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

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3rd June 2025

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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.

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%(1)
E-cigarettes : 9.1%(2)

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%)(2)

Data taken from:
[1]Smoking profile for England: statistical commentary,, Office for Health Improvement & Disparities, UK Gov, October 2024 update.
[2]Use of e-cigarettes (vapes) among adults in Great Britain, Action on Smoking Health (ASH), 2023.

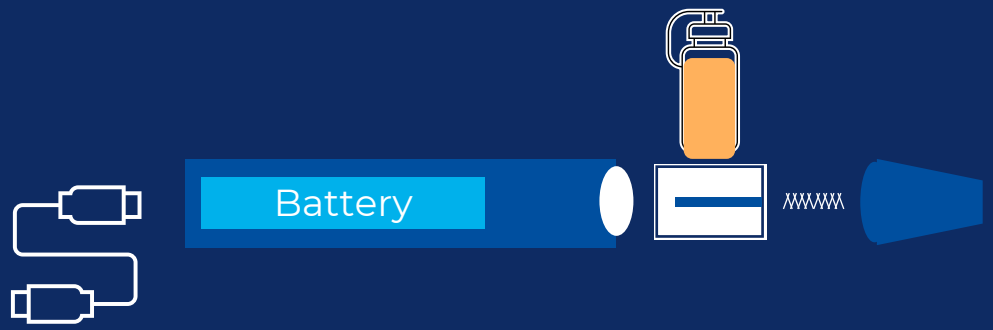
The different e-cigarette types

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 (Ω,) 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

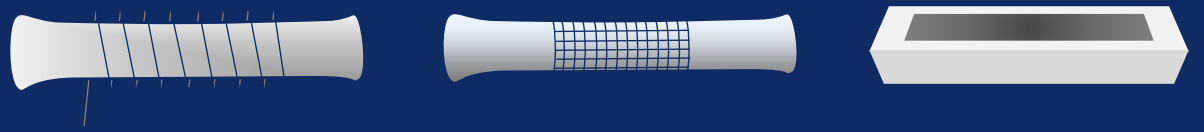
VG customises sensory aspects (sweetness/rich and Cloud formation)

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

Viscosity	VG > PG
Boiling Point	VG (287 ℃) > PG (188℃)

The e-cigarette atomiser

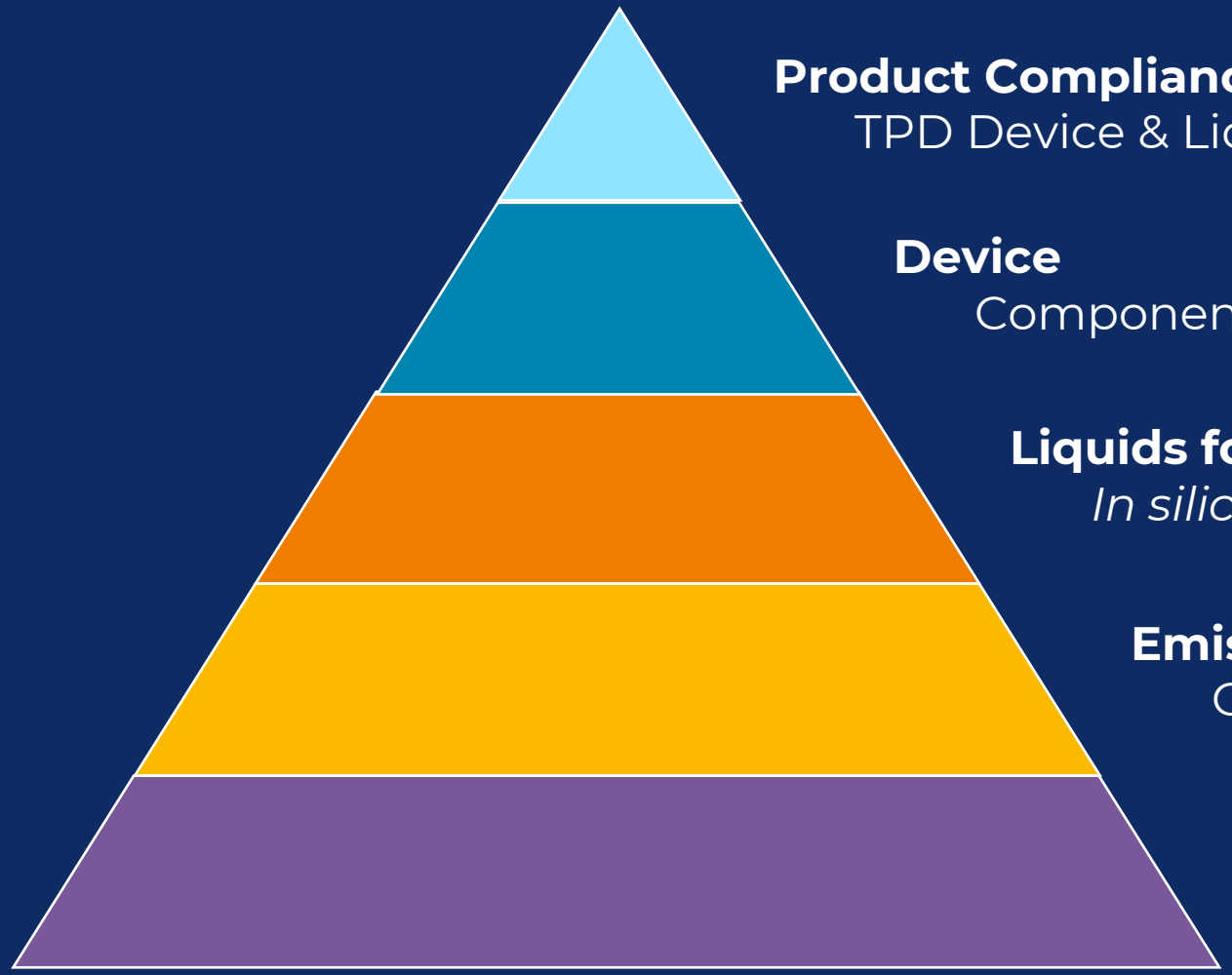
Microporous substrate or wick draws the e-liquid into heating atomiser



