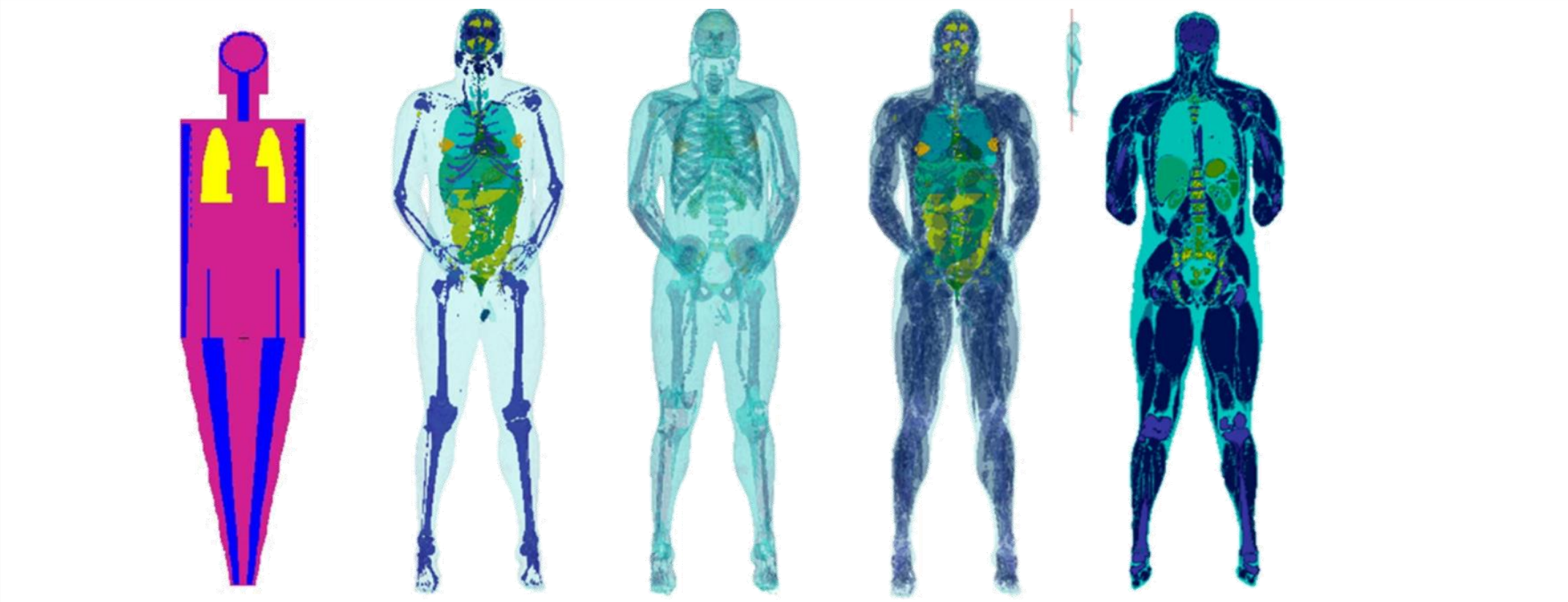

IIVS workshop 6 : Why is dosimetry important?

Liam Simms Imperial Brands

Roman Wieczorek Imperial Brands

Leon Stankowski Charles River Laboratories

IMPERIAL BRANDS SCIENCE

Why is dosimetry important?

Overview

- **What is dosimetry and why is it important?**
- Use of 2D submerged cultures vs 3D ALI exposed tissues
- Factors to consider in dosimetry
- Analytical Techniques to measure delivered dose
- SAEIVS: Smoke Aerosol Exposure In Vitro System characterisation
- How can you use *in vitro* dosimetry data?
- Summary

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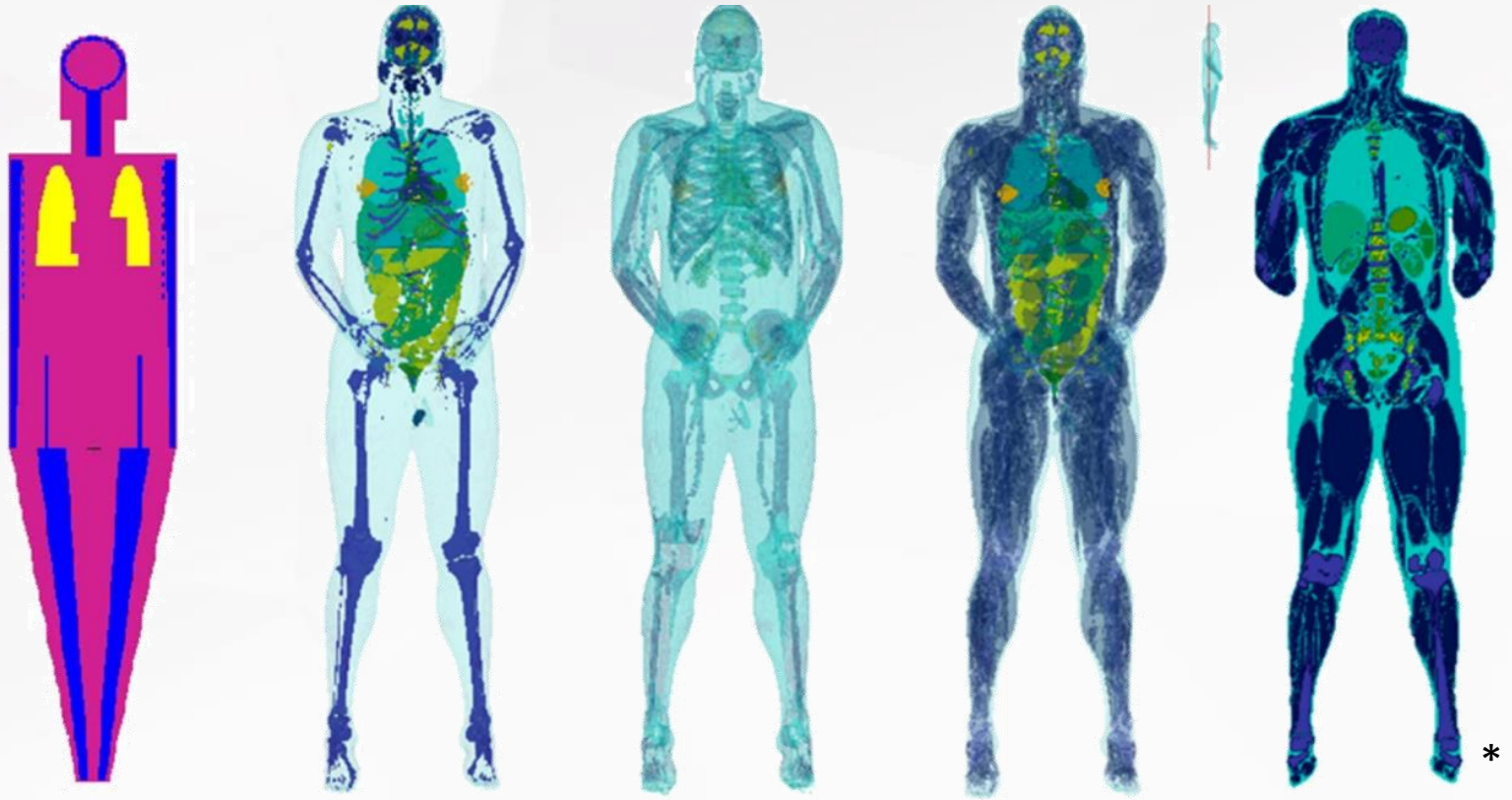
What is dosimetry?



*

Dosimetry is “*the study and practice of measuring or estimating the internal dose of a substance in individuals or a population. Dosimetry thus provides an essential link to understanding the relationship between an external exposure and a biological response*”.

*Talaat, K.,et al., 2019. Radiation dosimetry of inhaled radioactive aerosols: CFPD and MCNP transport simulations of radionuclides in the lung. *Scientific reports*, *9*(1), pp.1-21.

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Paracelsus the forefather of toxicology

"All things are poison, and nothing is without poison; the dosage alone makes it so a thing is not a poison”
Paracelsus 1538.

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What is *in vitro* dosimetry?

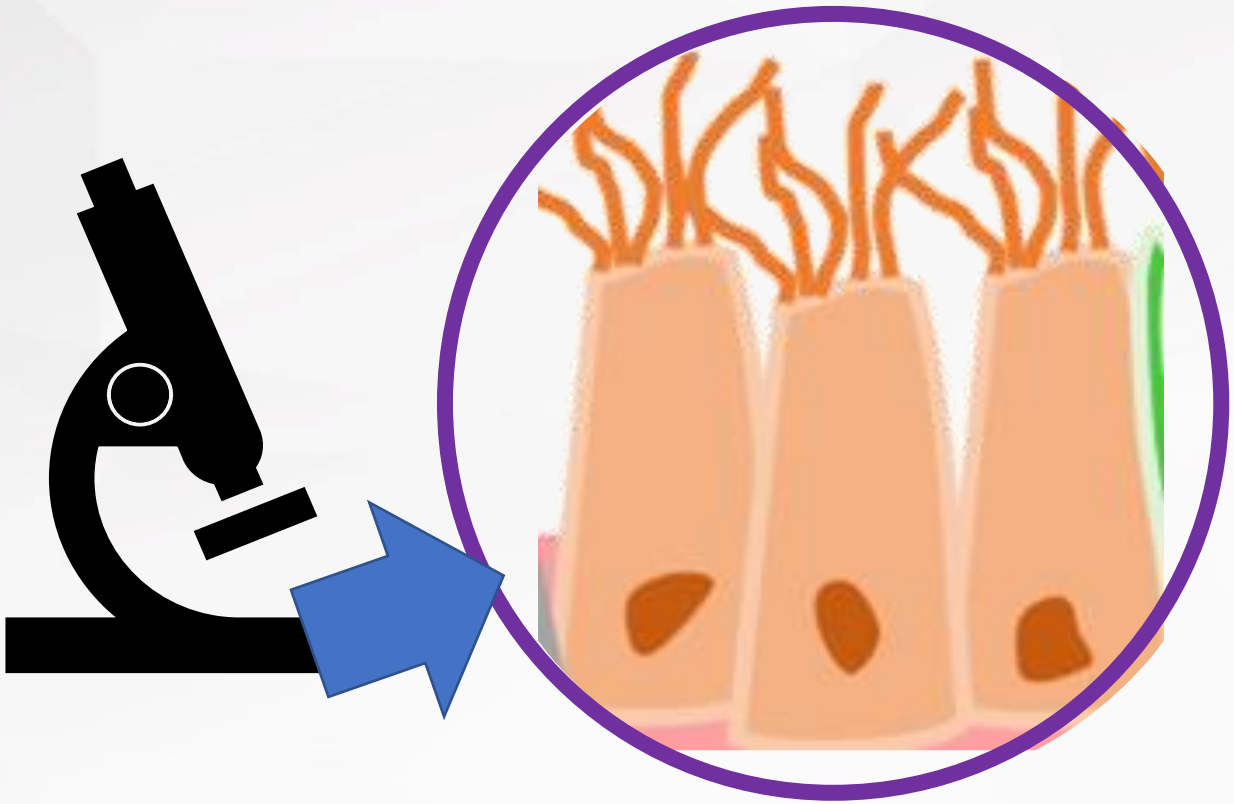

Illustration of ciliated cells

- Measuring the amount (concentration) of a substance that enters the cell.
  - Using markers such as nicotine
  - Indirectly: looking at deposited mass, measuring mass loss of a pod for example in EVPs for ALI exposures
  - Directly: lysing cells and measuring the compounds of interest inside the cell
- Determining dose, we can more accurately understanding the cellular response to an exposure and it physiological relevance

