A PRODUCT STEWARDSHIP ASSESSMENT STRATEGY FOR E-LIQUIDS

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This presentation is the opinions of the author and is for discussion purposes only, it may not necessarily reflect the opinions of Imperial Brands plc.

IMPERIAL BRANDS ADOPT A SCIENTIFIC APPROACH TO ASSESSING PRODUCTS WITHOUT TESTING ON ANIMALS

We have a duty to understand how our products interact and behave with consumer health, and any potential risks that may materialise:

- Consumer safety is key
- Regulatory compliance for market of sale

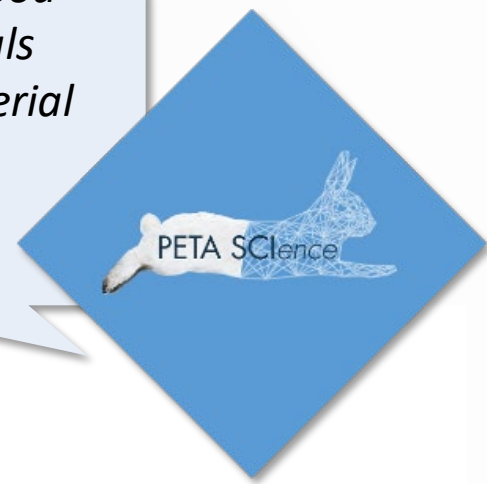
Imperial Brands PLC does not commission or conduct research involving animals and would not undertake such research unless formally required to do so by governments or by recognised regulatory authorities.

IB has an Alternatives to Animal Testing approach which involves using human relevant *in vitro* and *in silico* techniques

- These additional assays provide a weight of evidence and can help clarify results of routine assays.
- Our *in vitro* approach has been endorsed by PETA science

"We are a proponent of good science and sparing animals from toxicity tests, as Imperial has done."

Dr Andreas Stucki, PETA Science Consortium International

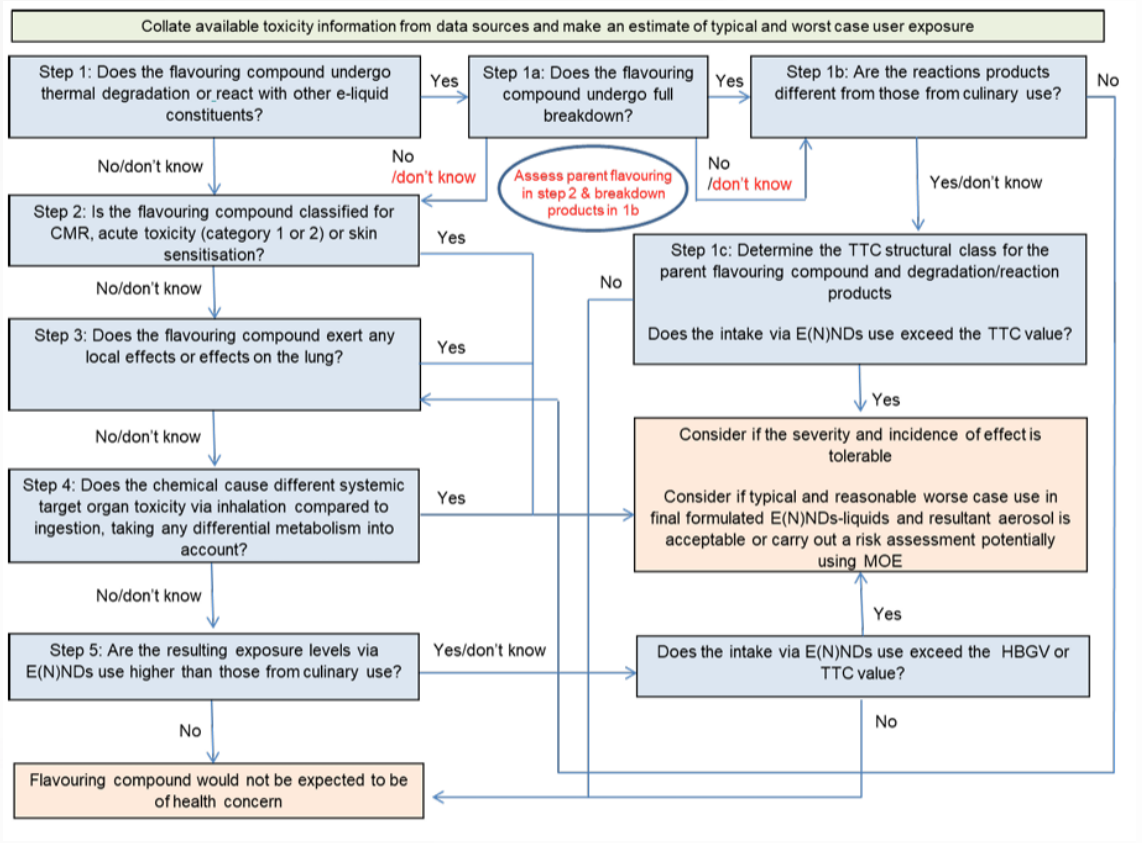


PRODUCT STEWARDSHIP ASSESSMENTS STRATEGIES

- Various manufacturers have published their EVP aerosol assessment strategies
- The only independent scientific group to write one, is the UK's Committee of Toxicology (COT)
- The guidance suggests a desk-based review, using quantitative risk assessment, history of use and Thresholds of Toxicological concern to assess individual ingredients.
- We broadly follow this framework however focus on the *in vitro* testing of the liquids/aerosols from the device they will be marketed in

