NEW CONCEPTS IN INHALATION TOXICOLOGY: THE IN VITRO APPROACH

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In vitro test systems

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TOPICS

- Why ALI testing
  - Biological relevance
  - Methodological considerations
  - The solution: P.R.I.T.ExpoCube
- Acute in vitro inhalation toxicity testing
  - Volatile organic compounds (VOCs)
  - Pesticides applied as dry powder aerosol
- Prediction of systemic availability
  - Determination of Papp coefficients
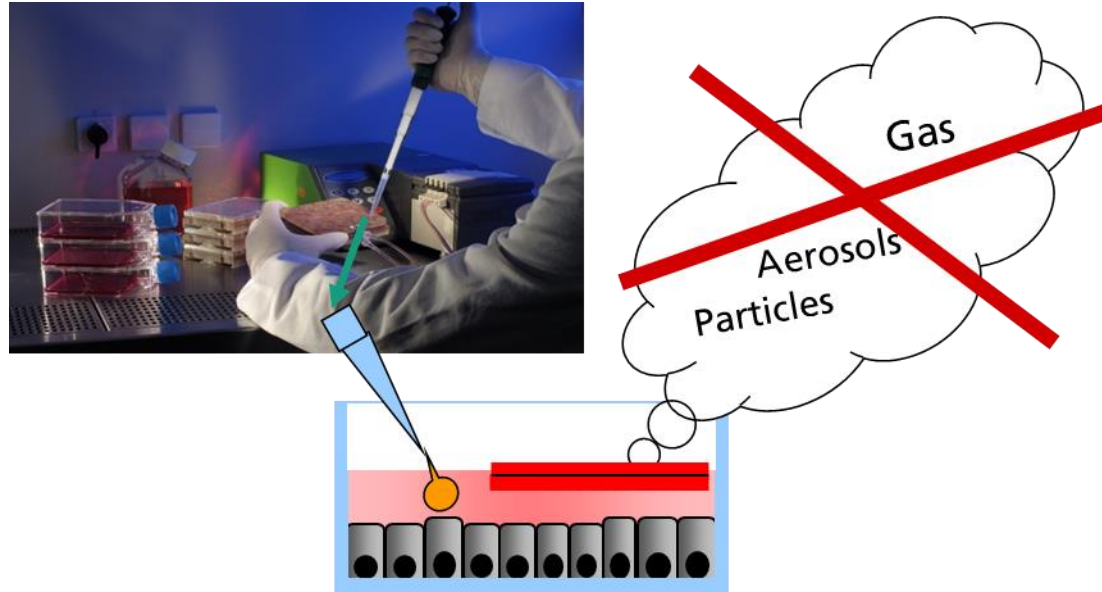 - PBPK modelling
- Summary and conclusion

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Why ALI?

In vitro barrier model "bridges" in vivo -> in vitro

inhalable compounds „air"

"blood" / liquid

Biological relevance: Best possible
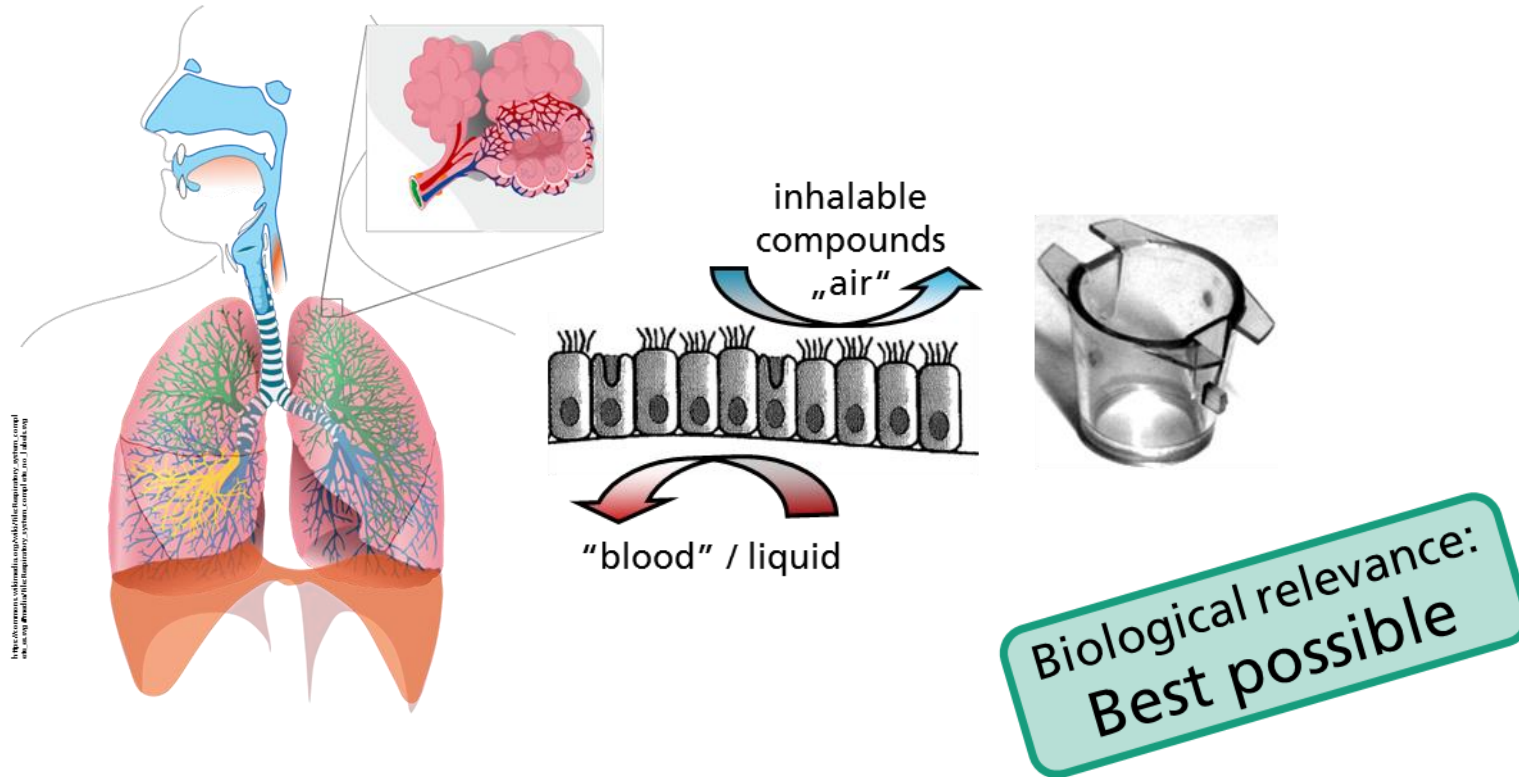
Why ALI?

