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NEXT GENERATION PRODUCTS

Next generation State of the Art Air Liquid Interface (ALI) In Vitro Exposure System for Fresh Smoke and Aerosols

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Conflict of interest statement

- I am a paid employee of Imperial Brands PLC, a manufacturer of tobacco and nicotine products
- The research described within this presentation was solely funded by Imperial Brands PLC
- The views expressed in the document are those of the author and do not necessarily reflect the opinions of Imperial Brands PLC

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Introduction of SAEIVS

The Smoke Aerosol Exposure In Vitro System (SAEIVS)

- Developed at Reemtsma 1999
- Refined at Imperial Brands
- Designed to support efficient high-throughput laboratory testing.

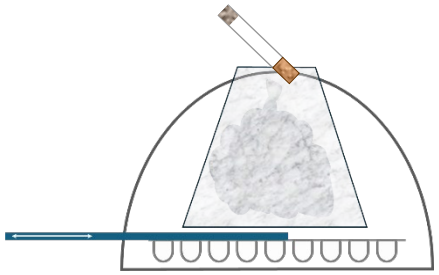
Development focused on enabling puff-specific dose adjustment of mainstream smoke and aerosol exposures while maintaining high sensitivity and precise, reproducible smoke dilution.

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Evolution of Smoke / Aerosol exposure machines

SAEIVS2 was built to address the increasing need for aerosol *in vitro* exposure.

The original smoke-testing systems were modified to support aerosol exposure.



First concept in 1999:
puff-specific smoke exposure in multiwell plate (MWP) under a dome



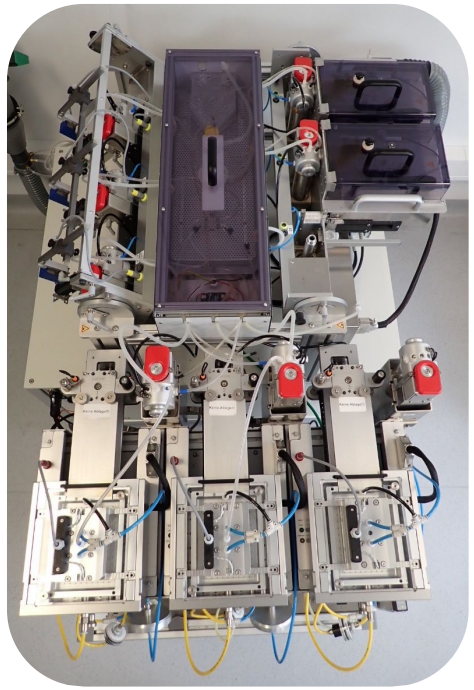
Bt020
(2002)



MSB-01
(2011)



SAEIVS
(2016)



SAEIVS2
(2025)



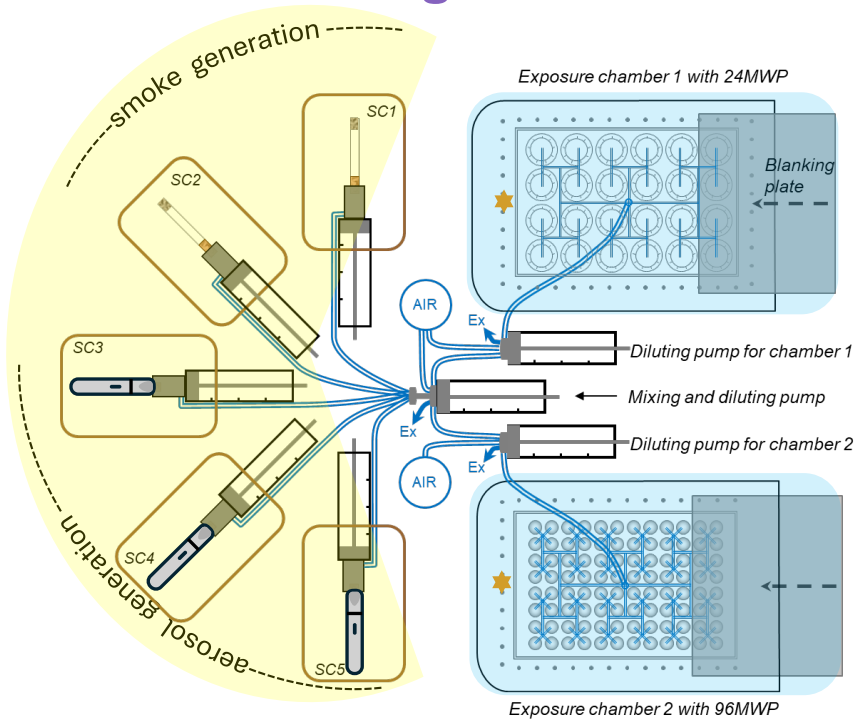


This is what 25 years of technological evolution looks like

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Optimisation of exposure for improved aerosol testing

