The Cultex® Exposure Modules – a universal approach for studying atmospheres *in vitro*

30 years of experience in the development of *in vitro* exposure facilities
and
experimental lung cell research

Air-liquid interface exposure

Exposure of cellular-based systems for studying the toxicological effects of inhalable substances

Preconditions

Direct contact: cells – atmospheric compounds

Homogeneous distribution of the test atmosphere on the cell surface

Relevant epithelial cell systems of the respiratory tract

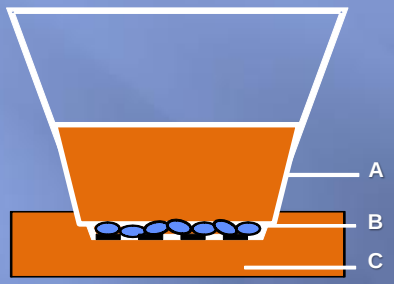
Implementation

Reliable exposure system characterized by:

- **A homogeneous distribution and deposition of inhalable compounds**
- **Maintenance of cell viability during exposure resulting in**
- **Stable and reproducible data**

Strategy

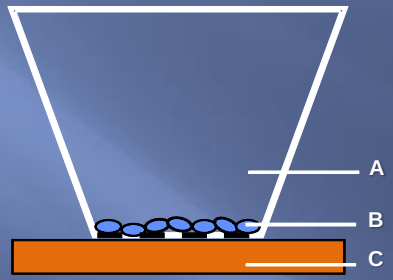
Cell cultivation and exposure



A Submerged culture conditions

B Cell growth of microporous membranes

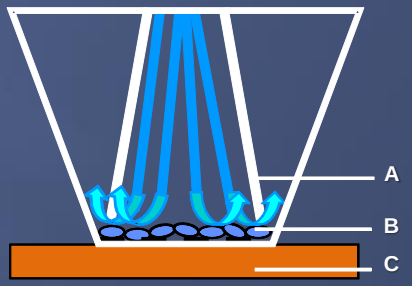
C Basolateral medium supply



A Incubator atmosphere

B Cell growth of microporous membranes

C Basolateral medium supply



A Test atmosphere (gas, aerosol)

B Cell growth of microporous membranes

C Basolateral medium supply

Submerged-culture (Incubator)	Air-lifted culture (Incubator)	Air-liquid interface (ALI) exposure (Exposure device)
Exposure to soluble compounds	Static exposure to airborne material	Dynamic exposure to airborne material

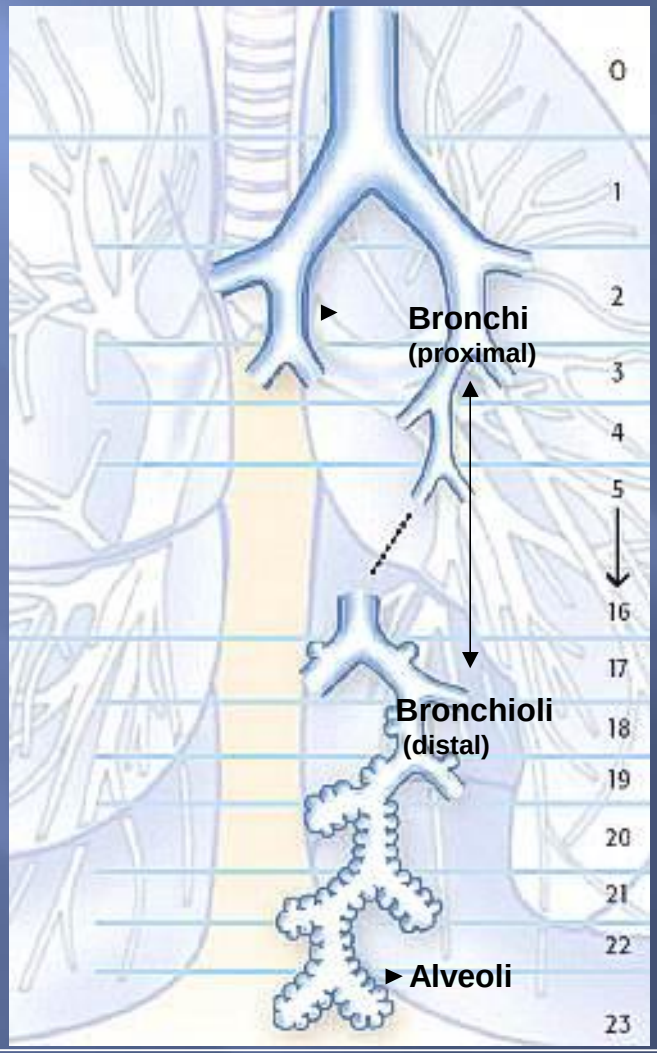
In vitro inhalation toxicology

Anatomic site

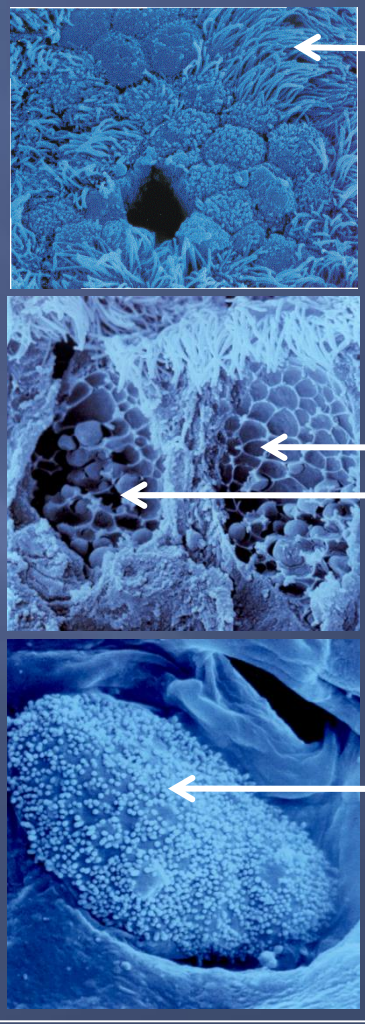
Bronchi
Ciliated and
Goblet cells
Basal cells
Progenitor cells

Bronchioli
Ciliated,
Goblet and
Club cells
Progenitor cells
(Stem cells?)