Depending on the physicochemical properties, some chemicals/particles cannot be tested in submerse setting
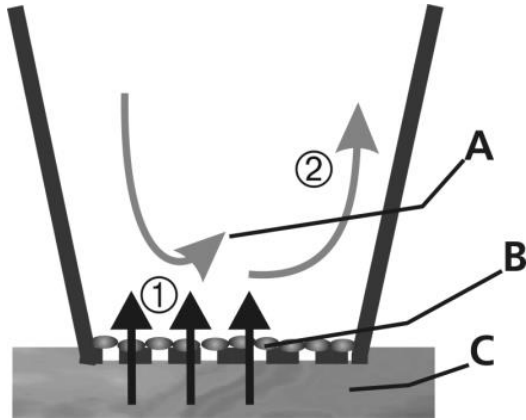

Challenges for submerse testing:
- Particle suspensions may be unstable (corona formation)
- Chemicals may be hydrophobic
- Volatility of the test chemical may cause dosing uncertainty
- Gases my be reactive

Basic ALI exposure conditions and considerations

ALI „microclimate"

A   exposure atmosphere (gas, aerosol)

B   ALI cell culture

C   culture medium

① mass transport (nutrition, humidification)

② fluid transport (evaporation, flow, stress)

Controlled by …cell-specific characteristics (cell-cell contact),…pore size and density,…culture media (osmolarity, viscosity),… pressures (liquid / air),… flow rate,… humidification of exposure atmosphere, …
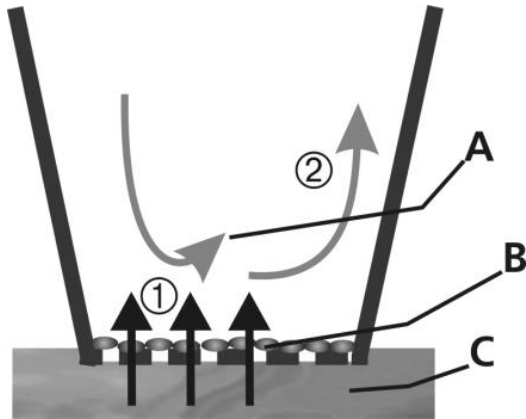
IN control

equilibrium, kinetics

① ≈ ②

- **high** exposure **efficiency**
- **good** cellular **viability**

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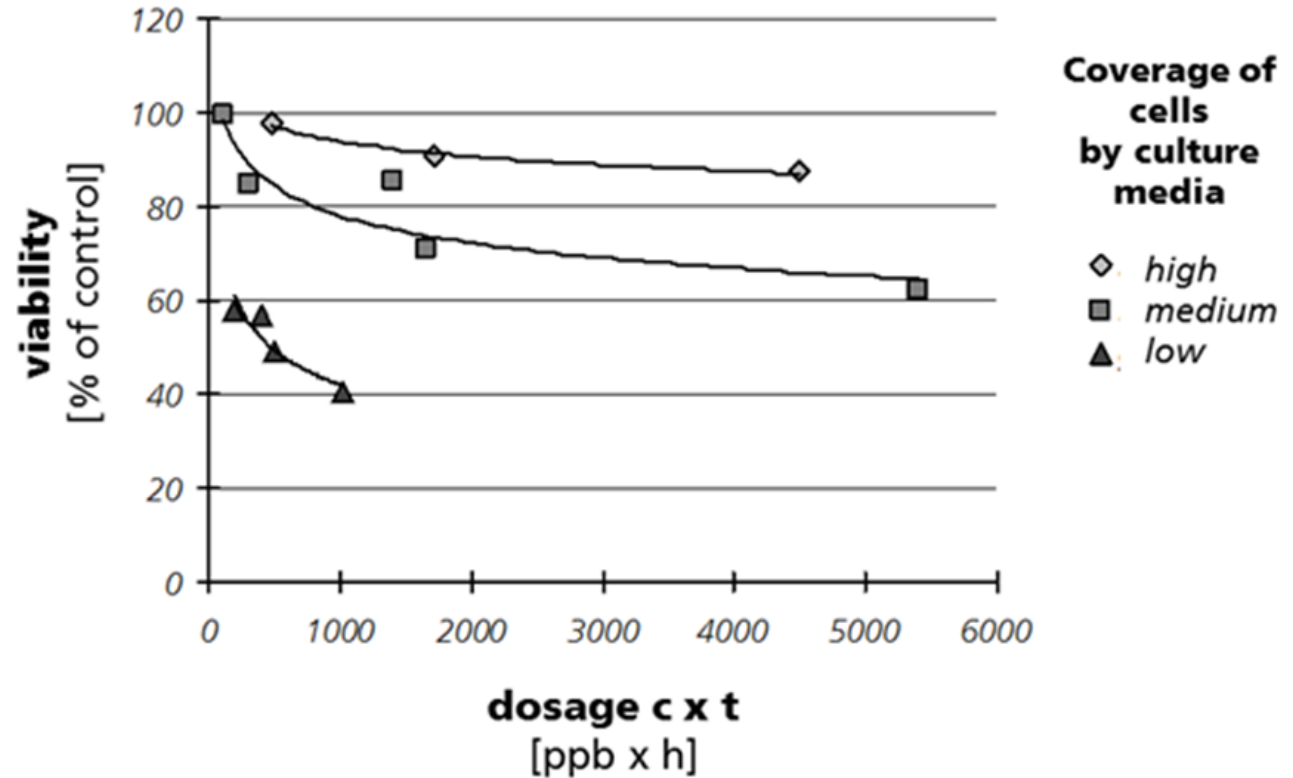# Basic ALI exposure conditions and considerations

ALI „microclimate"

① mass transport
(nutrition,
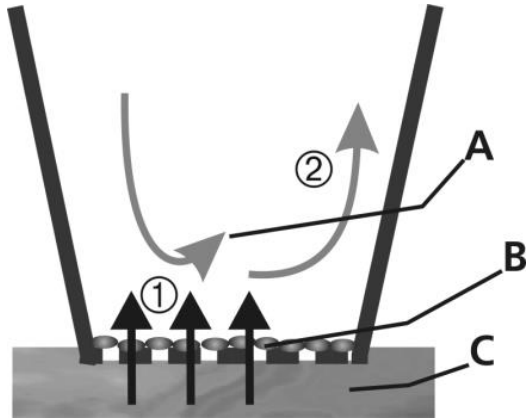humidification)

② fluid transport
(evaporation, flow,
stress)

Exposure to ozone
human lung fibroblasts

**Coverage of
cells
by culture
media**

◇ high
■ medium
▲ low

Ritter et al. (2001)

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Basic ALI exposure conditions and considerations

ALI „microclimate"

① mass transport
(nutrition,
humidification)

② fluid transport
(evaporation, flow,
stress)

