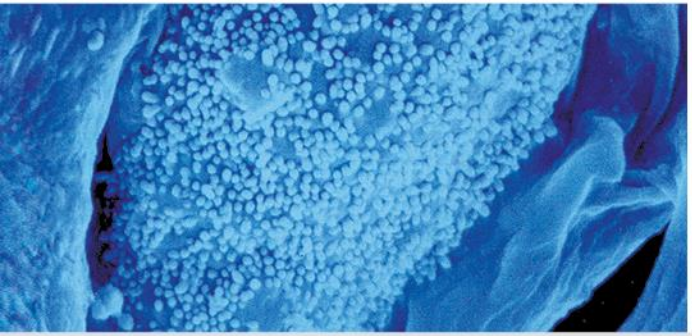
VITROCELL® in vitro Exposure Technology

Aerosol exposure devices
for *in vitro* toxicity testing at the air/liquid interface

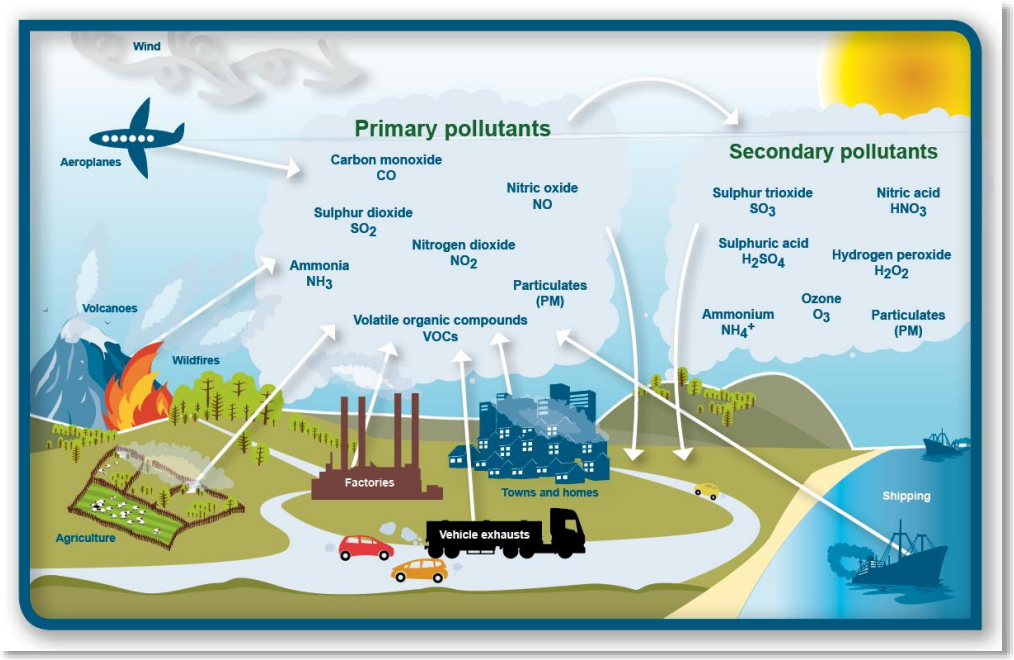


VITROCELL® SYSTEMS

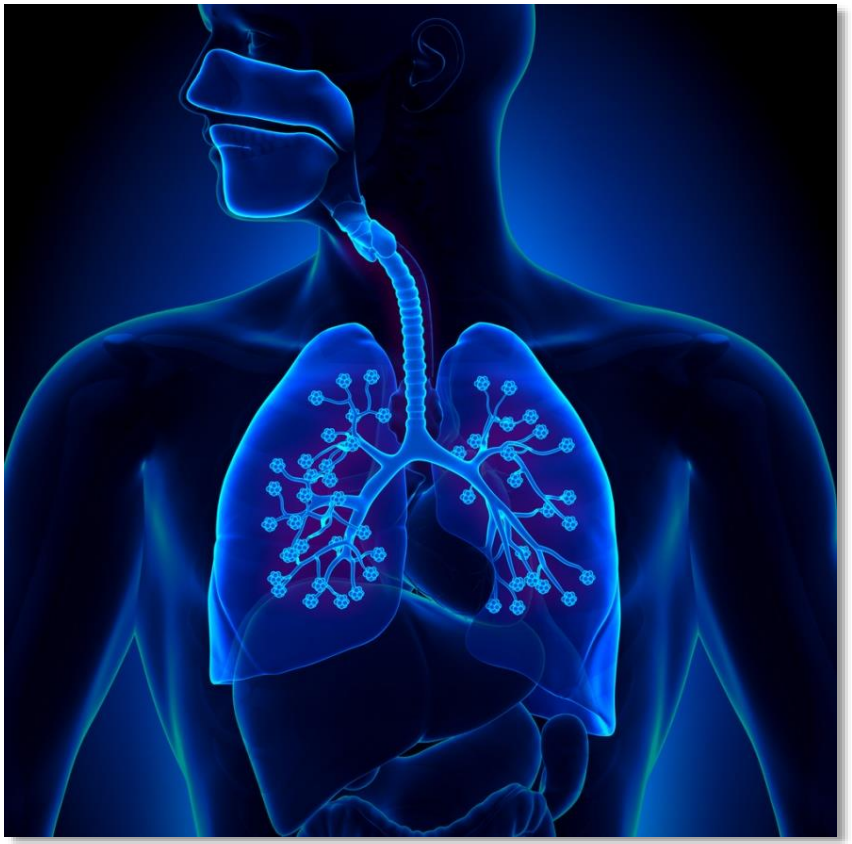
Aerosol exposure devices for *in vitro* toxicity testing at the air/liquid interface

Mission: use of turnkey in vitro exposure systems incl. online dosimetry tools

Gases
Complex Mixtures
Airborne Particles (Nano Particles)

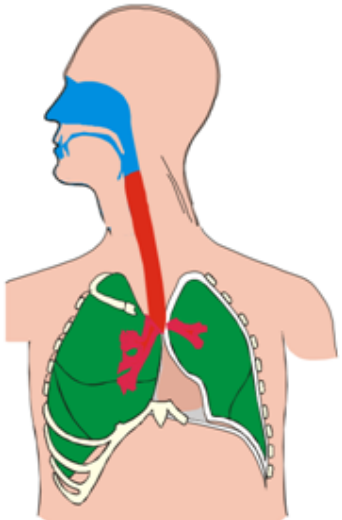


source: environment.scotland.gov.uk



source: cysticfibrosisnewstoday.com

Cell Types of Respiratory Tract

Function	Zone	respiratory tract scheme	Anatomy	Airway Surface	No. Of Airways	Air space/Liquid layer	Particle Diameter	Particle size	Cell Types
Air conditioning, temperature and humidity, cleaning. Fast particle clearance, air conduction	Extrapulmonary	 source: W. Koch, Fraunhofer ITEM, Hannover	Nose, Mouth, Esophargus	$2.4x10^{-5}m^2$	1	18 mm/ 10µm	5-10µm	Dust, Mist, fly-ash, Pollen, Spores	Ciliated Cells, non-ciliated cells (Globet/Mucous/Serous/Brush/Endocrine/Basal/Intermediate Cells)
	Pulmonary		Trachea	$4.5x10^{-2}m^2$	1		3-5µm	Fog, Mold, Bacteria, Exhaust gases	
			Main Bronchi/Bronchi	$3x10^{-2}m^2$	511		2-3µm		
Air conditioning, gas exchange, slow particle clearance			Bronchioles/terminal bronchioles	$2.6x10^{-4}m^2$	$6,5x10^4$	0.5-1mm/ 3µm	1-2µm		Respiratory epithelium (ciliated and non-ciliated cells with clara cells (without globet cells)
			Respiratory bronchioles	$7.5m^2$	$4,6x10^5$				Respiratory epithelium with maily secretory cells and few ciliated cells
gas exchange, very slow particle clearance			Alveolar ducts and sacs	$140m^2$	$4,5x10^7$	0.4mm/ 0.1µm	0.01-1µm	Fumes, Tobacco smoke, viruses, Oil smoke	Alveolar epithelial cells