Coil & Wick | Mesh & Wick | Ceramic

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

Product Assessment



Product Compliance
TPD Device & Liquids

Device
Component make up and compatibility

Liquids formulation assessment
In silico, ingredient inclusion and concentration

Emission
Chemical and dosimetry

Biological Testing

Direct testing of e-liquids

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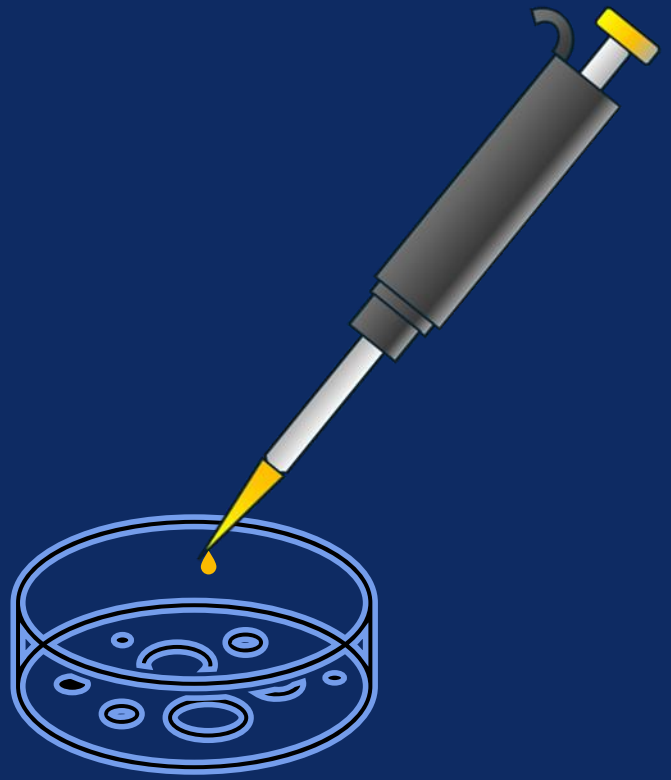
Direct dosing

Pros

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

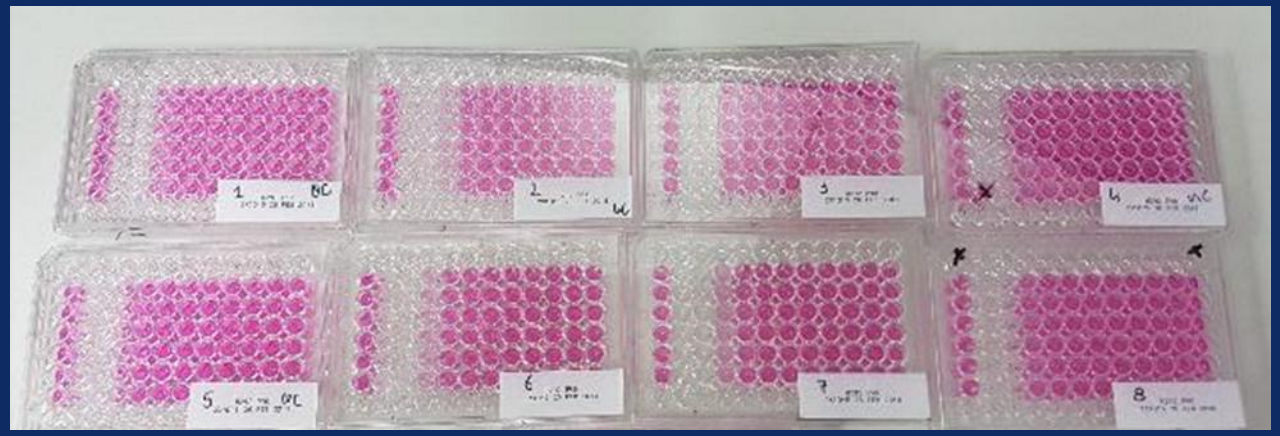
Cons

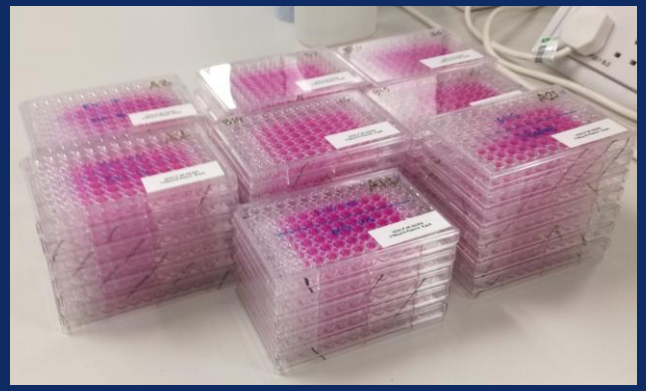
- No information on compatibility with device
- No information on consumer use or exposure
- No *in vitro* assessment of chemical changes during heating (i.e. device / liquid interaction)
- Not suitable for some physiological endpoints due to non-compatibility with air liquid interface models



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.



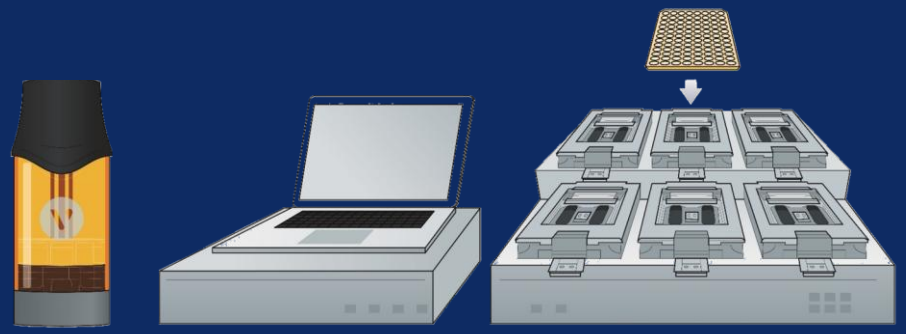


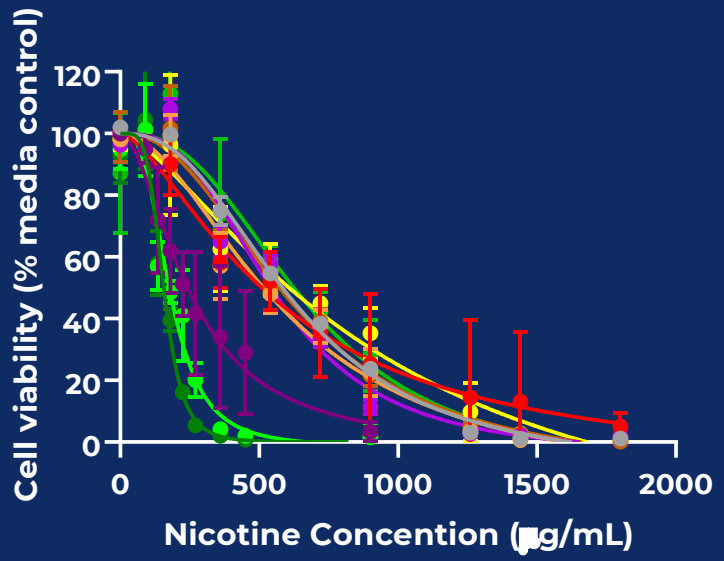
You can compare large numbers of products rapidly