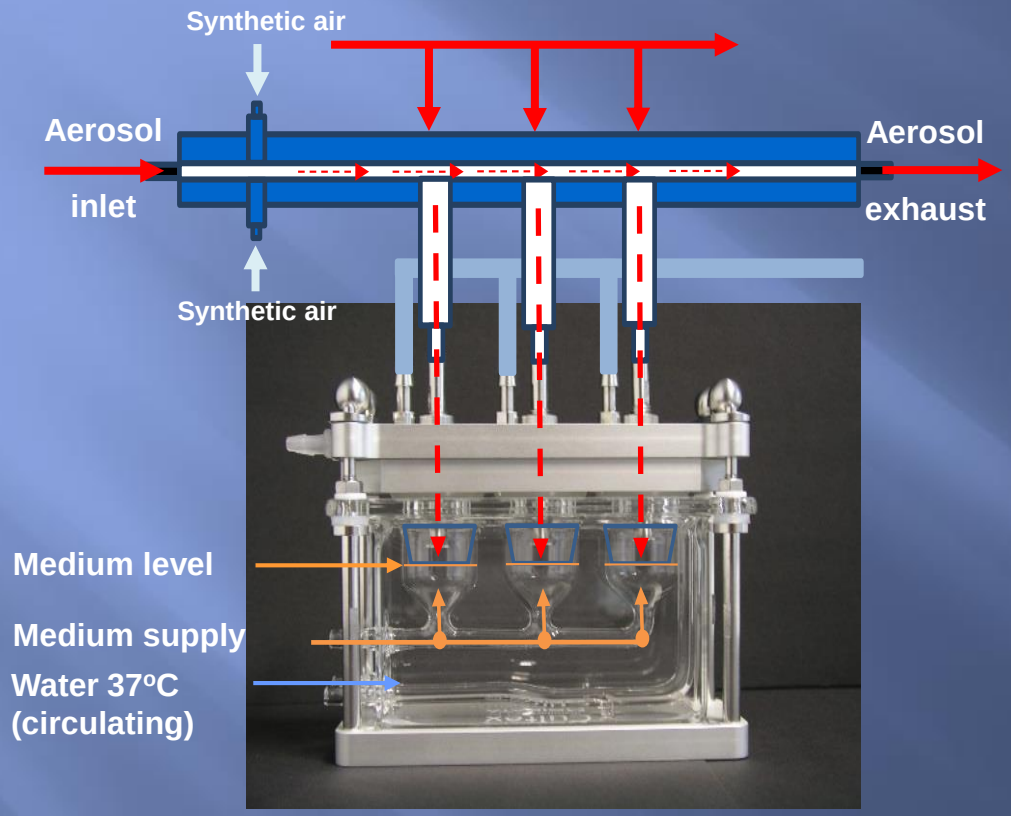
Alveoli
Type I and
Type II cells



Target cells e.g.



CULTEX® - patented ALI exposure systems



1st Generation of patented Cultex exposure modules
Linear aerosol distribution

General characteristics:

- Exposure of air-liquid cultures (ALI)
- Direct and continuous flow of the aerosol to the cells
- Medium supply and tempering (37°C)

Aerosol flow

- 3 sampling points from a
- Linear-guided aerosol flow

- Instability in particle distribution/deposition and thus reproducibility of results

CULTEX® RFS – ALI Exposure System

2nd Generation of Cultex exposure modules were optimized concerning the aspects:

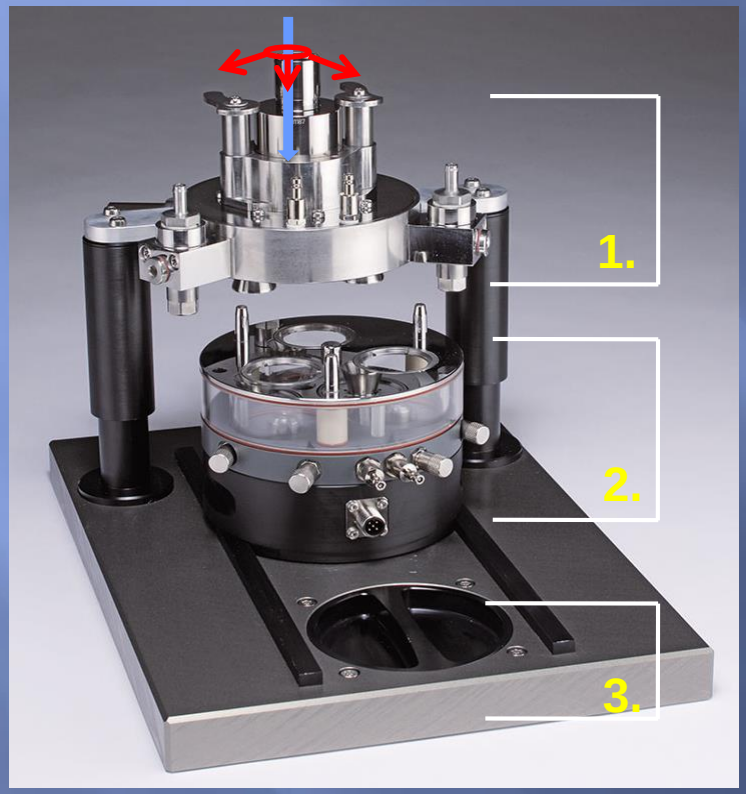
- Aerosol flow
- Flexibility
- Stability and
- Validity of the results