Source: Encyclopaedia of Occupational Health and Safety , Chapter 10 - Respiratory System

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Examples of cell culture inserts

Human Cell Culture Models

Airway epithelial cells

Calu-3 (ATCCHTB-55)
16HBE14o- (can be obtained from D.C. Gruenert)
BEAS-2B (ATCC CRL-9609)
NuLi-1 (ATCC CRL-4011)

Primary airway epithelial cells → Commercially available from Epithelix (CH), MatTek (USA)

hBEpC

Alveolar epithelial cell lines

A549 (ATCC CL-185): ATII phenotype
Immortalized human ATII cells with ATI phenotype → Commercially available from inscreenex (D)
NCI-H441 (ATCCHTB-174): ATII and Clara cell phenotype

Primary alveolar epithelial cells

hAEpC: ATII cells that differentiate in vitro into ATI-like morphology

3D cultures

3D aggregates of A549 cells
Bilayer co-culture model: epithelial & endothelial cells
Triple cell co-culture model: epithelial cells, macrophages, dendritic cells
Double, triple and quadruple cell co-culture models: epithelial cells, endothelial cells, mast cells, macrophages

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Examples of cell culture inserts

AT = alveolartype

Source: Barbara Rothen-Rutishauser, Advanced in vitro lung models in nanotoxicology reserch – advantages and limitations, Adolphe Merkle Institute
University of Fribourg Marly, Switzerland
Jud et al. Swiss Med Wkly 143:0 (2013).

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Triple cell co-culture model: epithelial cells, macrophages, dendritic cells
Double, triple and quadruple cell co-culture models: epithelial cells, endothelial cells, mast cells, macrophages

Biomimetic microsystems

Breathing lung-on-a-chip: epithelial & endothelial cells → Alveolix (CH), Emulate (USA)
Perfused chip: epithelial & endothelial cells

AT = alveolartype

Source: Barbara Rothen-Rutishauser, Advanced in vitro lung models in nanotoxicology reserch – advantages and limitations, Adolphe Merkle Institute
University of Fribourg Marly, Switzerland
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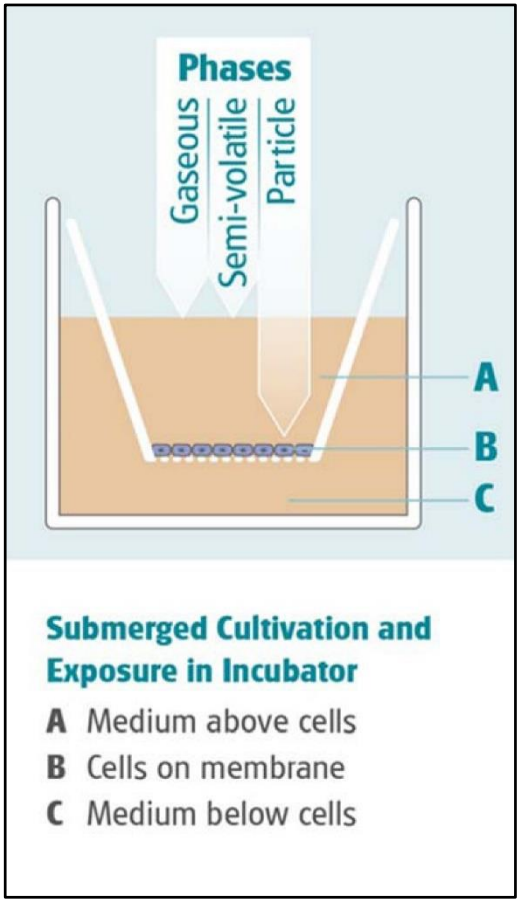
Examples of cell culture inserts

AlveoliX

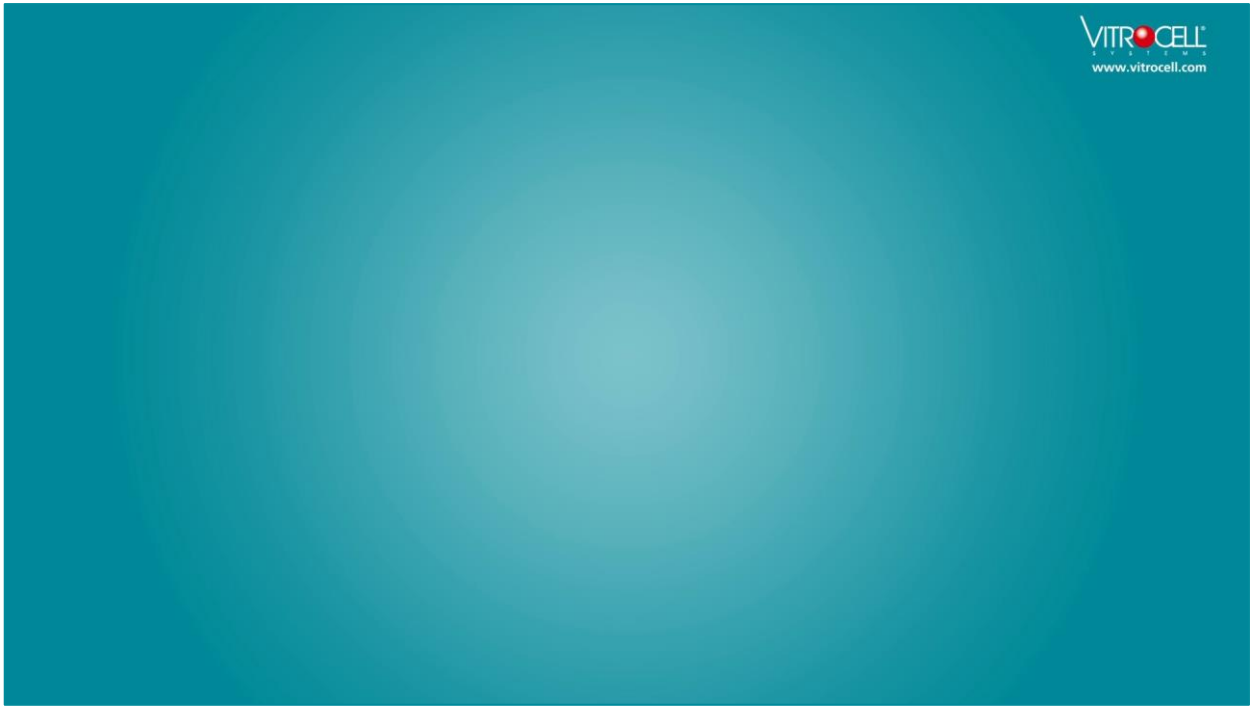
Emulate

Cooperation VITROCELL

Disadvantages Submerged Exposure

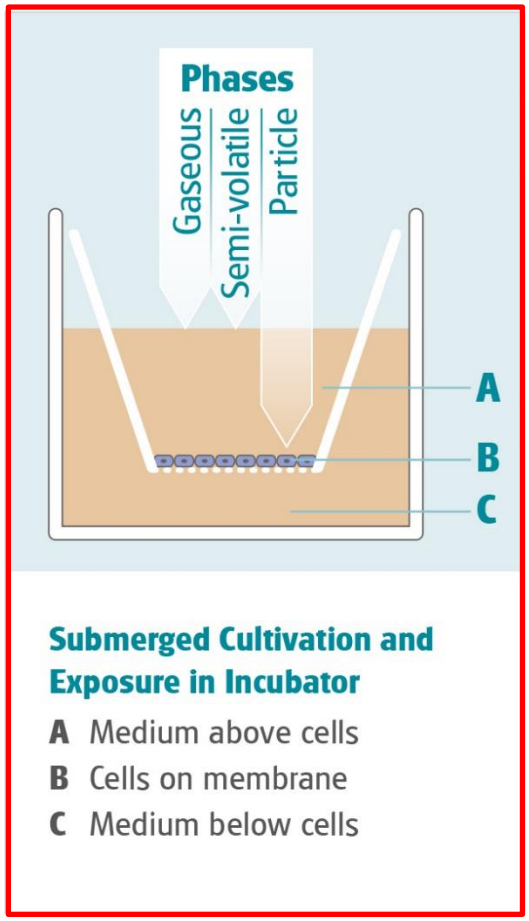


Submerged Exposure
Low sensitivity due to contact with media
> Undefined Dose



- **Different sedimentation times**
- **Upward diffusion**
- **Buoyant behaviour**