IN control
equilibrium, kinetics

① ≈ ②

- **high** exposure **efficiency**
- **good** cellular **viability**

OUT OF CONTROL

① >> ②
LACK of sensitivity

① << ②
Cell death

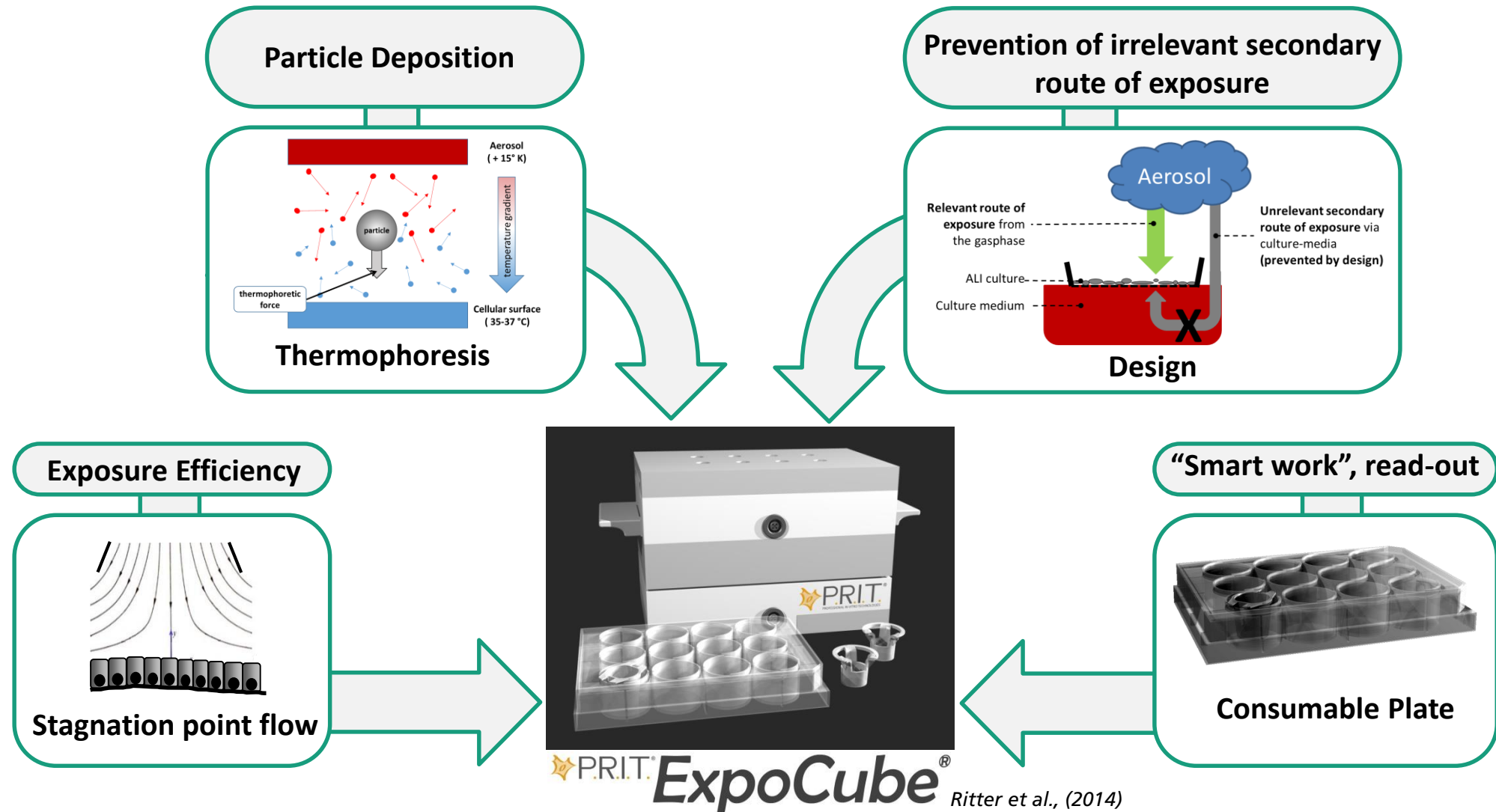**Systems need to be defined
for cell-/setup- specific conditions**

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The solution

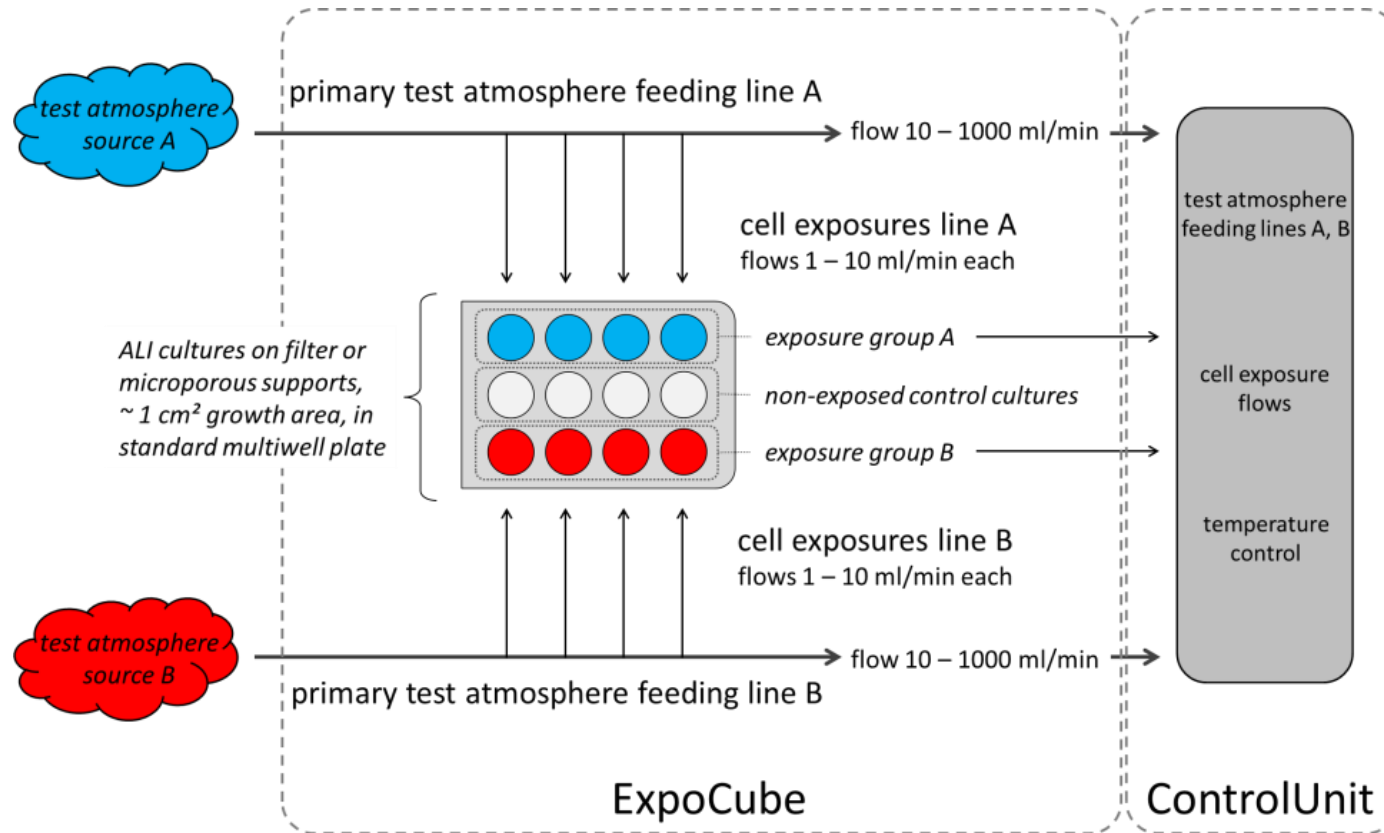The P.R.I.T.® ExpoCube® as an optimized approach in inhalation testing in vitro