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How do you expose cells to smoke/aerosol and some considerations to take in to account

- What fractions of smoke/aerosol can cells be exposed to
- 2D submerged cells vs whole smoke/ aerosol exposure
- Examples of common exposure systems

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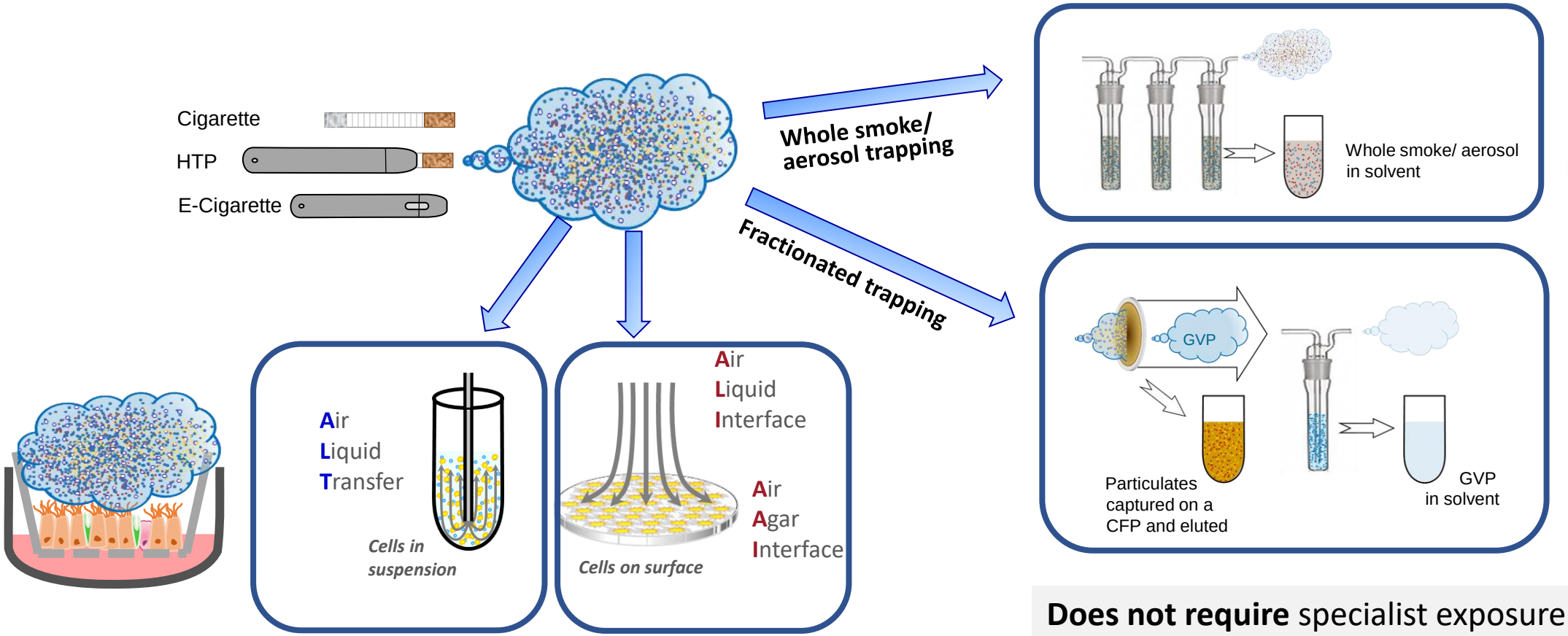
Different fractions of smoke/aerosol can be trapped or alternatively cells can be exposed to whole smoke aerosol



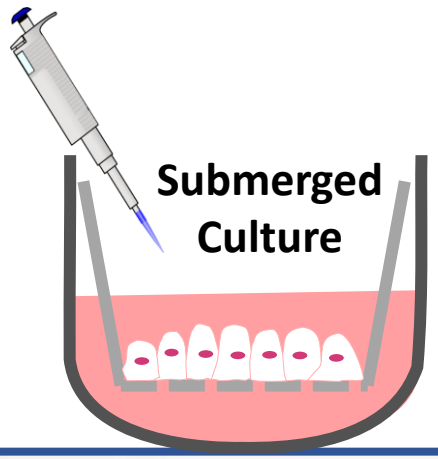
Direct cell exposure **requires** technical expertise and equipment to enable cell exposure

Does not require specialist exposure chambers and delivery systems. Doses are added directly to cell media

Pratte, P.*et al.*, 2017. *Human & experimental toxicology* 36, no. 11: 1115-1120

Various techniques are used to expose cells to chemicals

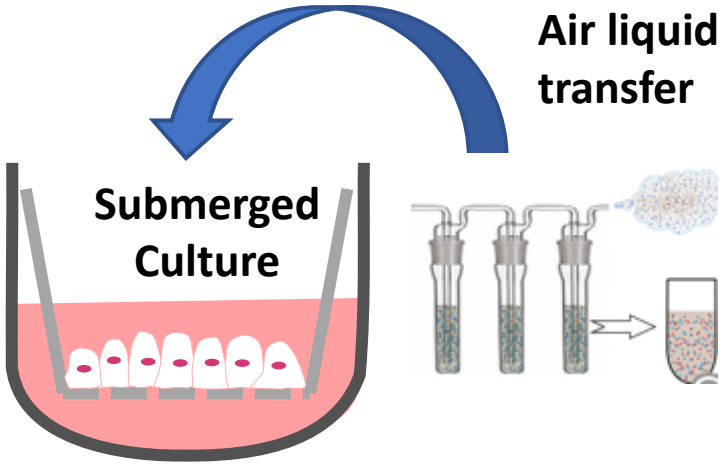
A: Submerged 2D cultures



Dose added to cells media is accurately defined

Dose which are exposed to cells cells are cannot be measured easily without killing cells

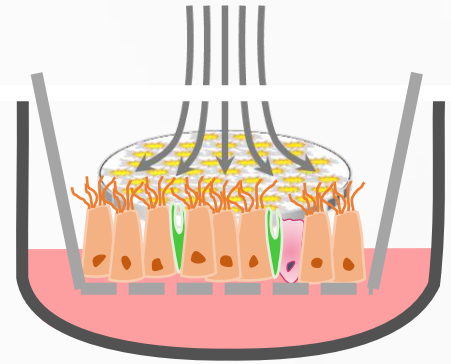
B: 2D tissues exposed to trapped samples



Choice of solvents can influence the types of compounds trapped.

Some solvents can only be added in limited concentrations e.g Ethanol, DMSO*

C: Air –liquid interface



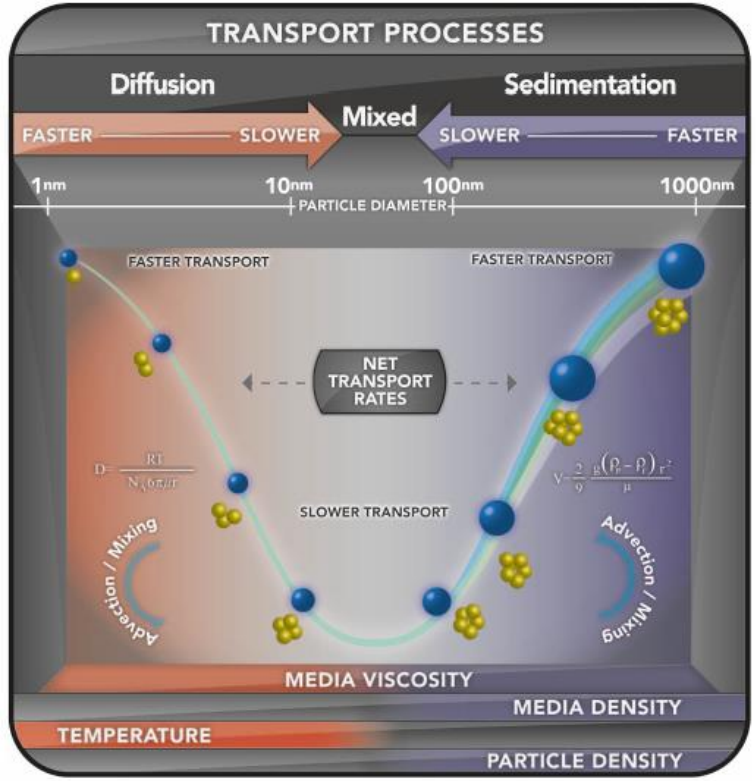
Exposure requires specialised equipment and expertise.

* Choice of solvent may limit trapping efficiency or amount that can be added to the test system

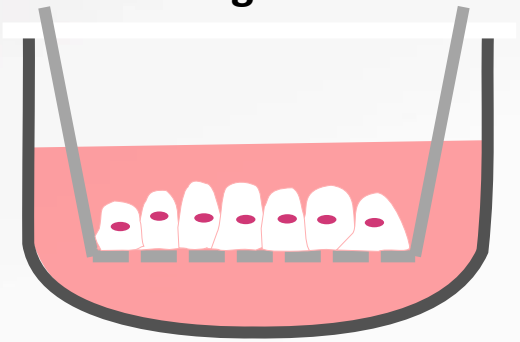
Exposure of solid particles is different for submerged cultures and via the Air liquid interface

For submerged culture, solid particle size affects transport rates to the cell surface.