Resulting in the patented modular system:

CULTEX ® Radial Flow System (RFS)

CULTEX® RFS - Exposure System

Modular System



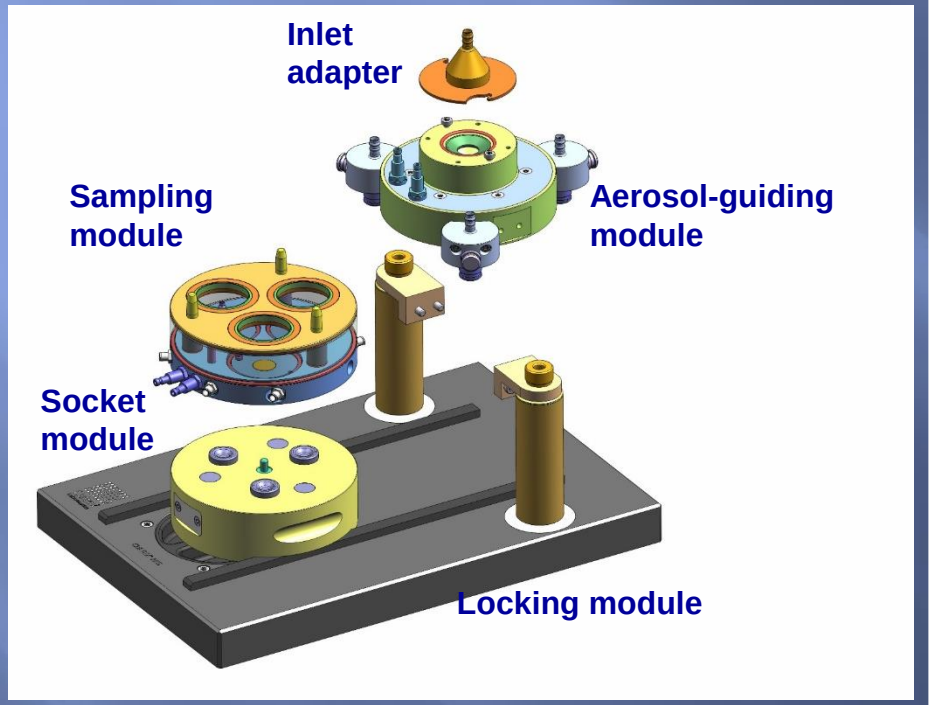
1. Aerosol-guiding module including Inlet adapter
2. Sampling module and basic module
3. Locking module

Aerosol flow

- with a central aerosol flow through the module
- one central sampling for all insert cultures
- homogenous & reproducible distribution and deposition of particles

CULTEX® RFS – ALI Exposure System

Flexibility



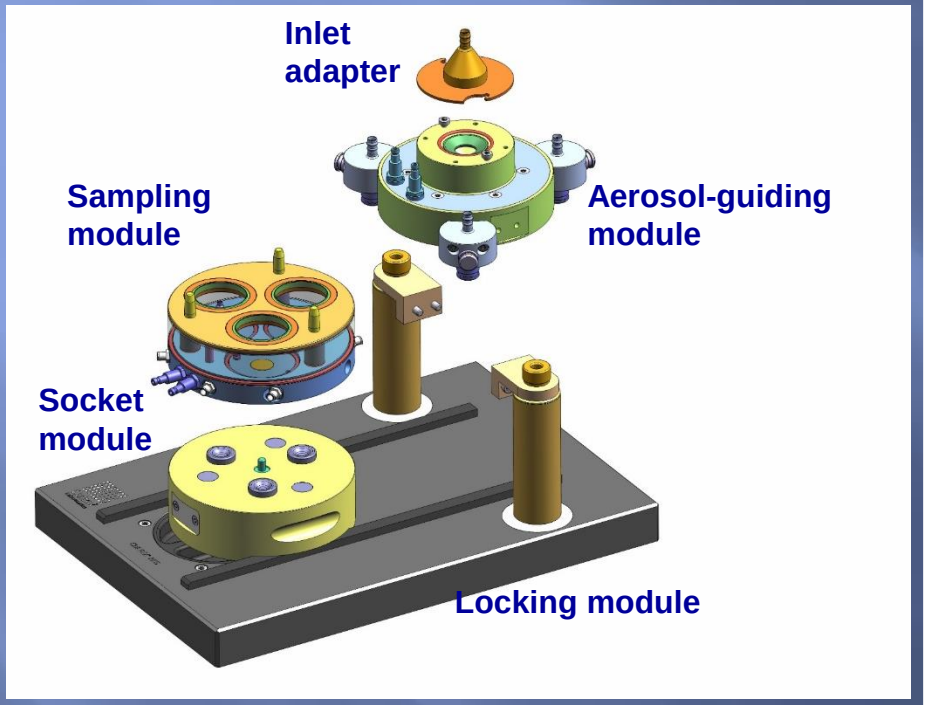
Aerosol-guiding module

- Patented nozzle into the inlet adapter (uniform distribution of particles)
- Integration of a charger for the electrostatic deposition (inlet adapter)
- Radial distribution of the aerosol from the sampling point
- Emission ducts for different insert types and size (6.5, 12 and 24mm including Petri dishes - Ames test)