Particle Deposition

Thermophoresis

Prevention of irrelevant secondary route of exposure

Design

Exposure Efficiency

Stagnation point flow

"Smart work", read-out

Consumable Plate

P.R.I.T. ExpoCube® *Ritter et al., (2014)*

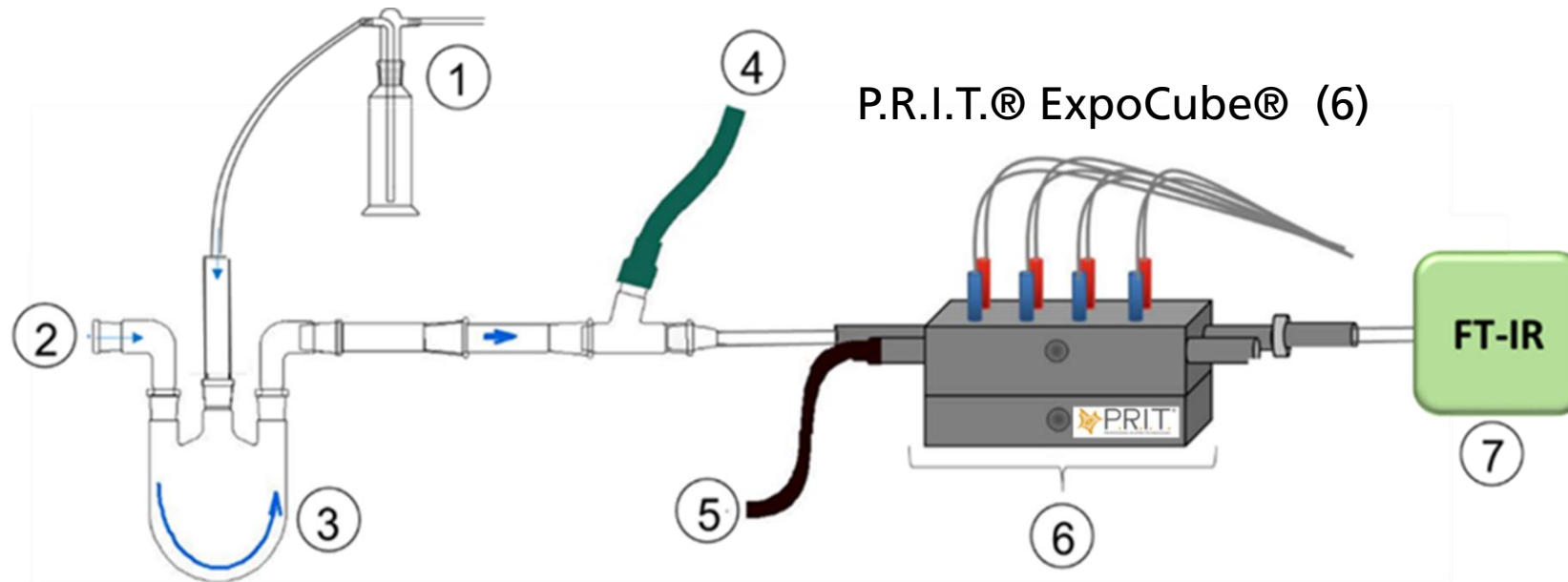Fraunhofer ITEM

- ALI cell cultures exposed in commercial standard plates
  - no change of culture medium / cell environment before/after exposure
- Different test groups in one plate

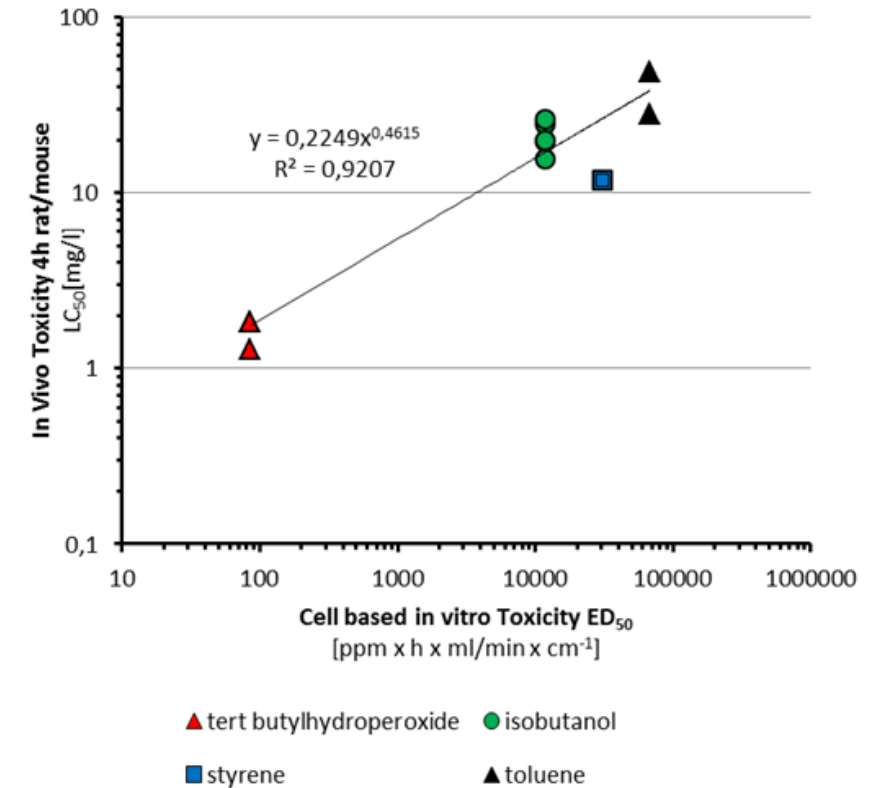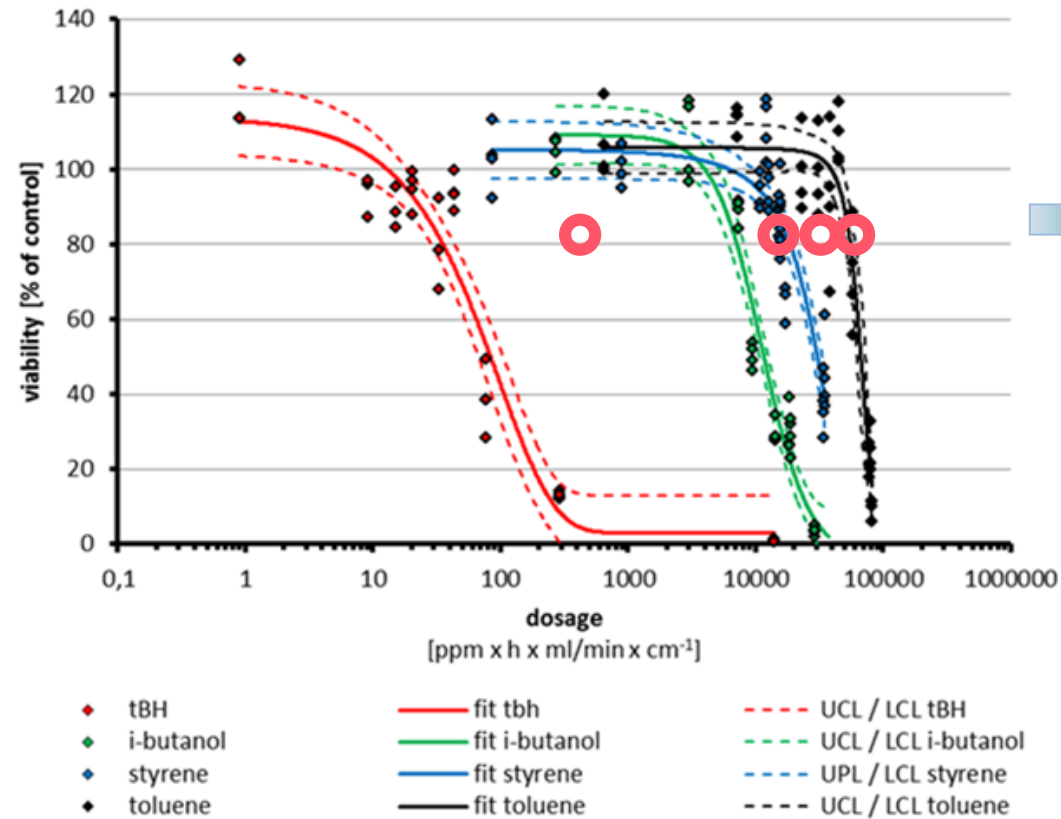# Experimental setup for gases/vapors