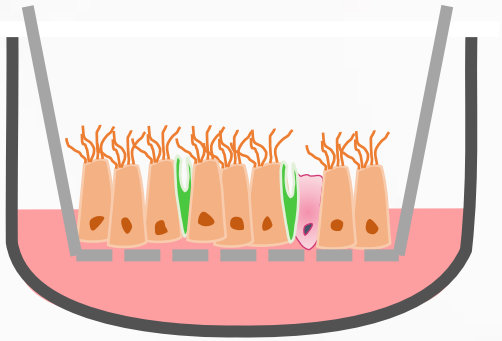
- ≤~10 nm: relatively fast; controlled mainly by diffusion
- ≥~200 nm: relatively fast, particularly for dense particles like the metals (due to sedimentation)
- 10 -100 nm: slower transport; controlled by diffusion and sedimentation (neither particularly effective)



Submerged Culture



Air –liquid interface



Hinderliter, P.M.,et al., 2010. ISDD: A computational model of particle sedimentation, diffusion and target cell dosimetry for in vitro toxicity studies. *Particle and fibre toxicology*, *7*(1), pp.1-20.

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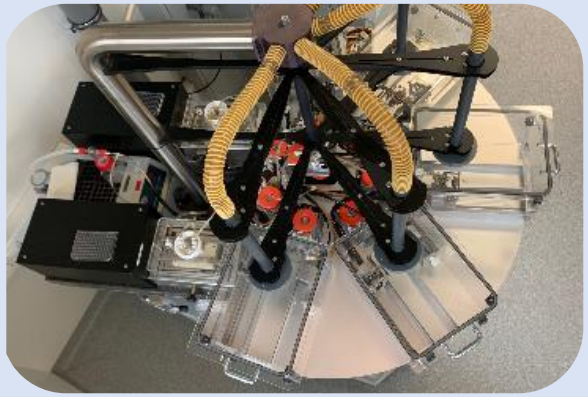
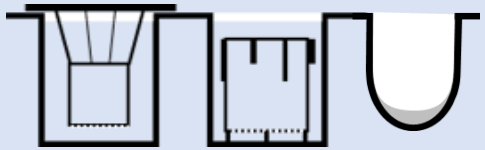
Multiple exposure systems are available for *in vitro* exposure of cells to whole smoke/aerosol at the ALI

IB's- SAEIVS - Smoke Aerosol Exposure In Vitro System
Integrated Smoking machine



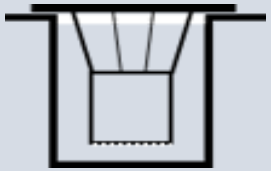


Transwell Insert 96 MWP

Borgwaldt smoking machine rm20S with BAT exposure system

www.bat-science.com

Vitrocell® smoke robot VC 10 S-TYPE with at 24/48 exposure system



Transwell

CULTEX exposure system

The smoke machines are connected to exposure chambers and represent the current state-of-the-art in ALI exposure to fresh smoke/ aerosol

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Factors to consider in dosimetry

- Fundamental differences in respiratory structure across animal species
- Various forces depending on particle size can affect particle deposition
- For hydrophilic particles, particle size can vary as you move along the respiratory tract
- As you move down the human respiratory tract cell types change, uptake depends on where in the lungs the particles are deposited

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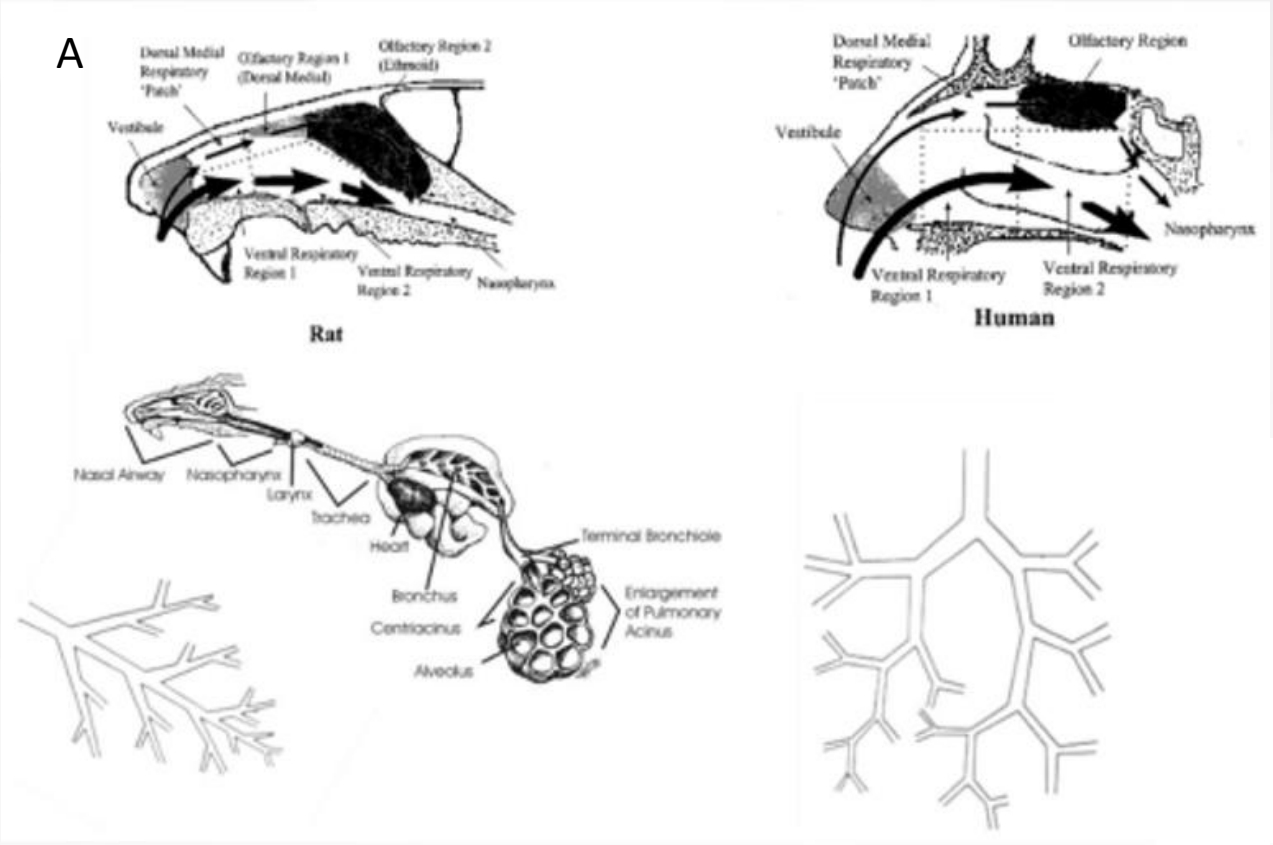
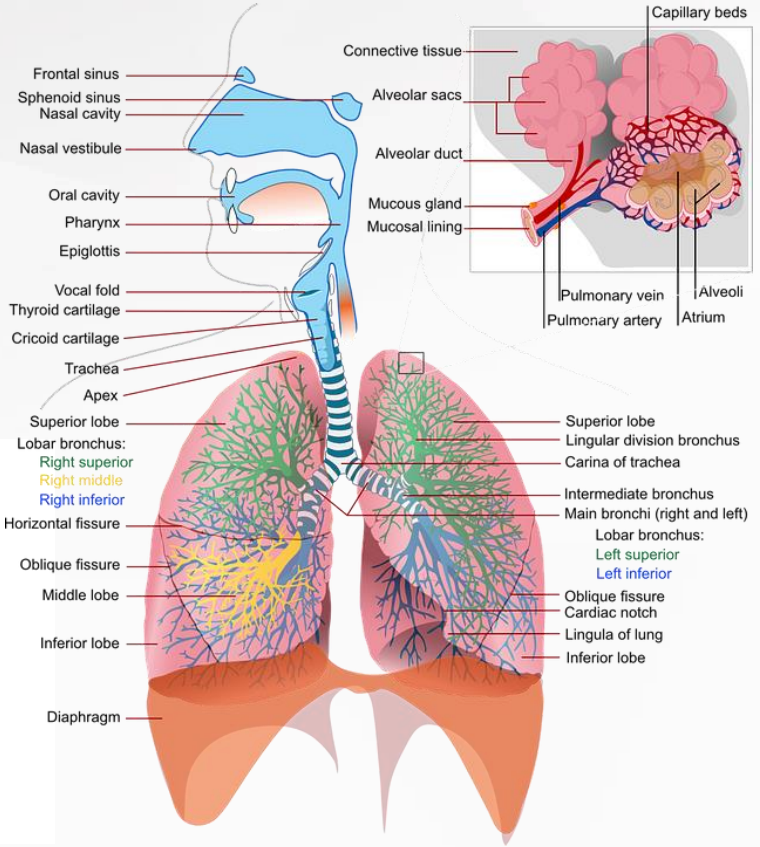
1. Fundamental differences in lung geometry across species:





Rats are obligate nose breathers

Rats are predominantly monopodal branching in the lung

Rats do not have respiratory bronchioles, alveolar sacs are reached after 3-13 branches of the lung

A: Clippinger, A.J. et al., 2018. Alternative approaches for acute inhalation toxicity testing to address global regulatory and non-regulatory data requirements: An international workshop report. *Toxicology In Vitro*, *48*, pp.53-70.

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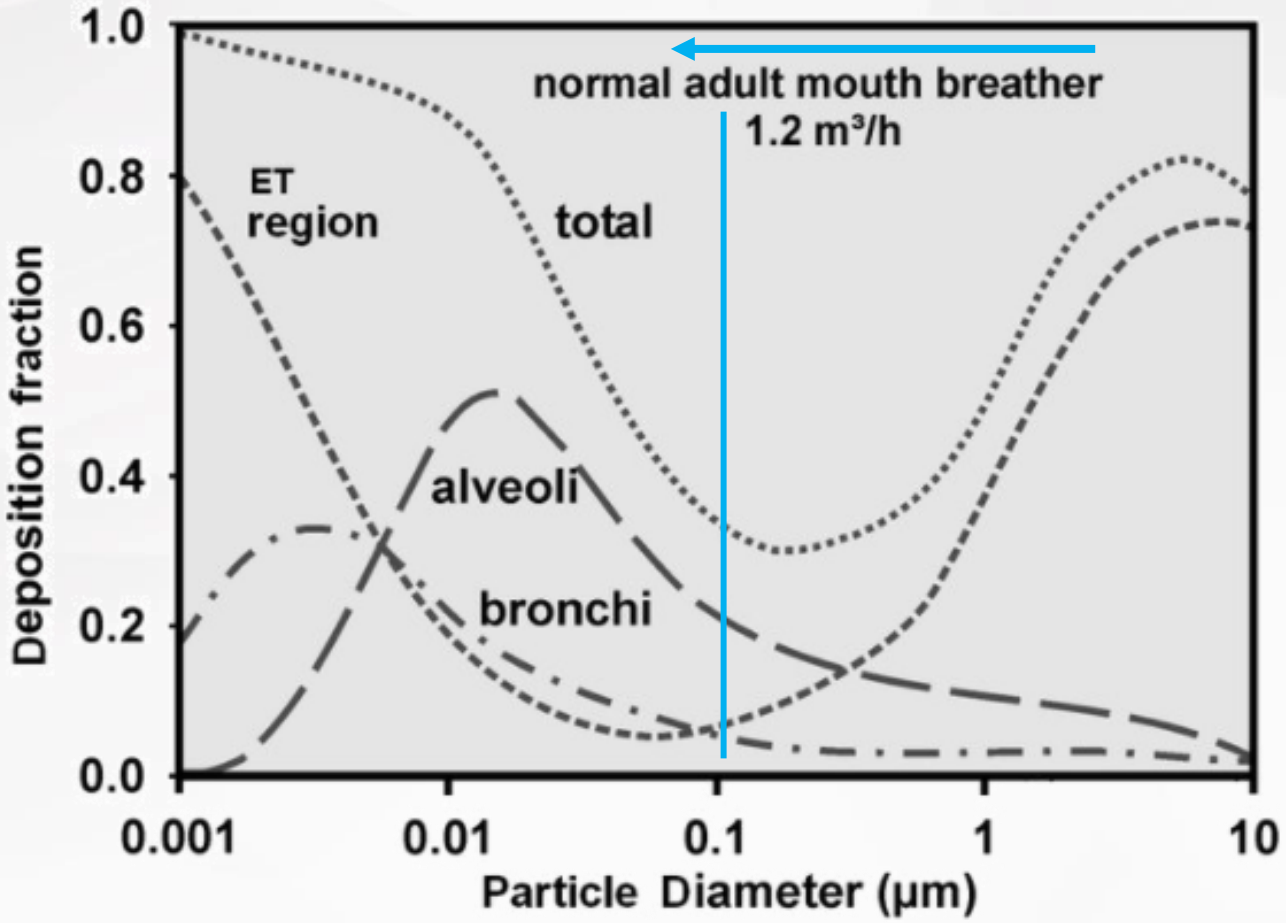
2. Various forces can affect particle distribution.

