A novel approach for the screening of E-cigarette aerosols using an Ames whole aerosol assay

David Thorne

CORESTA SSPT

ST64

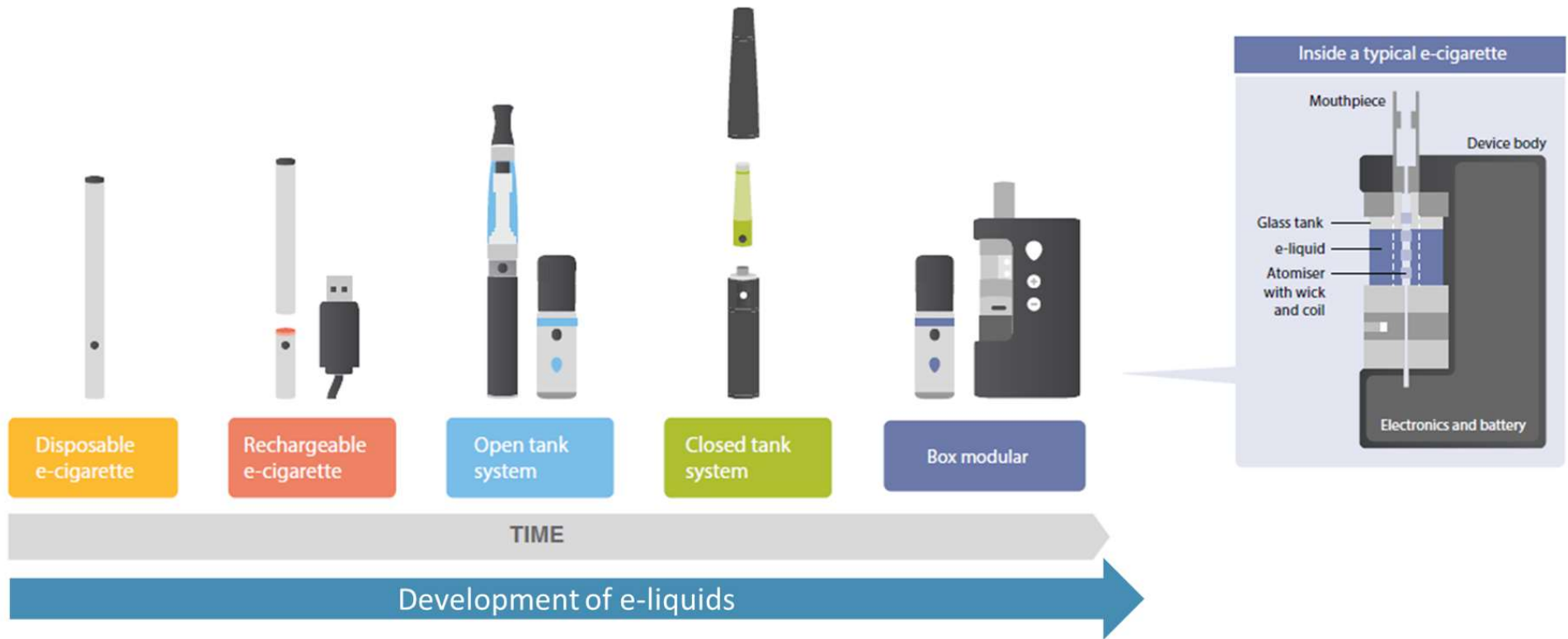**Kitzbühel, Austria 2017**

Oct 9th – 12th

Agenda

- Background

- Assessing the risk profile of e-cigarettes

- Whole aerosol assessment of e-cigarettes (AMES)

- Conclusions

E-cigarettes have evolved rapidly

Disposable e-cigarette	Rechargeable e-cigarette	Open tank system	Closed tank system	Box modular

TIME

Development of e-liquids

Inside a typical e-cigarette

Mouthpiece

Device body

Glass tank

e-liquid

Atomiser with wick and coil

Electronics and battery

In the UK there is a growing consensus on e-cigarette harm reduction potential

Public Health England

Kevin Fenton, Public Health Director of Health and Wellbeing:

"The wider body of evidence consistently finds that e-cigarettes are less harmful than smoking"