P.R.I.T.® ExpoCube® (6)

FT-IR

Generation by evaporation (1-4),
clean air control (5)

Online analysis by
FT-IR spectrometry (7)

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Relevance – *in vitro-in vivo* correlations for gases/vapors

Acute inhalation toxicity

Basic dosing considerations for ALI experiments with aerosols/particles

- Particle size distribution of the aerosols has to be known

  - Deposition is dependent on particle size (MMAD)

  - Physical forces: sedimentation + impaction (3 – 10 µm),
    sedimentation + diffusion (< 3 µm)

  - In vitro deposition rates for particles < 1µm are in the range of 1 -2 %

    ⇨ *Long exposure times needed to deposit a certain dose*

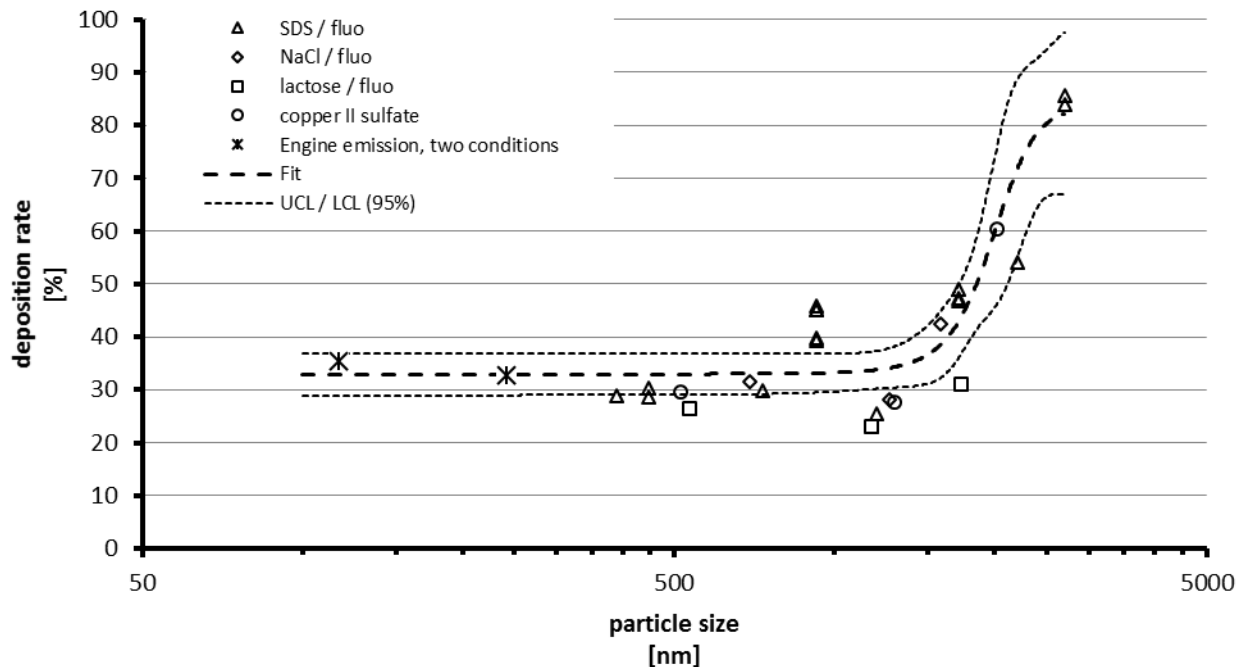Thermophoresis effect for efficient particle deposition from aerosols
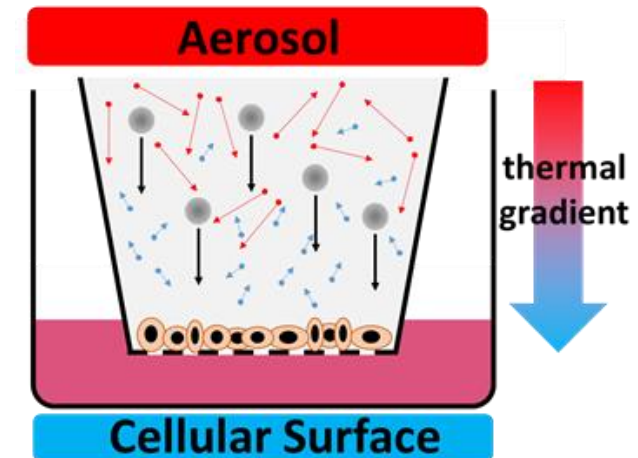
METHOD: In vitro dosimetry

- Test materials:

  - Dry particle aerosols from droplet aerosol generation
  - Engine exhausts
  - Dry particle aerosols from dust aerosol generation

- Methods:
  - CFD-Simulations
  - Fluorescence methods (tracing)
  - Analytical chemistry
  - Piezo balance

Characterization of size dependent particle deposition in ExpoCube® using thermophoresis conditions