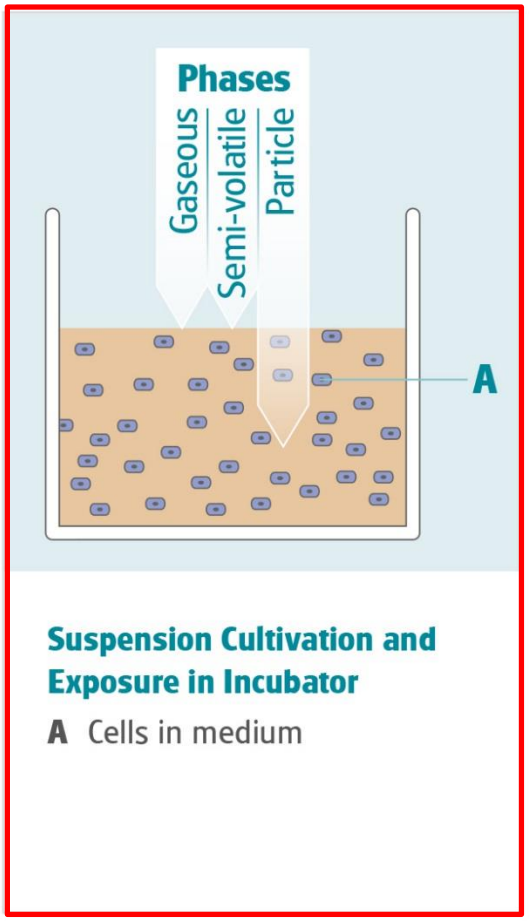
Advantages of Air/Liquid Exposure



Submerged Cultivation and Exposure in Incubator

A Medium above cells
B Cells on membrane
C Medium below cells

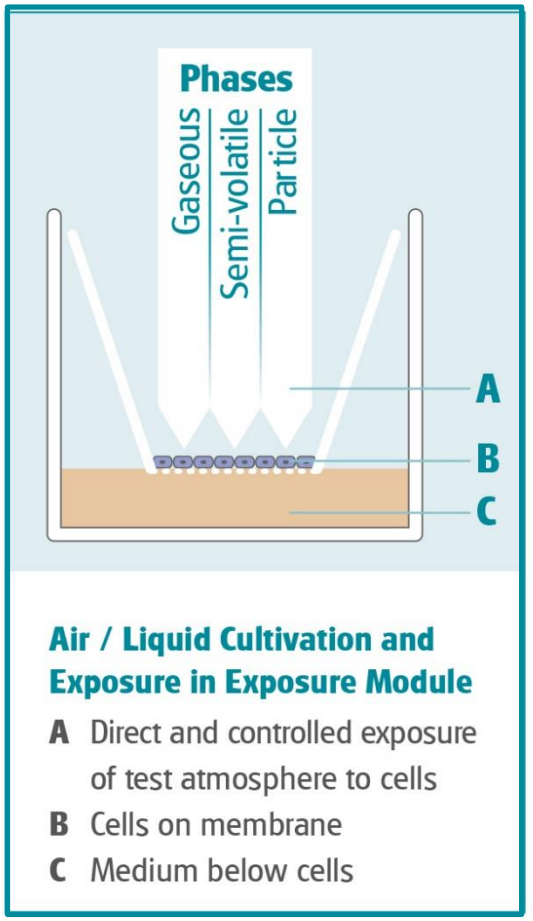
Low Sensitivity
Undefined Dose



Suspension Cultivation and Exposure in Incubator

A Cells in medium

Low Sensitivity
Undefined Dose



Air / Liquid Cultivation and Exposure in Exposure Module

A Direct and controlled exposure of test atmosphere to cells
B Cells on membrane
C Medium below cells

High Sensitivity
Defined Dose
Physiologically Relevant

How to test airborne substances *in vitro* at the air/liquid interface?

Gases

Environmental Atmospheres

Vehicle Emissions

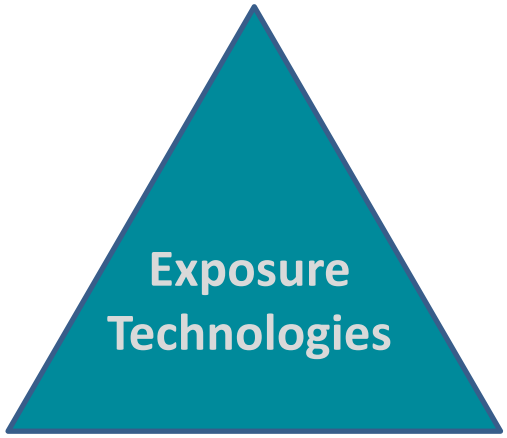
Cigarette Smoke & e-Cig Vapors

Nanomaterials

Medical Liquid Aerosols

Powders

Gases & Complex Mixtures



Liquid Aerosols

Dry Powders

Steps for ALI Exposure

- Provide the Test Substance
  - Aerosol generation (Lab)
  - Direct sampling (Environment)

→ Substance Characterisation

- Define Exposure Principle & Device
  > Continuous Flow
  > Single Droplet Sedimentation
  > Dry Powder

- Establish Dose / Response Range

- Dosimetry Tools
  - Gas phase
  - Particle phase

Critical System Elements for ALI Exposure

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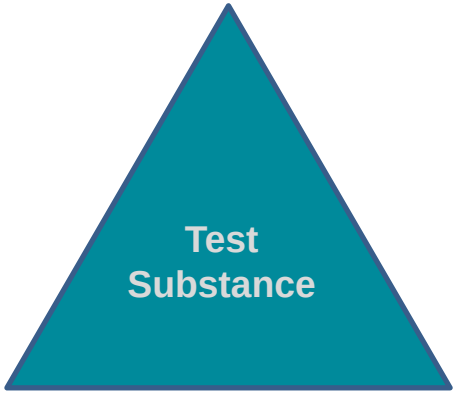
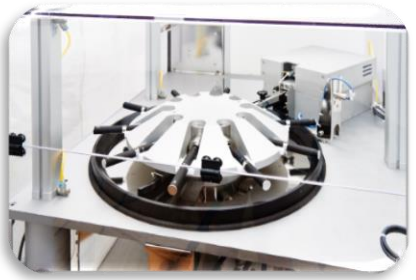
Providing the Test Substance > in the Lab