E-cigarettes: an evidence update

"The current best estimate is that e-cigarettes are around 95% less harmful than smoking"
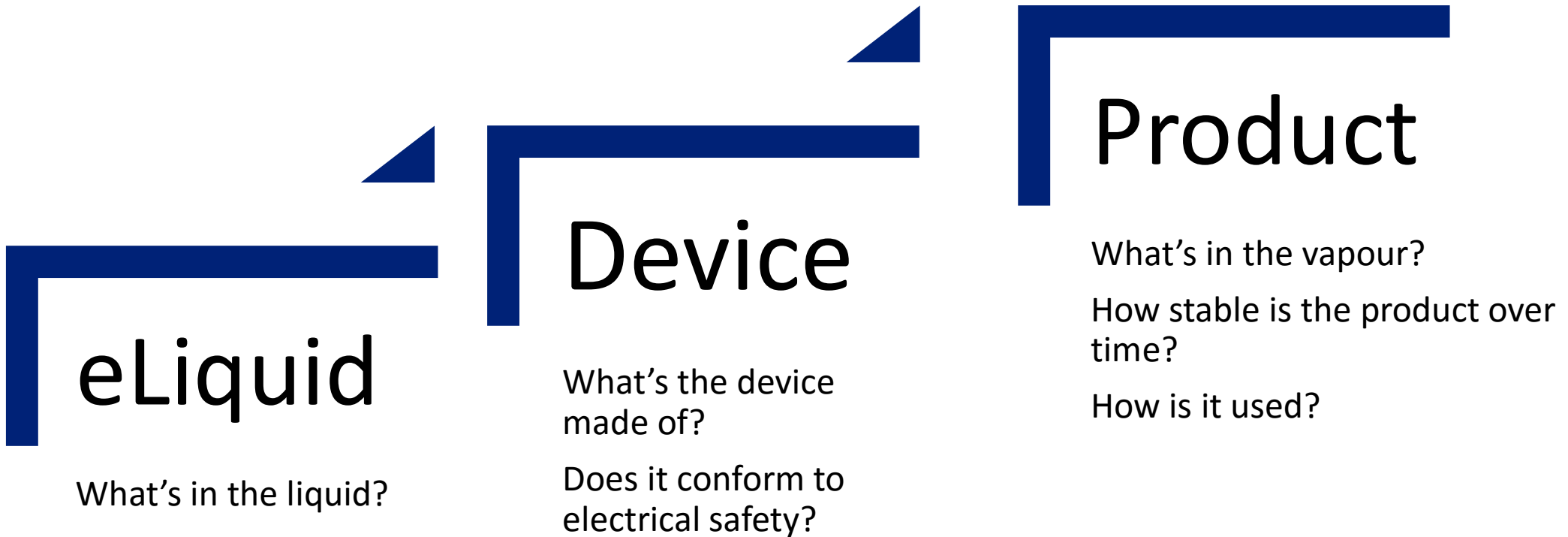
Royal College of Physicians

Nicotine without smoke: tobacco harm reduction

Promote e-cigarettes widely as substitute for smoking says new RCP report

ash. briefing
action on smoking and health

Electronic cigarettes (also known as vapourisers)

"Compared to tobacco products, electronic cigarettes are significantly safer"

BAT's approach to e-cigarette assessment

eLiquid + Device = Product

Product

What's in the vapour?

How stable is the product over time?

How is it used?

Device

What's the device made of?

Does it conform to electrical safety?

eLiquid

What's in the liquid?

Costigan and Meredith (2015) Regul Toxicol Pharmacol 72:361–369

BAT's approach to e-cigarette assessment

In vitro

01
EMISSIONS

- Untargeted emissions
- Targeted emissions
- Environmental emissions

in vitro reg tox

02
EXPOSURE

- Puffing behaviour
- Average daily consumption
- Clinical PK
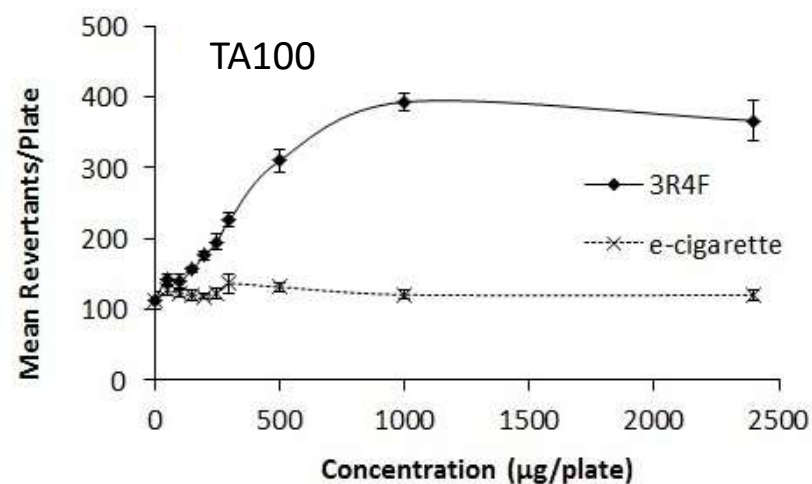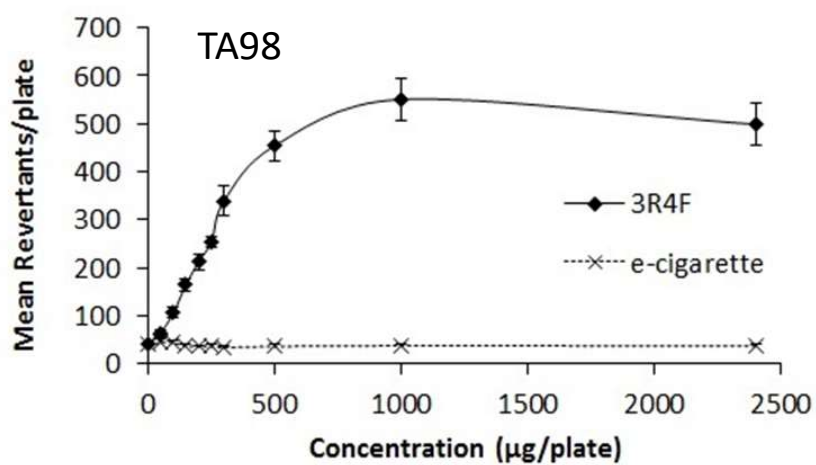- Clinical BoE