Flavour screening (Phase 1)

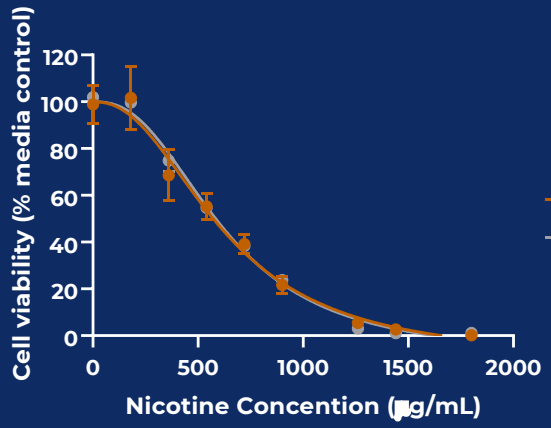
ePod e-liquid cartomiser

Real-time cell analyser (RTCA)

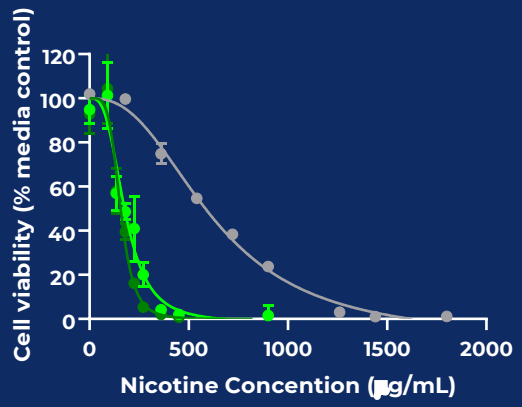




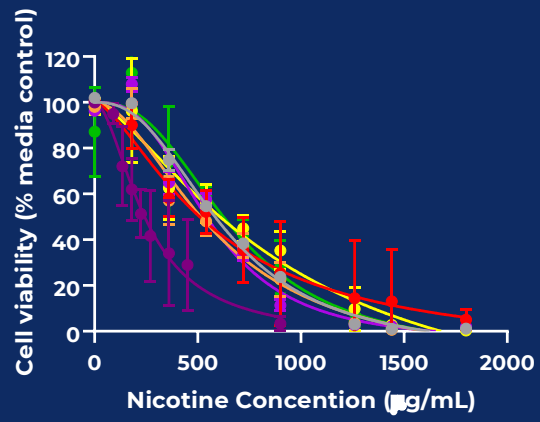
Very Berry
Tequila Sunrise
Garden Strawberry
Golden Tobacco
Tropical Mango
Vanillia Medley
Chilled Mint
Cucumber Mix
Peppermint Tobacco
Base Formulation



Golden Tobacco
Base Formulation



Chilled Mint
Peppermint Tobacco
Base Formulation



Very Berry
Tequila Sunrise
Garden Strawberry
Tropical Mango
Vanillia Medley
Cucumber Mix
Base Formulation

Bishop et al Tox Letters 2023

Testing of e-liquid and device combinations.

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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

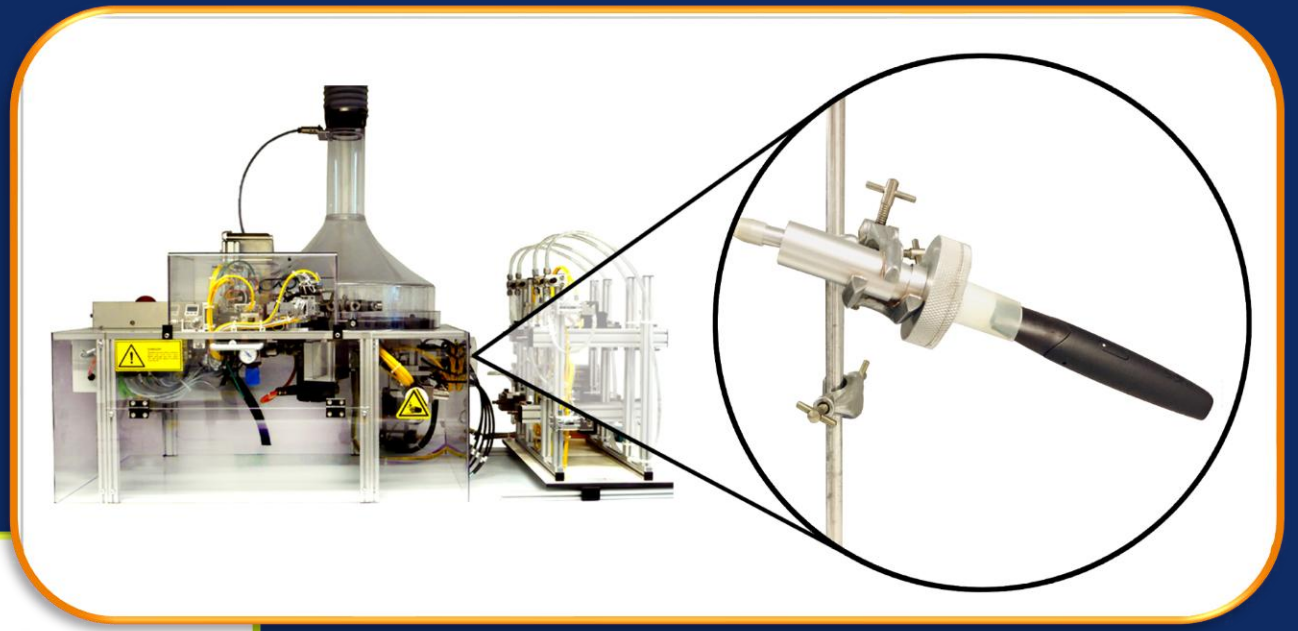
Devices have evolved to meet consumer preferences. This necessitates increased testing of actual consumer behaviour when using particular devices.