Drawing of SAEIVS



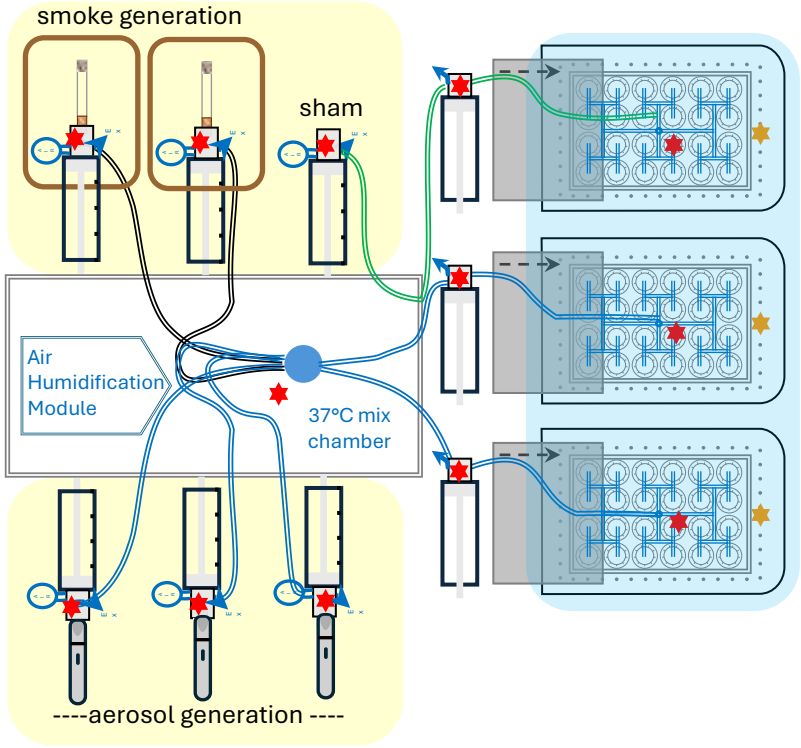
part heated at 25°C

part heated at 37°C

Smoke/aerosol generation

Exposure chambers

Drawing of SAEIVS2

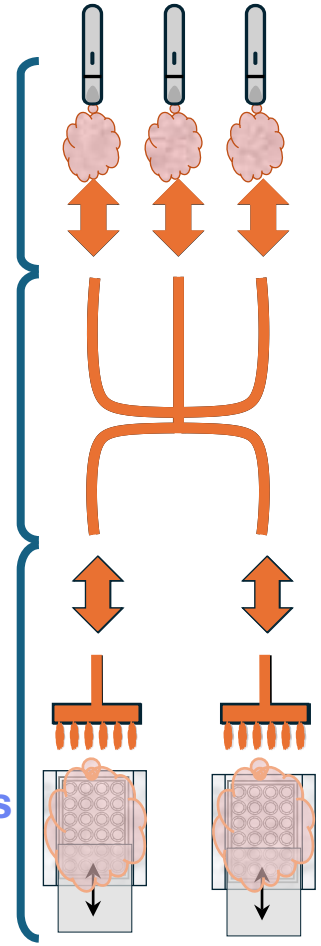


- Temperature control to reduce condensation and droplet formation of aerosols
- Additional exposure chamber for triplicate testing or duplicate testing with sham control
- Elimination of the mixing pump to minimize aerosol losses and maximize puff volume
- Reliable aerosol exposure in all three chambers with a puff volume of up to 130 ml

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Optimisation approaches for aerosol testing

Representative Test Setup for Three Aerosol Devices



Devices with pumps

Three devices (e.g., EVP and HTP) operated in parallel using individual pumps, generating a total aerosol volume of 165 mL (3 × 55 mL).

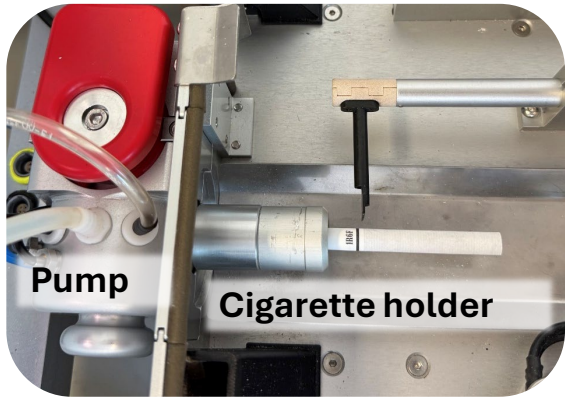
Tubing with mixing elements

Approximately 20 mL of the 165 mL aerosol generated was used to prime the tubing and valves of the pumps.
Subsequently, 110 mL of aerosol is distributed between the two exposure chamber pumps.

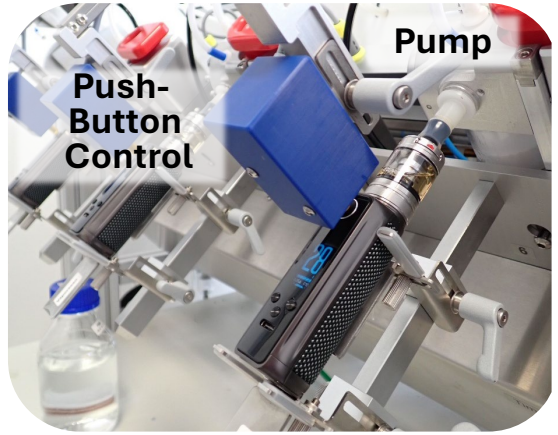
Exposure pumps connected to exposure chambers

After each puff, an exact puff aerosol volume (55 ml) was delivered to each exposure chamber at a constant flow rate.
Row of wells are sequentially covered to enable a dose response to be obtained.

Direct connection to the pumps



Cigarette holder on the pump.