in vitro disease models

03
RISK

- Clinical BoBE
- Risk perception
- Post market surveillance

in vitro systems science

Murphy, J (2017). Pan European Networks, Government 21:52-55 and Murphy J et al (2017) Reg Tox Pharm (in press)

Classical regulatory toxicology –TPM

OECD TG 471: Bacterial Reverse Mutation Test S. typhimurium TA98 & TA100

Exposure to reference cigarette smoke caused mutations in a dose dependent manner;
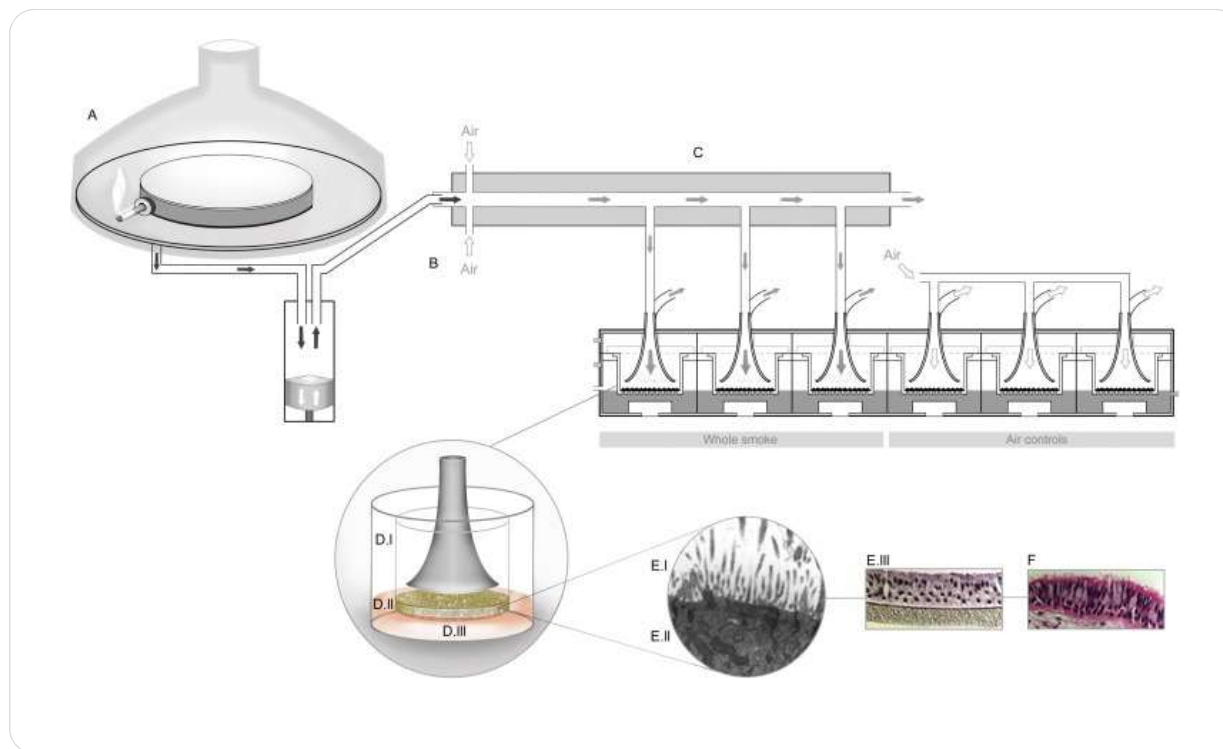e-cigarettes gave no response*