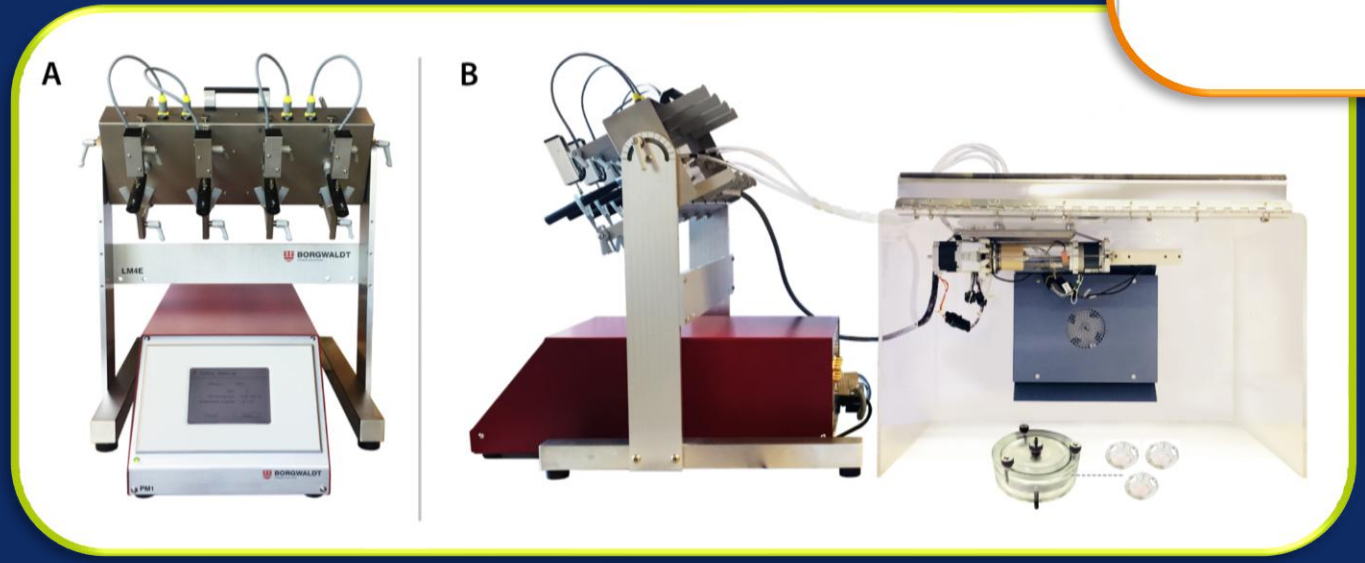
This implicates various parameters for evaluation including:

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

Mimicking human use with smoking robots

Vitrocell VC10 system



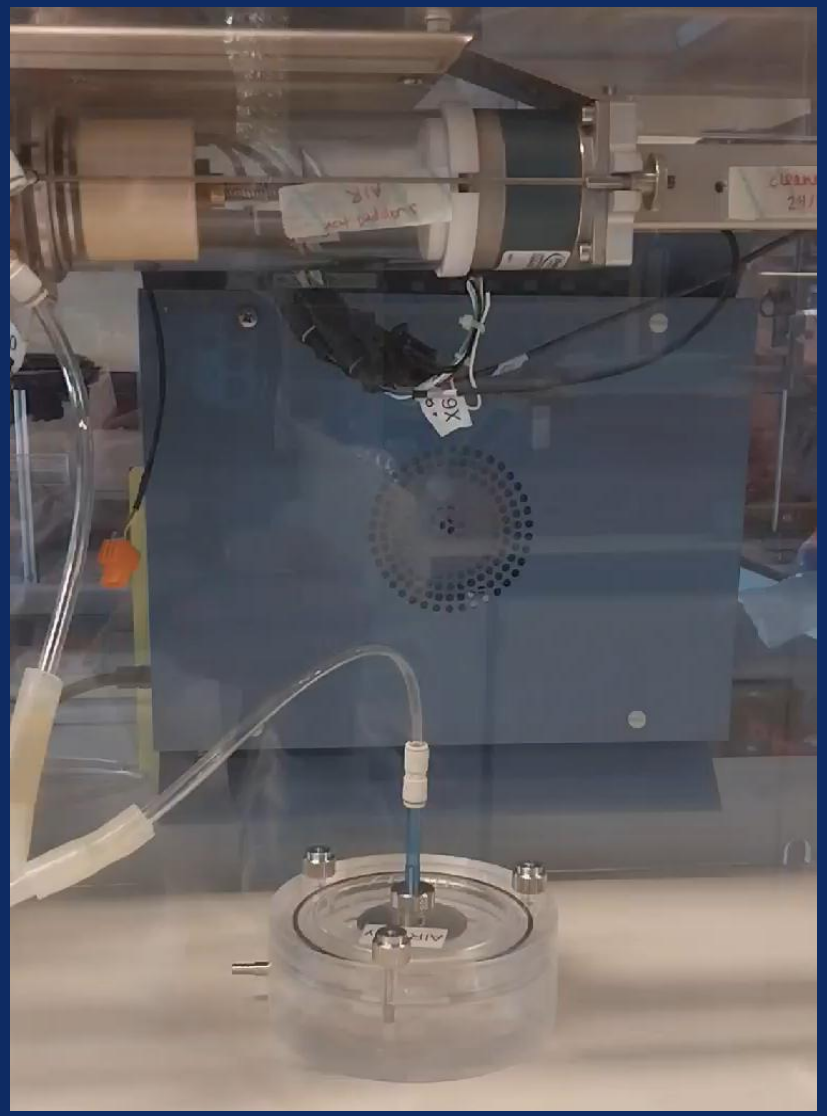


Körber LM4E system

Piston Power

Robots deliver human use puffing behavior:

Consistent volumes, durations and frequencies.