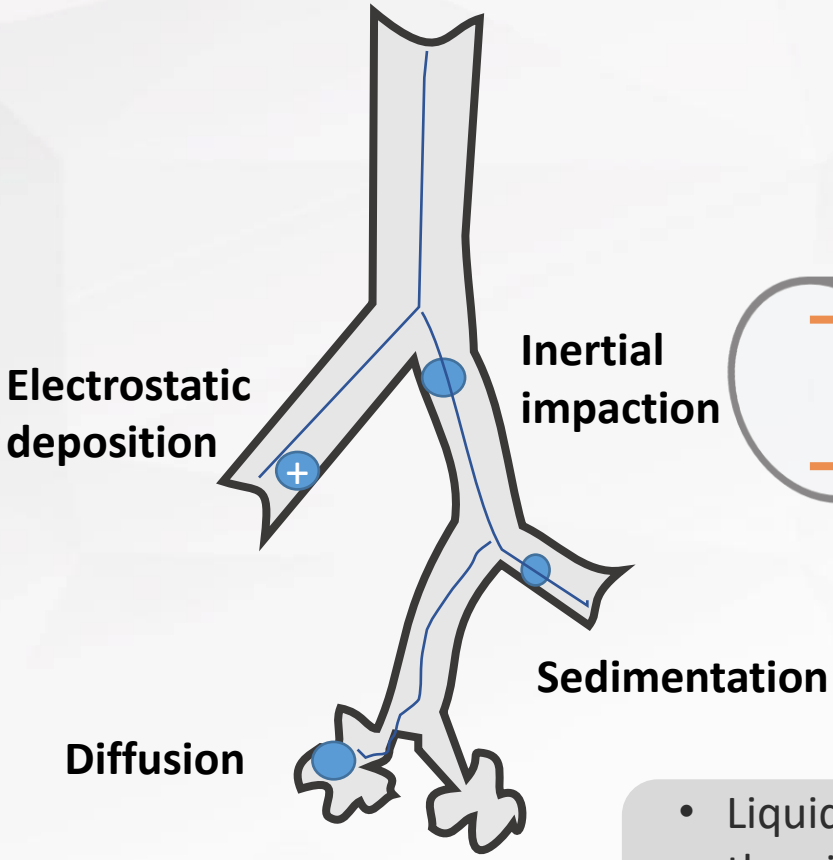


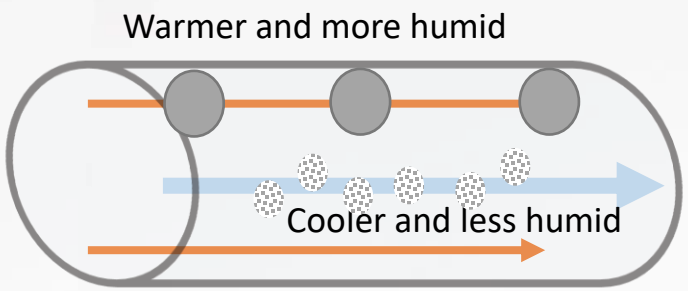
- Deposition depends on inhaled particle sizes, lung geometry and breathing pattern.
- Uptake of water by droplets depend on physical properties
- Sub-micron particles are mainly deposited by impaction and sedimentation
- Nano sized particles are mainly deposited by diffusion
- Deposition decreases with decreasing particle size (10-1 um). Then increases due to increased availability of particles at the alveoli.

ET= Extra thoracic region

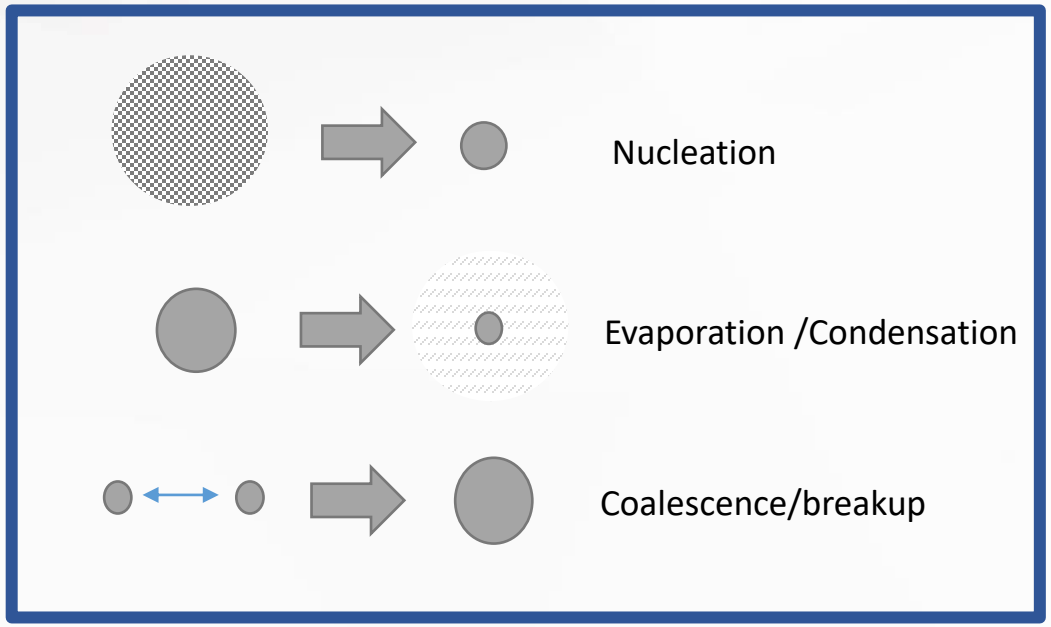
Hussain, M., et al., 2011. Lung deposition predictions of airborne particles and the emergence of contemporary diseases, Part-I. *Health*, *2*(2), pp.51-59.

3. Liquid particle size is not always constant and can change phase





Particles can change size as they move down the respiratory tract



- Liquid droplets close to airway walls only grow initially ; liquid droplets in the center of the airway first shrink, then grow
- Changes in droplet size distribution width depends on conditions during inhalation.

Modified from Grasmeijer, N.,et al., 2016. An adaptable model for growth and/or shrinkage of droplets in the respiratory tract during inhalation of aqueous particles. *Journal of Aerosol Science*, *93*, pp.21-34.

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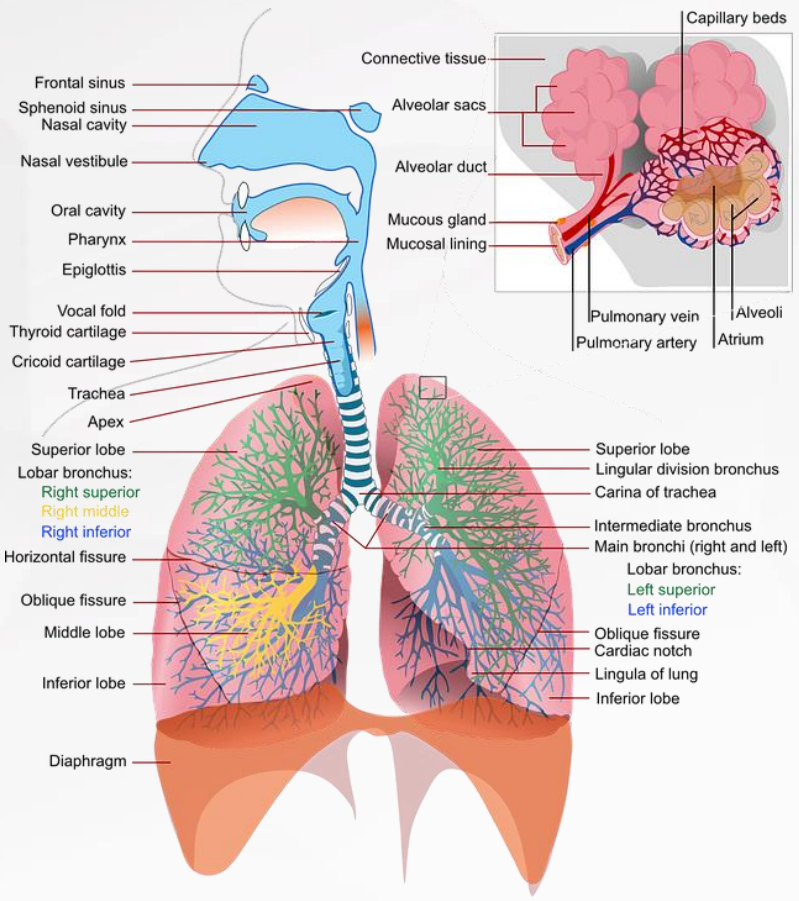
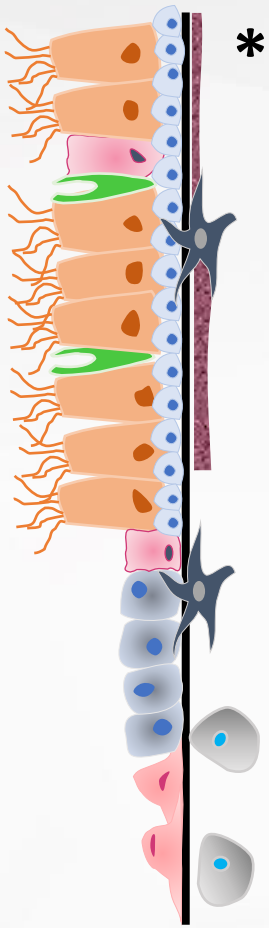
Cell types change as you move down the respiratory tract, which affects absorption

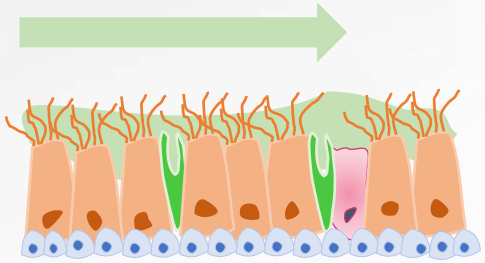


Upper respiratory track

Lower respiratory track



Mucociliary escalator



MATTEK A BICO COMPANY

EPITHELIX

- Basal epithelial cells
- Ciliated epithelial cells
- Secretory cells
- Goblet cells
- Cuboidal epithelial cells
- Dendritic cells
- Alveolar epithelial cells
- Smooth Muscle cells
- Alveolar macrophage

* Modified from Cao, X et al., (2020).. *In Vitro Cellular & Developmental Biology-Animal*, pp.1-29.