Committee on Toxicity

Framework for risk assessment of flavouring compounds via inhalation exposure



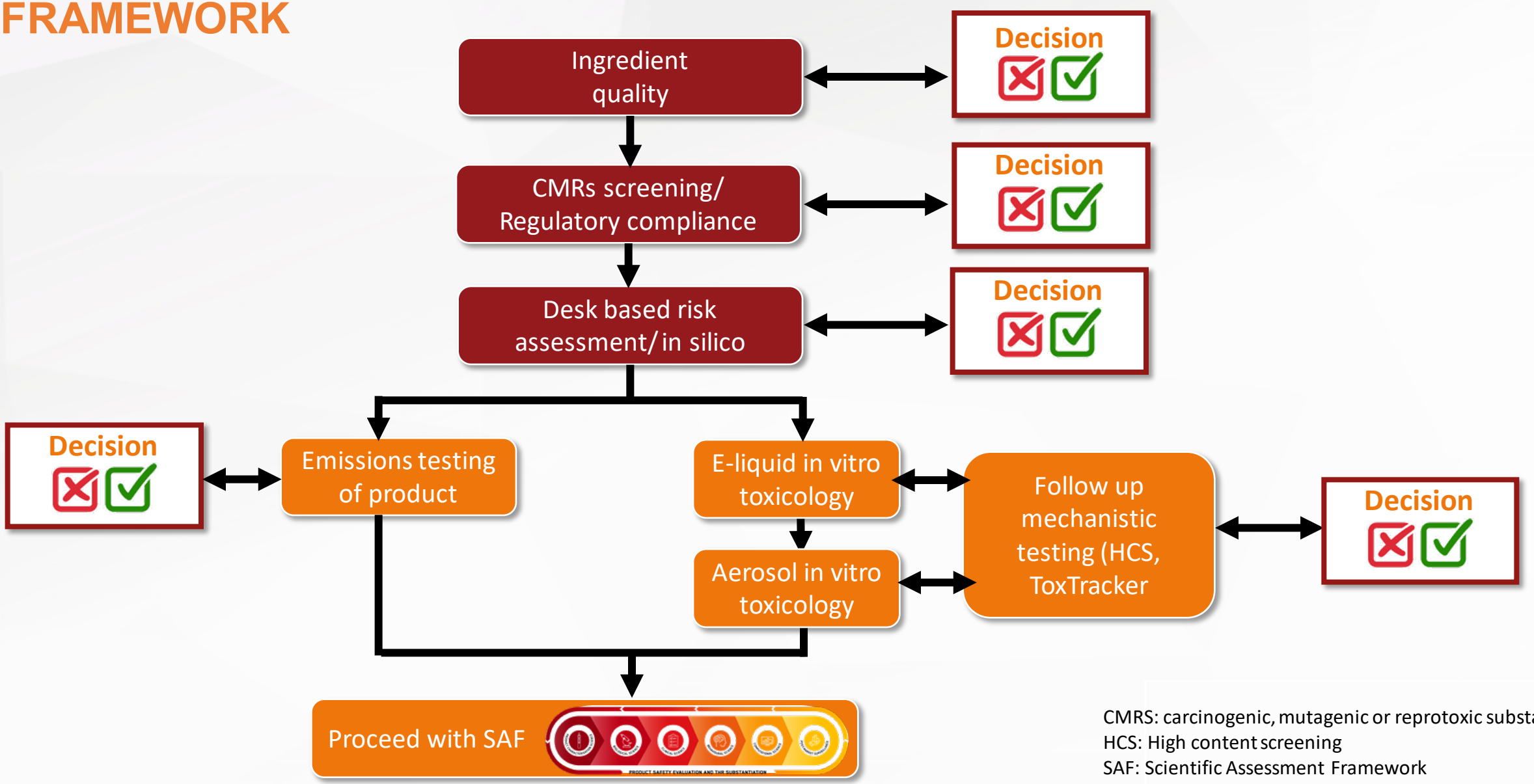
https://cot.food.gov.uk/

BIOLOGICAL TESTING; PART OF A SCIENTIFIC ASSESSMENT FRAMEWORK (SAF)

A multi-stage, multiyear testing and research programme designed to evaluate the potential harm reduction of each of our NGPs relative to combustible cigarettes.

https://imperialbrandsscience.com/

IMPERIAL BRANDS PRODUCT SAFETY TOXICOLOGICAL ASSESSMENT FRAMEWORK



CMRS: carcinogenic, mutagenic or reprotoxic substances
HCS: High content screening
SAF: Scientific Assessment Framework

DESK BASED TOXICOLOGICAL ASSESSMENT ACTIVITIES

Qualified Toxicologists determine quality and purity of the individual ingredients of an e-liquid, using a range of techniques:

- Review information from suppliers
- Existing scientific literature and Read-Across
- *In Silico* Predictions (e.g. Lhasa DEREK, ToxTree, OECD Toolbox)
- If these initial desk-based investigations are satisfactory we move the novel e-liquid into *in vitro* testing (liquid and aerosol) and HPHC analysis of the whole aerosol

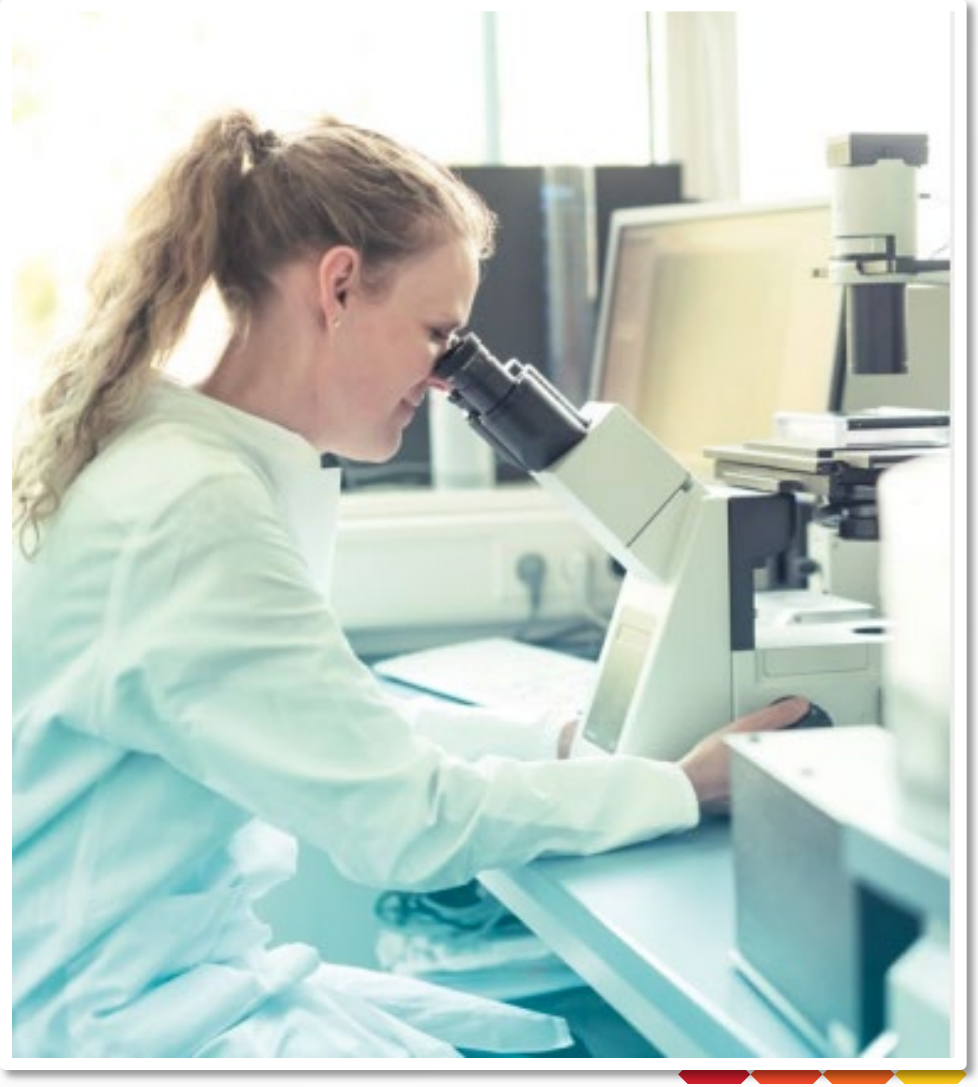
IN VITRO ASSESSMENT OF E-LIQUIDS AND WHOLE AEROSOLS

E-Liquids are initially assessed neat to determine the formulation mixture biological activity:

Neutral Red Uptake Assay	Human bronchial epithelium Beas-2B cells
Ames assay	5 Salmonella strains +/-S9
In Vitro Micronucleus	Human lymphoblastoid TK6 cells

To determine the impact of e-liquid aerosolization, cells are also exposed to whole aerosol in the following techniques:

Neutral Red Uptake Assay	Human bronchial epithelium Beas-2B cells
Ames assay	5 Salmonella strains +/-S9
In Vitro Micronucleus	Chinese hamster lung V79 cells) at ALI