Thorne *et al.* (2016) *Mutation Research* **812**:329–396   *These qualities do not necessarily mean this product produces less adverse health effects than tobacco products

Irritancy assessment of aerosols

## Comparison of cytotoxicity after cigarette and e-cigarette exposure using EpiAirway

BRITISH AMERICAN TOBACCO
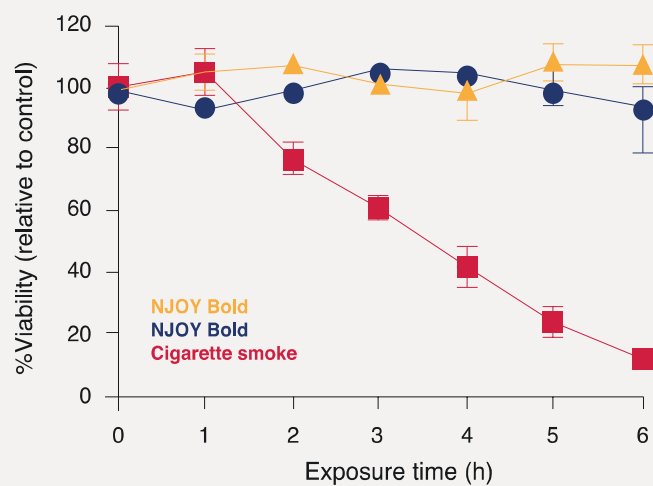
IRRITANCY
(WHOLE AEROSOL)

No cytotoxicity with e-cigarette in EpiAirway cultures*

*These qualities do not necessarily mean this product produces less adverse health effects than tobacco products

Neilson *et al* 2016 *Toxicol.* In Vitro; **29**(7):1952–1962

Comparing transcriptional perturbations in MucilAir

48, 854 genes/ RNA features screened

3R4F 8197 significant genes/RNA features
Vype ePen 49 significant genes/RNA features
Vype ePen* 113 significant genes/RNA features
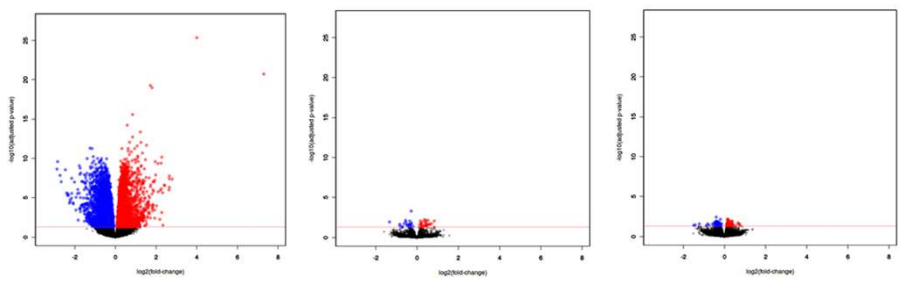
Toxicogenomics – RNA-seq differential gene expression*

RNA-seq data mapped onto 131 pathway-focussed gene sets with specific biological function and disease processes
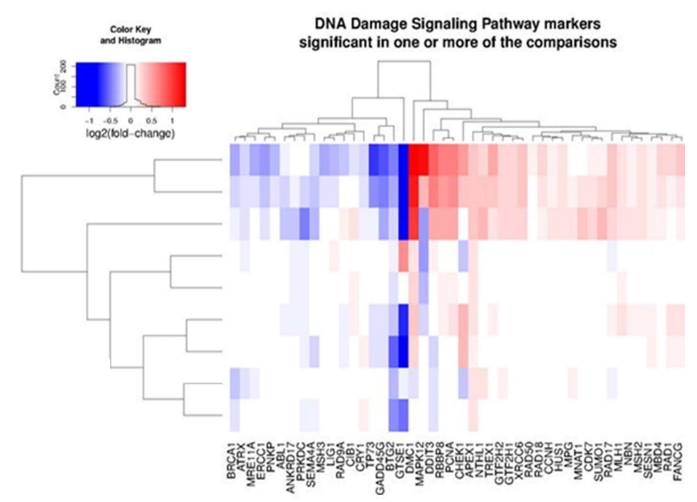
Gene enrichment analysis: heatmap indicating fold change for RNAs significant at pFDR<0.05