Tiltable EVP Pumps with Push-Button Control

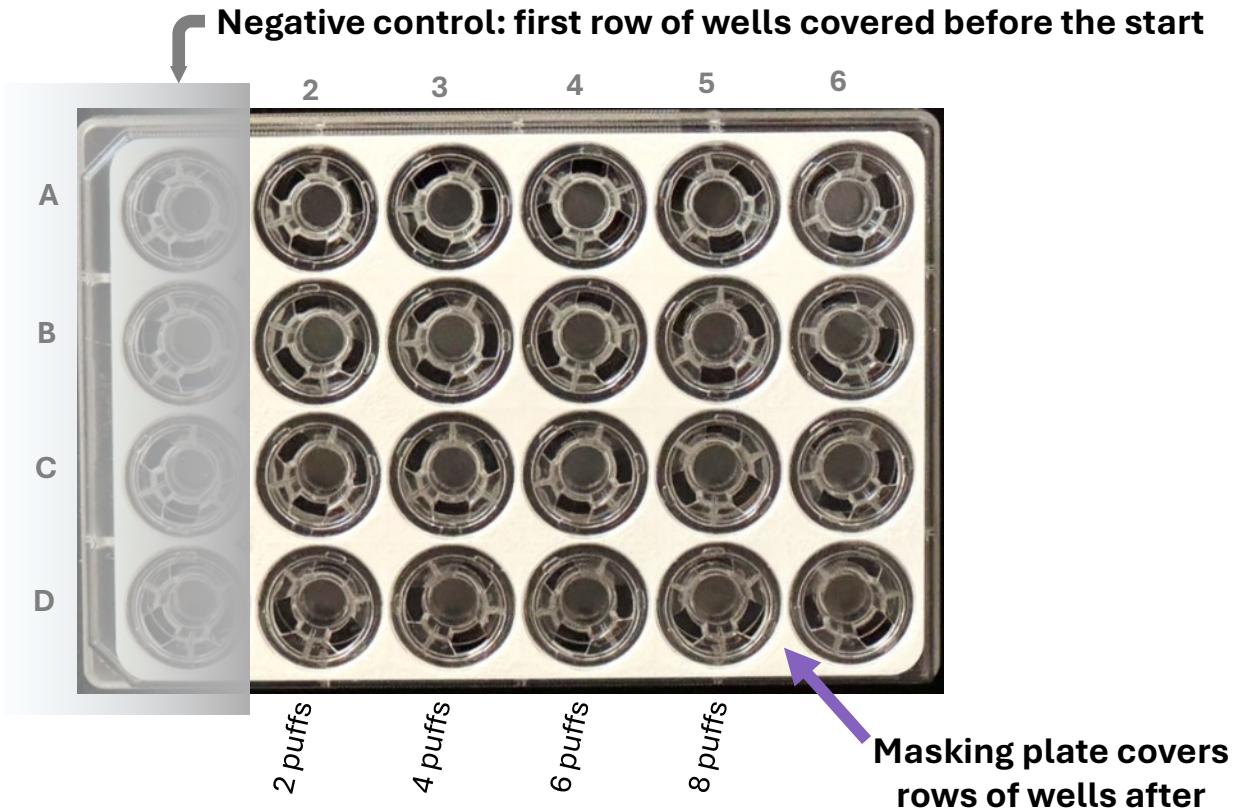
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24 Multiwell Plate - smoke distribution

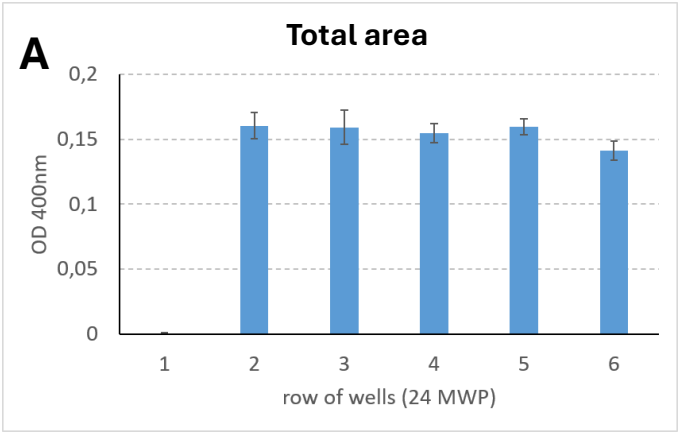
1R6F cigarette smoke was distributed across a 24 MWP with transwells in PBS
Optical density of PBS (400nm) reflected the TPM concentration in wells.

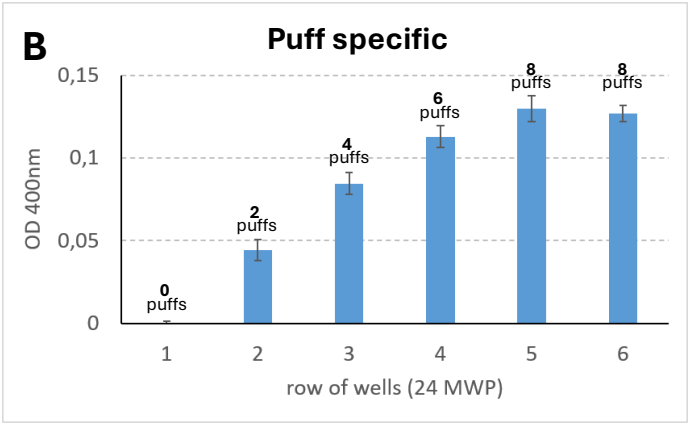
A) Uniform distribution of 1R6F cigarette smoke across the plate

B) Increasing puff-specific exposure (2-8 puffs)



Column-wise smoke distribution across the wells (n=4)