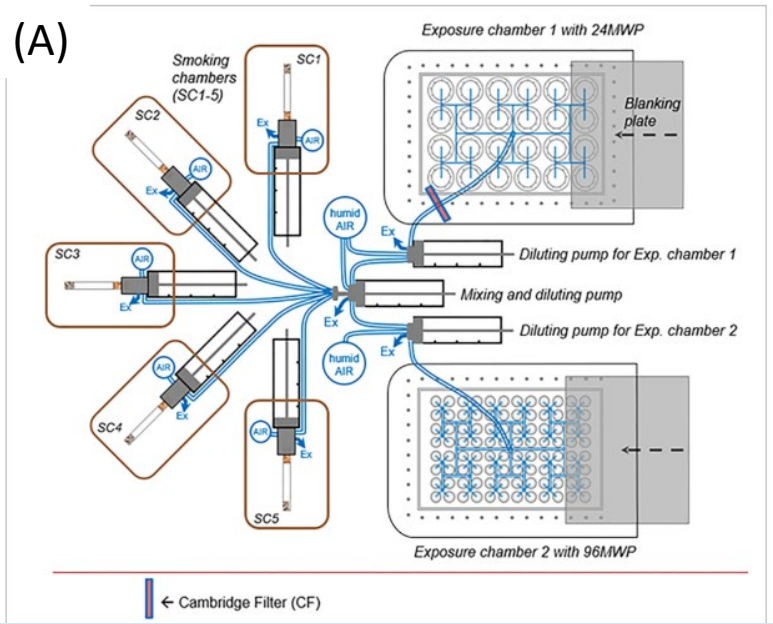


IN VITRO ASSESSMENT OF E-LIQUIDS AND WHOLE AEROSOLS CONT.

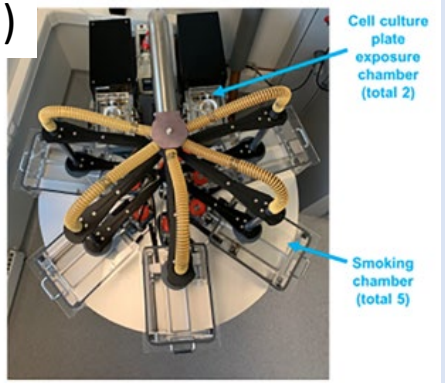
For smoke / aerosol generation, we use the “Smoke Aerosol Exposure In Vitro System” (SAEIVS), an in-house system to enable cells to be exposed at the ALI to whole smoke or aerosol.

Fig A and B : Schematic of SAEIVS:

(A)



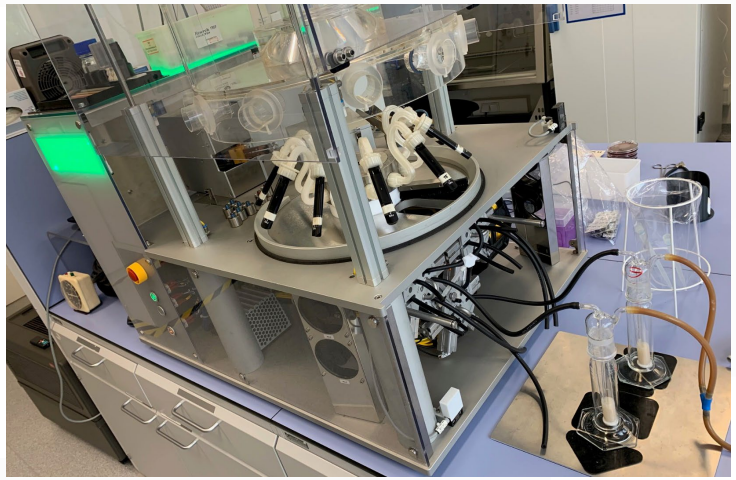
(B)



For Ames, the bacterial suspension is bubbled with smoke /aerosol prior to plating to increase the potential for exposure to the bacteria

Fig C: Bubbling of concentrated bacterial suspension for Ames test:

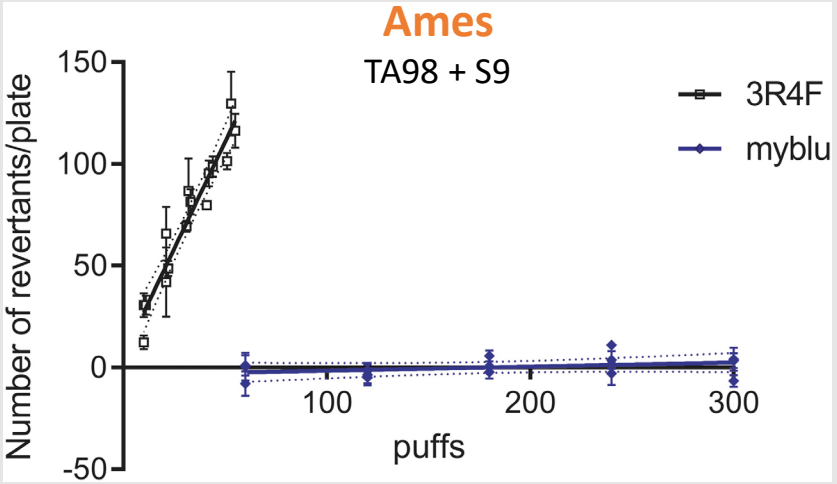
(C)