Gases & Complex Mixtures



Gas Cylinder



Smoking Robot

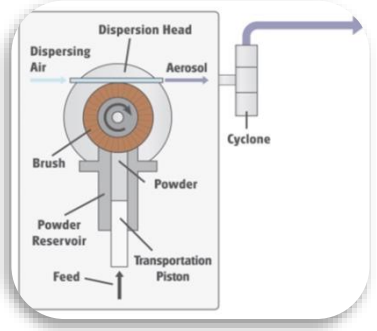
Automotive Engine

Liquid Aerosols

Dry Powders

Aerosol Generator

Cloud Aerosol Generation



Bulk Powder Aerosol Generation

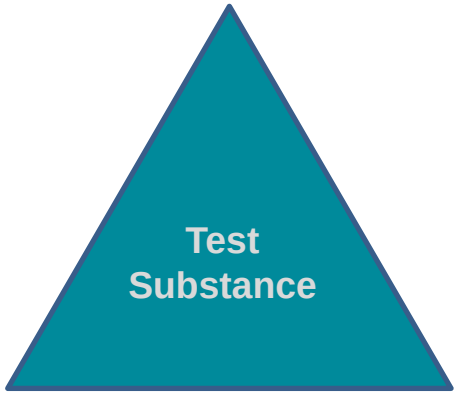
Small Quantity Powder Chamber

Providing the Test Substance > Sampling directly from Environment

Gases & Complex Mixtures

Mobile Test Lab

Automated Exposure Station
> Suitable for long-term exposures (24 h)



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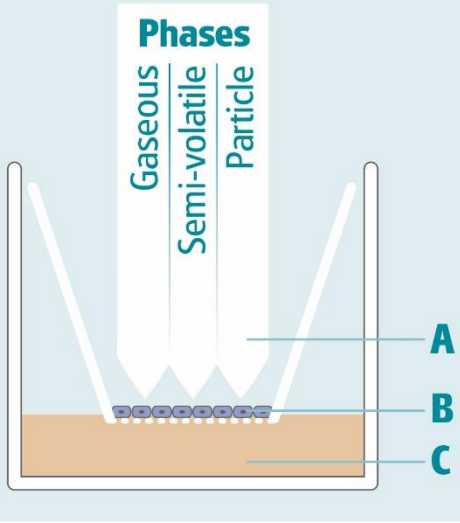
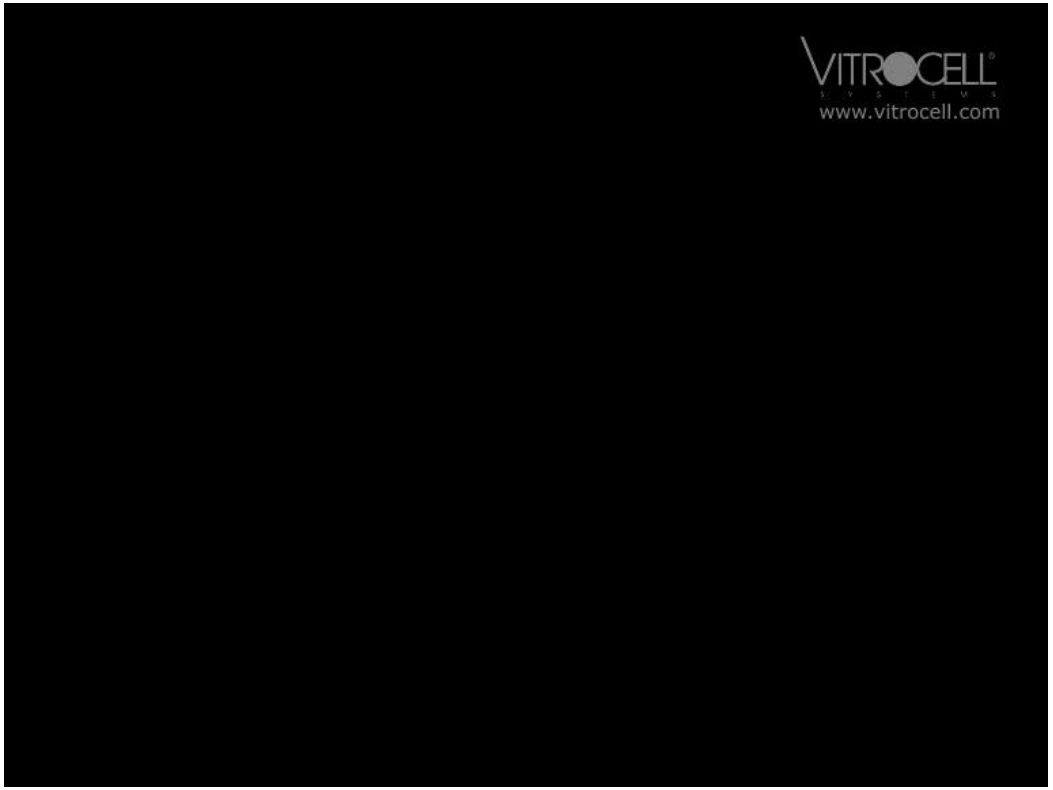
Exposure Principle 1: Continuous Flow



Air / Liquid Cultivation and Exposure in Exposure Module

A Direct and controlled exposure of test atmosphere to cells
B Cells on membrane
C Medium below cells

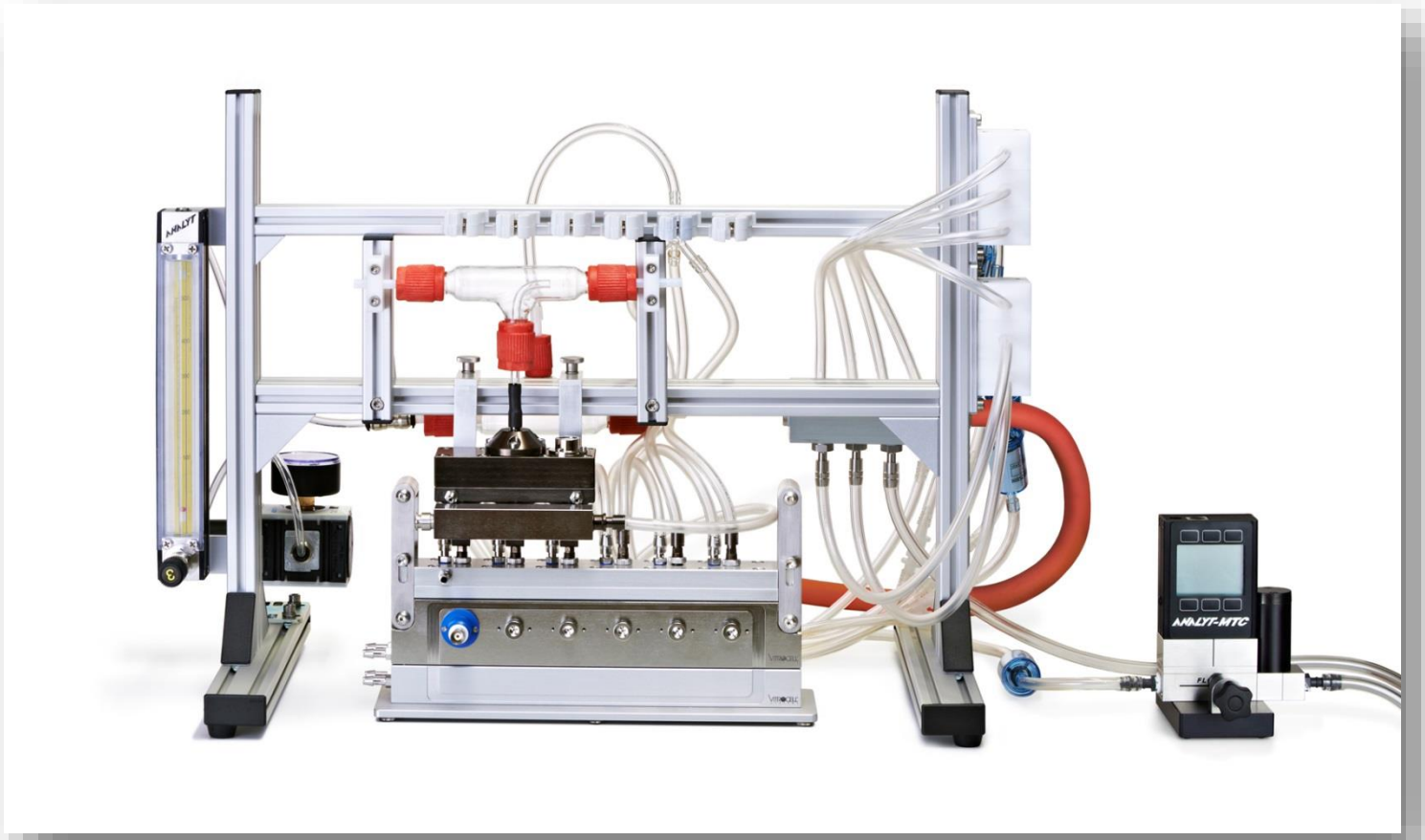
Air/Liquid Interface
High Sensitivity
Defined Dose