IMPERIAL BRANDS SCIENCE

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For measuring dose delivered, many possibilities are available

Concentration or dose of individual constituents or total mass

- PG, Glycerol , nicotine, carbonyls, flavours, TPM etc
- Trapping in DMSO, cell culture media, PBS

Collected on to glass coverslips or cell culture inserts

- uses standard Analytical Chemical techniques HPLC, GC ±MS, LC ±MS

Directly weigh solid particulate matter on Quartz Crystal Microbalance (QCM)

Particle concentration and/or size distribution

Using Laser photometer and a cascade impactor

- CO, CO_2, other gases
- NDIR, FTIR gas analysers

QCM

Cascade impactor

Typical aerosol exposure measurements are generally limited to one or a few analytes analysed in one of the replicate wells (e.g. nicotine, glycerol, TPM) or by using in-line (laser photometers) to monitor equipment.

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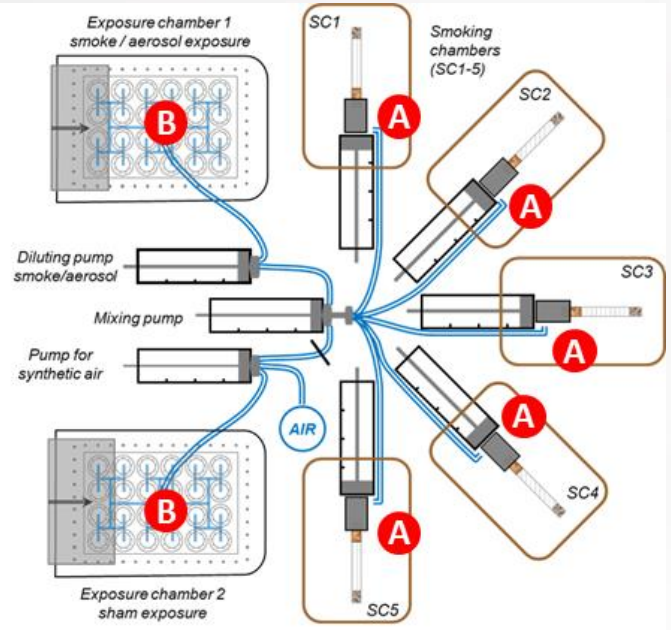
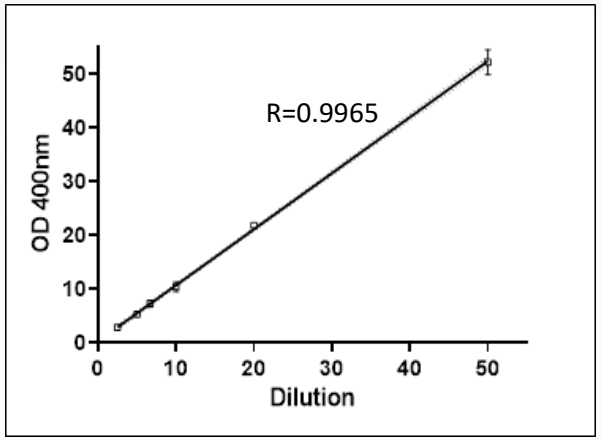
SAEIVS (Smoke Aerosol Exposure In Vitro System) efficiently delivers smoke to cells.

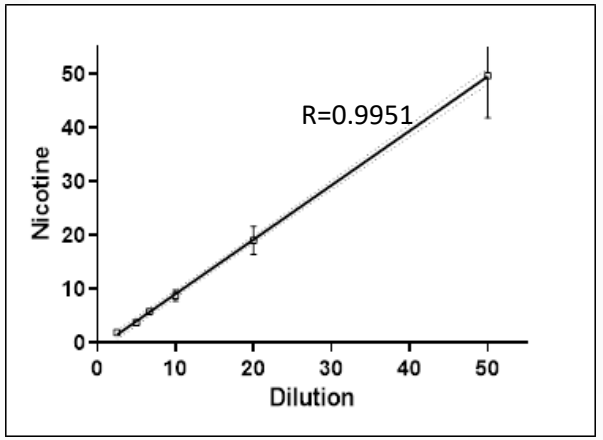


SAEIVS: ~20% loss of smoke between cigarette (A) and exposure chamber (B); aerial view.

For 100% smoke delivery, differences between measurements at the smoking (**A**) and exposure (**B**) chambers were calculated



C



D

Correlation between OD400 and nicotine deposition in wells exposed to the diluted smoke on the 96 MWP

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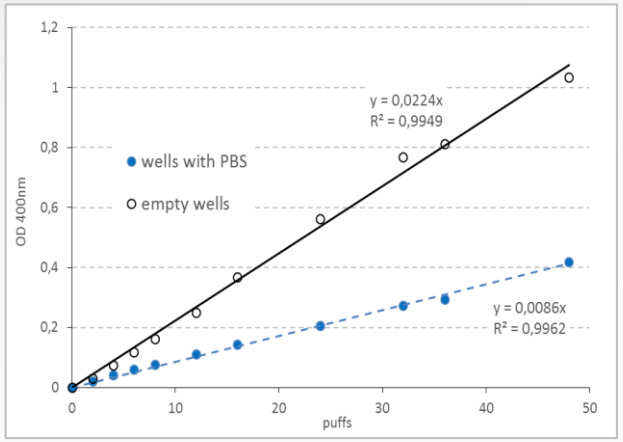
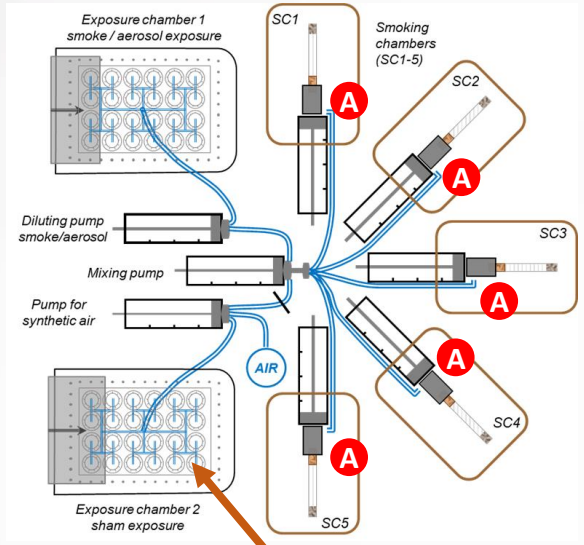
Minimal effects of cell surface on deposition rate of particles on to glass

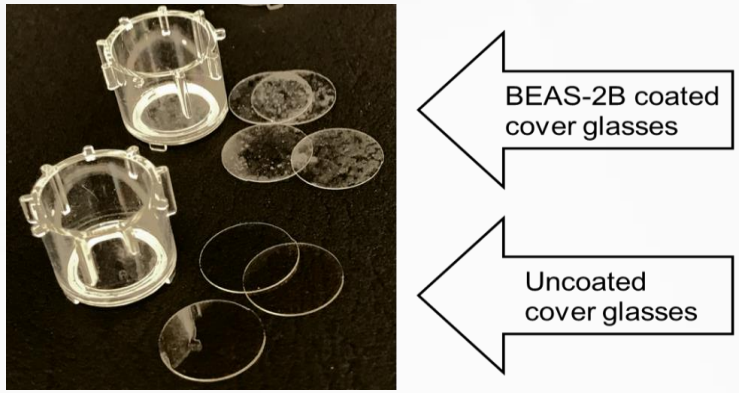
- Empty wells had a higher deposition of nicotine possibly due to static attraction
- The surface of glass slides added to the trans wells had minimal effects on nicotine deposition

A





B



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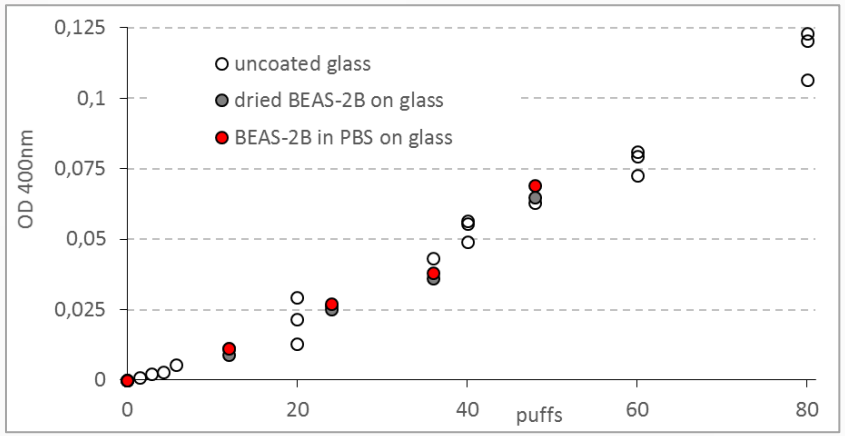
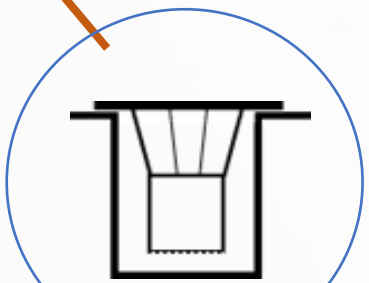
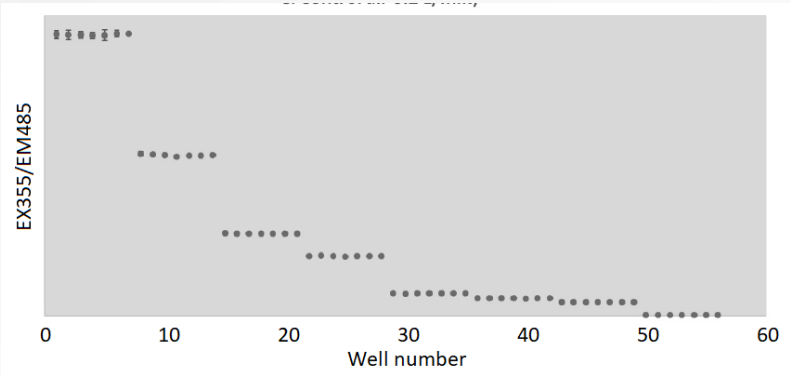




