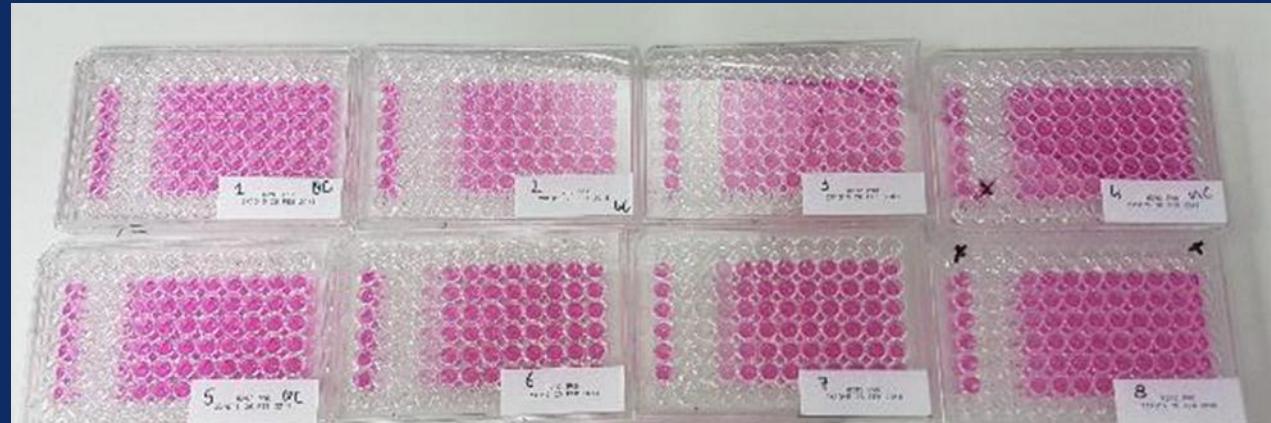
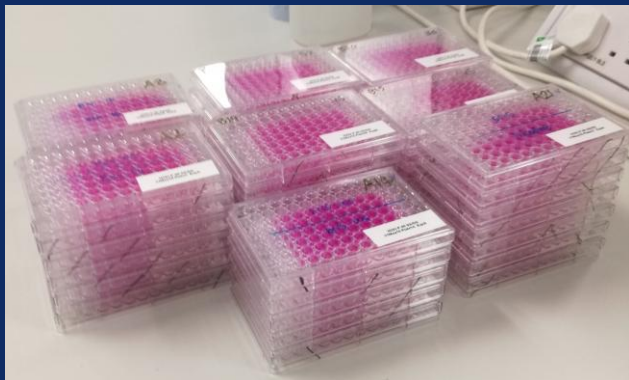
## Cons

- No information on compatibility with device
- No information on consumer use or exposure
- No *in vitro* assessment of chemical changes during heating (i.e. device / liquid interaction)
- Not suitable for some physiological endpoints due to non-compatibility with air liquid interface models



# The simplest method of assessment

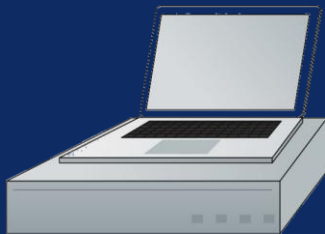
Direct addition of e-liquids in 2D cell culture studies offers opportunities to assess the impact of changes in formulation on cytotoxicity or hazard ID.