- Aerosol maintains reactivity of original compounds
- Physiologically relevant
- Possibility to integrate online dosimetry tools

Continuous Flow Device - VITROCELL® 12/6 CF

VITROCELL® 12/6 CF exposure module rack for 12- and 24- well sized inserts



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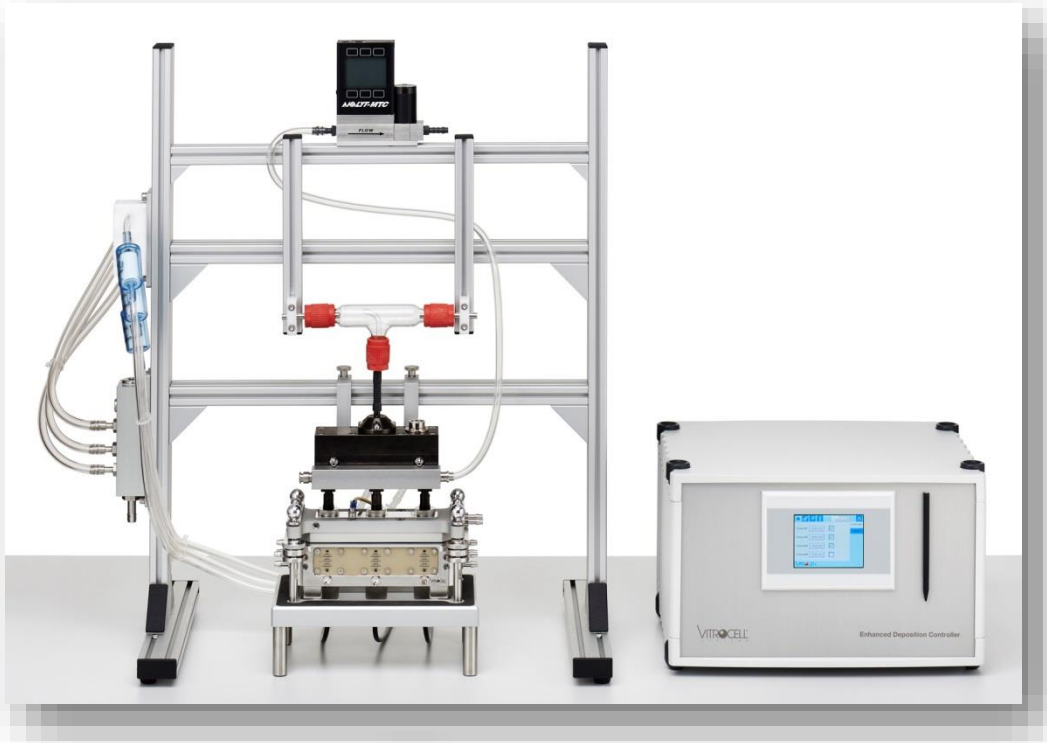
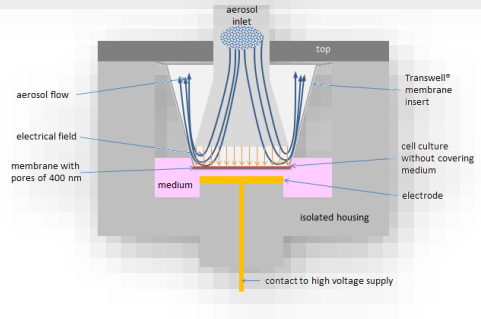
Continuous Flow Device - VITROCELL® 6/4 and 6/3

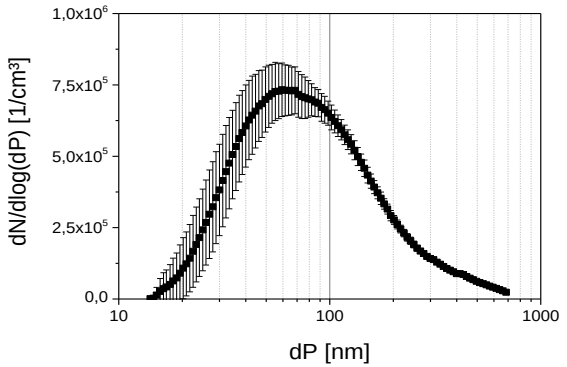
VITROCELL® 6/4 CF and 6/3 CF exposure modules for 6-, 12- and 24- well sized inserts

Optional Standalone Deposition Enhancement

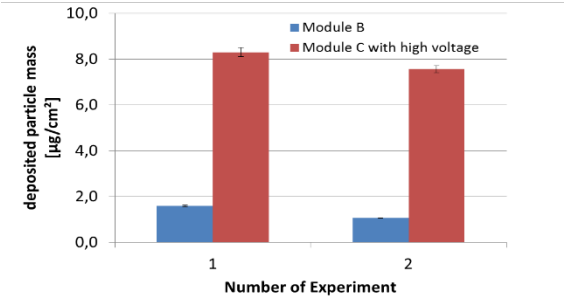




- Working by electrical field
- Approx. 6….8-fold increase of deposition



Number size distribution of fluorescein sodium in the ALI aerosol measured by SMPS (mean of 29), d_{modal} = 65 nm, s_g = 2.0



Data courtesy of KIT, Karlsruhe, Germany

Automated Exposure Stations

- Automated process control
- System is entirely heated (37°C)
- Integrated humidification of aerosol and clean air (85% r.H.)
- Aerosol flow control by mass flow controllers
- Online dose measurement (QCM)