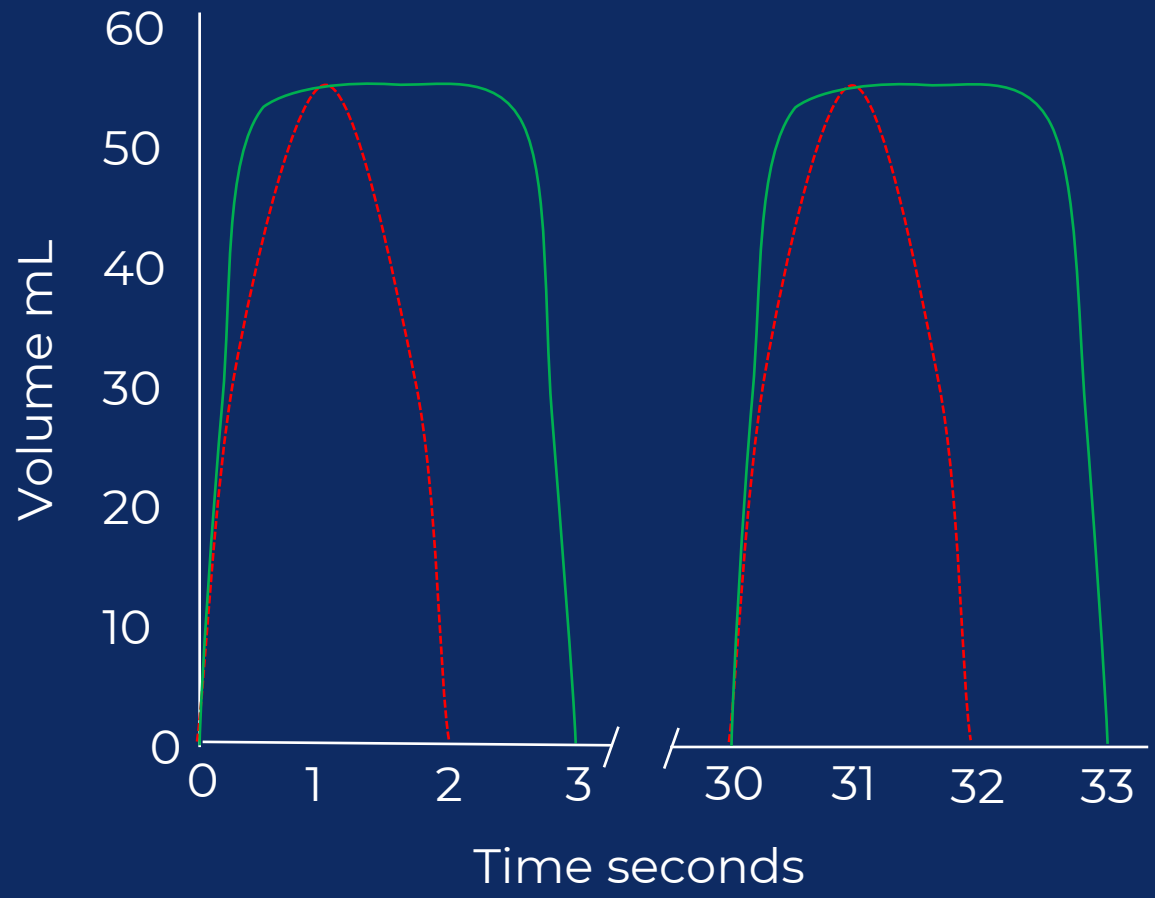
Puffing regimens modified for e-cigarette use