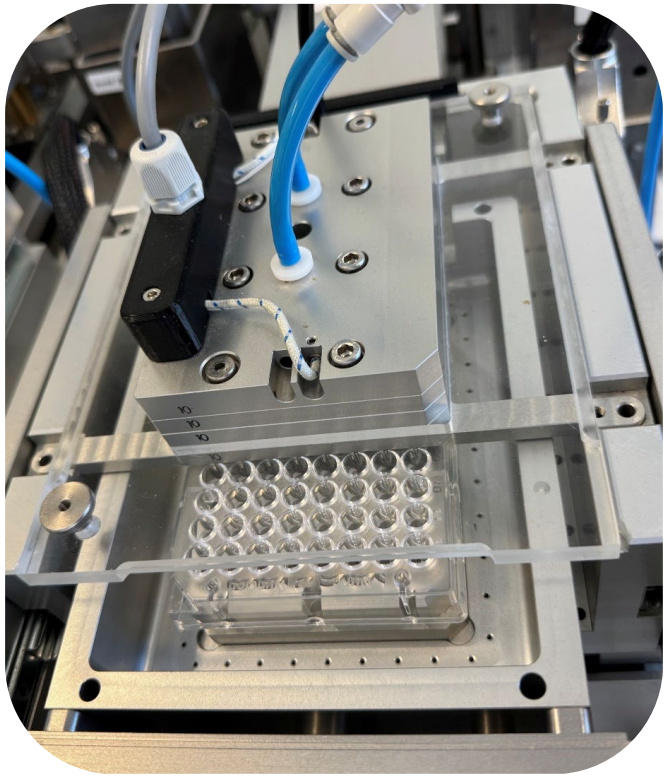




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96 Multiwell Plate - smoke distribution

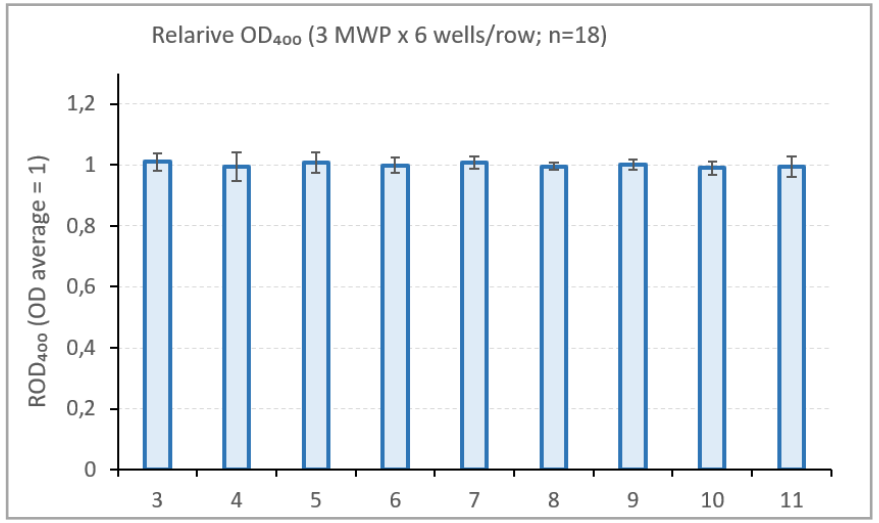
1R6F cigarette smoke was distributed across a 96 MWP (50µl DMSO/well)
TPM optical density in PBS reflected the exposure distribution.

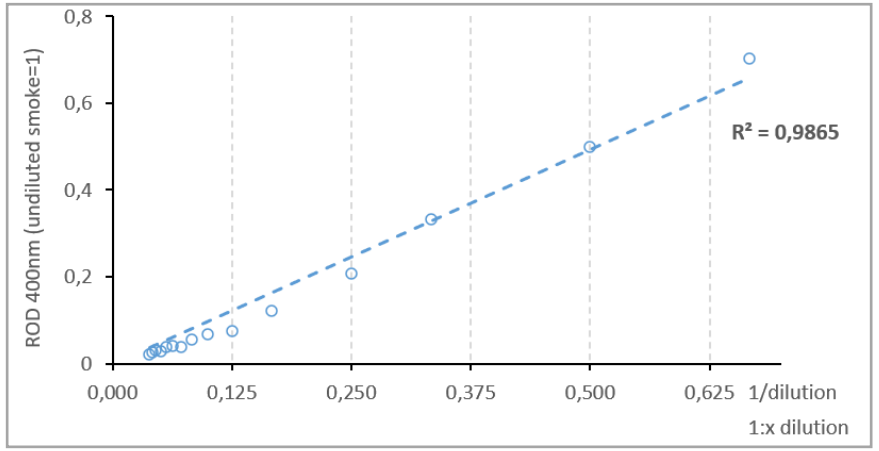


Partially open exposure chamber with a 96 MWP

Uniform smoke distribution across the wells, column by column
[4x 1R6F smoke (dilution 1/8)]