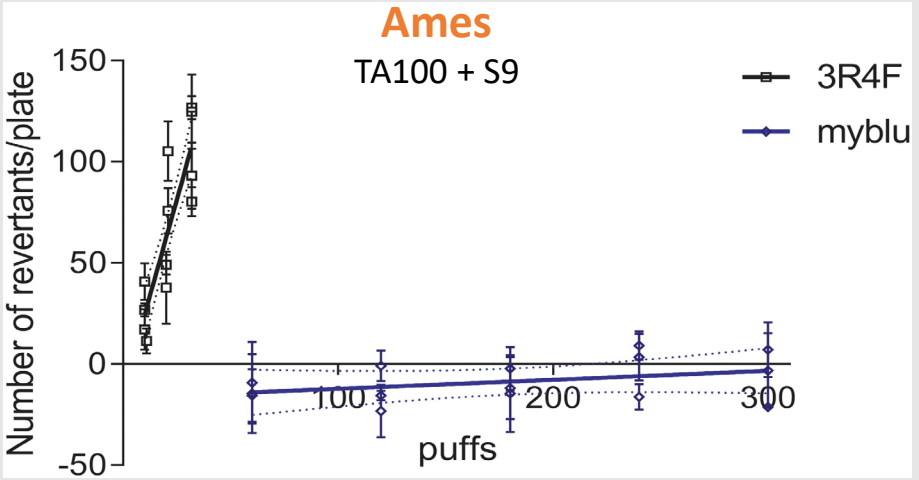
Wieczorek, R., et al., 2023. Characterisation of a smoke/aerosol exposure in vitro system (SAEIVS) for delivery of complex mixtures directly to cells at the air-liquid interface. *Journal of Applied Toxicology*.

EXAMPLE RESULTS COMPARING A POD-BASED E-CIGARETTE TO 3R4F REFERENCE CIGARETTE IN

A



B



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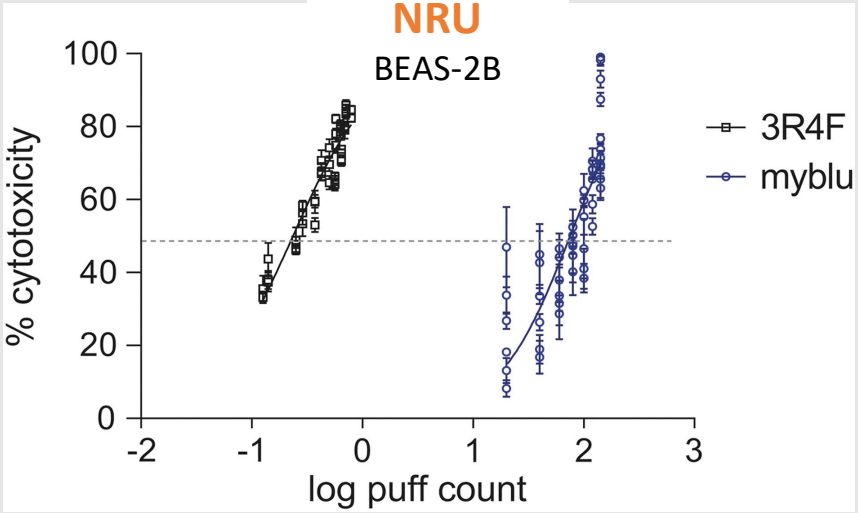
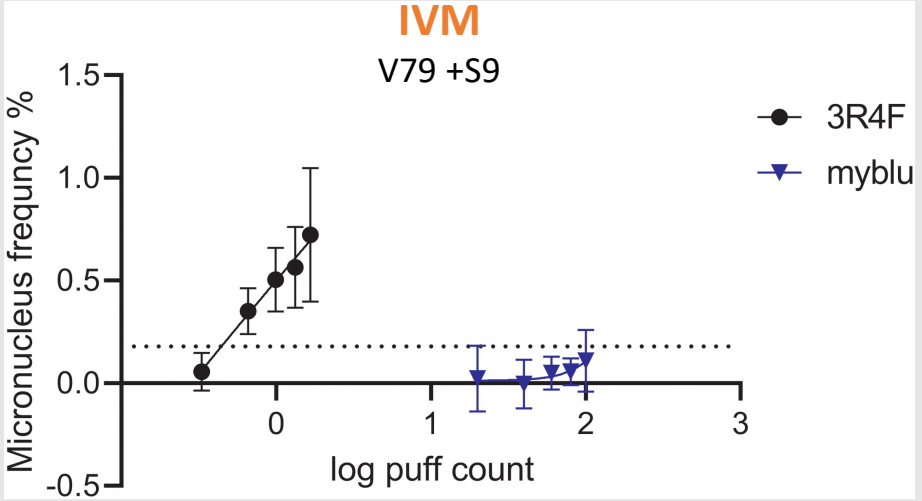


D

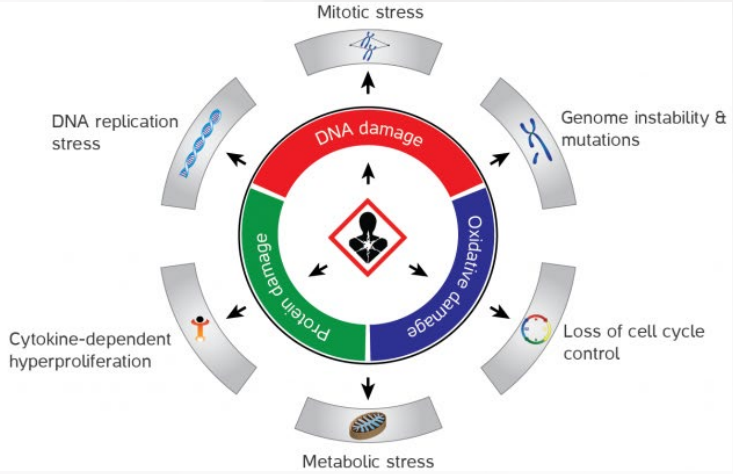


Rudd, K., et al., (2020). Chemical composition and in vitro toxicity profile of a pod-based e-cigarette aerosol compared to cigarette smoke. *Applied In Vitro Toxicology*, *6*(1), pp.11-41.