Haswell, L.E. et al (2017) Sci Reports, 7(1), 888

*These qualities do not necessarily mean this product produces less adverse health effects than tobacco products
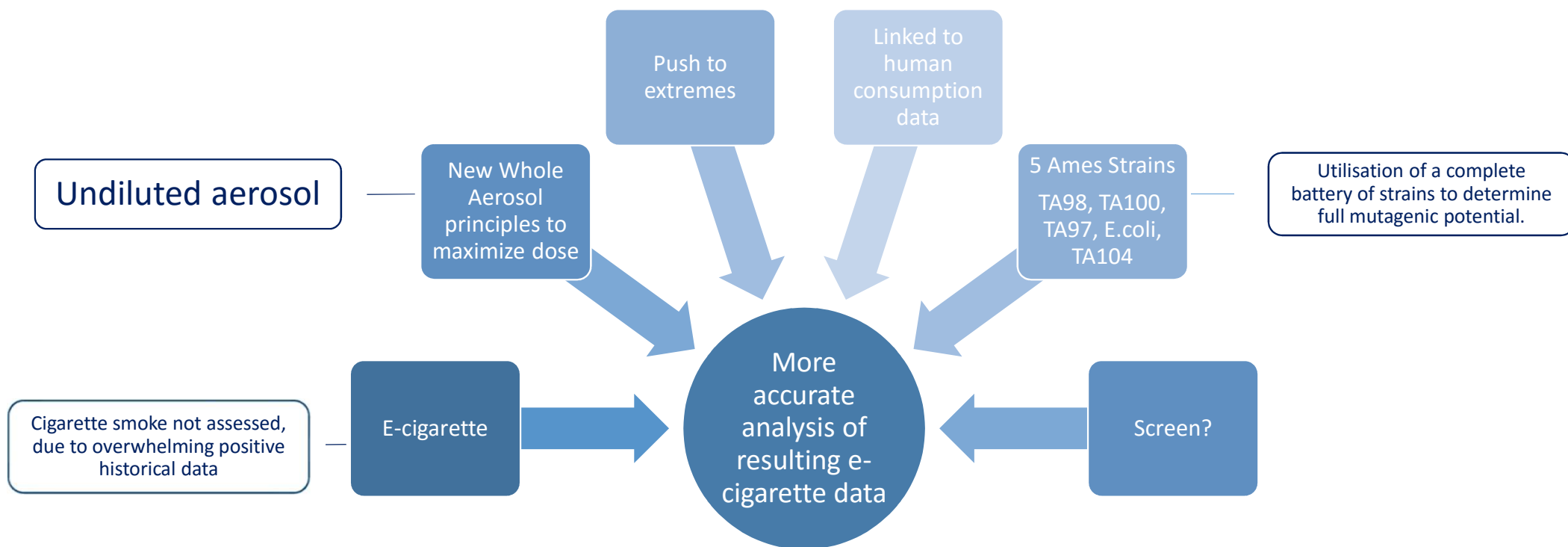
How do you confirm a negative response?

Public Health
England

"The wider body of evidence consistently finds that e-cigarettes are less harmful than smoking"

"The current best estimate is that e-cigarettes are around 95% less harmful than smoking"

Experimental Design

Study Outline

Undiluted aerosol

New Whole Aerosol principles to maximize dose

Push to extremes

Linked to human consumption data

5 Ames Strains
TA98, TA100, TA97, E.coli, TA104

Utilisation of a complete battery of strains to determine full mutagenic potential.

More accurate analysis of resulting e-cigarette data

Cigarette smoke not assessed, due to overwhelming positive historical data

E-cigarette

Screen?

Experimental Design
Product

| Product	Description	Aerosol Generation	Standardised Method for Aerosol Generation
	A closed system vaping device (Vype ePen, 18mg/ml Blended Tobacco)	Aeroionisation of an e-liquid formulation	CORESTA regime (CR81) [*] 55 ml puff volume; 3 sec puff duration; 30 sec interval between puff Square Wave puff profile

* CORESTA Recommended Method No 81, 2015. Routine analytical machine for e-cigarette aerosol generation and collection – Definitions and Standard Conditions. CRM No 81, 2015.

Experimental Design

Exposure set up - Diluted vs. Undiluted

A

diluting airflow

exhaust

diluting airflow   diluted aerosol   vacuum pump
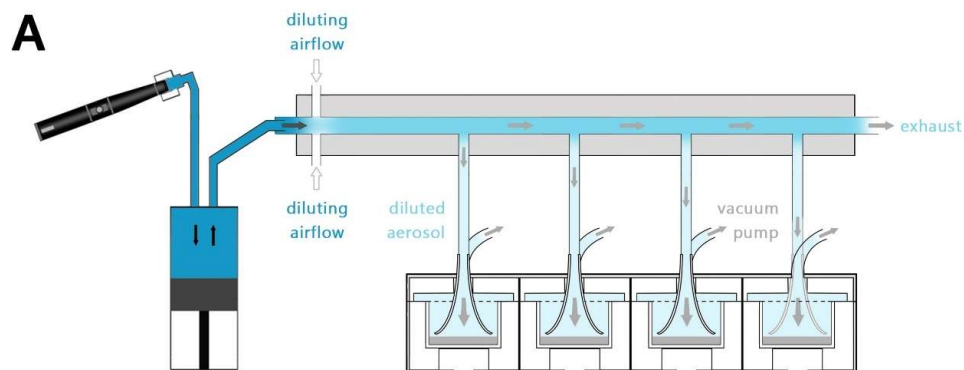
[A] Under standard operating parameters, the generated aerosol is delivered to the dilution system, where an airflow (L/min) is added to the aerosol to create a diluted aerosol stream. Under a negative pressure (vacuum, mL/min) a portion of the aerosol stream is drawn into the exposure module. Under standard conditions, only a small portion of the total generated aerosol reaches the biological surface.