Smoke dilution gradient from factor 1/1.5 to 1/26

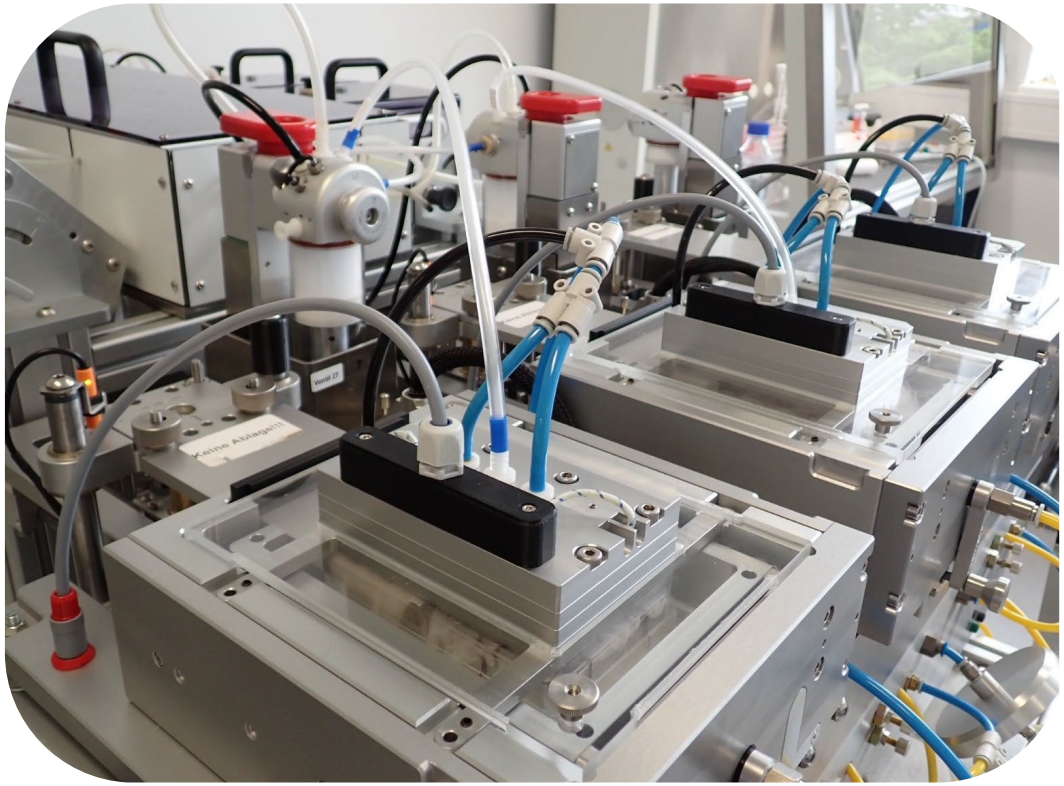




1:8 1:4 1:2.7 1:2 1:1.6

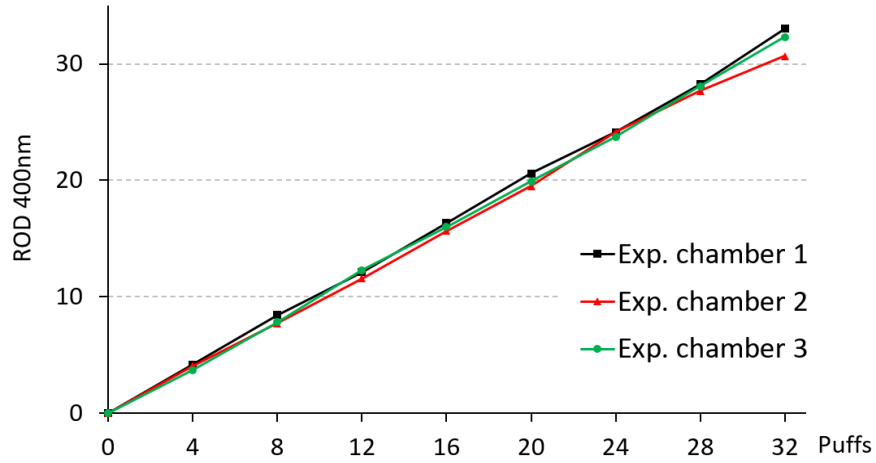
Comparability of Exposure Chambers

Near-identical TPM deposition profiles demonstrate uniform puff-specific smoke distribution across all three exposure chambers (ROD_{400}).



Three exposure chambers

Visualization of Relative Optical Density (ROD) increases at 400 nm in three exposure chambers using diagram and heat map



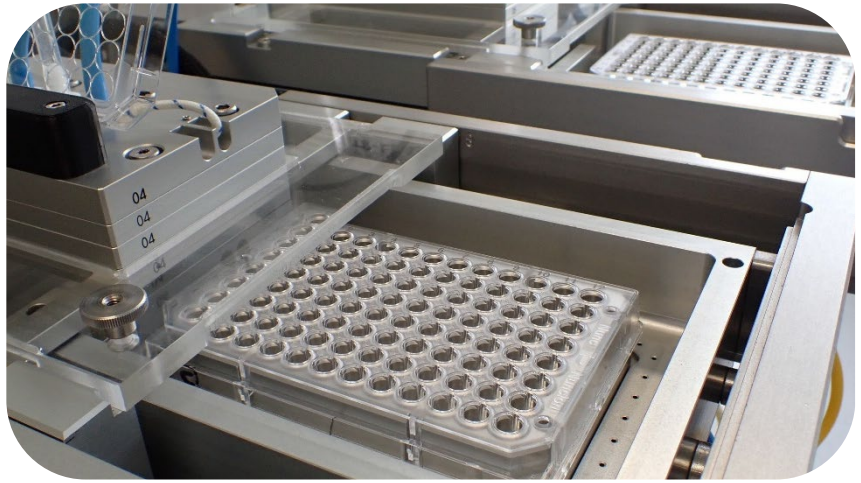
		control area		exposure area								
96MWP	1	2	3	4	5	6	7	8	9	10	11	12
A	0,055	0,051	0,051	0,058	0,066	0,073	0,079	0,087	0,094	0,098	0,093	0,116
B	0,051	0,051	0,051	0,058	0,066	0,073	0,080	0,089	0,097	0,104	0,115	0,121
C	0,051	0,052	0,051	0,059	0,067	0,074	0,083	0,093	0,101	0,108	0,115	0,117
D	0,051	0,051	0,051	0,060	0,067	0,074	0,083	0,093	0,101	0,107	0,114	0,115
E	0,051	0,052	0,051	0,059	0,067	0,075	0,084	0,094	0,100	0,107	0,115	0,115
F	0,052	0,053	0,052	0,059	0,067	0,074	0,084	0,093	0,100	0,110	0,116	0,120
G	0,052	0,053	0,052	0,059	0,070	0,074	0,082	0,093	0,099	0,106	0,115	0,120
H	0,055	0,053	0,052	0,060	0,069	0,079	0,083	0,093	0,099	0,104	0,114	0,121
		0	**0**	**4**	**8**	**12**	**16**	**20**	**24**	**28**	**32**	**puffs**