Characterisation of other Multi chamber exposure systems

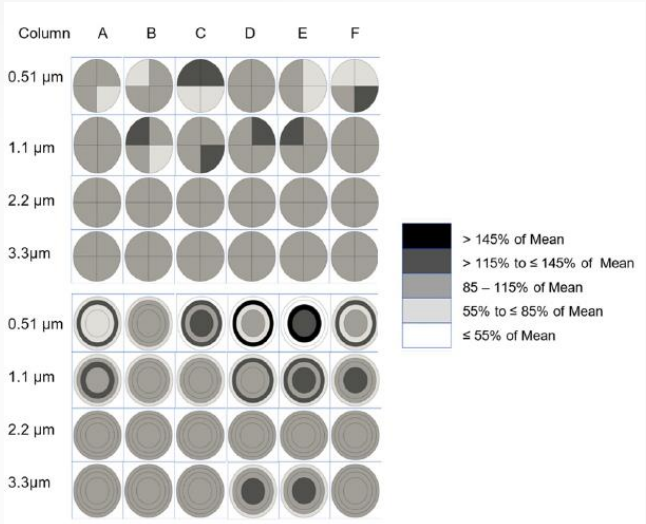
- Initial instrument qualification/validation is critical
  - Reproducibility/variability between replicate exposure wells
    - Ideally not more than ±15% across replicates
    - %CV generally inversely proportional to concentration
  - Reproducibility across experiments

Repeatability of TPM delivered and effects of dilution (1R6F TPM trapped in DMSO)



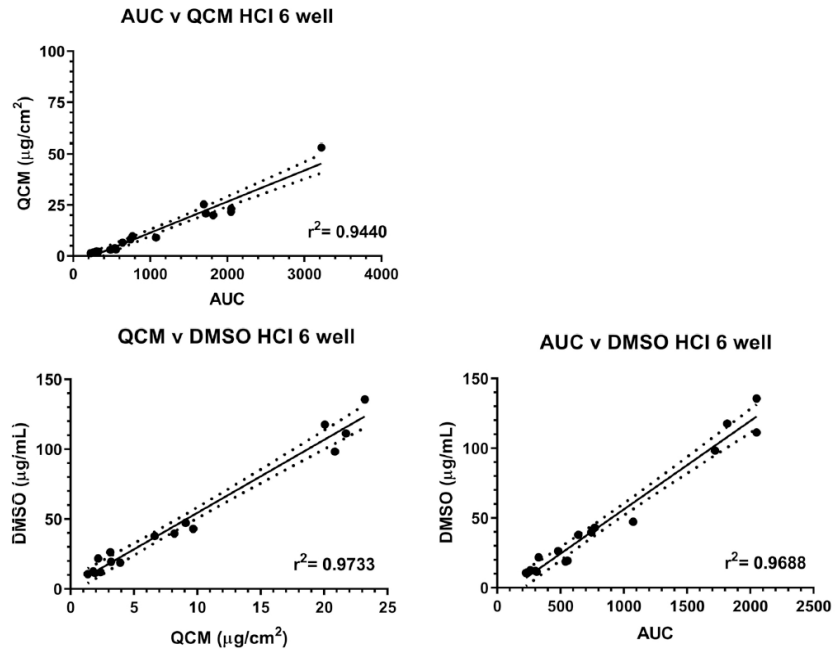
Ahmad (2020) Charles River Study Number 00999545

Deposition uniformity of monodisperse polystyrene fluorescent particles



Oldham et al (2020)
https://doi.org/10.1080/02786826.2019.1676877

Different measurements should be correlated (3R4F TPM in DMSO; AUC vs QCM vs TPM)



Keyser et al (2019) https://doi.org/10.1016/j.toxrep.2019.10.011

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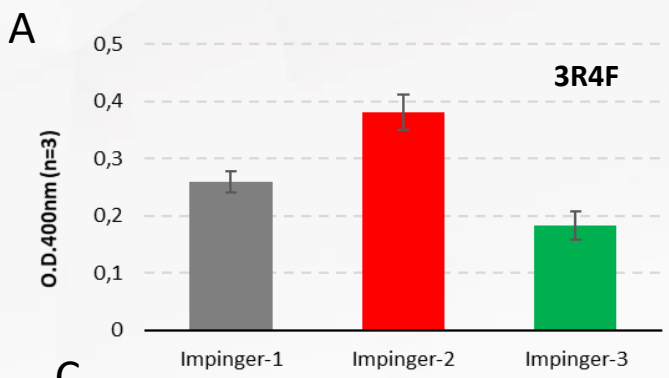
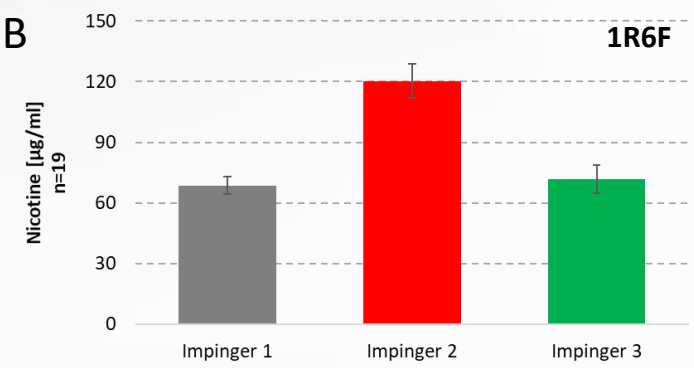
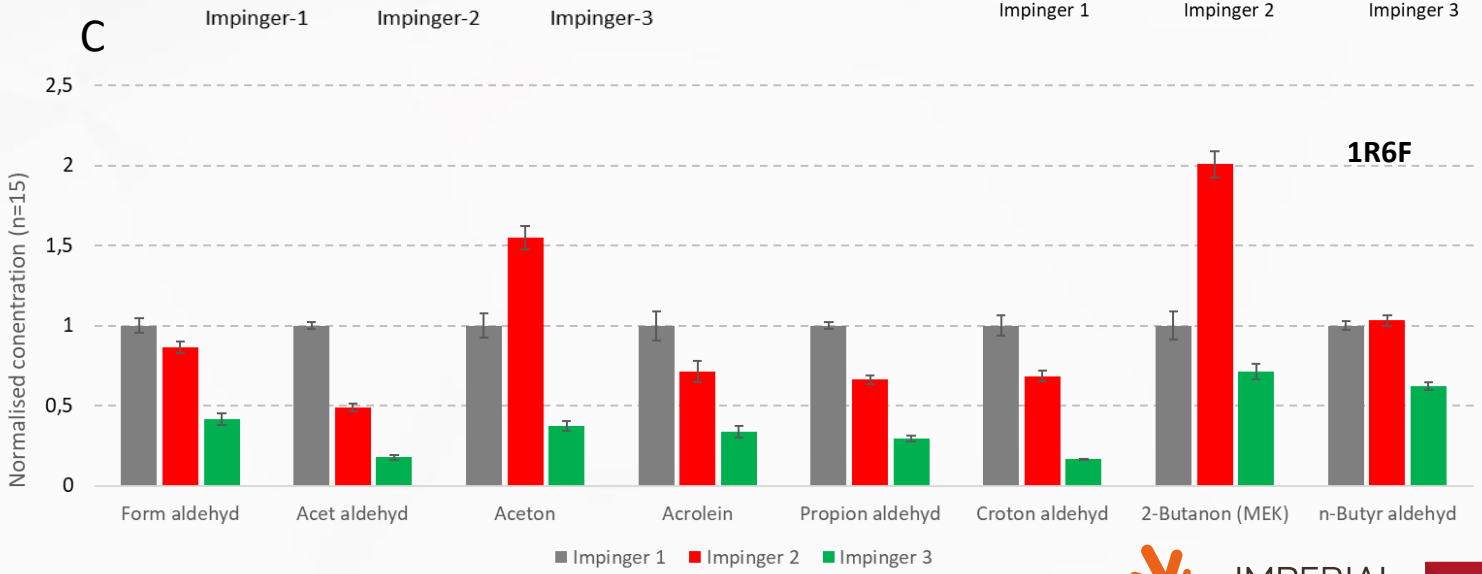
Use of a series of impingers to trap chemicals from smoke / aerosol for submerged cultures

Trapping of particulate mass from 3R4F and nicotine and carbonyls from 1R6F in 3 connected impingers







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How can you use *in vitro* dosimetry data in risk assessment ?