- Connection to a variety of aerosol generation sources for
  - gases, volatile compounds, particles, complex mixtures
- Medical devices
- Spray devices
- In- and outdoor atmospheres
- Working place atmospheres

CULTEX® RFS – ALI Exposure System

Flexibility

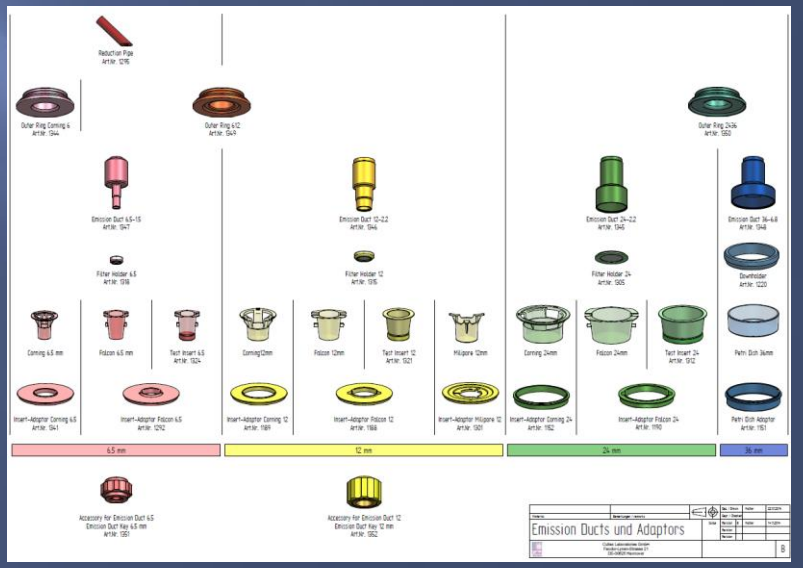


Aerosol-guiding module

- Condensate trap for humidified aerosols
- Integration of sensors for humidity and temperature

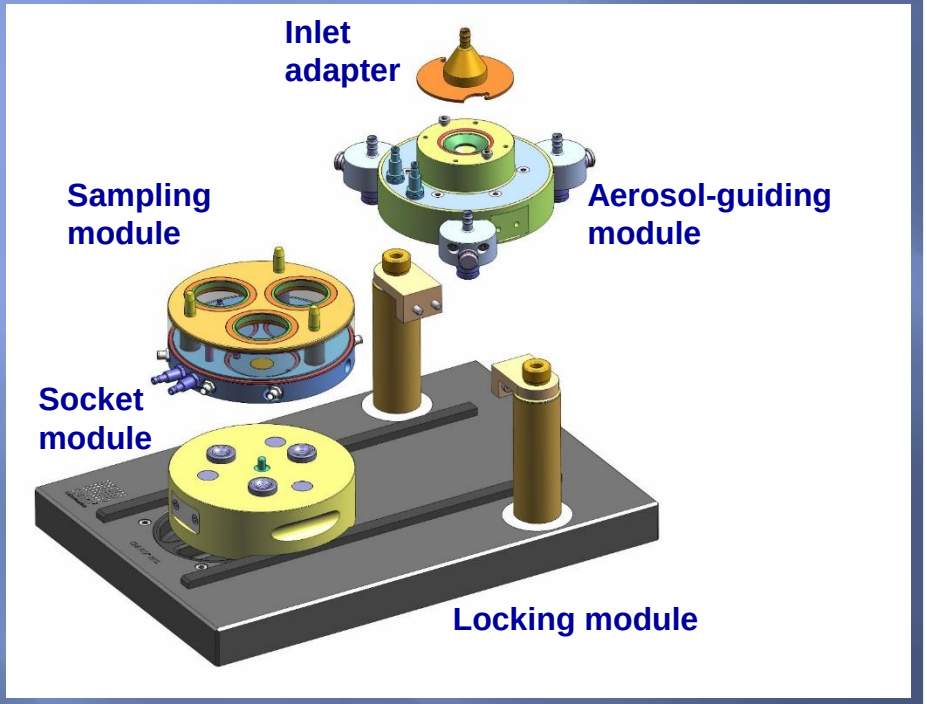
Sampling module

- Adjustment of the module to inserts of 6.5, 12 and 24mm and different types including Petri dishes (Ames test) by special adapters



CULTEX® RFS – ALI Exposure System

Flexibility



Sampling module

- Individual medium in- and outlets
- Precise medium level control
- Single medium exchange or continuous medium exchange
- Tempering of the module