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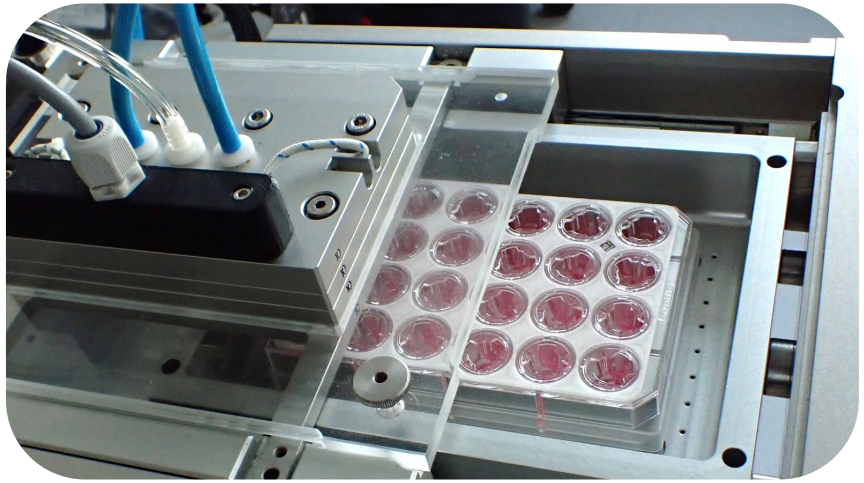
Comparison of biological data SAEIVS vs. SAEIVS2

The SAEIVS are primarily used for in vitro exposure studies in the IVM and NRU assays.

- NRU assay: Performed in 96-well U-MWP using a collagen matrix and BEAS-2B cells.
- IVM assay: Performed in 24-well MWPs with Transwell® inserts and V79 cells.



BEAS-2B on collagen in 96 U-MWP for NRU exposure

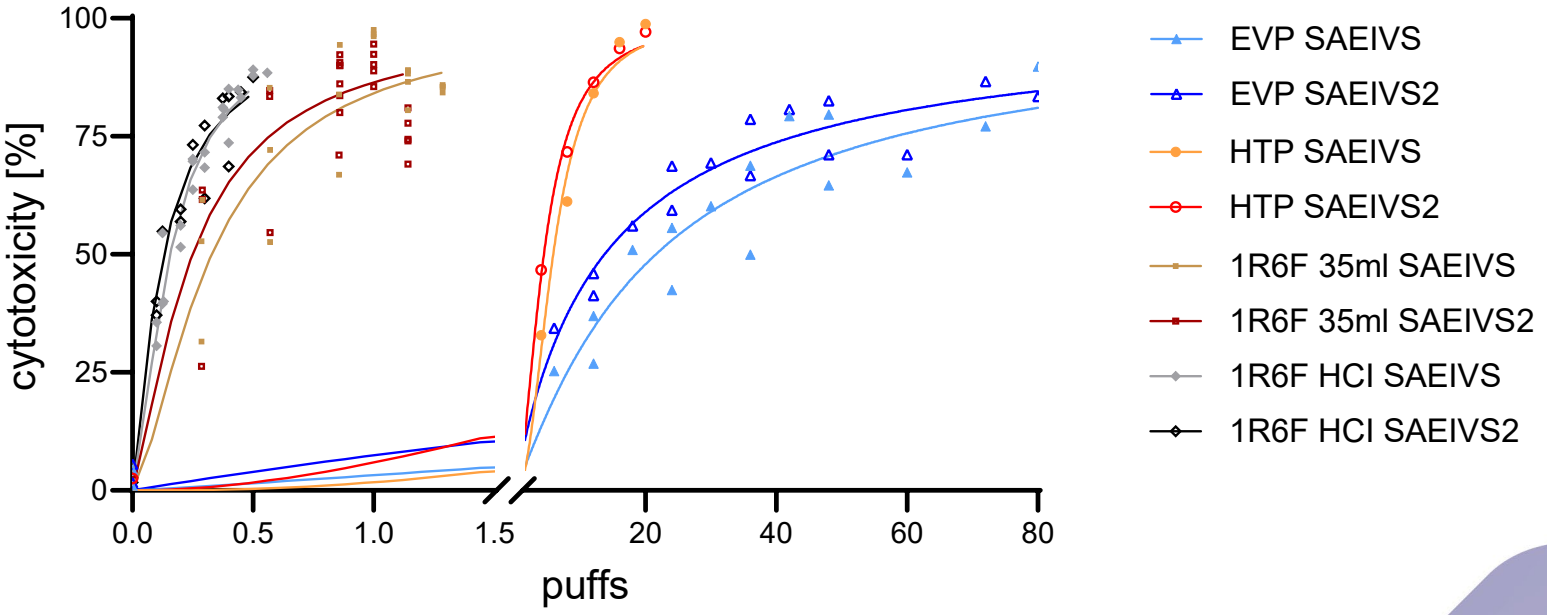


Transwells in 24 MWP for IVM exposure

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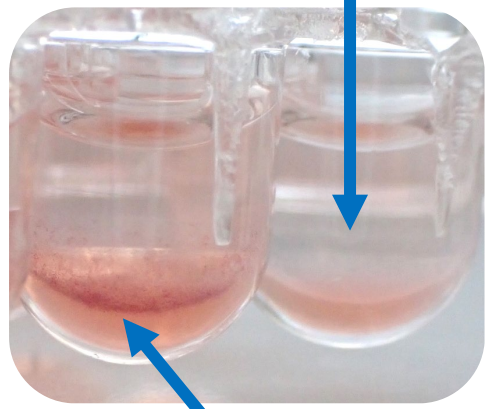
Biological Validation in NRU assay

- BEAS-2B cells cultured on collagen I reached EC50 within ≤100 puffs for all ALI smoke and aerosol exposures, including the least toxic EVP aerosol tested.
- NRU data from both versions of SAEIVS were comparable. HTP aerosol and cigarette smoke induced similar responses, whereas EVP aerosol appeared to reach the cells more efficiently.