- Dosimetry is key linking exposures to Adverse Outcome Pathways (AOPs)
- Using *in vitro* doses to model human adverse concentrations
- Finally an example of a 3D repeated study

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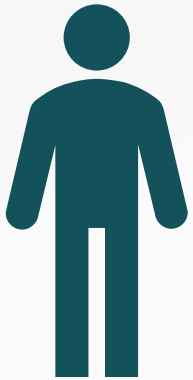
Dosimetry is key linking exposures to Adverse Outcome Pathways (AOPs)

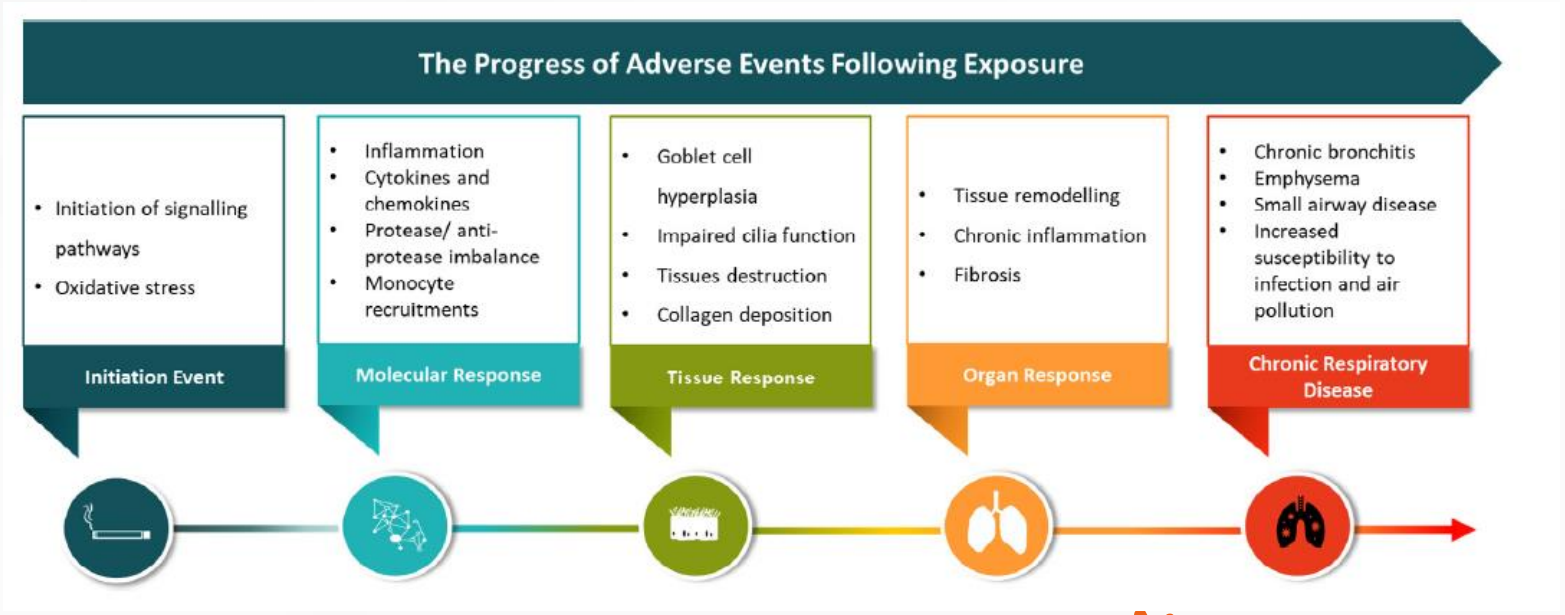
External Exposure → Internal Exposure → Dosimetry

Target site exposure

- Solubility
- Particle size, density, deposition
- Chemical reactivity
- ADME at exposure site

"Accurate dosimetry characterization requires determining the amount, rate, distribution, and form of a substance delivered to the target tissue of interest" (Kuempel et al., 2015).





Czekala et al., 2021 Current Research in Toxicology, *2*, pp.99-115.2021; Kuempel et al., 2015.. J. Occup.Environ. Hyg. 12 (Suppl. 1), S18–40.

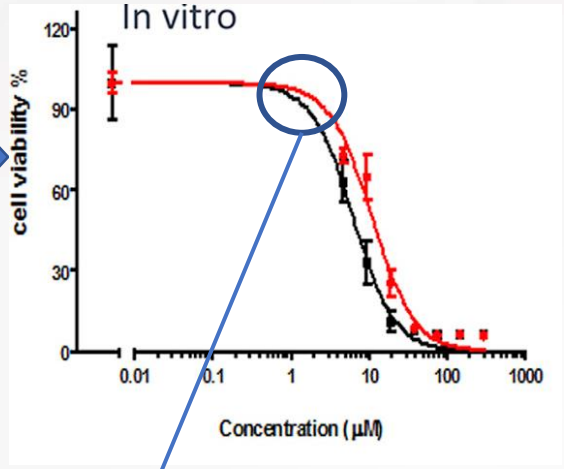
Using *in vitro* doses to model human adverse concentrations

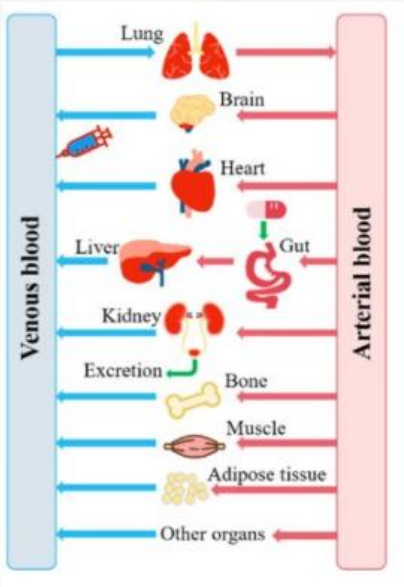
Point of departure (POD) from in vitro assay (µM)

QIVIVE

Human Exposure mg/kg bw

In vitro assay of human relevance





POD from in vitro study/correlated to cellular end point

Modelling corresponding human concentration *

Comparing modeled exposure to actual human exposure

QIVIVE (Quantitative in vitro to in vivo extrapolation) –using kinetic modeling, to link the measured in vitro POD to the corresponding vivo exposure that would be expected to result in an adverse health effects. * Peng, Y.,et al., 2021., In Vitro and In Silico Approaches. *Metabolites*, *11*(2), p.75.

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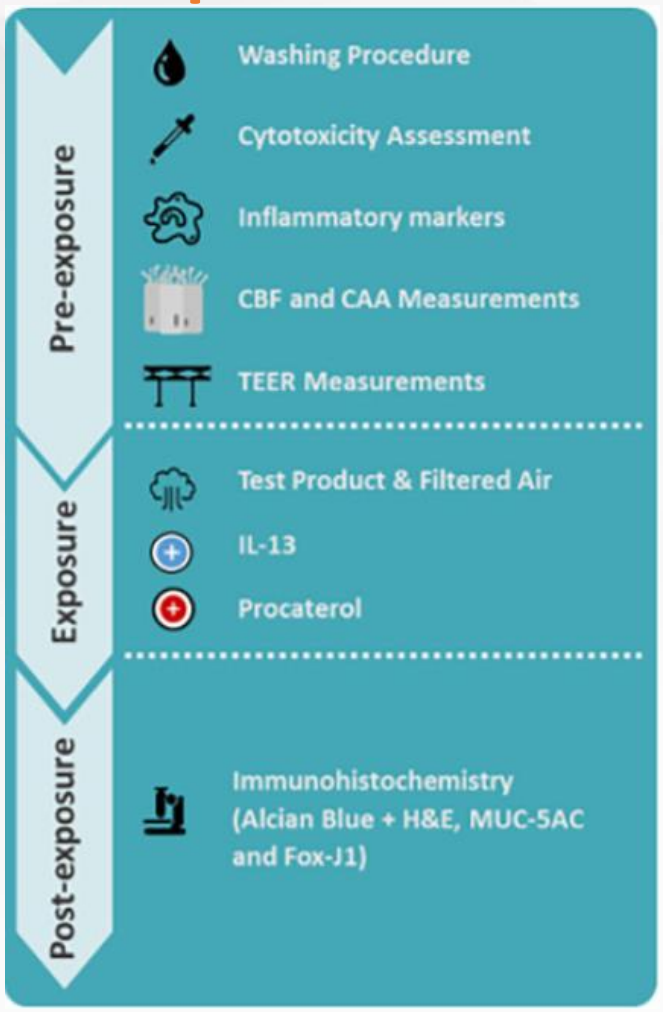
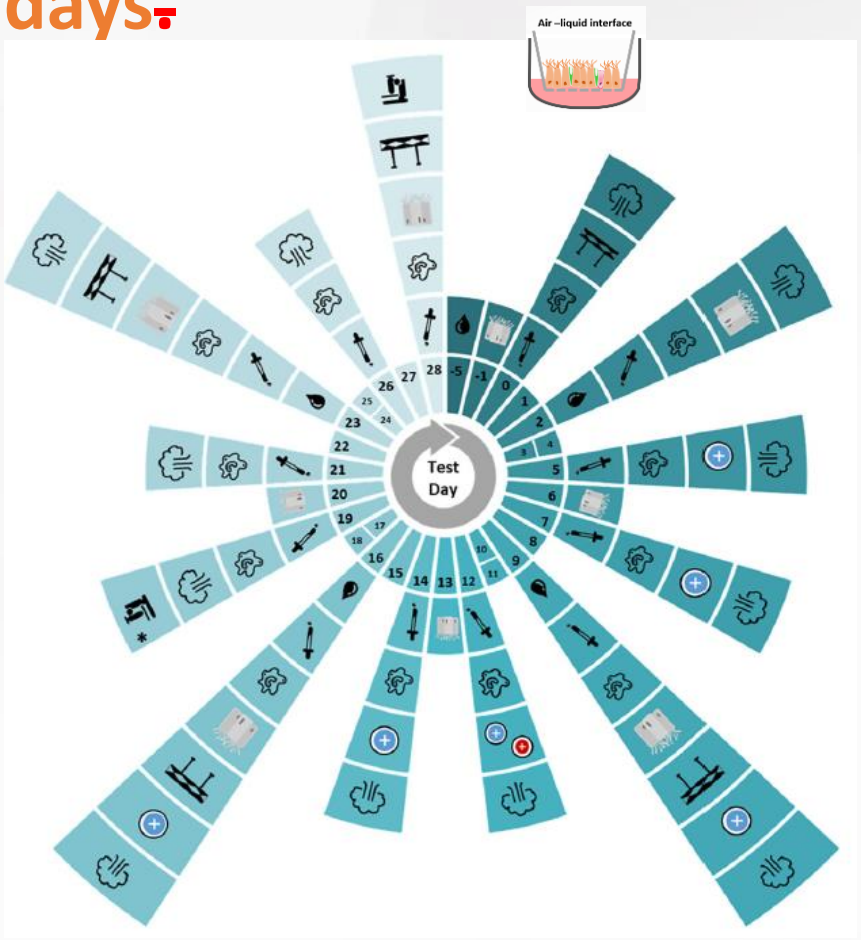
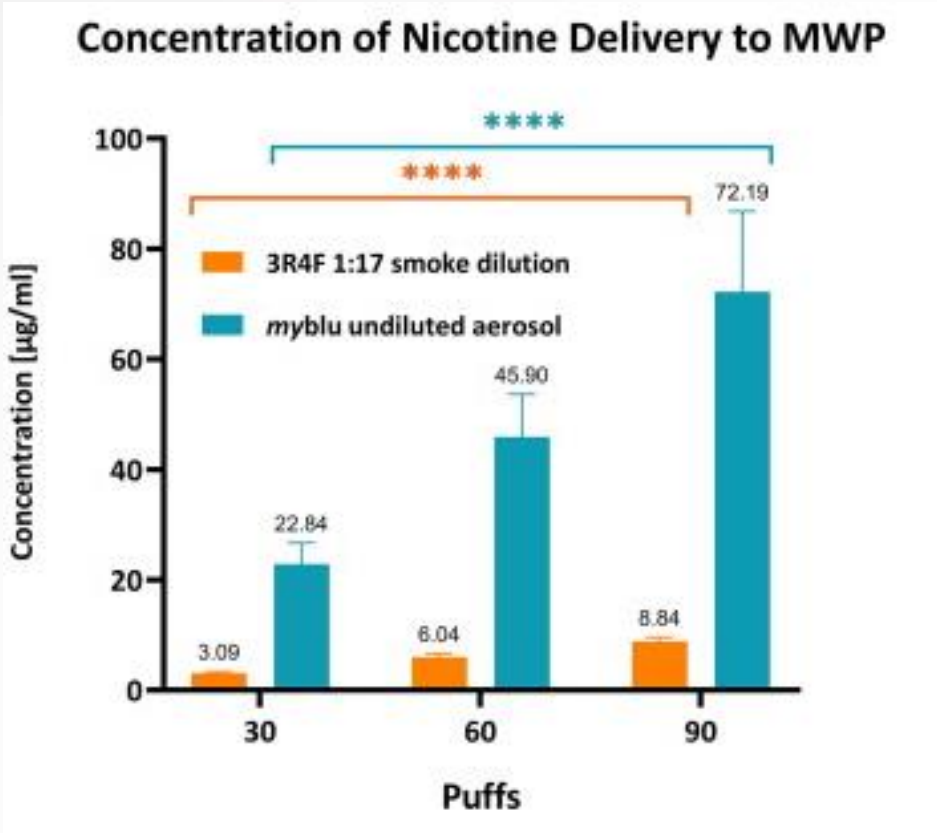
Imperial example: Using ALI grown NHBE (MUCILAIR™) human cultures demonstrated reduced toxicity of EVP compared to CC, repeated exposures for 28 days.