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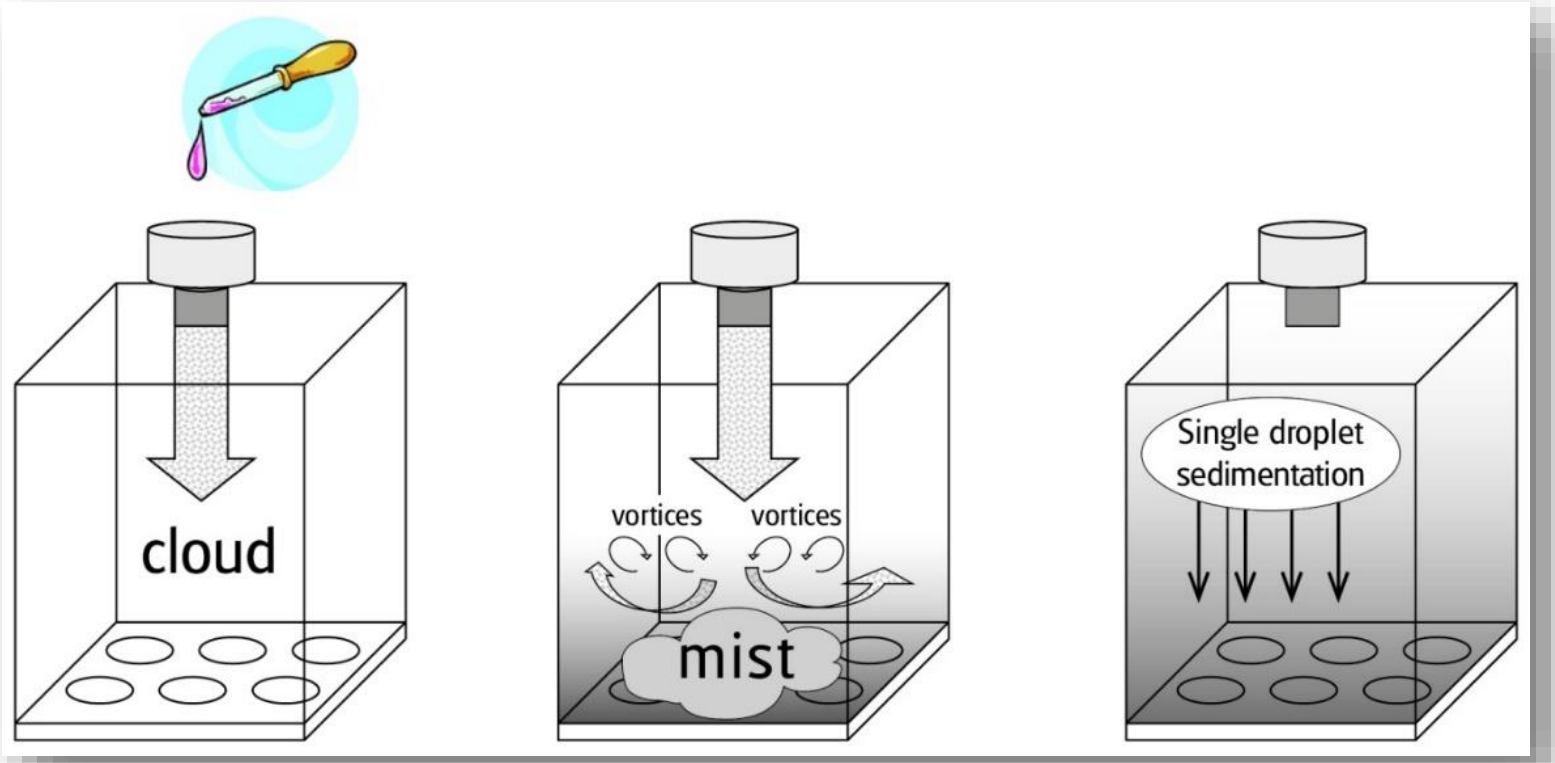
Exposure Principle 2:
Cloud Single Droplet Sedimentation

- Exposure systems for small quantities of liquid aerosols (200 µl)
- High droplet output rate – Cloud dynamics with high deposition efficiency
- Easy-to-use and clinically relevant



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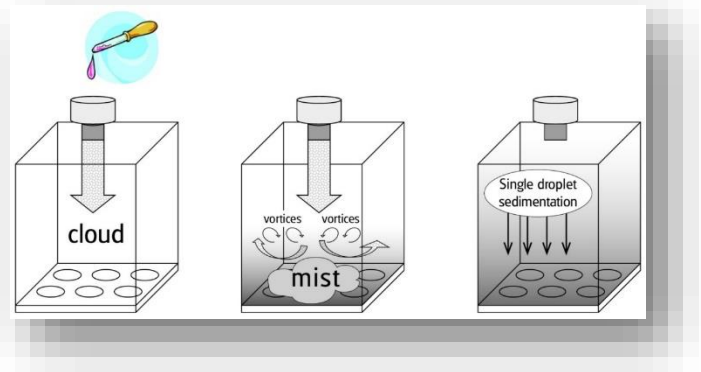
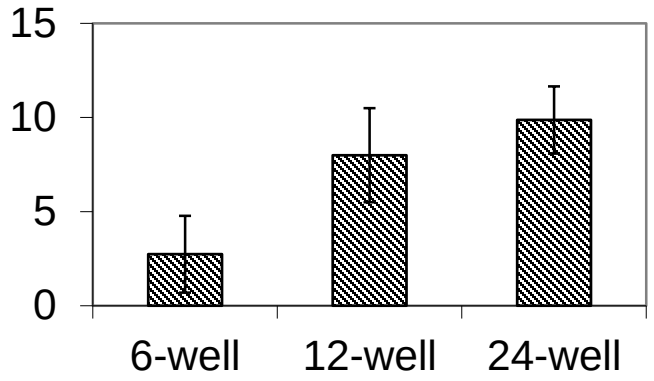
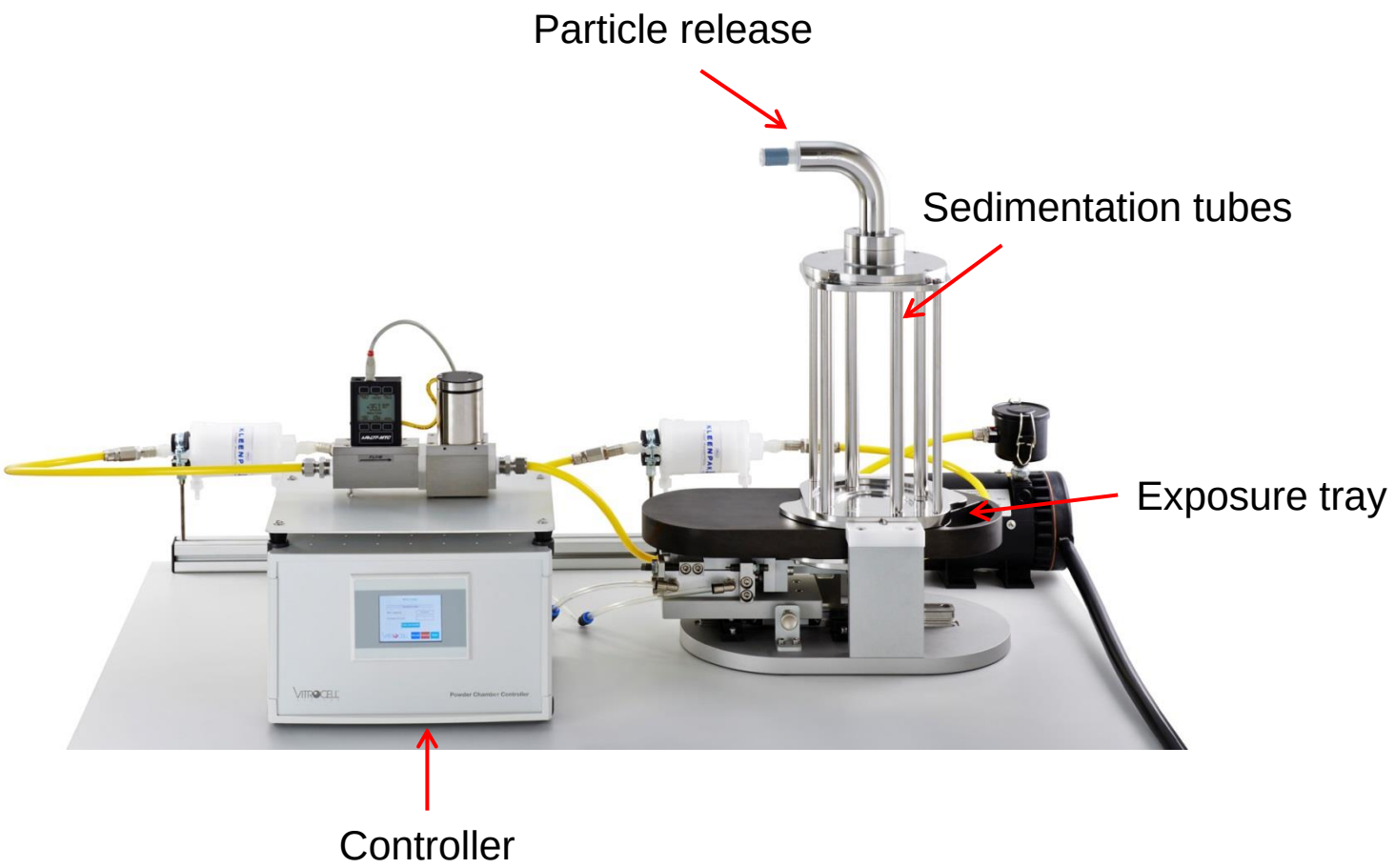