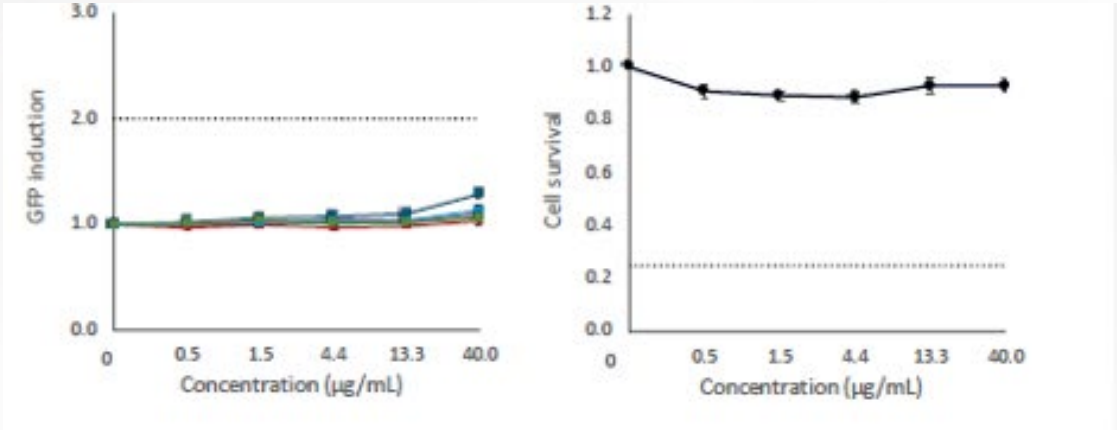
FURTHER MECHANISTIC INSIGHTS CAN BE OBTAINED FOR GENOTOXIC/ MUTAGENIC RESPONSES

Biological damage	Biomarkers
DNA damage	Bscl2, Rtkn
Oxidative stress	Srxn1, Blvrb
Protein damage	Ddit3
Cellular stress	Btg2

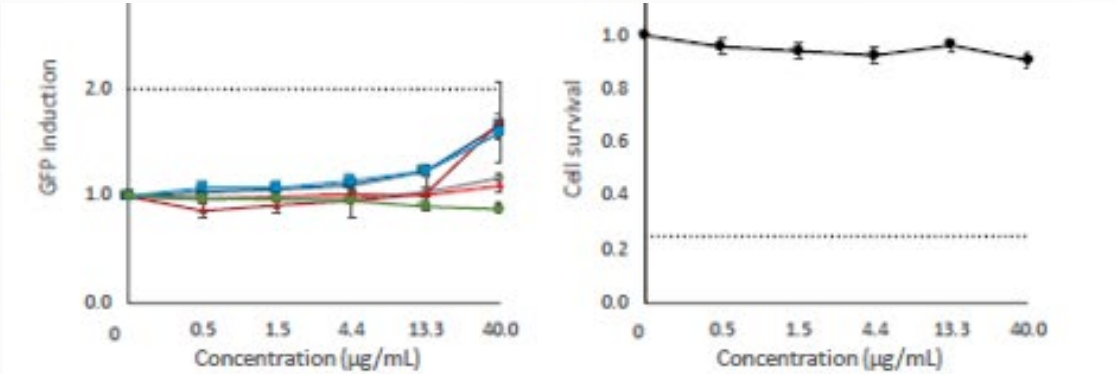
toxys



Example E-Liquid A –S9 (Fig A)



Example E-Liquid A +S9 (Fig B)



Czekala, L., et al.,(2021) . The in vitro ToxTracker and Aneugen Clastogen Evaluation extension assay as a tool in the assessment of relative genotoxic potential of e-liquids and their aerosols. *Mutagenesis*, *36*(2), pp.129-142.

HIGH CONTENT SCREENING FOR ADDITIONAL ENDPOINTS: INFLAMMATION (NFKB)

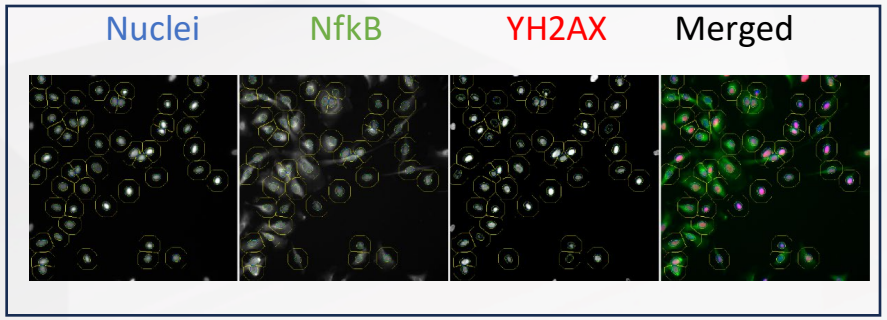


Fig 1: HCS images with Etoposide treated cells for the red and green fluorescence intensity indicating the level of γH2AX (circle regions; red) and amount of NfκB respectively (circle and ring regions, green).