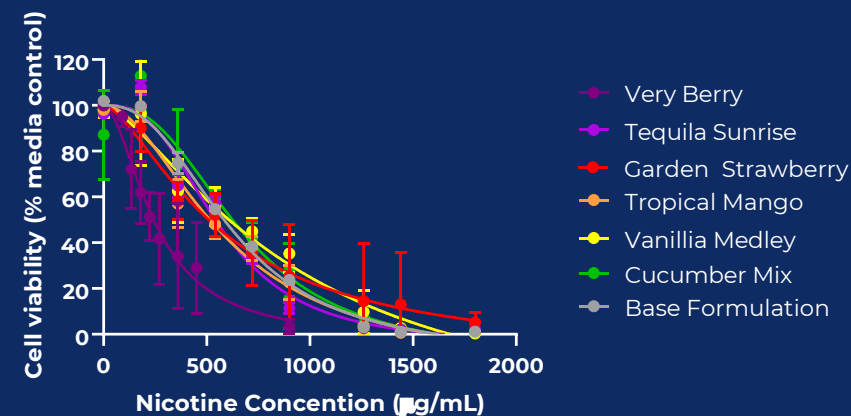
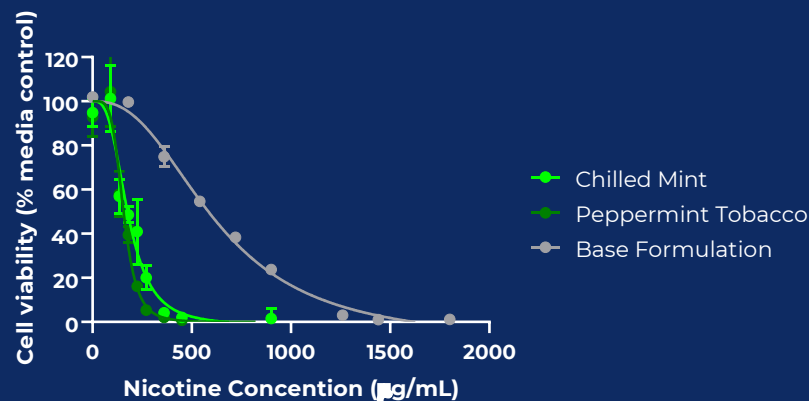
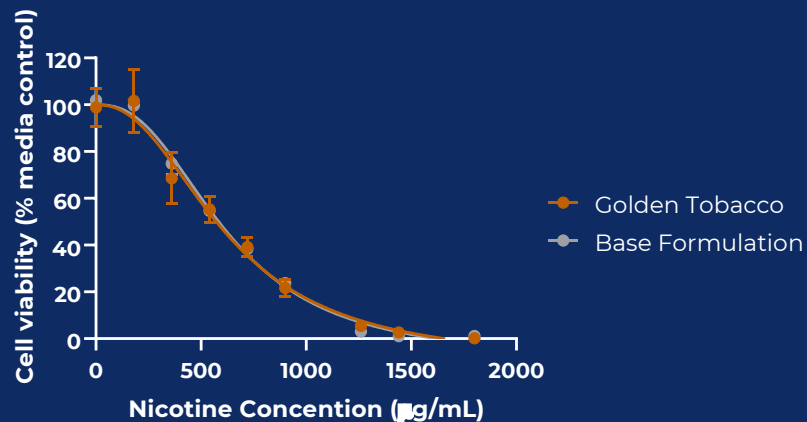
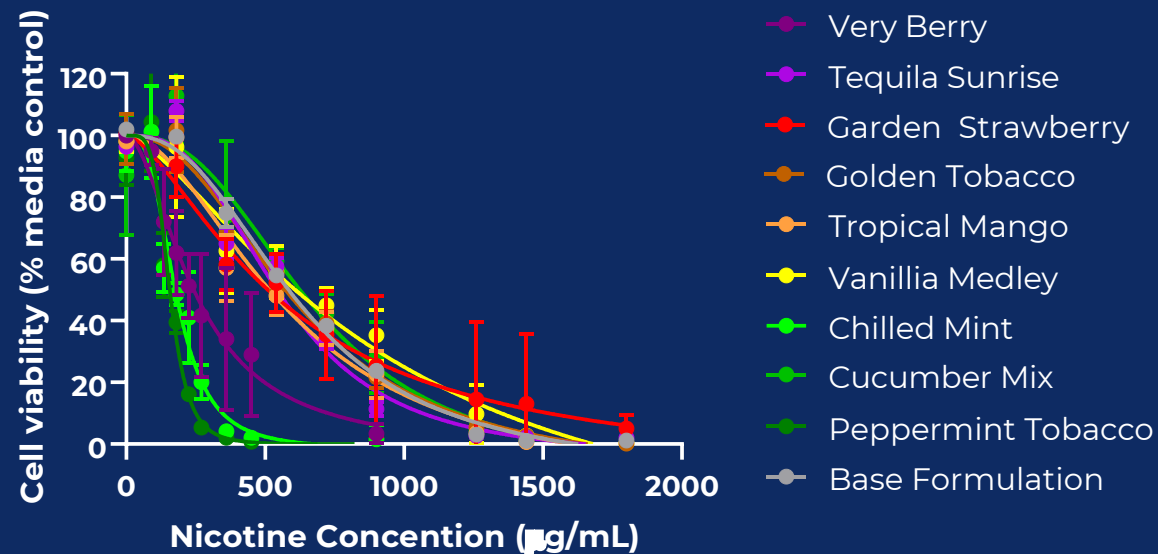
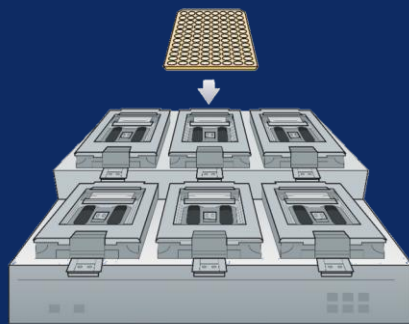
You can compare large numbers of products rapidly

# Flavour screening (Phase 1)

ePod e-liquid  
cartomiser



Real-time cell  
analyser (RTCA)



Bishop et al Tox Letters 2023

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# Testing of e-liquid and device combinations.



# Testing device and e-liquid together

## Pros

- Compatibility and performance of device and liquid
- Impact of heating liquid on emission
- E-liquid delivery and exposure
- The effect of human use behaviour

## Cons

- More labour and time intensive
- Requires specialist equipment



# The importance of device use behaviour

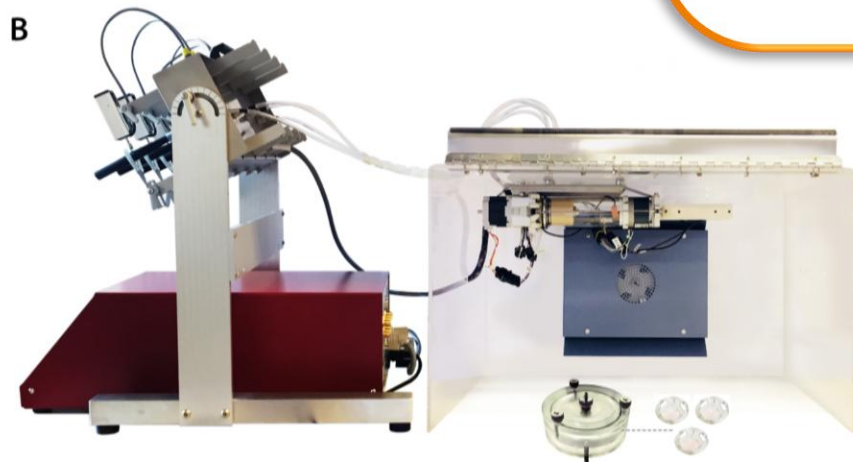
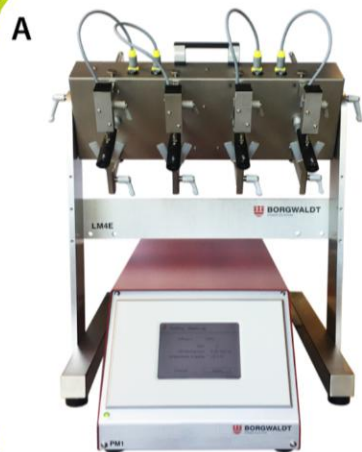
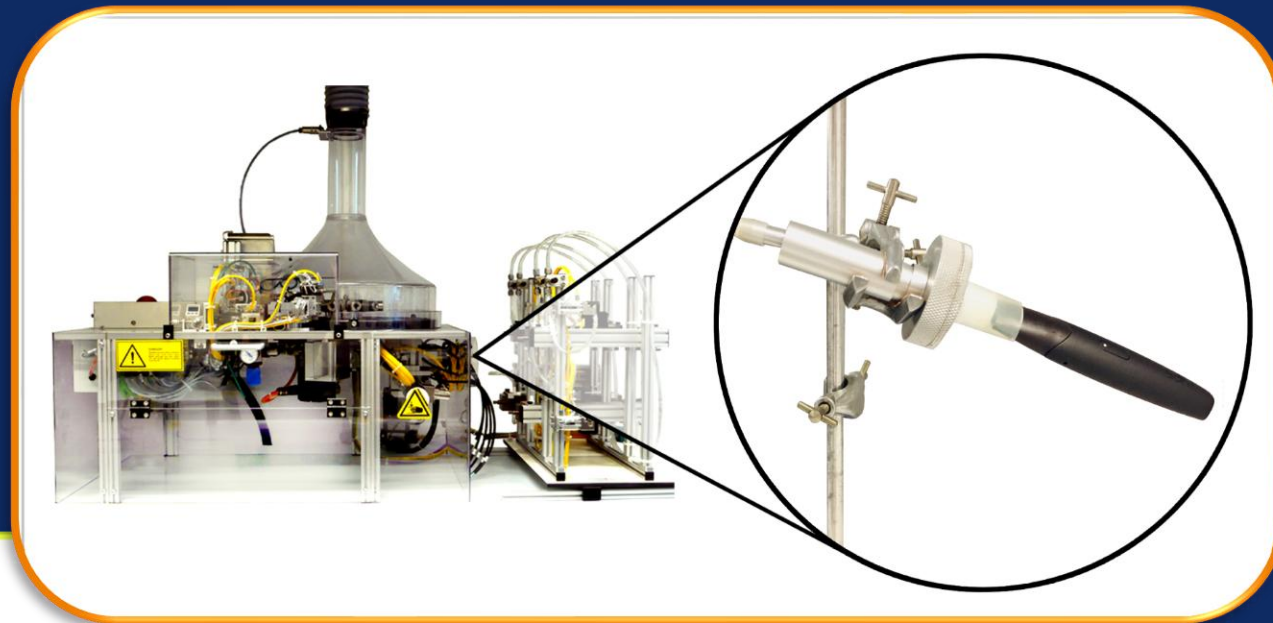
Devices have evolved to meet consumer preferences. This necessitates increased testing of actual consumer behaviour when using particular devices.