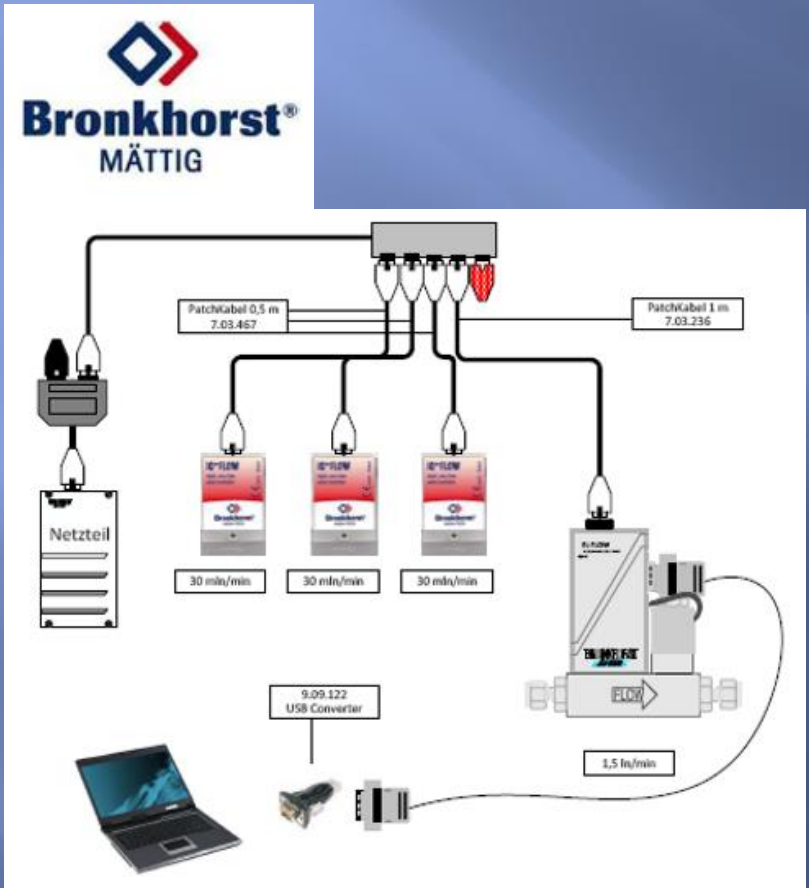
Socket module

- Integration of the precipitator for the electrostatic deposition device
- Guide module for exact positioning of the sampling module below the aerosol-guiding module

Locking module

- By simply turning the handwheel, the aerosol-guiding module is sealed tight with the sampling module (including socket module) or opened

CULTEX® RFS – ALI Exposure System



Stability

CULTEX® RFS

- A precision mechanical instrument made of materials tested for cell compatibility

Mass Flow Controller (MFC)

Computer-controlled aerosol flow according to

- air humidity and
- temperature

Characteristics of the MFCs:

- MFCs controlled by a control box, no manual readjustment
- Real-time monitoring of the parameters on the PC
- Short response time and high accuracy
- High stability and reliability
- Space-saving design

CULTEX® RFS – ALI Exposure System

Validity

Validation of the CULTEX® RFS procedure and optimization of a prediction model for the assessment of the acute inhalational toxicity of dusts

BMBF* Projects: 0315710
031A581 (project work completed 30.06.2018)

Bundeswehr Institute of Pharmacology and Toxicology, Munich , Germany

Walther Straub Institute of Pharmacology and Toxicology, University Munich, Germany

seh consulting + services, Paderborn, Germany

Cultex Laboratories GmbH, Hanover , Germany

* Federal Ministry of Education and Research

CULTEX® RFS – ALI Exposure System

Validity

Validation of the CULTEX® RFS procedure and optimization of a prediction model for the assessment of the acute inhalational toxicity of dusts

Test material: 20 chemicals (dust atmosphere) with relevant *in vivo* reference data
Test procedure: investigation of the coded compounds in 3 laboratories (13 of the 20 were tested in 3 independent experiments).

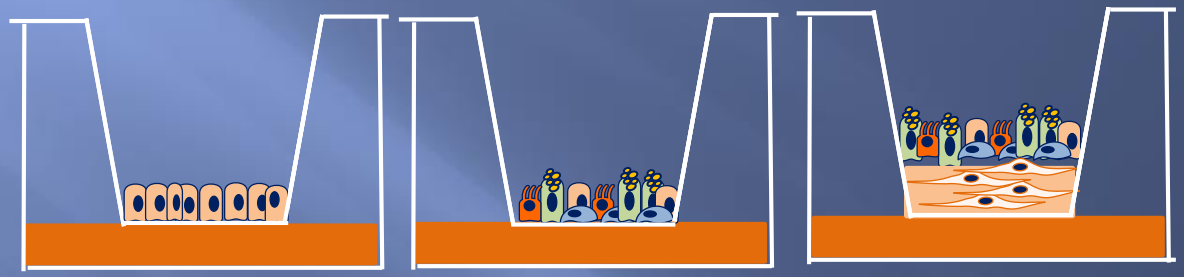
Intra-laboratory reproducibility: on average at least 92%
Inter-laboratory reproducibility: 90%
Overall concordance: 85%

The CULTEX® RFS procedure for the direct exposure of cultivated cells at the air-liquid interface is a

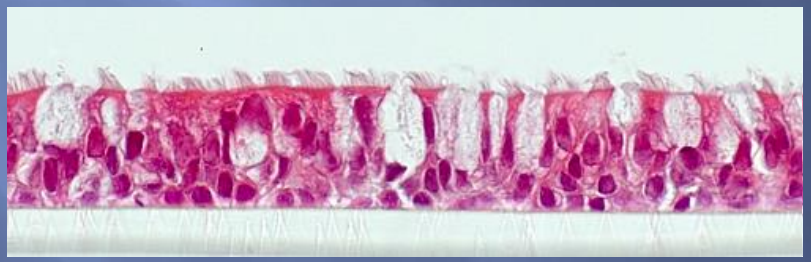
- **robust**
- **transferable**
- **reproducible and**
- **predictive test system for**
- **the acute inhalational toxicity of dusts**

Cell-based studies

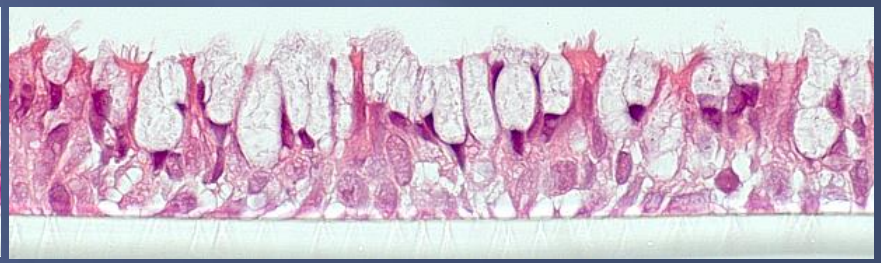
Mono- and Co-cultures



- **Permanent cell lines (tumor & immortalized cells)**
- **Primary cells:**
  - **Undifferentiated, predifferentiated and differentiated cells**



Primary normal bronchial epithelial cells (# 048 parent cells) differentiated at the air-liquid interface for 24 days.