Versions for 6-, 12- and 24-well sized inserts

Well-to-well variability between inserts
Range 3 and 10% (1σ)



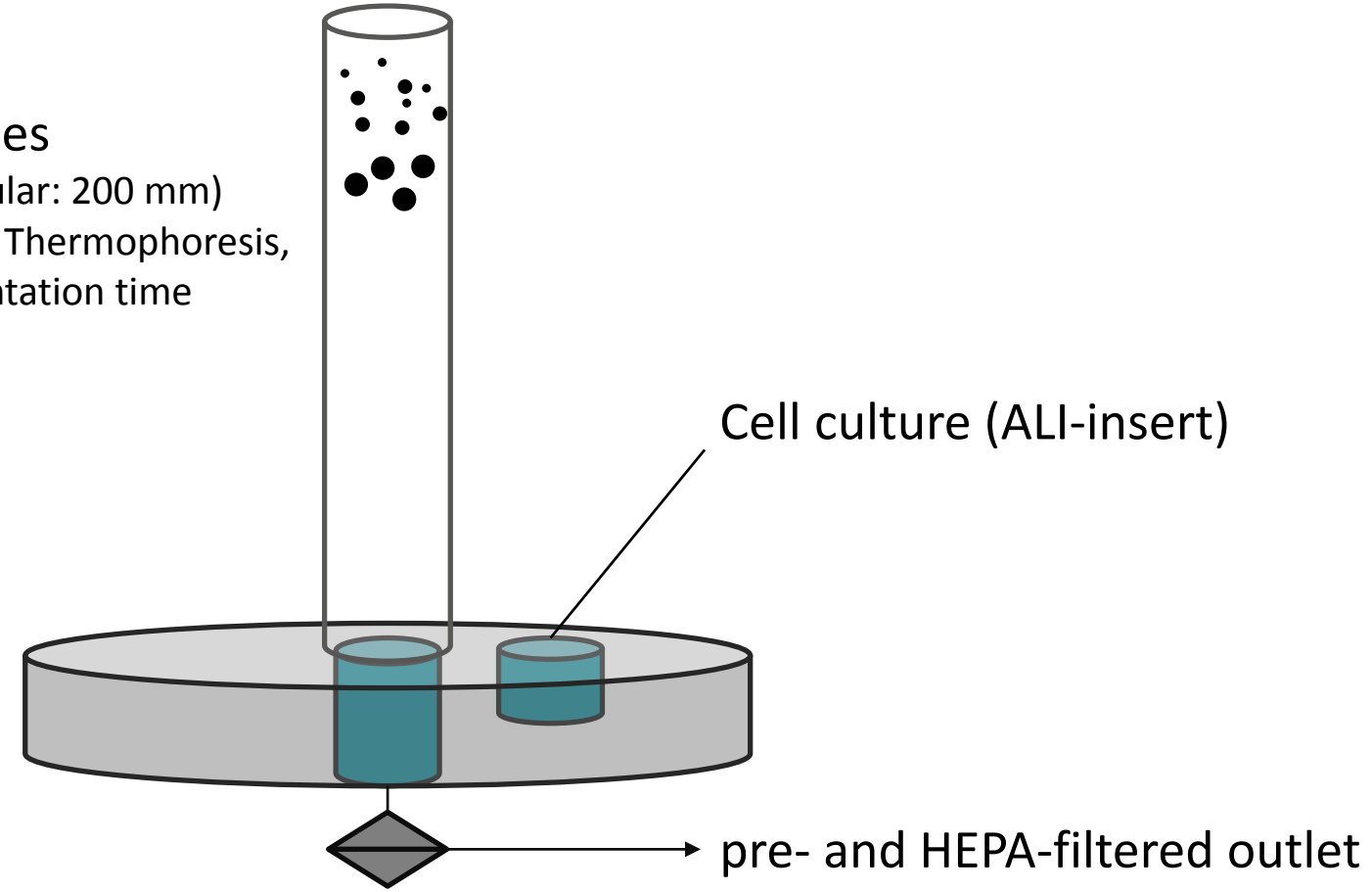
Exposure Principle 3:
Small Quantities of Dry Powder / Powder Chamber



Powder Chamber Process

Sedimentation Tubes

- Variable length (regular: 200 mm)
- Optional heating for Thermophoresis, to decrease sedimentation time



Exposure Tray

- Heated to 37 °C

Step 1: Powder Loading – usual mass of 20 mg per exposure

Substances / Preferred Exposure Principles

Substances	Exposure Principle / Module Type
 Gases	Continuous Flow  
 Environmental Atmospheres	Continuous Flow  
 Vehicle Emissions	Continuous Flow 
 Cigarette Smoke & e-Cig Vapors	Continuous Flow  
 Particles & Nanomaterials	Continuous Flow Cloud Powder Chamber   
 Medical Liquid Aerosols	Cloud 
 Powders	Continuous Flow Powder Chamber  

Critical System Elements for ALI Exposure

- Provide the Test Substance
  - Aerosol generation (Lab)
  - Direct sampling (Environment)

→ Substance Characterisation

- Define Exposure Principle & Device
  > Continuous Flow
  > Single Droplet Sedimentation
  > Dry Powder

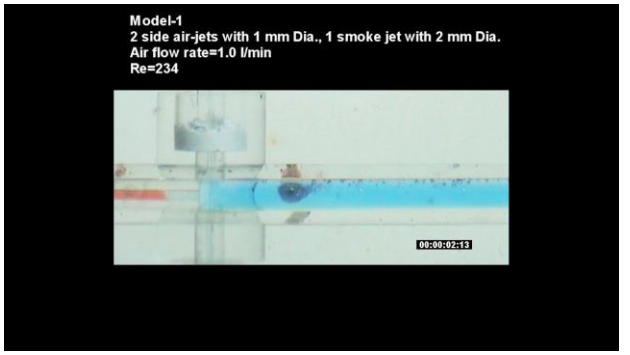
- Establish Dose / Response Range

- Dosimetry Tools
  - Gas phase
  - Particle phase

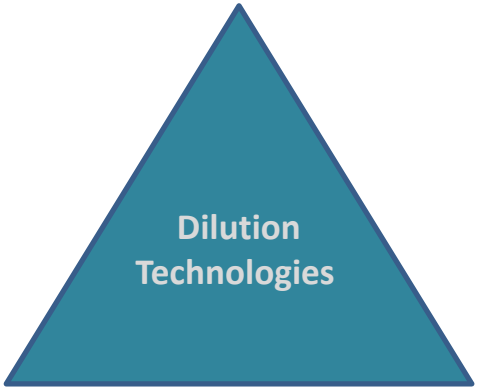
Dose/Response Relations

- Time-based exposure at same concentration

- High to low exposure concentration levels



Gases & Complex Mixtures → **Dilution Air**



Liquid Aerosols

→ **Dilution Air**

→ **Concentration in Liquid**

Dry Powders

→ **Dilution Air**

→ **Concentration Mix of Substance / Carrier Material**

Critical System Elements for ALI Exposure

- Provide the Test Substance
  - Aerosol generation (Lab)
  - Direct sampling (Environment)