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

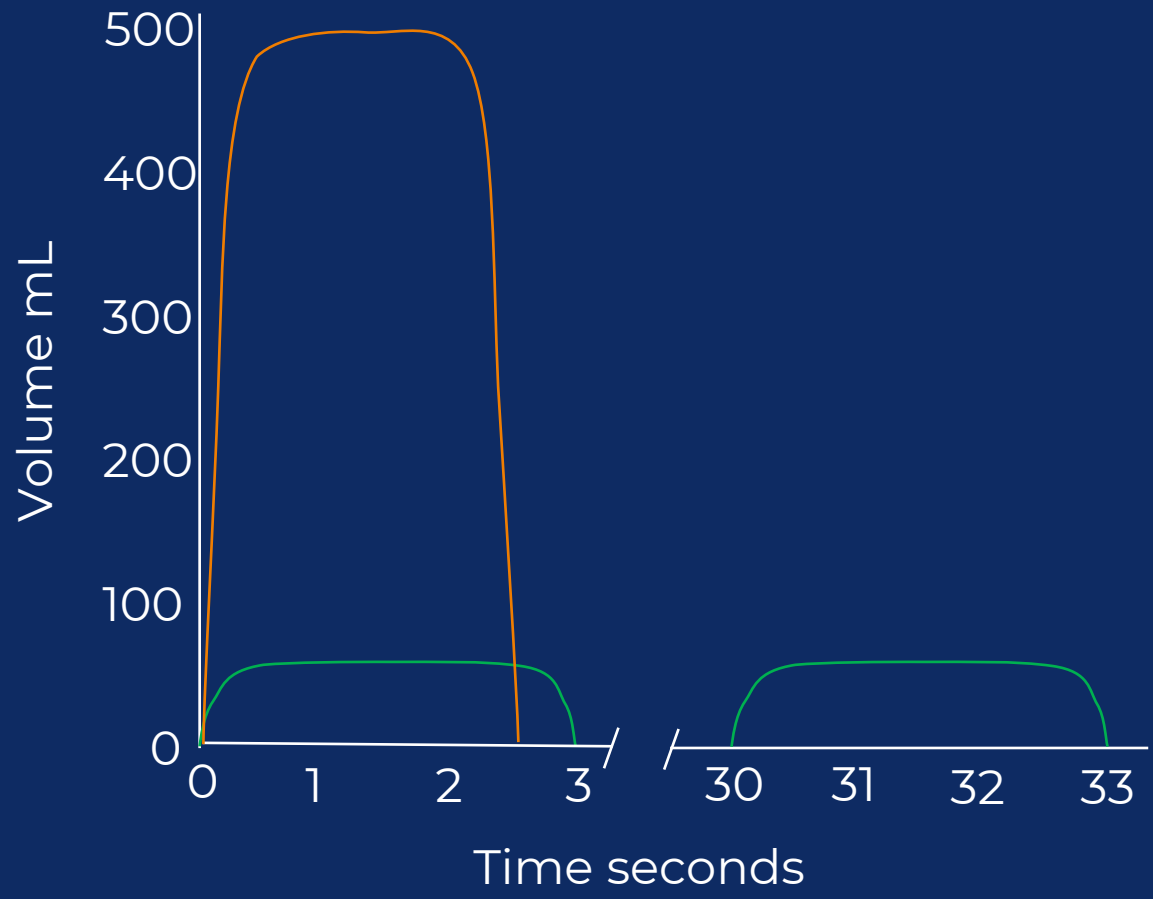


Informed by human use studies - directly impacting on e-cigarette function

Evolution of e-cigarette puffing regimens

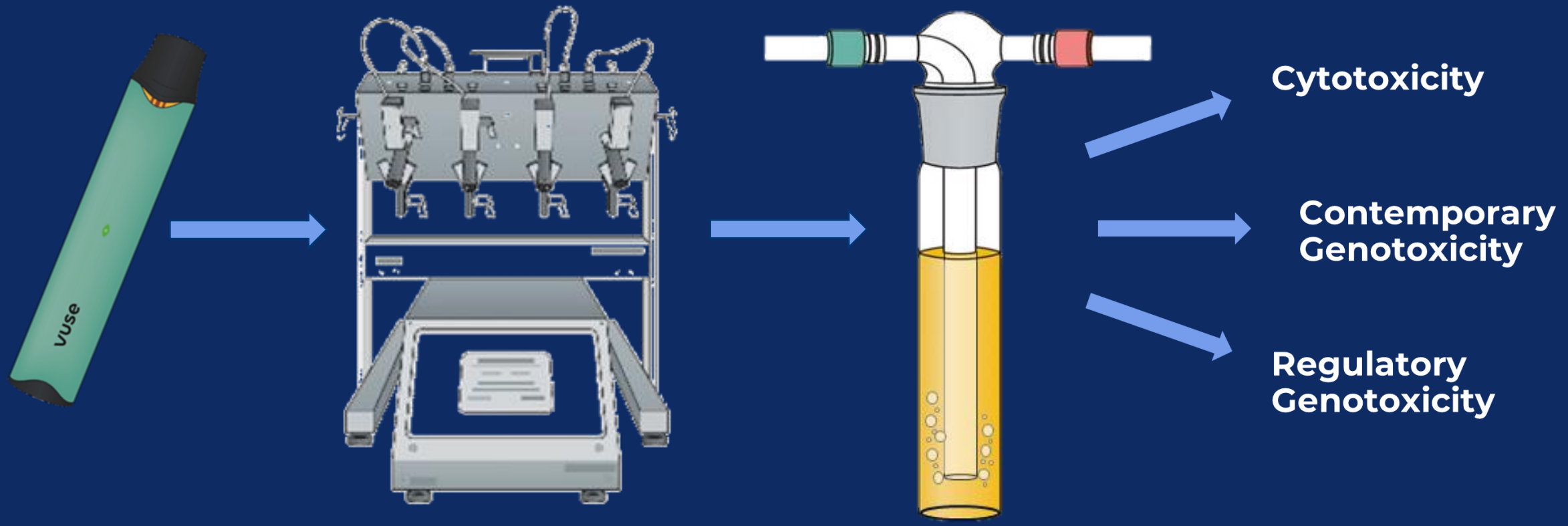
(ISO 20768:2018)
CORESTA Recommended Method 81
55ml Puff Volume
3 s Puff Duration (Square wave)
30 s Puff interval, Twice per minute

(EN 17957:2024)
Direct to lung
Puff volume 500 ml ± 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

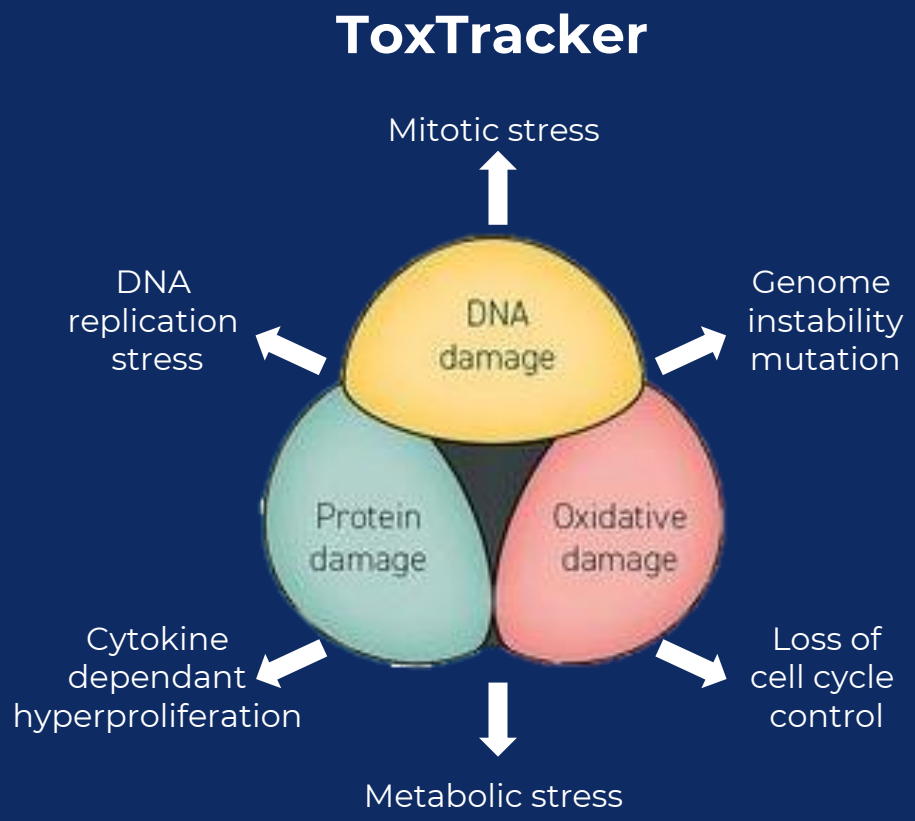
Aqueous extract (AqE) production



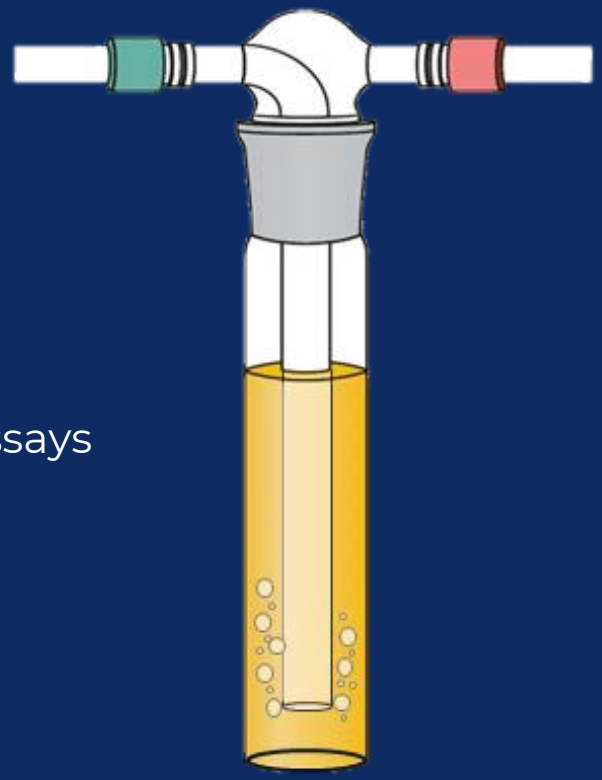
Genotoxic screening assay ToxTracker®

- Stem cell-based reporter assay that provides mechanistic insights into the mode-of-action.
- Assay is compatible with S9 metabolism

- DNA Damage
  - Bscl2 (Ames), Rtkn (Micronucleus)
- Oxidative Stress
  - Srnx1 (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