This implicates various parameters for evaluation including:

- Vapour particle size
- Vapour quantity
- Liquid, Wick & coil interaction and performance
- Emissions
- Dosing and exposure

# Mimicking human use with smoking robots

## Vitrocell VC10 system



## Körber LM4E system

# Piston Power

Robots deliver human use puffing behavior:

Consistent volumes, durations and frequencies.



# Puffing regimens modified for e-cigarette use

## Cigarette

### Health Canada Intense (HCI)

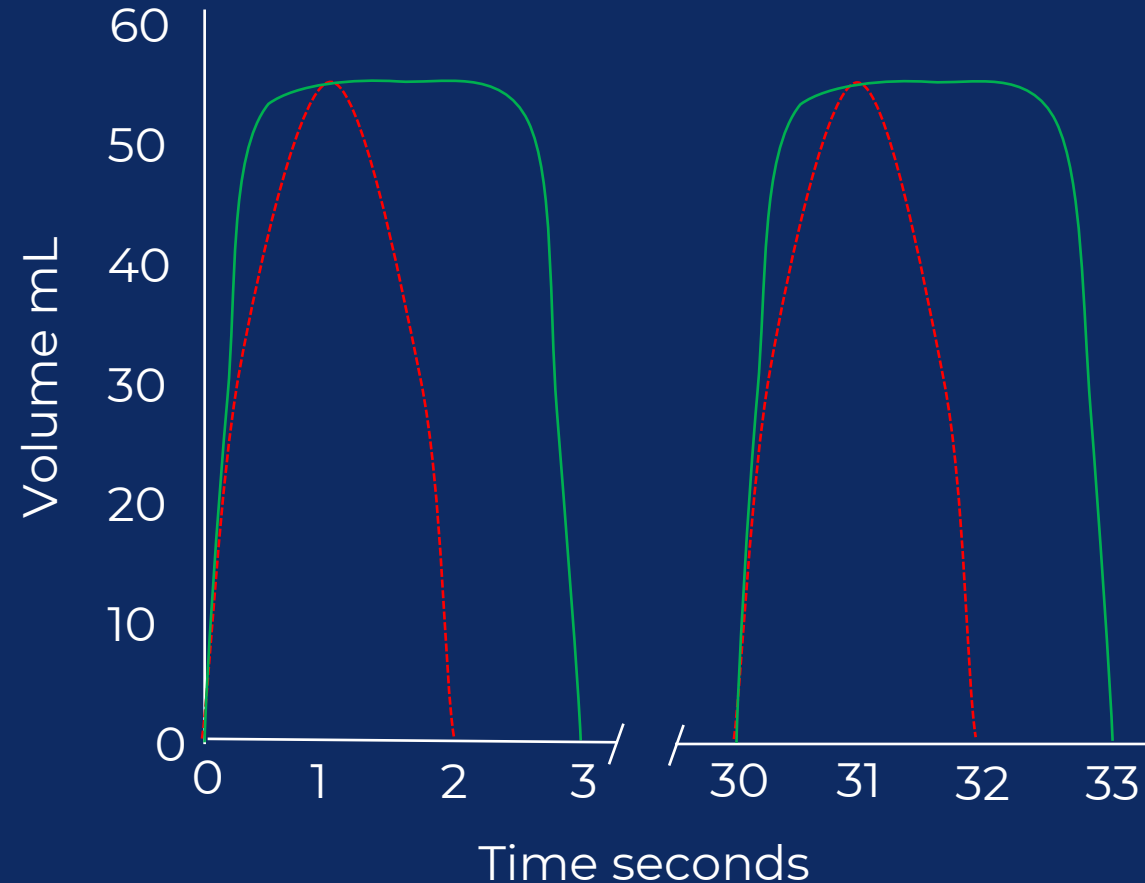
100% Vent Blocking  
55ml Puff Volume  
2 s Puff Duration  
30 s Puff interval, Twice per minute