→ Substance Characterisation

- Define Exposure Principle & Device
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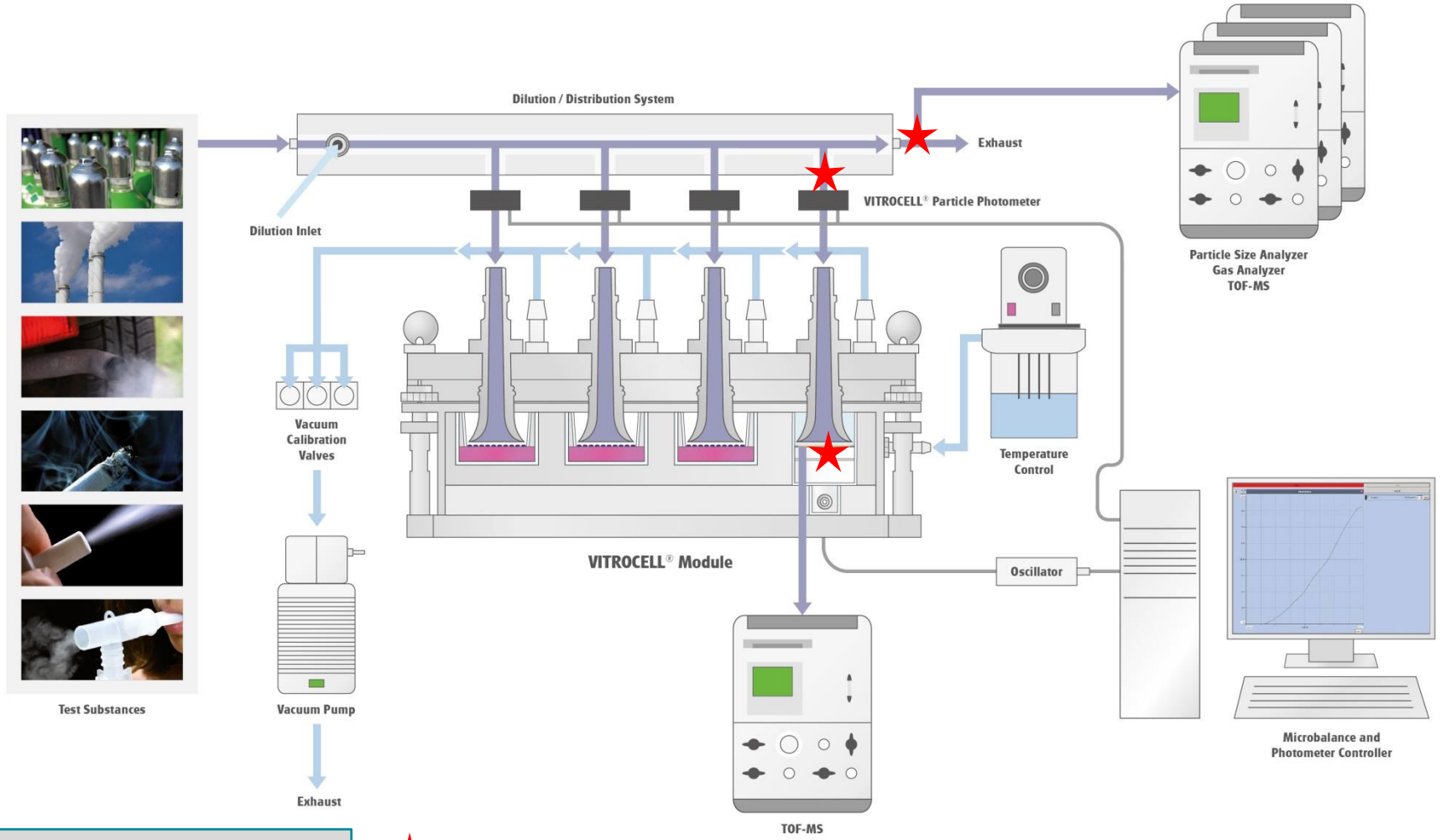
  - Particle phase

System Element: Online Dosimetry Tools

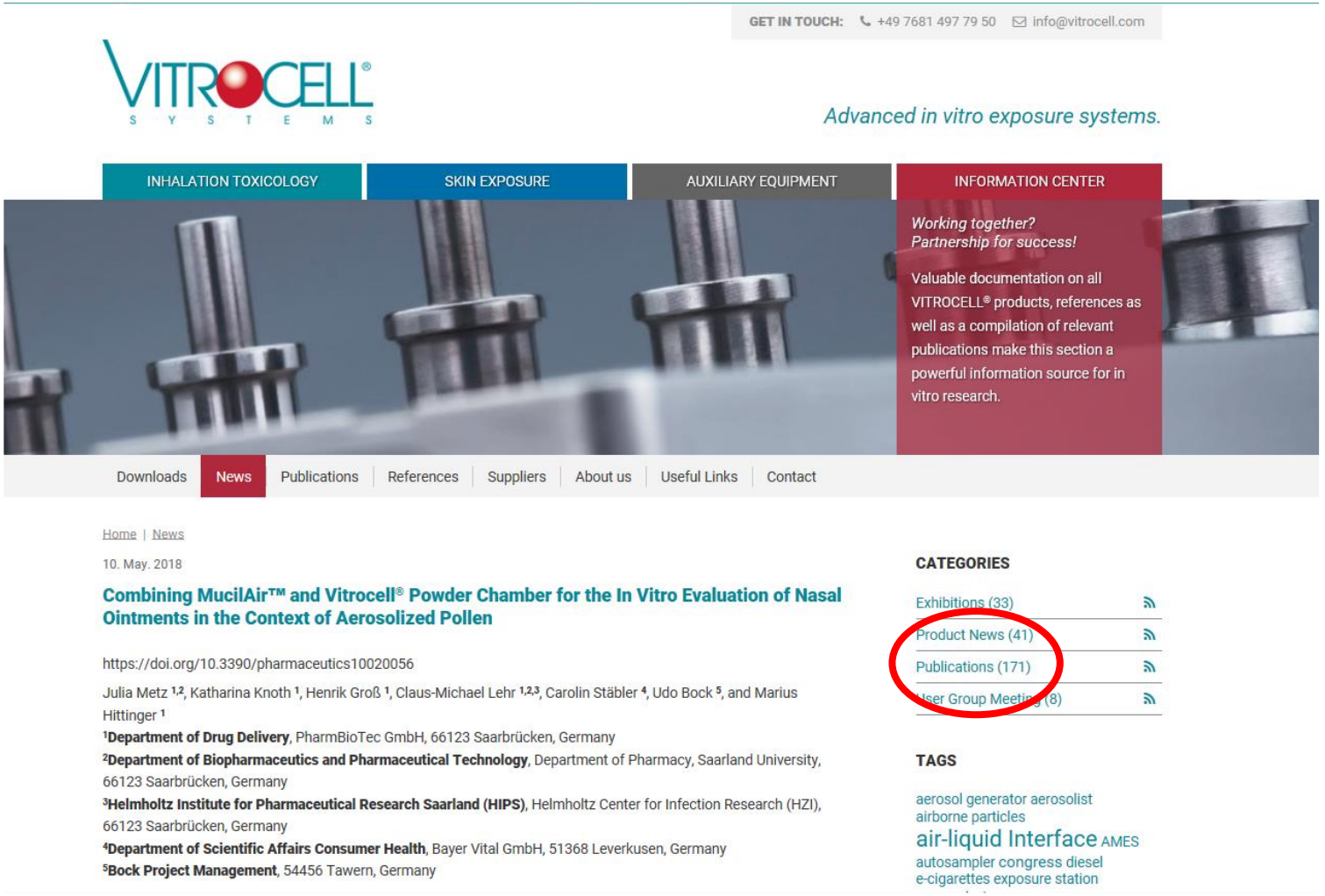


Online dosimetry hotspots ★

Dose Monitoring with Microbalance Technology

VITROCELL® Publications

Please visit our website vitrocell.com and the „Publications“ chapter with over 170 documents



Thank you for your attention !