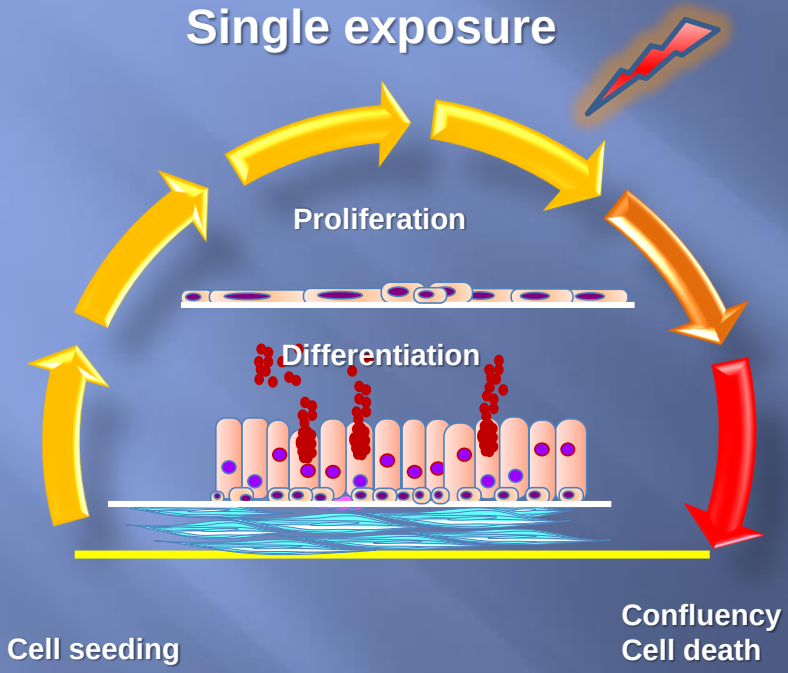


Transduced cells (CL-1548) derived from # 48 parent cells, differentiated at the air-liquid interface for 24 days.