## E-Cigarette

### (ISO 20768:2018)

CORESTA Recommended Method 81

55ml Puff Volume  
3 s Puff Duration (Square wave)  
30 s Puff interval, Twice per minute



Informed by human use studies - directly impacting on e-cigarette function

# Evolution of e-cigarette puffing regimens

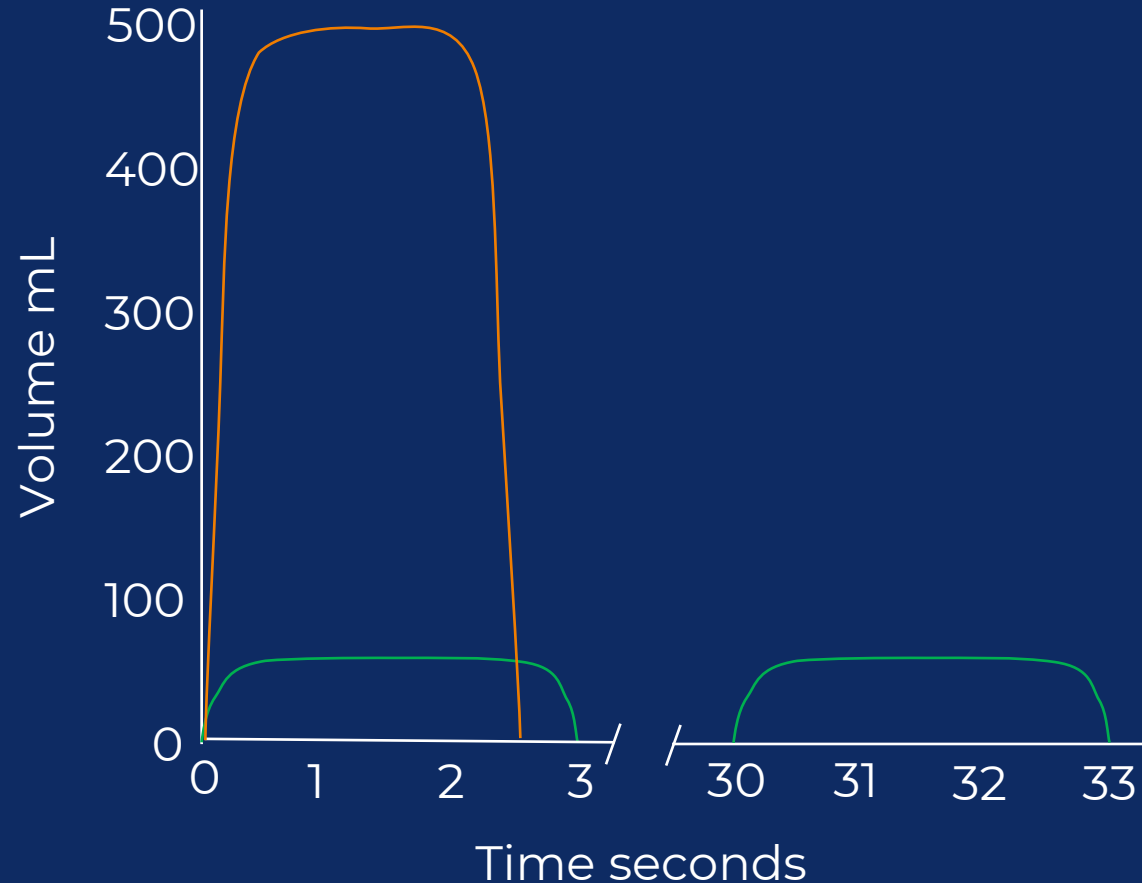
(ISO 20768:2018)

CORESTA Recommended Method 81

55ml Puff Volume

3 s Puff Duration (Square wave)

30 s Puff interval, Twice per minute



(EN 17957:2024)

Direct to lung

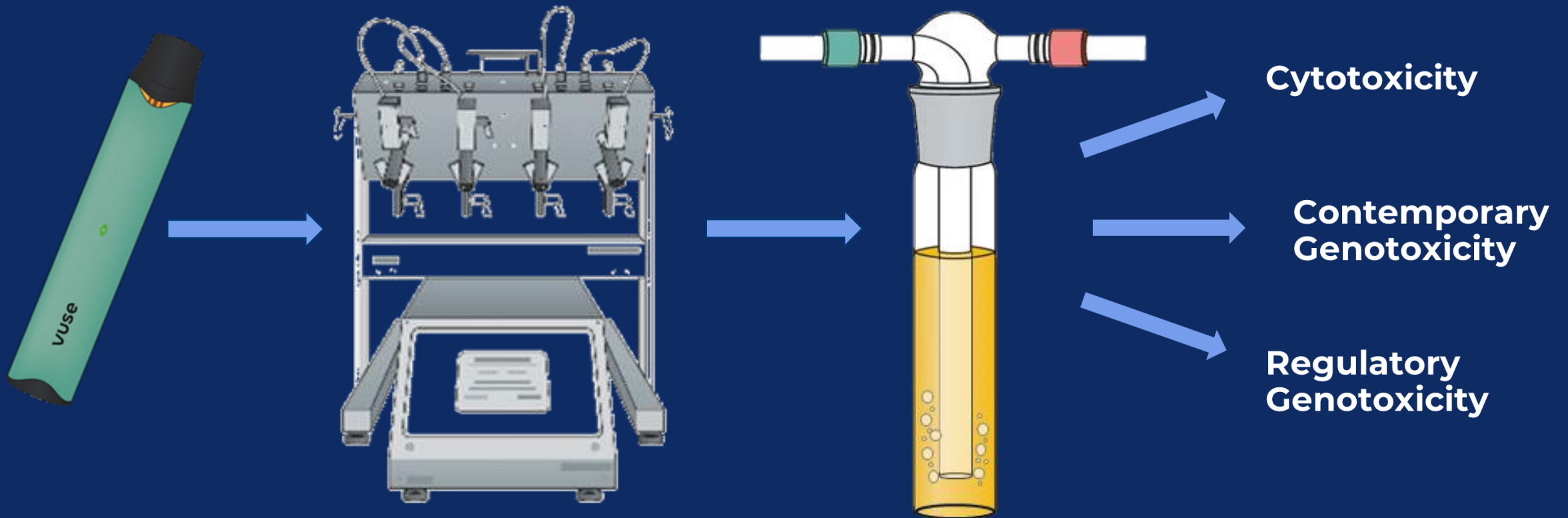
Puff volume 500 ml  $\pm$  6 ml.