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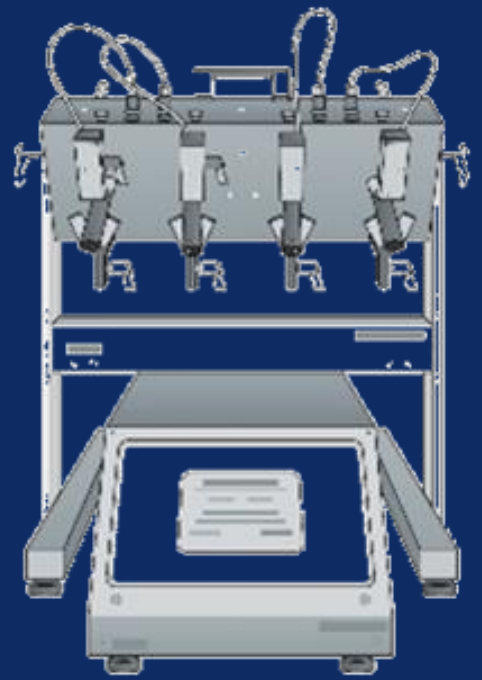
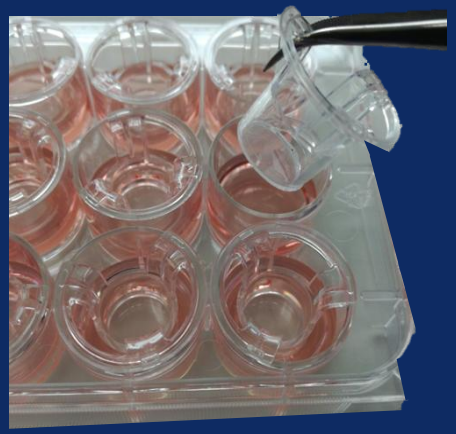
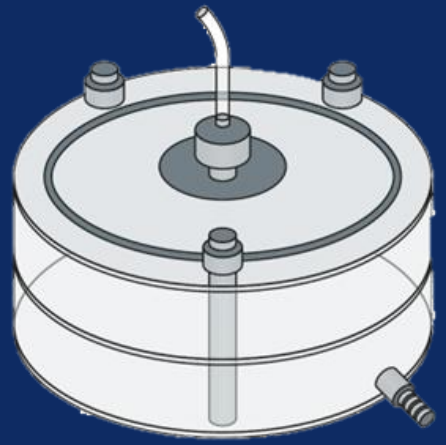
Whole aerosol exposures







Pros

3D Human tissues

Most representative vapour exposure

Comparative dosimetry

Disease contextualised endpoints

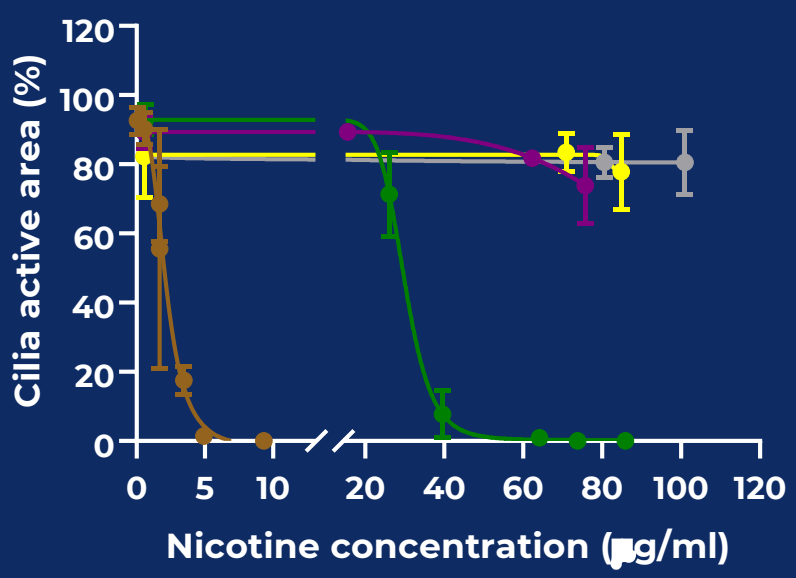
Can be used with standard genetox assays (e.g. Ames)