"Deposition Rate"

$$DR\ [\%] = \frac{mass\ deposited\ on\ cells\,[\mu g]}{mass\ conducted\ over\ cells\,[\mu g]} * 100$$

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Aim of the study: Acute Inhalation Toxicity of Chemicals

- Acute inhalation toxicity (OECD Test Guideline 403)

- Group of materials: crop protection agents

  - Highly, poorly or non-solvable in water

  - Small amounts of testing material available (< 1g)

  - Short testing periods

- Relevant exposure scenario for inhalation

- Characterization of the relevance of results

- "Alternative" (3R-principle) testing approach to animal in vivo inhalation experimentation

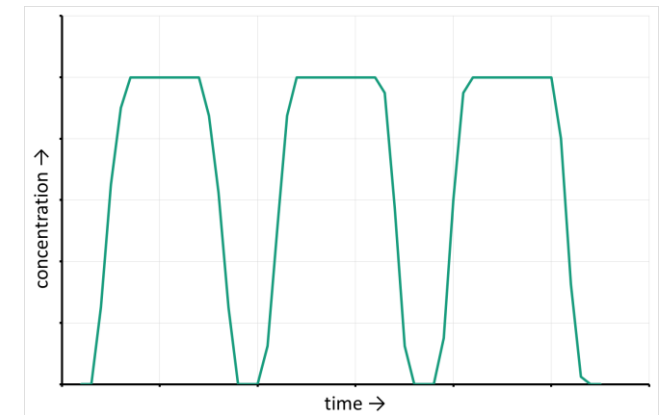# METHOD: Dry-particle aerosol generation from powders

PreciseInhale®
(Inhalation Sciences AB, Novum, SE)

Gerde, P. 1999a. Dust gun—Aerosol generator and generation.
U.S. Patent 6,003,512.
www.preciseinhale.com

- Disaggregation of powder material by application of high pressure

- Highly concentrated aerosol generation (up to 25 mg/l)

  - Aerosol generation in "shots" during 100s periods
  - Particle size distributions by impactor
  - Dosages were analyzed by light scattering photometer / filter

- Definition of exposure dose by

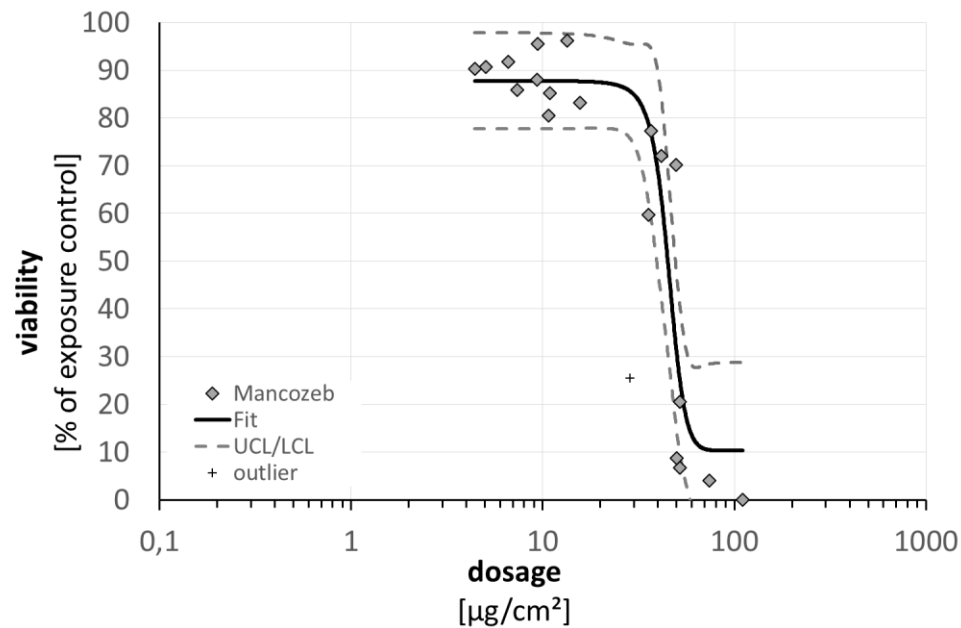 - Aerosol concentration (mass per "shot")
  - Number of shots

➜ Discontinuous aerosol exposure
(air -> aerosol -> air -> aerosol …)

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■ Mancozeb

■ Chlorothalonil

■ ED_{50}: 45.02 µg/cm² *(545 mg consumption)*   ■ ED_{50}: 6.76 µg/cm² *(256 mg consumption)*

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In vitro testing of test materials – Crop Agents   Results

■ Fosetyl-AL
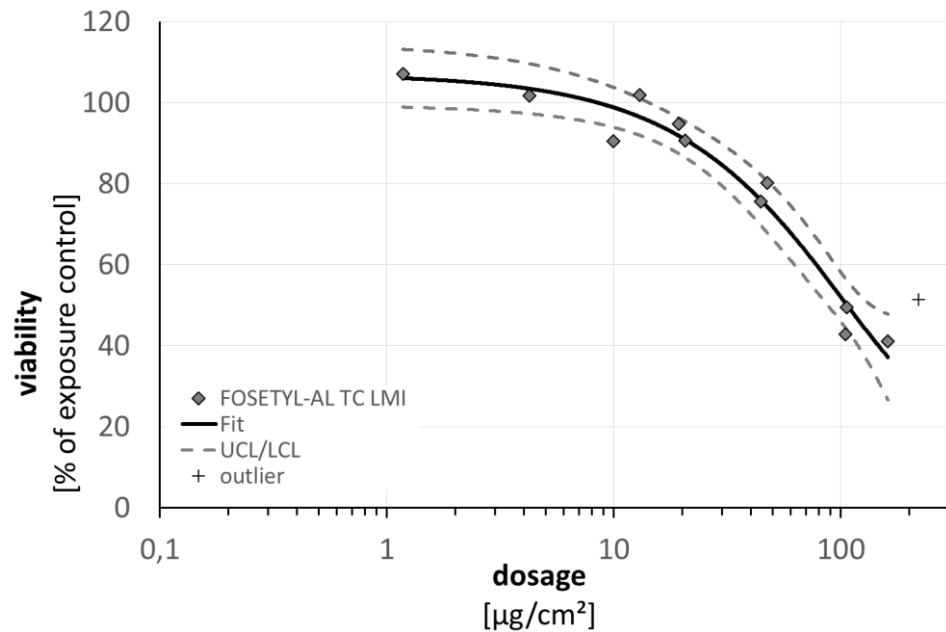
■ Ethiprole
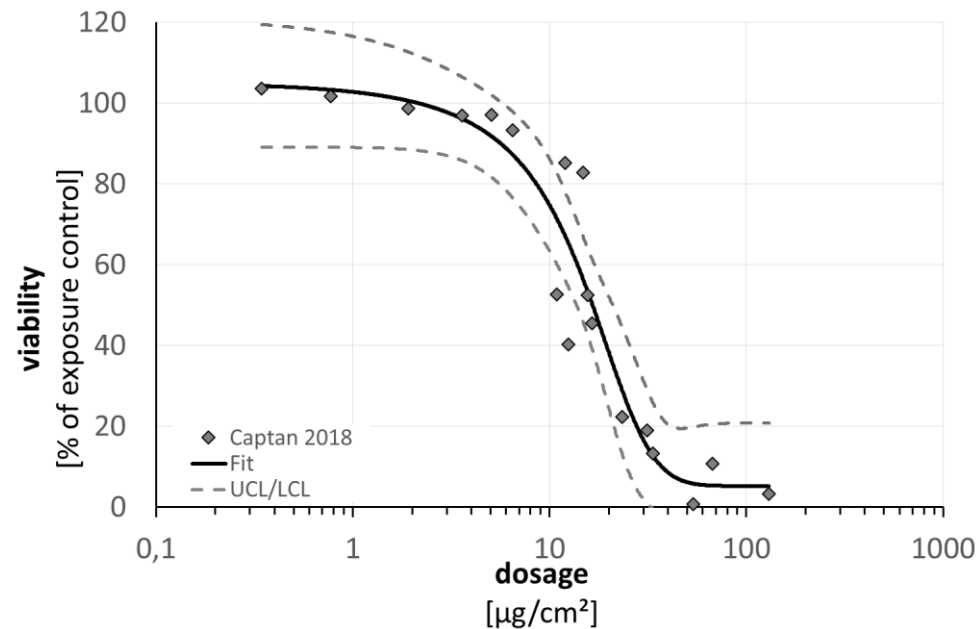
■ ED$_{50}$: 106.56 µg/cm²   *(235 mg consumption)*   ■ ED$_{50}$: 71.02 µg/cm²   *(495 mg consumption)*