2.5 s Puff duration

60 s Puff interval, once per minute

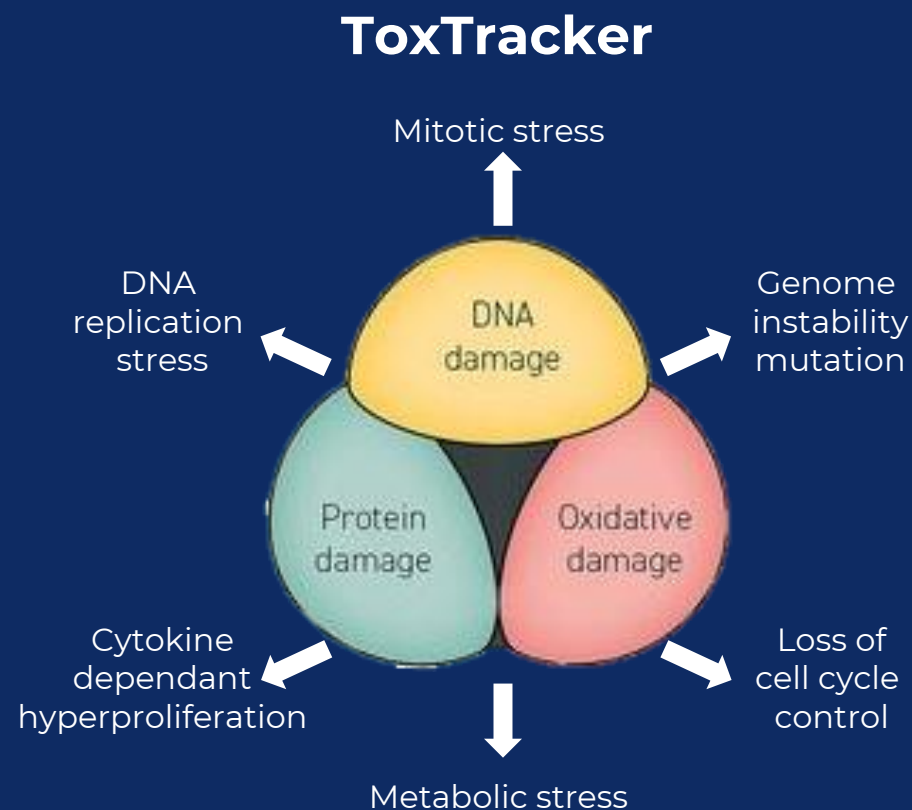
Informed by human use studies - directly impacting on e-cigarette function

# Aqueous extract (AqE) production



# Genotoxic screening assay ToxTracker®

- Stem cell-based reporter assay that provides mechanistic insights into the mode-of-action.
- Assay is compatible with S9 metabolism
- DNA Damage
  - Bsc12 (Ames), Rtnk (Micronucleus)
- Oxidative Stress
  - Srxn1 (Nrf2 Dependant), Blvrb (Nrf2 independent)
- Protein Damage
  - Ddit3
- Cell Stress
  - Btg2