U-wells with collagen I after NR staining

Control well: collagen I matrix without cells at the edge of the MTP plate.

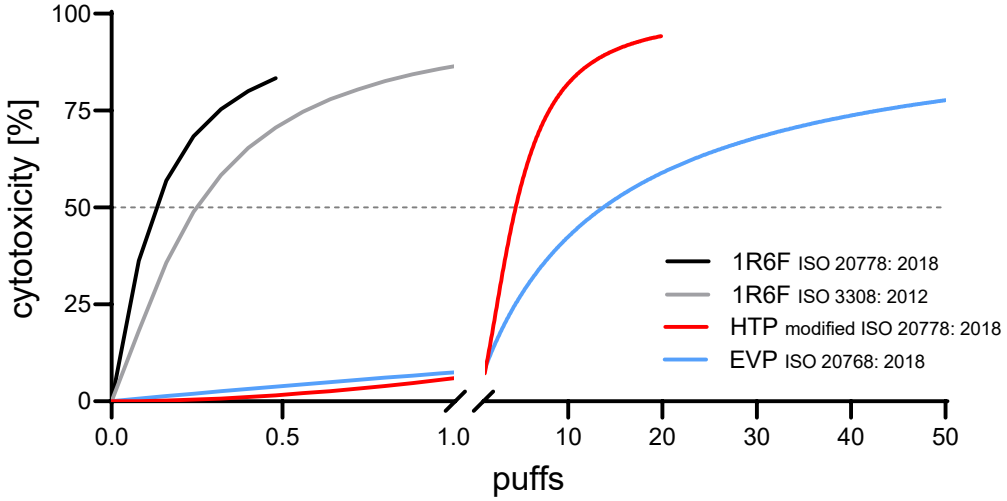


Well with BEAS-2B cells on collagen I in the inner wells

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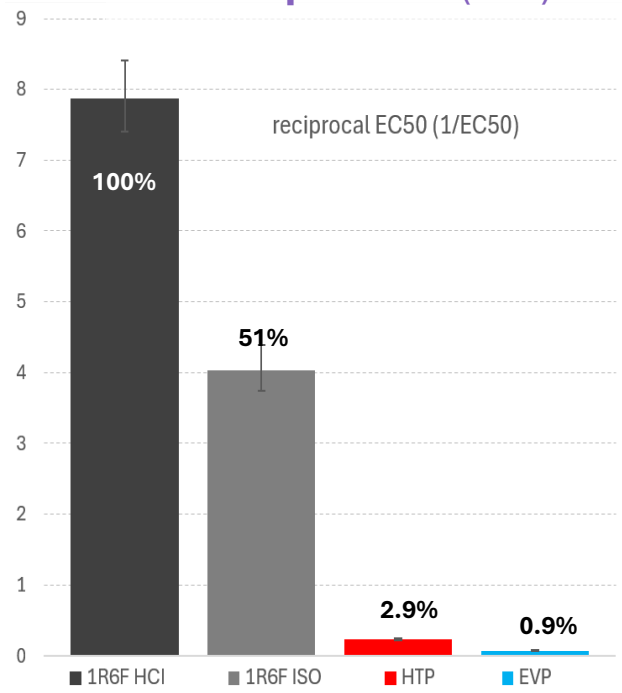
NRU assay with SAEIVS2

- Minimal droplet formation through temperature-controlled aerosol delivery
- High reproducibility with stable exposure conditions and exact defined dilution
- The redesigned setup enabled faster cleaning and smoke-to-aerosol changeover, allowing all tests below to be completed by one person in a single day.



ISO 3308: 2012 35/2/60/bell shape/no vent block
ISO 20778: 2018 (HCI) 55/2/30/bell shape/vent block
ISO 20778: 2018 (HCI modified) 55/2/30/bell shape/no vent block
ISO 20768: 2018 (CRM N°81) 55/3/30/square shape profile

Direct cytotoxicity comparison (n=3)