Culture types

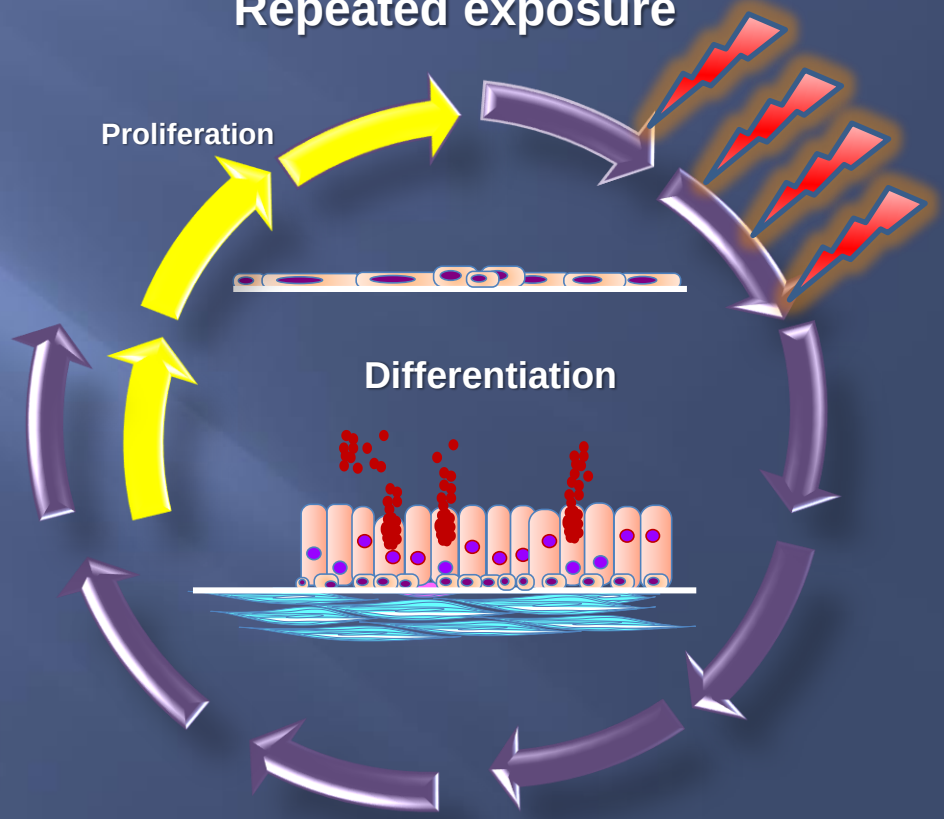
Short-term culture

Single exposure



Long-term culture

Repeated exposure

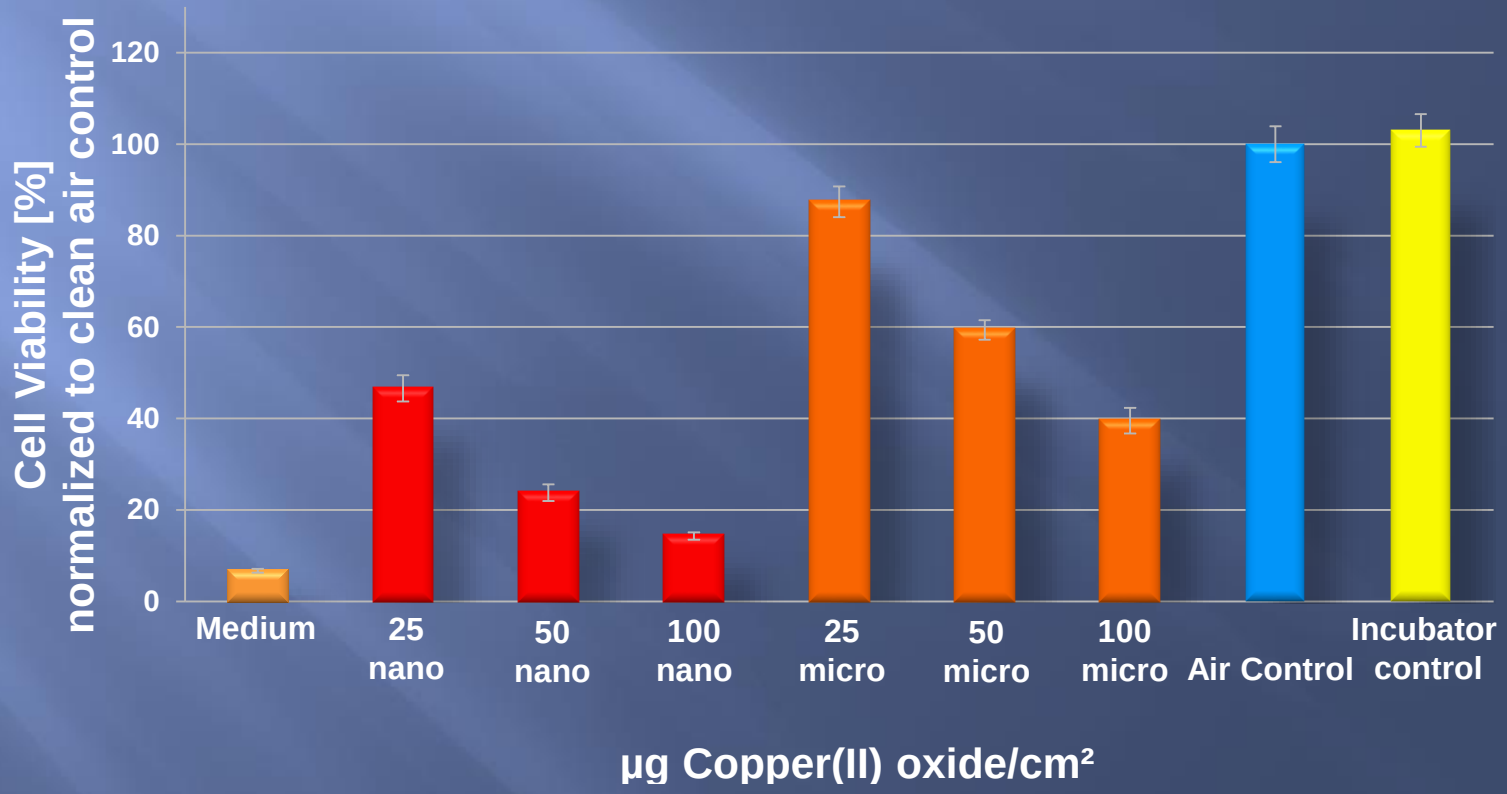


Acute toxicity:
Single exposure
Dose-response relationship

Subacute effects:
Persistent damage and phenotypical and functional modifications

Acute toxicity

Exposure of A549 cells to Copper(II) oxide:
Copper(II) oxide nano (40-80 nm)
Copper(II) oxide micro (5 µm)



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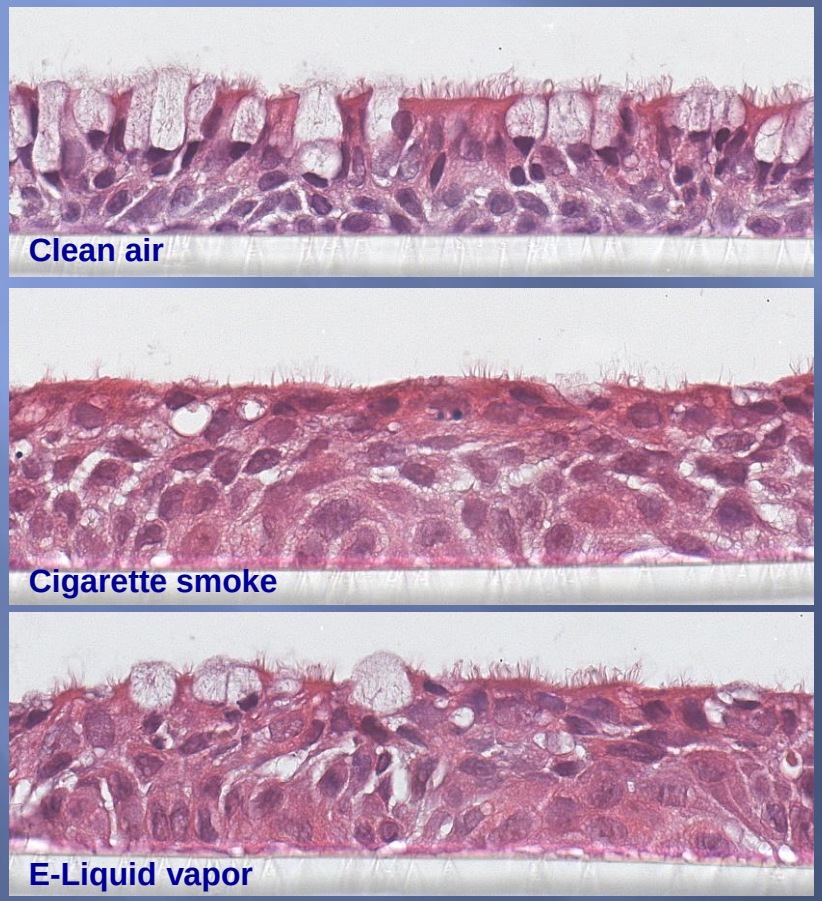
Subacute toxicity