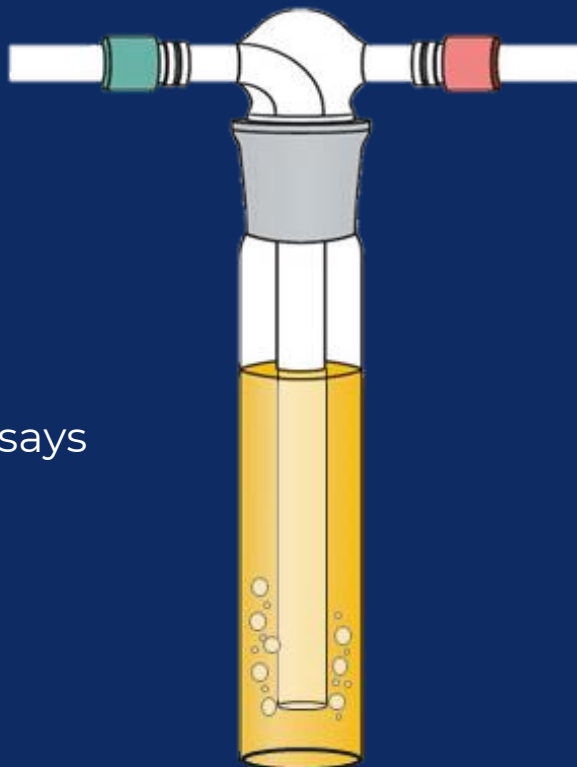
# Aqueous extract Pros and Cons

## Pros

Relatively easy to produce

Flexible testing matrix

Suitable for a range of 2D assays



## Cons

Struggles with insoluble chemicals

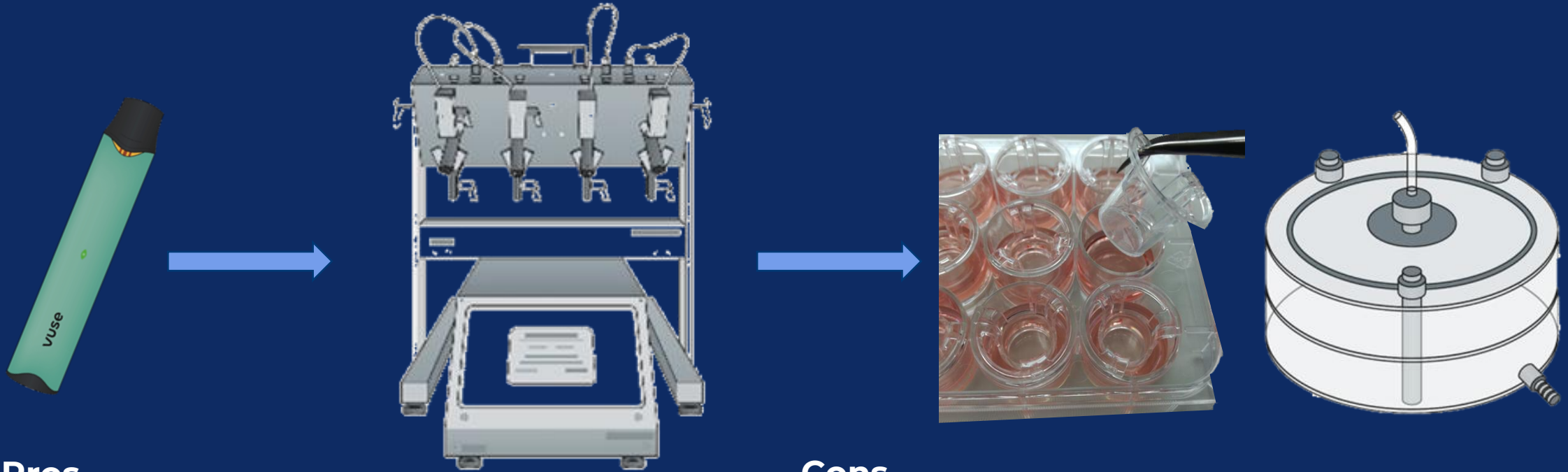
Volatiles can be short lived

Not suitable for air/liquid interface 3D lung models

# Whole aerosol exposure



# Whole aerosol exposures



## Pros

3D Human tissues

Most representative vapour exposure

Comparative dosimetry

Disease contextualised endpoints

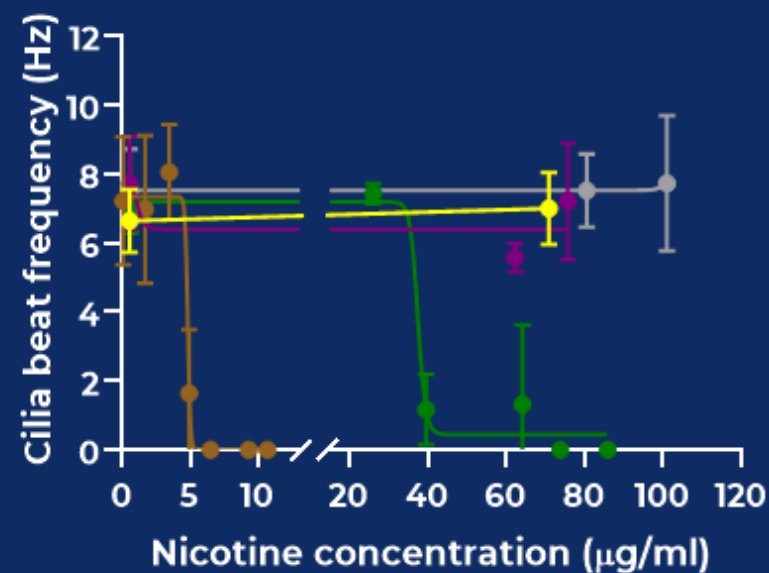
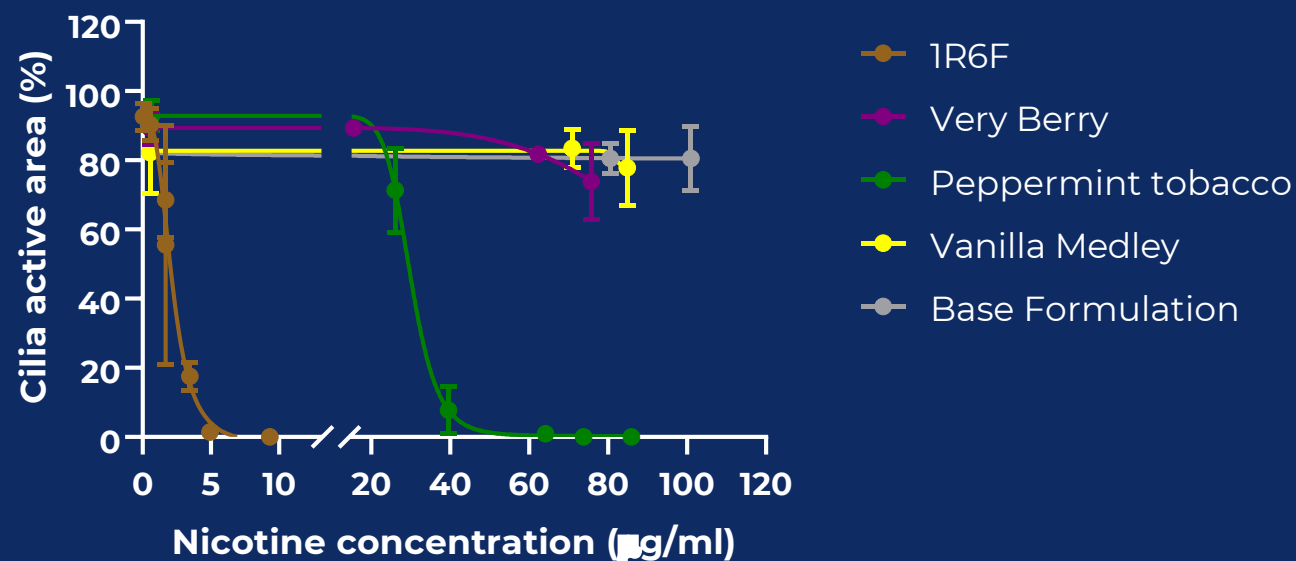
Can be used with standard genetox assays (e.g. Ames)