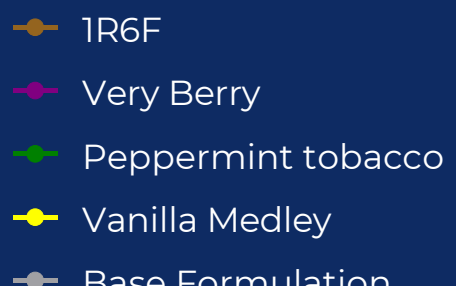
Cons

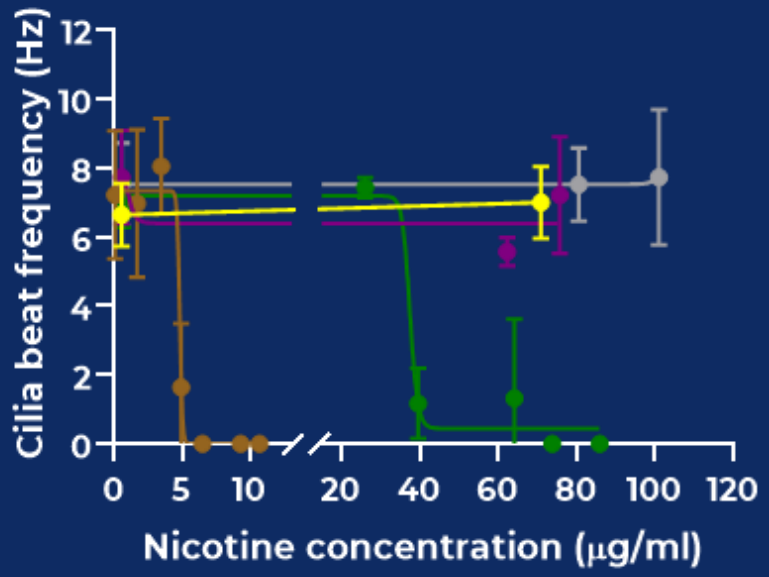
Needs to be performed in real time

Complicated and expensive

Flavour screening Phase 2







All Liquids Demonstrate ≥95% Reduction in *in vitro* Toxicity

Bishop et al Tox Letters 2023

Whole aerosol endpoints

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

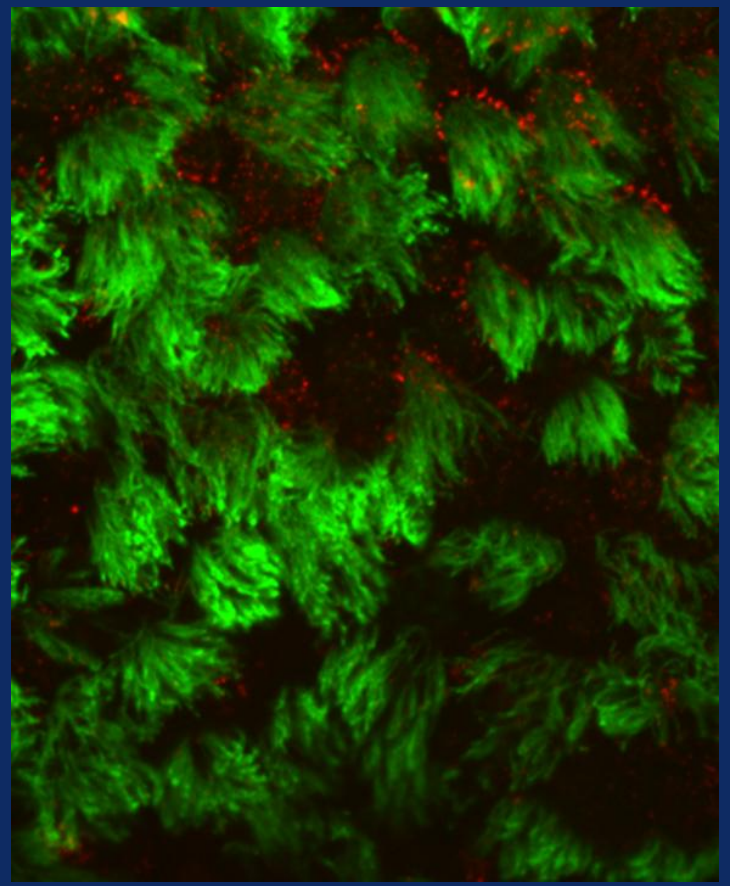
Cytotoxicity

Oxidative Stress

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

Cilia beat frequency (CBF)

Cilia active area.



Future Directions

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Bridging the gap

In Vitro
Hazard ID

Risk
Assessment

Adverse Outcome Pathway (AOP)

A structured representation of biological events leading to adverse health effects

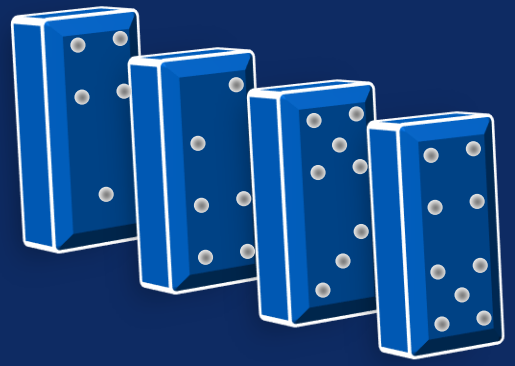
- DNA Binding
- Protein Oxidation
- Chemical stressor

- Protein Production
- Altered signalling
- Cell-cell interactions
- Altered Tissue Function
- Altered Tissue development

Disease
Impairment

Adverse Outcome Pathway (AOP)

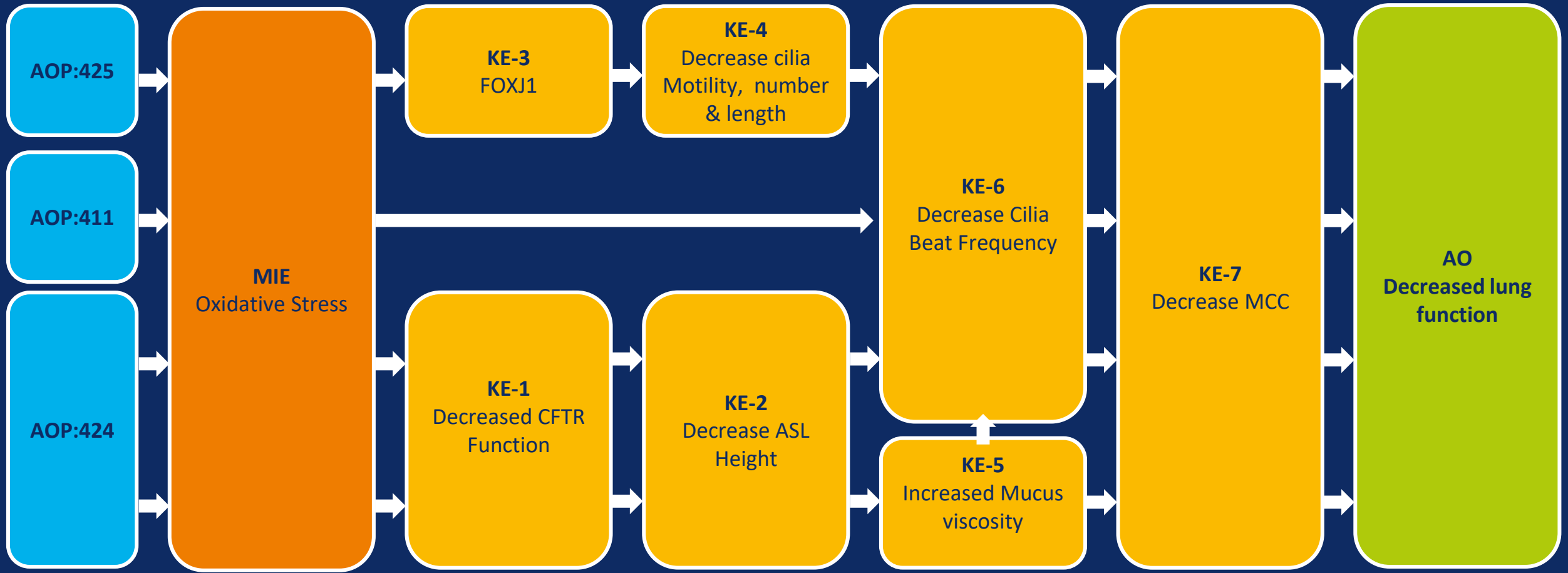
- Non-animal alternative
- Link clinical biomarkers potential outcomes / mechanistic narrative
- Potential biomarker evaluation
- Cost effective product screening
- Independent review and acceptance process (OECD)



AOP Oxidative Stress - Decreased Lung Function

AOP 411, 424 & 425

Experimental targets



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

Events leading to COPD can be mimicked *in vitro*

	Oxidative Stress	Cilia formation & altered fluid uptake	Airway surface liquid height, cilia length	Cilia number Beat freq.	Mucus viscosity	Mucociliary clearance
Clinic	Isoprostane	Nasal brushing, FOXJ1, CFTR			Nasal brushing MUC5AC MUC5B	Carbon black or saccharin tracking
In vitro	Srxn, Blvrb, Nrf2, HMOX1 Isoprostane	FOXJ1, CFTR		TEER Cilia beat frequency Active area	MUC5AC MUC5B	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?