Repeated exposure of an immortalized cell line CL-1548 NHBE cells to cigarette smoke (K3R4F) and e-liquid vapor without nicotine



Exposure conditions

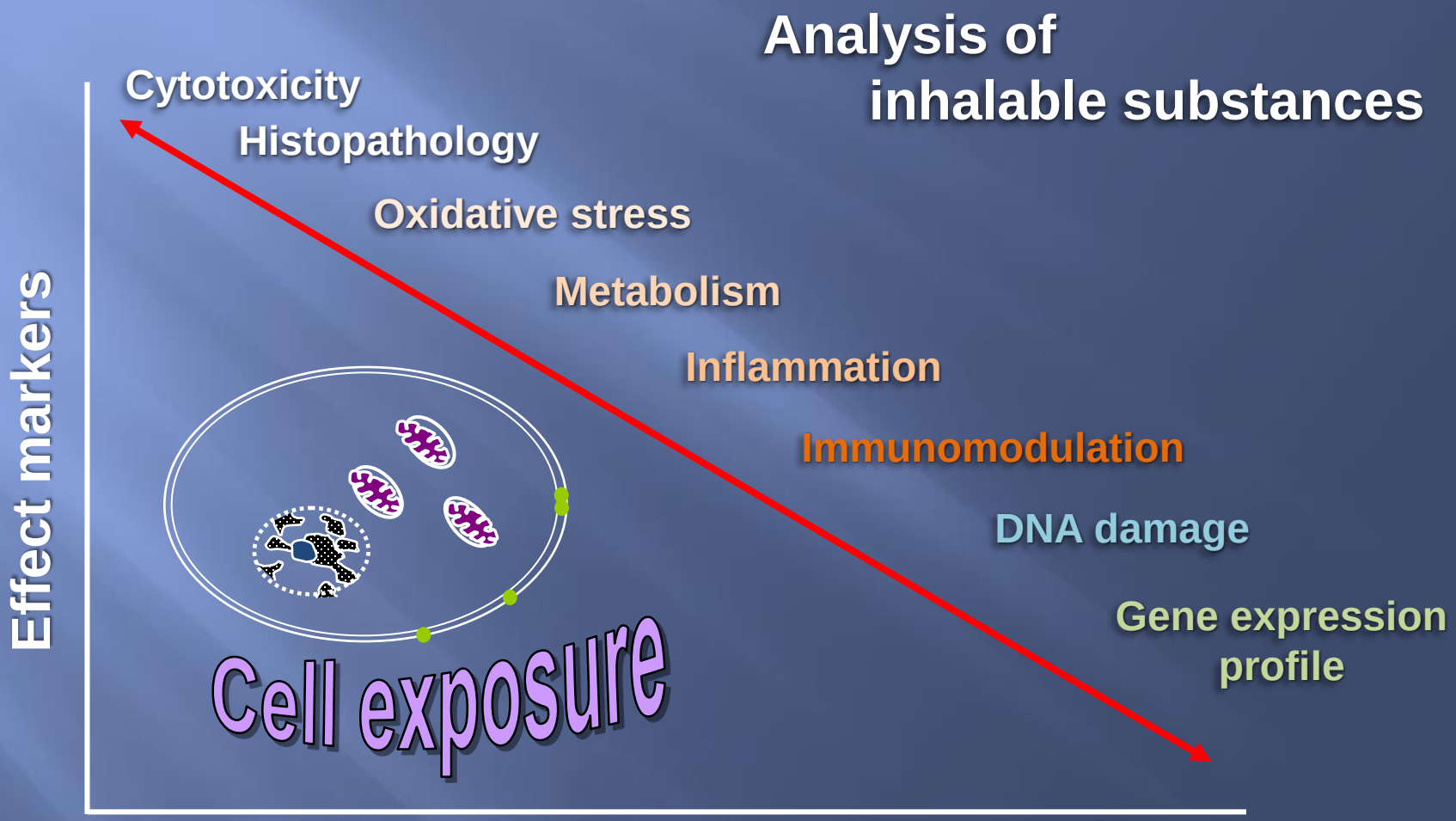
Exposure in the non-toxic range

- 4 K3R4F cigarettes/10 times
- 50 puffs e-liquid vapor /10 times

Toxicological Effects

- Cilia toxicity
  - Ciliary beat frequency
  - Loss of ciliated phenotype
  - Shortening of cilia length (inhibition of cilia growth)
- Reduction of mucus-producing cells

Biological effects



Summary

The Cultex® Exposure modules and method represent a universal approach for studying airborne atmospheres

1. Acute toxicity (single exposure to different test substance concentrations)

- **Screening of inhalable substances**
  - **Gases**
  - **Particles**
  - **Complex mixtures (e.g. combustion products)**
- **Calculation of dose-response relationships**

2. „Subacute“ effects by repeated exposure

- **Exposure in the non-toxic range to build up a permanent burden for the cells**
- **Modulation of cellular morphology and function – comparable with effects *in vivo***

Thank you for your attention

www.cultex-technology.com