## Cons

Needs to be performed in real time

Complicated and expensive

# Flavour screening Phase 2



All Liquids Demonstrate  $\geq 95\%$  Reduction in *in vitro* Toxicity

Bishop et al Tox Letters 2023

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# Whole aerosol endpoints

## Whole aerosol exposure to 3D tissues and assessment of disease related endpoints:

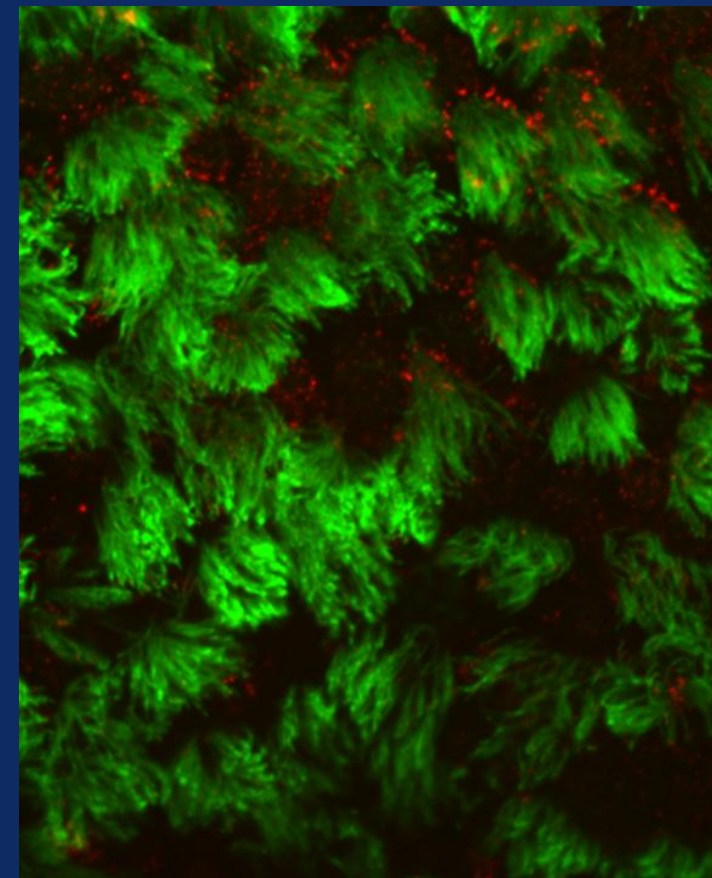
Cytotoxicity

Oxidative Stress

Tight Junction Barrier Integrity / Trans epithelial electrical resistance (TEER)

Cilia beat frequency (CBF)

Cilia active area.





# Future Directions



# Bridging the gap

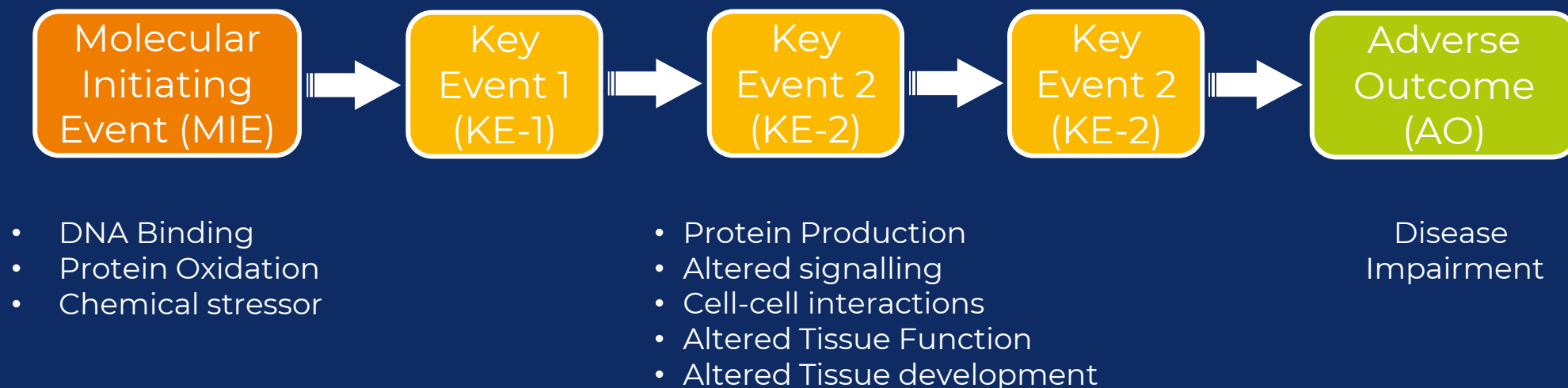
**In Vitro  
Hazard ID**



**Risk  
Assessment**

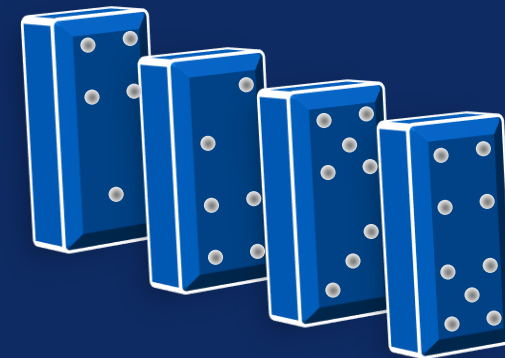
# Adverse Outcome Pathway (AOP)

A structured representation of biological events leading to adverse health effects