- Captan

- Captan   validation (+ 12 months)
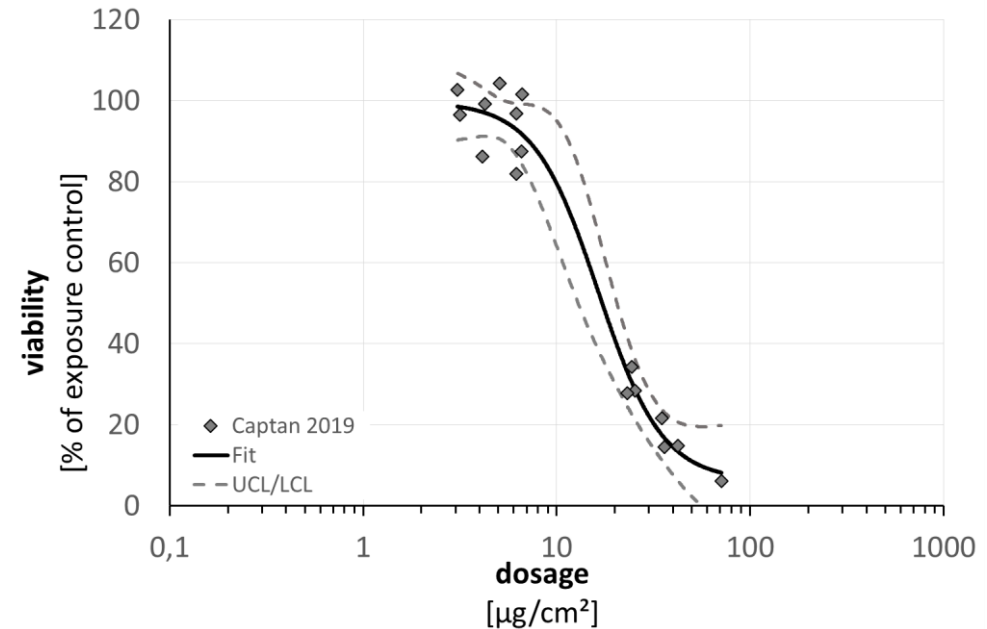
- ED_{50}: 16.48 µg/cm²   *(140 mg consumption)* ED_{50}: 17.21 µg/cm²   *(54 mg consumption)*

RESULTS: In vitro – in vivo correlation

$y = 2{,}3432\ln(x) - 4{,}9701$
$R^2 = 0{,}9056$

X-axis: **in vitro** ("inhalation", human lung cells) ED50 [µg/cm²]

Y-axis: **in vivo** (inhalation, rats) LD50 [mg/l]

Legend:
- ■ Chlorothalonil
- ◆ Mancozeb
- ▲ Captan
- ○ Fosetyl-AL
- ⊠ SDS
- ● Ethiprole
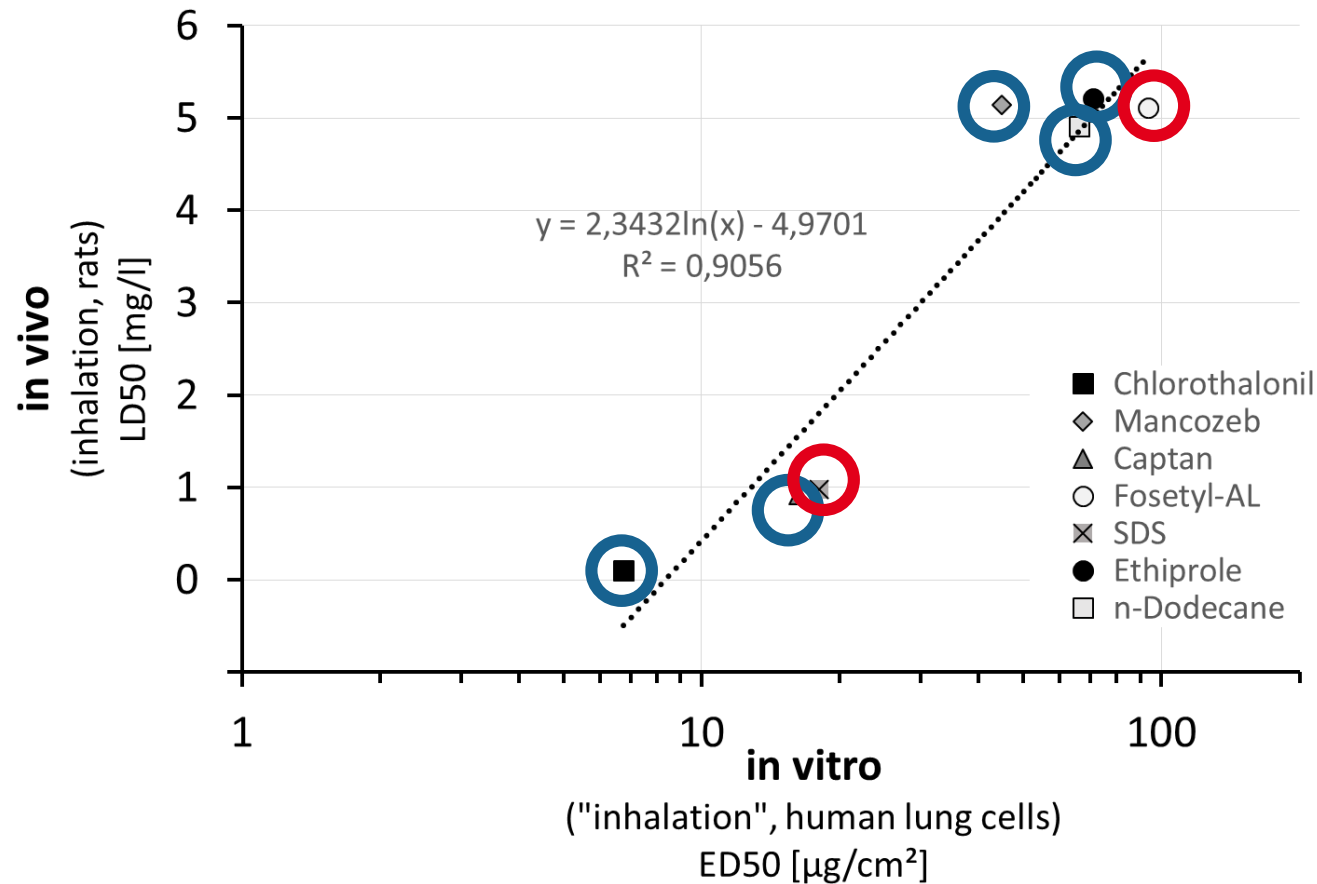- □ n-Dodecane