B

airflow blocked

exhaust blocked

airflow blocked   undiluted aerosol

Increased aerosol exposure
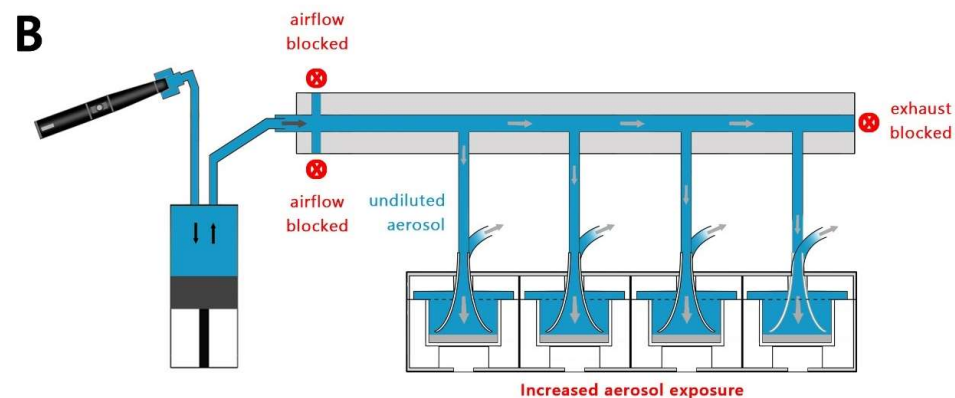
[B] In a modified approach, the VC 10 was adapted to significantly increase exposure conditions using an undiluted aerosol stream that is transferred in its totality to the exposure module. To achieve this, the two diluting air ports and the exhaust port were deliberately blocked (X) and the vacuum disabled, the resulting undiluted aerosol was forced entirely into the exposure module under positive pressure.

Thorne *et al* (2017) Mut. Res. In submission

Dosimetry assessment of the two dilution techniques

Dose clarification
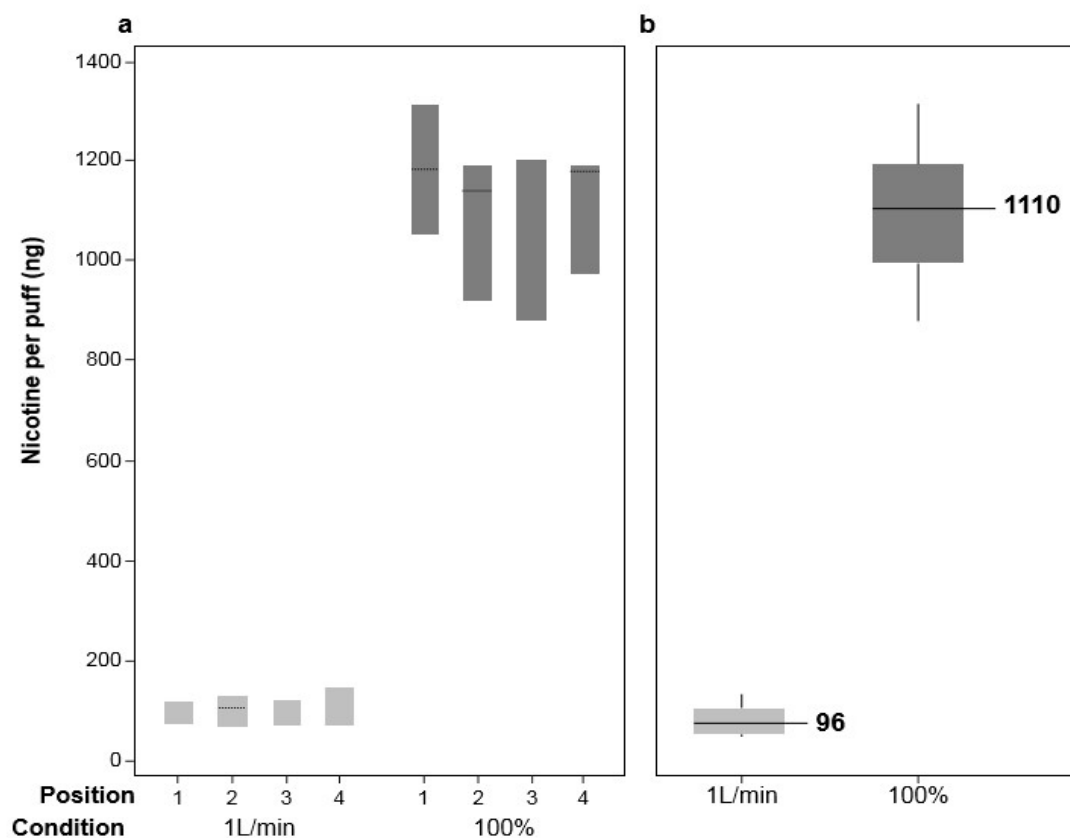
[a] Vype ePen nicotine delivery to the module using both diluted (1 L/min) and undiluted techniques assessing positional variability