Fig A: E liquid A NFkB 4 hours

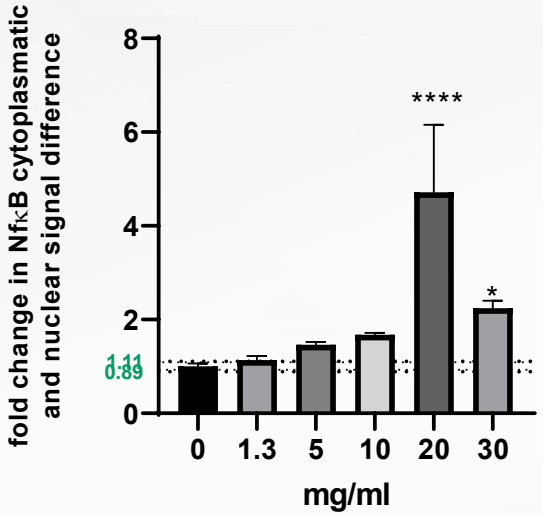


Fig B: E liquid A NFkB 24 hours

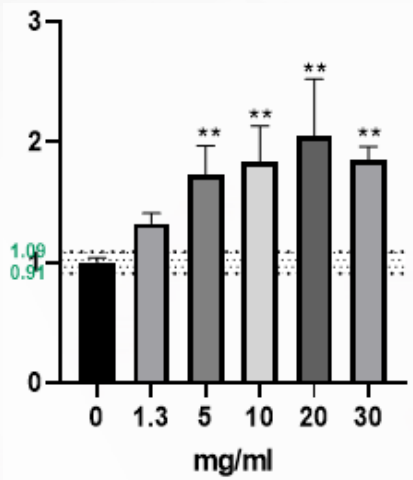


Table 1 Summary of MEC and AC50 responses for 3R4F CSC and different e-liquids *

	High Content Screening - Biological Response Heat Map															
	3R4F (0.001 – 0.02%)		BL 0% (0.5, 1.0, 3%)		BL 1.2% (0.0313 – 3%)		BL 2.4% (0.0313 – 3%)		Blueberry 2.4% (0.0313 – 3%)		Tobacco 2.4% (0.0313 – 3%)		Menthol 2.4% (0.0313 – 3%)		Vanilla 2.4% (0.0313 – 3%)	
	MEC	AC50%	MEC	AC50%	MEC	AC50	MEC	AC50	MEC	AC50	MEC	AC50	MEC	AC50	MEC	AC50
Cell count	↓0.001	0.008	NR	NR	NR	NR	↓2.1	>3I	↓1.06	1.78	↓1.36	2.08	↓0.36	0.885	↓1.48	1.89
Cell Cycle Arrest	↑0.001	>0.005	NR	NR	↑1.69	2.0	↑0.718	0.982	↑0.377	0.598	↑0.363	0.548	↑0.0551	0.096	↑0.406	0.573
Cell Membrane Permeability	↑0.001	0.02I	NR	NR	↑0.345 (NS)	>3I (NS)	↑0.149	>3I	↑0.783	1.22	↑1.52	2.05	↑0.659	0.855	↑1.64	2.06
Caspase 3/7 Intensity	↑0.001	>0.015I	NR	NR	NR	NR	NR	NR	↑0.596	>3I	↑0.652	>3I	↑0.106	>2I	↑1.17	>3I
NF-κB	↑0.005	>0.02I (NS)	NR	NR	NR	NR	NR	NR	↑1.12	1.86	↑1.86	2.54	↑0.63	0.991	↑1.9	2.42
Mitochondrial Mass	↑0.006	>0.02I	NR	NR	NR	NR	↓0.8 (NS)	>3I (NS)	NR	NR	↑1.88	>3I	↑0.88	>1I	↑2.98	>3I
Mitochondrial Membrane Potential (Δψm)	↓0.005	0.012	NR	NR	NR	NR	NR	NR	↓0.865	1.05	↓0.88	1.94	↓0.689	1.36	↓1.16	2.32
Oxidative Stress	↓0.005	0.009	NR	NR	NR	NR	NR	NR	NR	NR	↓0.836	1.61	↓0.833	1.63	↓1.08	1.96
Glutathione Content	↓0.008	0.008	NR	NR	NR	NR	NR	NR	↓1.35	1.65	↓1.77	2.14	↓0.735	0.816	↓1.9	2.15
Cellular ATP	↑0.006	>0.02I	↓2.59	>3	↓1.55 (NS)	>3I (NS)	↓1.02	1.82	↓0.952	1.31	↓1.76	1.76	↓0.602	0.773	↓1.62	1.71

*Czekala, L., et al., 2019. High Content Screening in NHBE cells shows significantly reduced biological activity of flavoured e-liquids, when compared to cigarette smoke condensate. *Toxicology in vitro*, *58*, pp.86-96. NHBE Normal human bronchiole epithelial cells

RECAP OF PROCESS TO ASSESS E-LIQUIDS

Ingredients and materials of sufficient quality/on any negative lists?