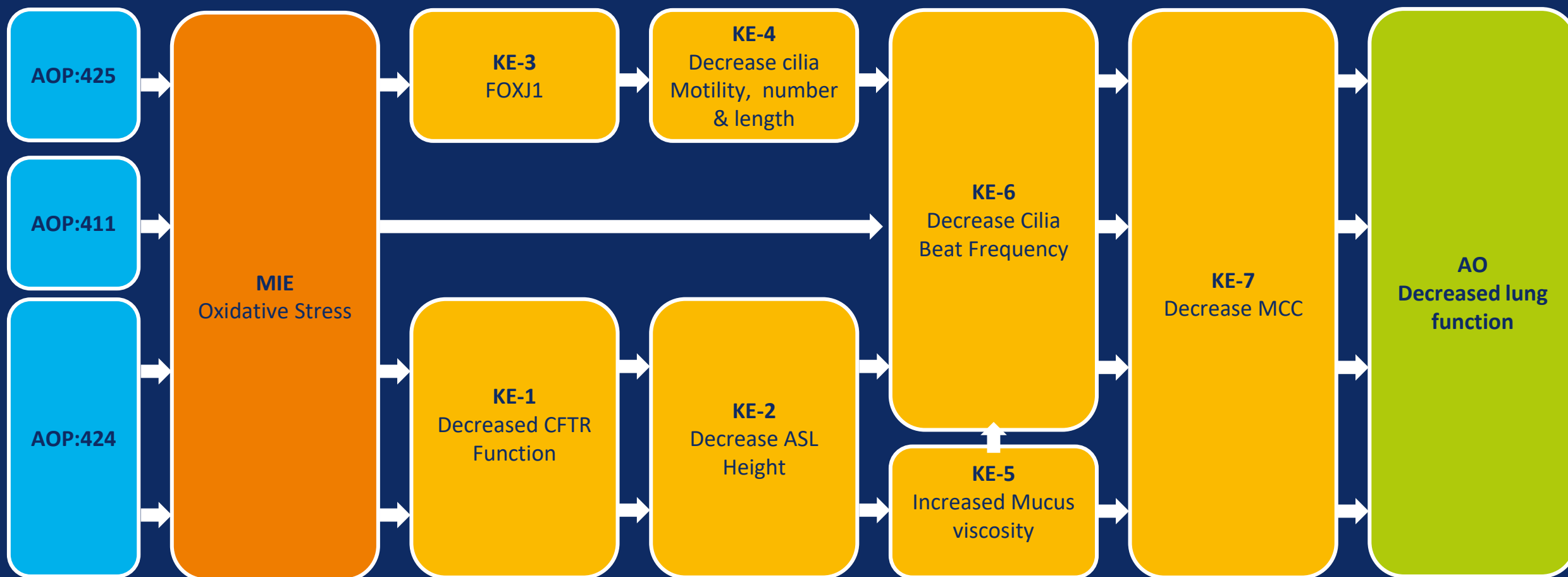
# Adverse Outcome Pathway (AOP)

- Non-animal alternative
- Link clinical biomarkers potential outcomes / mechanistic narrative
- Potential biomarker evaluation
- Cost effective product screening
- Independent review and acceptance process (OECD)



# AOP Oxidative Stress - Decreased Lung Function

## AOP 411, 424 & 425 Experimental targets



Luetlich *et al.*, Front. Toxicol. 2021

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# Events leading to COPD can be mimicked *in vitro*

Clinic

*In vitro*

Oxidative Stress



Isoprostane

Srxn, Blvrb, Nrf2,  
HMOX1  
Isoprostane

Cilia formation  
& altered fluid uptake



Nasal brushing,  
FOXJ1, CFTR

FOXJ1, CFTR

Airway surface  
liquid height,  
cilia length



Cilia number  
Beat freq.



TEER  
Cilia beat  
frequency  
Active area

Mucus  
viscosity



Nasal brushing  
MUC5AC  
MUC5B

MUC5AC  
MUC5B

Mucociliary  
clearance



Carbon black  
or saccharin  
tracking

Bead tracking

Cellular level

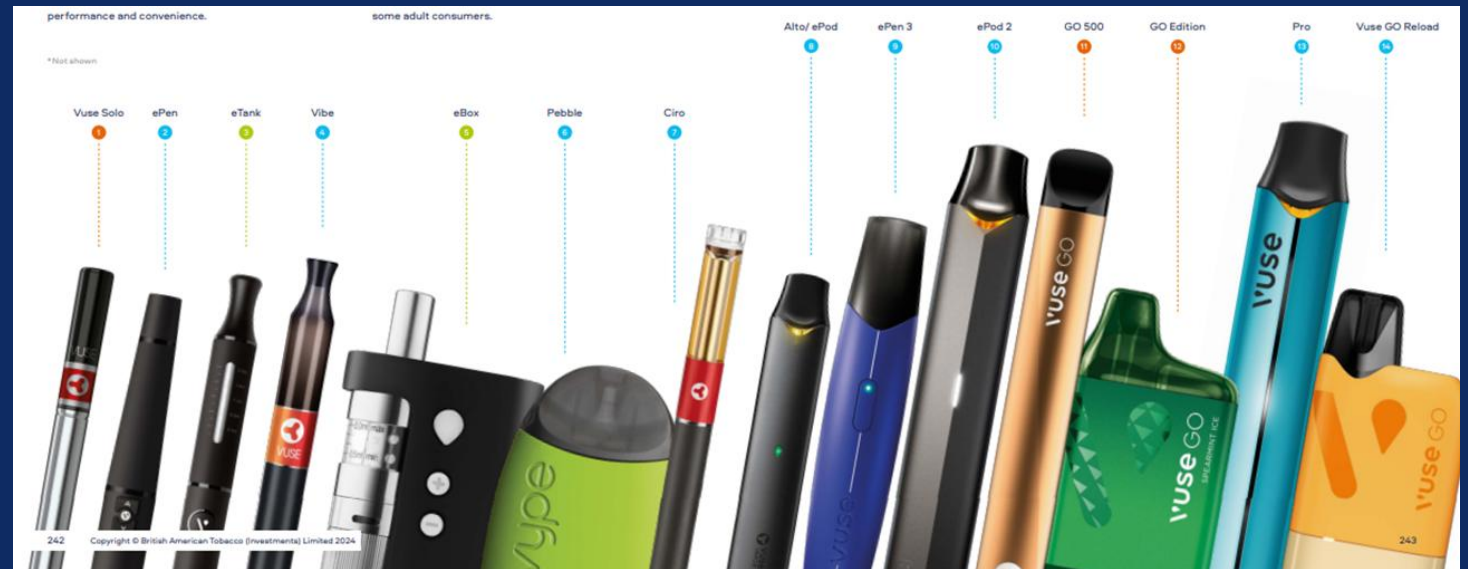
Tissue function

# Summary

- While not covered here we have had some success in developing these in vitro assessments.
- New approach methodologies (NAMs) offer new opportunities to reduce the requirement of animal models.
- Future developments of current AOPs to allow a quantitative approach with clinical measure may offer opportunities to address the question of absolute risk.

# Conclusions

- E-cigarette devices have evolved and designs have multiplied in the past decade.
- There is a necessity to ensure safety and quality standards, as well as a reduced risk profile.
- As a result, the methods of testing devices and liquids needs to evolve too.



# Thank you for Listening

## Questions?