[b] Mean comparison of Vype ePen nicotine delivery to the module using both diluted and undiluted techniques. The difference in deposited nicotine per puff between diluted and undiluted techniques is 11.5-fold
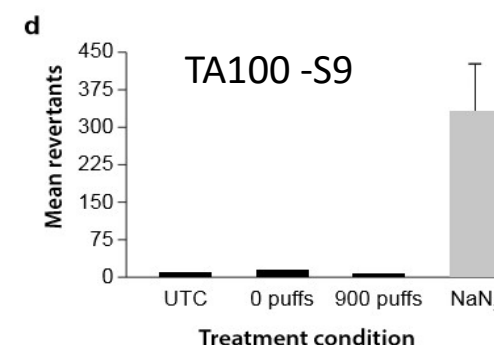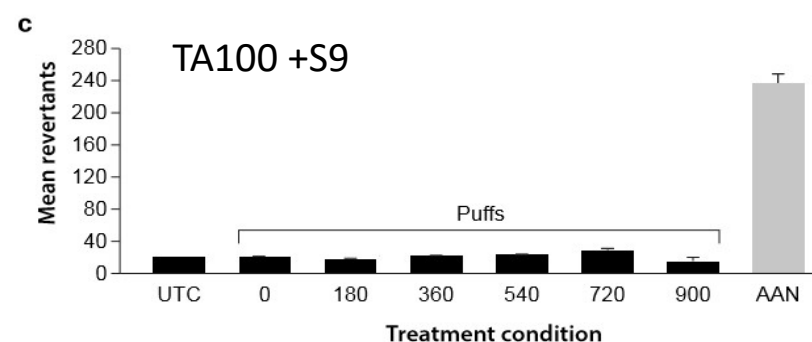
Thorne *et al* (2017) Mut. Res. In submission

The Data

TA98 and TA100 after 0 - 900 puffs from an e-cigarette
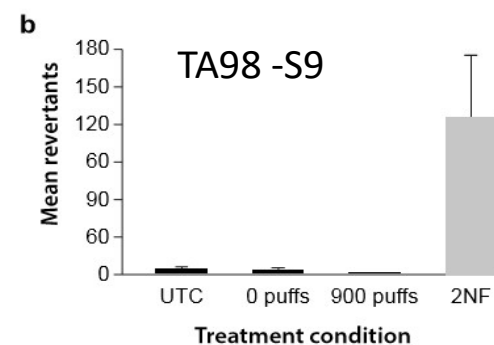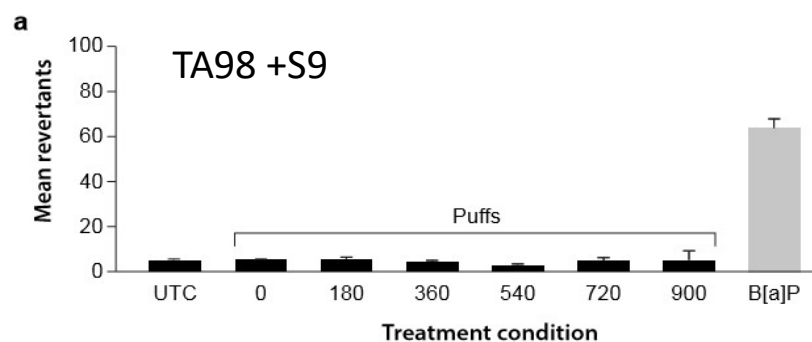
BRITISH AMERICAN TOBACCO

UTC = untreated control
0 puff = air control
B[a]P = benzo[a]pyrene
2NF= 2-nitrofluorene
AAN = 2-aminoanthracene
NaN3 = sodium azide