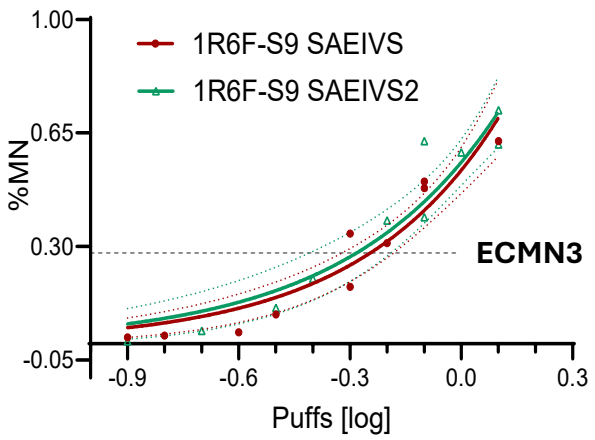


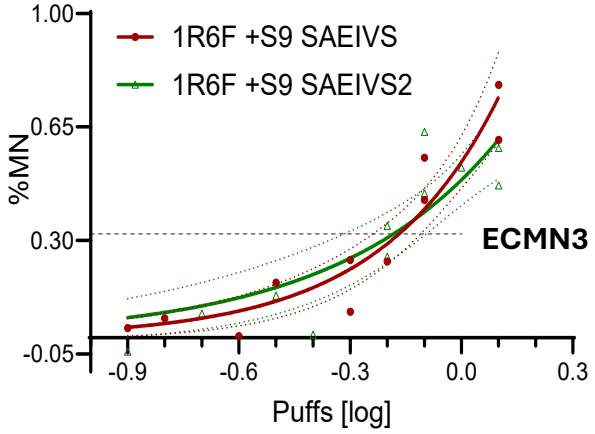
Higher bars = higher cytotoxicity

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IVM assay comparison in 24 MWP

Micronucleus increase were similar across both exposure systems, with no significant differences observed in the presence or absence of S9.





Direct comparison of smoke genotoxicity between SAEIVS and SAEIVS2 (n=4)



ECMN3 is the effective concentration required to induce a 3-fold increase in micronucleus frequency above background levels

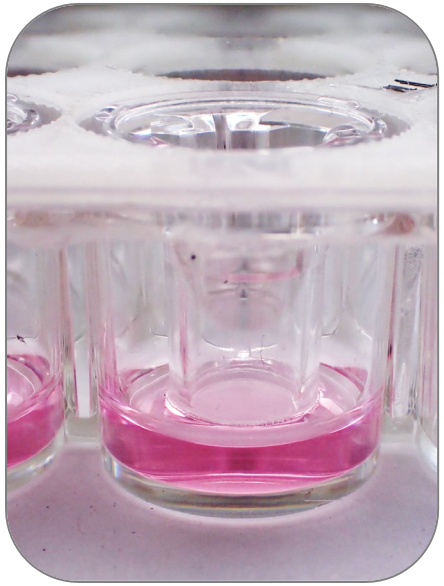
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Dosimetry in transwell

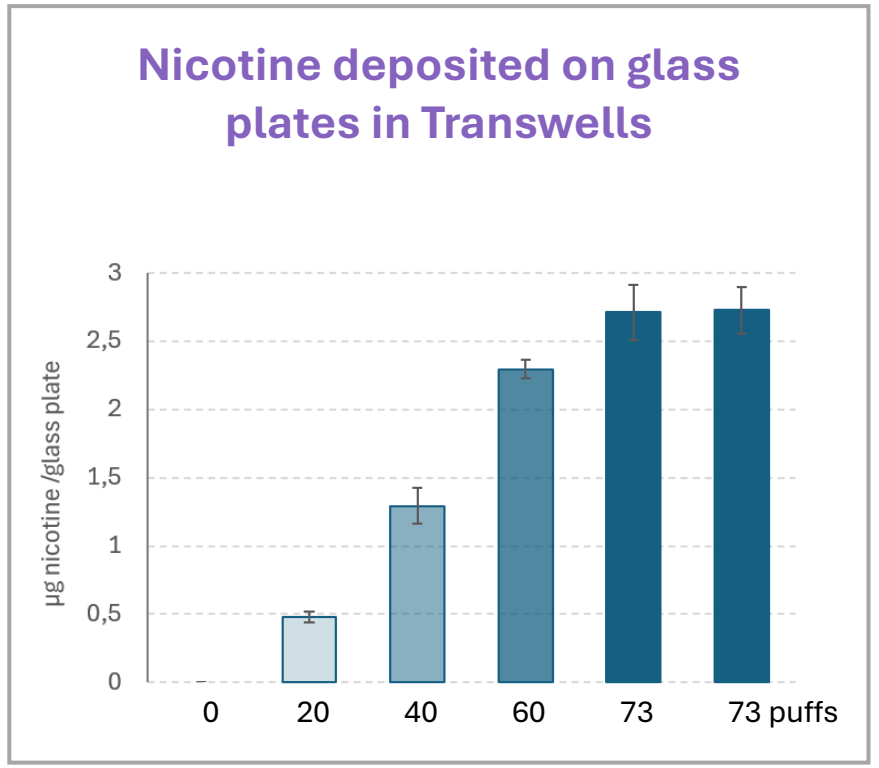
- Glass discs placed in sealed Transwell inserts simulate the cell exposure surface for nicotine deposition.
- Nicotine levels increased with puff number, indicating a clear dose-response relationship
- Replicate exposures at 73 puffs showed nearly identical nicotine levels



Material for dosimetry: 24 MWP with masking plate, transwells and glass plates



Transwell with glass plate in well with medium



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Summary

- The most significant SAEIVS2 enhancements include a dead volume of <0.5 mL, aerosol temperature control, and the addition of a third exposure chamber.
- Uniform TPM deposition was achieved across the exposed well surface, between row-specific well positions, and across all three exposure chambers for both 24- and 96-well plate formats.
- Comparable toxicity responses to cigarette smoke and heated tobacco product aerosol were observed in SAEIVS and SAEIVS2.
- Improved EVP aerosol delivery, together with reduced droplet formation in the transfer tubing, increased aerosol bioactivity and resulted in greater cytotoxicity in the temperature-controlled SAEIVS2 system.
- The combination of upgraded pumps and temperature-controlled aerosol transport provides highly stable exposure conditions and reliable, reproducible results.
- SAEIVS2 demonstrates both high robustness and sensitivity, with EVP aerosols achieving EC50 values in fewer than 100 puffs

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