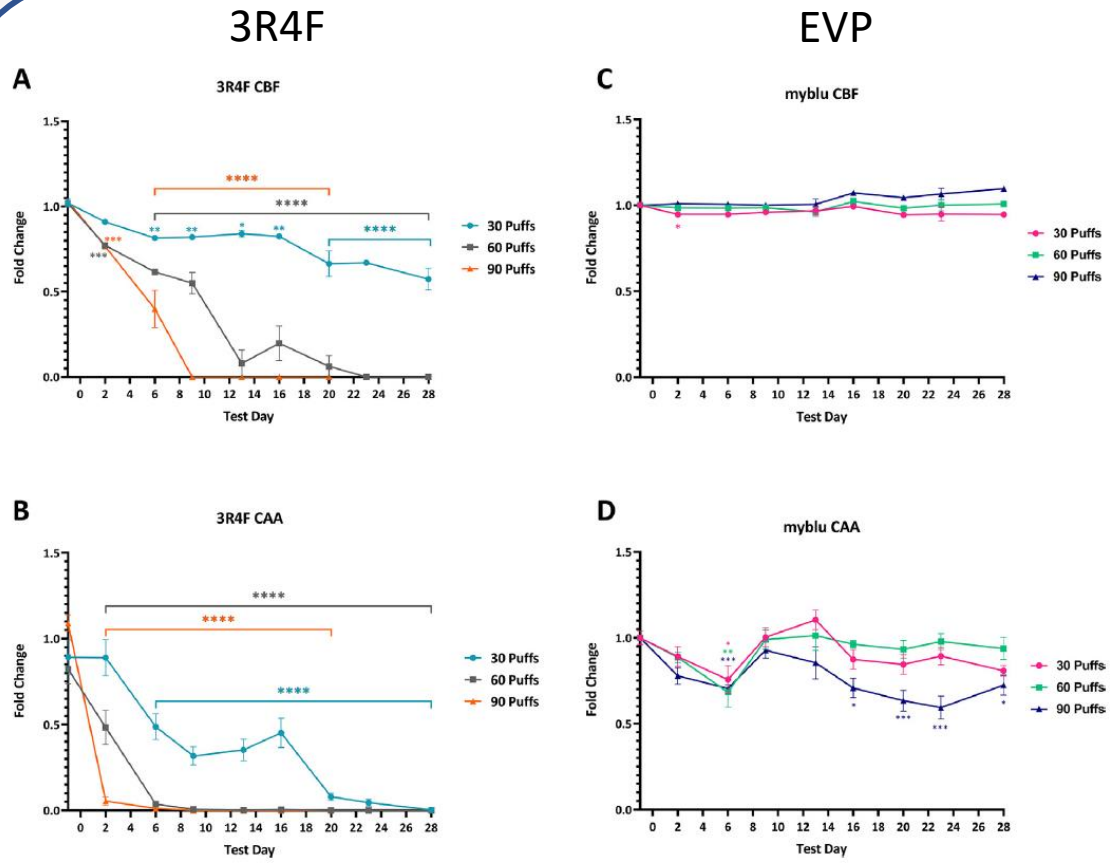


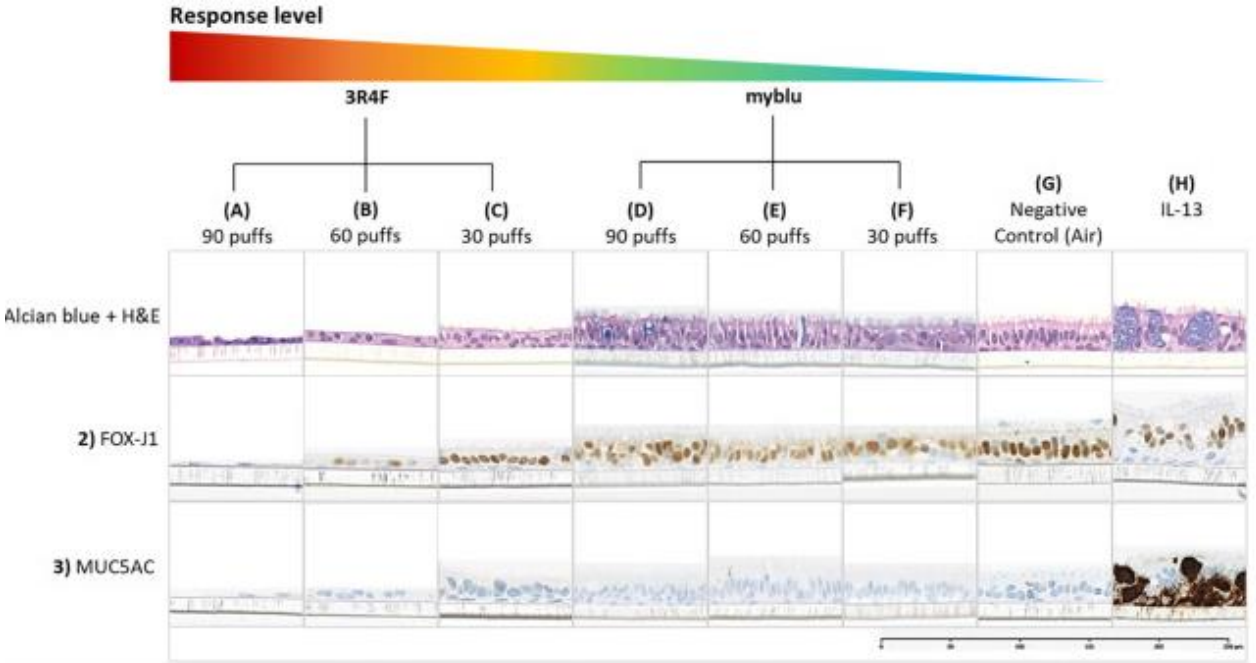




Czekala, L., et al., 2021.. *Current Research in Toxicology*, *2*, pp.99-115.

28 Day repeated study marked difference in EVP vs CC in histology, Cilia Beat frequency (CBF), Active Area (AA)





Test Article	3R4F			myblu			Negative Control: Air	IL-13
Puffs	90	60	30	90	60	30		
Alcian blue x̄ (%)	/	0.08	0.06	4.0	5.1	3.3	3.7	48
FOX-J1 x̄ (%)	/	6.9	50	51	55	53	53	43
MUCSAC x̄ (%)	0.21	0.49	0.49	0.66	0.51	0.46	0.36	65

Graphs A and C, CBF; B and D CAA. 3R4F was diluted 1:17; EVP was undiluted

Czekala, L., et al., 2021.. *Current Research in Toxicology*, *2*, pp.99-115.

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Summary:

- Dosimetry is key to better understanding biological responses in *in vitro* assays. This better understanding allows the determination of physiological relevance of results
- Chemical composition and deposition of aerosols in the respiratory tract depend on many factors including particle size (gas–liquid partitioning is important)
- Physiochemical characteristics of the chemicals and ADME at the site of exposure are important.
- Different ENDs products and Tobacco require different markers
- To ultimately extrapolate *in vitro* doses to human relevant doses requires PBPK modelling which is beyond the scope of this session.

ADME - Absorption, Distribution, Metabolism, Excretion
PBPK - Physiologically Based PharmacoKinetic modelling

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I would like to thank

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- Biological Toxicology Laboratory Hamburg
- The non routine chemistry department

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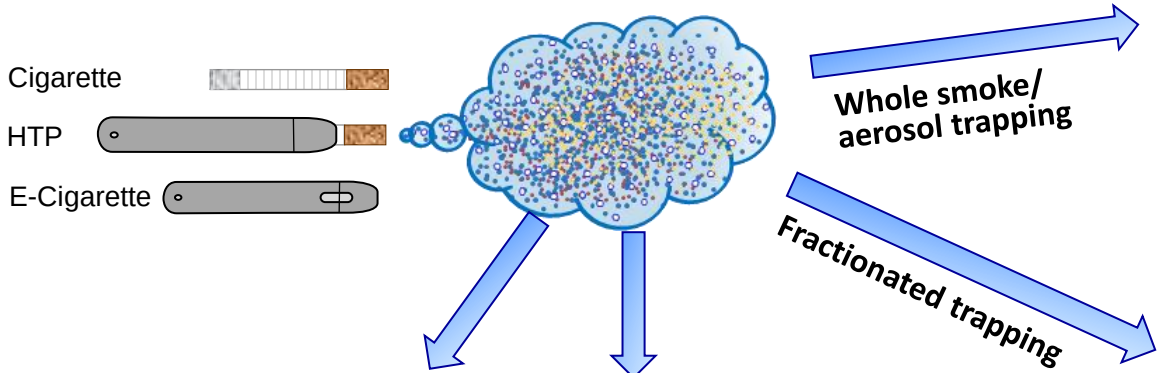
Different fractions of smoke/aerosol can be trapped or alternatively cells can be exposed to whole smoke aerosol



Direct cell exposure **requires** technical expertise and equipment to enable cell exposure

Does not require specialist exposure chambers and delivery systems. Doses are added directly to cell media

Pratte, P.*et al.*, 2017. *Human & experimental toxicology* 36, no. 11: 1115-1120