Average puffs per day ~163 ± 138 puffs per day

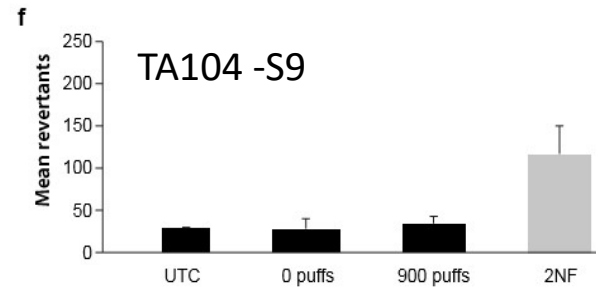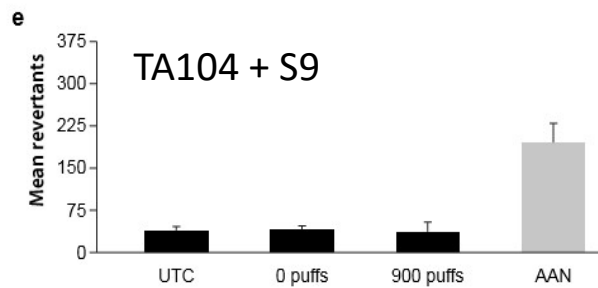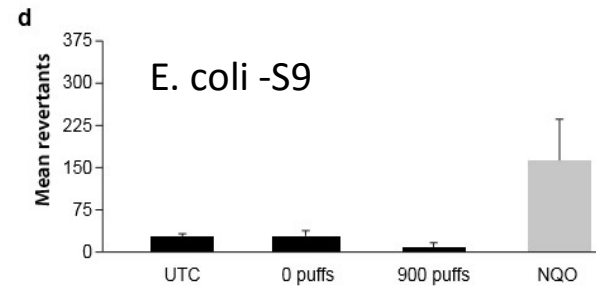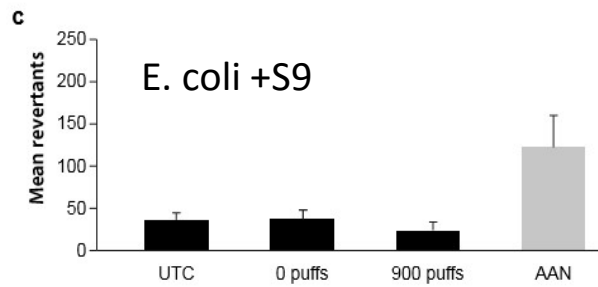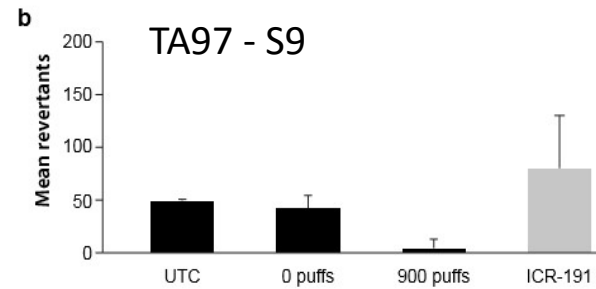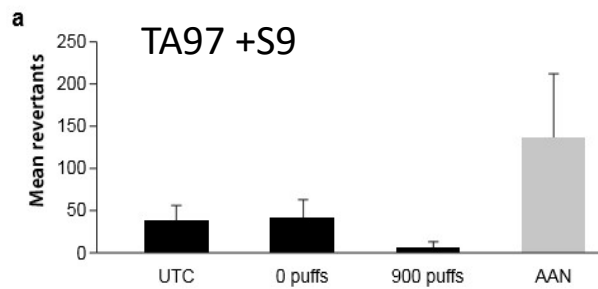99% of users puffed less than 560 puffs per day

Dautzenberg 2015 "the 1 million puffs study" 2015

Thorne *et al* (2017) Mut. Res. In submission

The Data
TA97, E.coli, TA104 - Screening approach?

BRITISH AMERICAN TOBACCO

UTC = untreated control
0 puff = air control
B[a]P = benzo[a]pyrene
2NF= 2-nitrofluorene
AAN = 2-aminoanthracene
NaN3 = sodium azide
NQO = 4-nitroquinoline 1-oxide

Thorne *et al* (2017) Mut. Res. In submission

SUMMARY

- Demonstrated a new way of utilising and conducting WA exposures, linked to human consumption and maximising dose

- Despite extreme whole aerosol exposure, up to 900 puffs, mutagenicity was not observed in any strain

- Pre-clinical assessment shows that e-cigarettes have the potential to reduce risk relative to cigarettes

- We have confirmed a negative!

- Data currently under Review in Mutation research (Peer-Review Comments)

Thank You and Acknowledgements

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