Compliance with limits of purity and any contaminants/any country specific regulatory restrictions

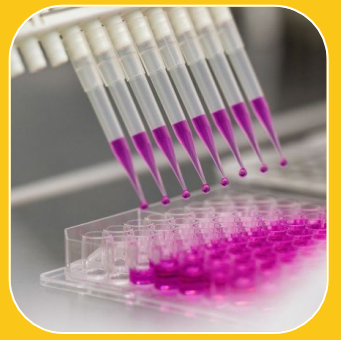
'Desk-based' risk assessment using existing scientific information

Screening of Ingredients for CMRS activity

Screening for Carcinogenic, Mutagenic, Reproductive and Respiratory/skin Sensitisation properties

Laboratory testing using a regulatory battery of tests

Assessment of the EVP mixture, as both liquid and whole aerosol



Any Additional testing based on results e.g., Toxys, HCS, 3D tissues

Explore ToxTracker/HCS to look at possible mechanism of action

Decision

Considering all data including smoke chemistry data and comparison to historical products

CONCLUSIONS:

Publications and Presentations available at:
https://imperialbrandsscience.com/

- We hold a robust toxicological assessment process, utilising a variety of different alternative to animal testing approaches (desk based, *in silico* and *in vitro*)
- We use expert toxicologists to assess our materials and products, to ensure only suitable products are released
- We will continue to develop our Toxicological techniques to facilitate quicker and better decision making for our NGPs

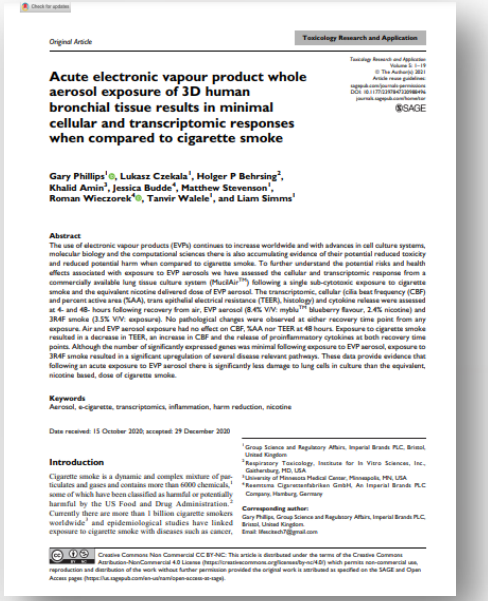

frontiers in Toxicology

ORIGINAL RESEARCH

Use of Human Induced Pluripotent Stem Cell-Derived Cardiomyocytes to Predict the Cardiotoxicity Potential of Next Generation Nicotine Products


APPLIED IN VITRO TOXICOLOGY

Preclinical Assessment of Tobacco-Free Nicotine Pouches Demonstrates Reduced *In Vitro* Toxicity Compared with Tobacco Snus and Combustible Cigarette Smoke

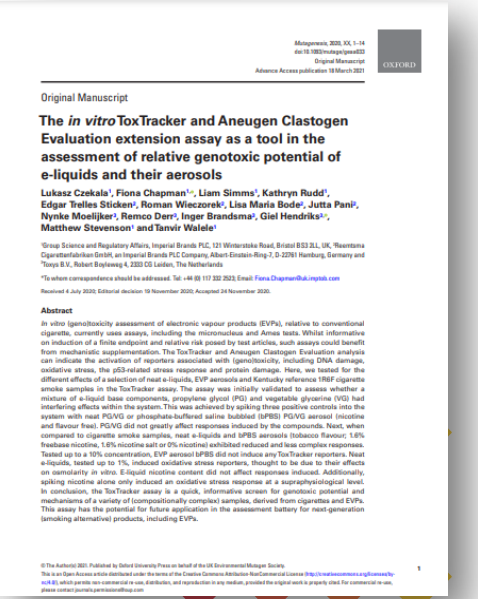

Original Article

Toxicology Research and Application

Acute electronic vapour product whole aerosol exposure of 3D human bronchial tissue results in minimal cellular and transcriptomic responses when compared to cigarette smoke


Current Research in Toxicology

Multi-endpoint analysis of human 3D airway epithelium following repeated exposure to whole electronic vapor product aerosol or cigarette smoke


Original Manuscript

The *in vitro* ToxTracker and Aneugen Clastogen Evaluation extension assay as a tool in the assessment of relative genotoxic potential of e-liquids and their aerosols

ACKNOWLEDGMENTS

- Matthew Stevenson, Scientific Substantiation and Engagement
- Biological Toxicology Laboratory and Toxys for conducting the *in vitro* studies
- Co-authors on the various manuscripts cited in this presentation