Correlation to aerosol concentration

- Clear distinction of material with / without concern
- "Quantitative" relationship
- "no concern" substances still differ in vitro
  (in vivo testing range is limited to 5 mg/l)
- Solubility does NOT interfere with toxicity

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RESULTS: In vitro – in vivo correlation

Chart — y-axis: **in vivo** (inhalation, rats) LD50 [mg/l]; x-axis: **in vitro** ("inhalation", human lung cells) ED50 [µg/cm²]

$y = 2{,}3432\ln(x) - 4{,}9701$
$R^2 = 0{,}9056$

Legend:
- ■ Chlorothalonil
- ◆ Mancozeb
- ▲ Captan
- ○ Fosetyl-AL
- ⊠ SDS
- ● Ethiprole
- ☐ n-Dodecane

Correlation to aerosol concentration

- Clear distinction of material with / without concern

- "Quantitative" relationship

- "no concern" substances still differ in vitro
  (in vivo testing range is limited to 5 mg/l)

- Solubility does NOT interfere with toxicity

○ High solubility
○ Low solubility

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RESULTS: In vitro – in vivo correlation
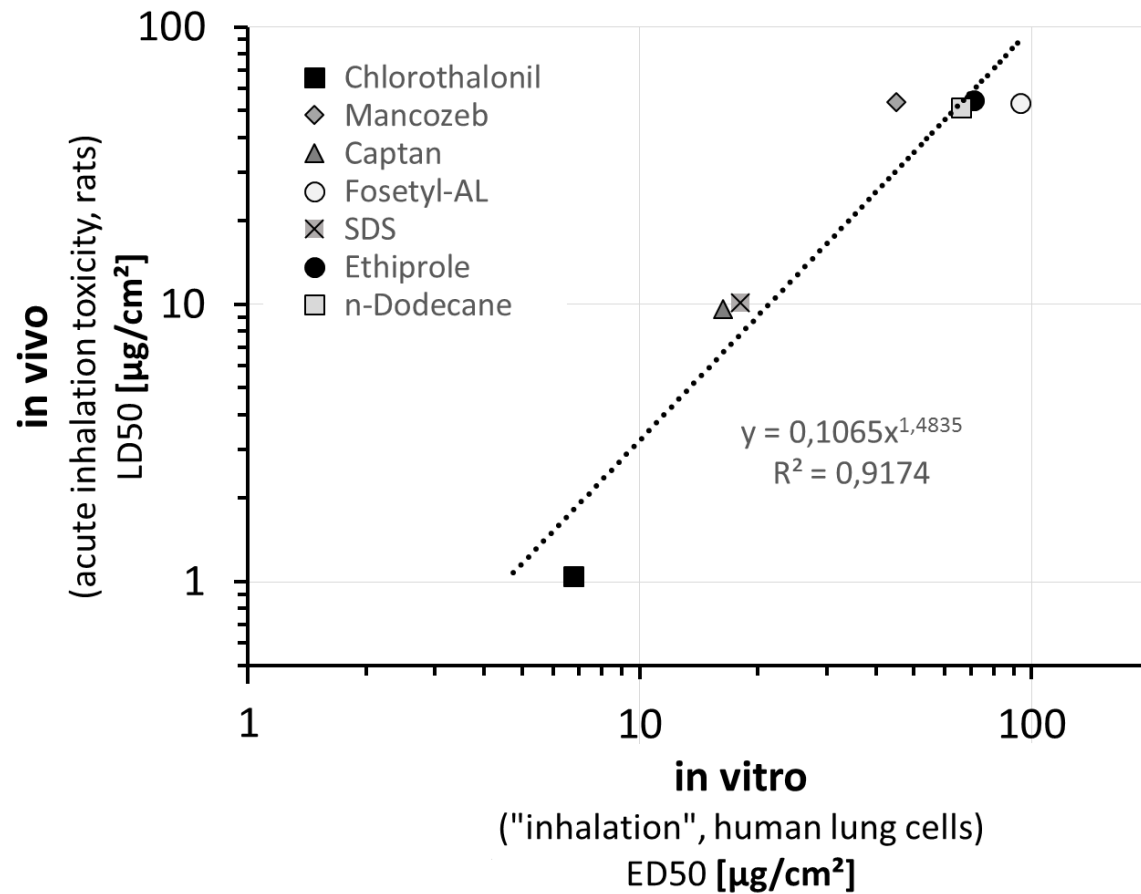
Correlation to lung surface load

- MPPD model (rat):
  - whole resp. tract deposition
  - 2.5 µm MMAD
  - 3000 cm² inner lung surface

- Same dose range in vitro <-> in vivo

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Summary und Conclusion Part 1

Aim	Result
• Acute local toxicity	• Dose-response relationships
• Aerosol generation and application	• Successful by short-time exposure strategy
• Relevant test system	• ALI culture of human lung cells
• Low amounts of test material needed	• ~ 500 mg / test item
• Short-term experiments	• 24 h per test series, ~ 12 – 18 exposures = 3 test series per substance
• Relevance of results	• Promising in vivo (rat) in vitro correlation • "quantitative" correlation, relevant dosages

→ The individual aims of this study could be realized

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Part 2: Prediction of systemic availability upon inhalation - Aim of the study

inhalable ciprofloxacin

ciprofloxacin analytics (LC-MS/MS)

- Investigate the transport of an inhalable version of the antibiotic ciprofloxacin HCl monohydrate (CHM)

  - Pulmonary barrier models (airway and alveolar)

  - Calculate P_{app} coefficients

P_{app}

- Simulating ADME processes in the human body using the PBPK model with P_{app} coefficients obtained in vitro

Calculation of blood levels

- Calculation of blood levels and comparison with existing human data
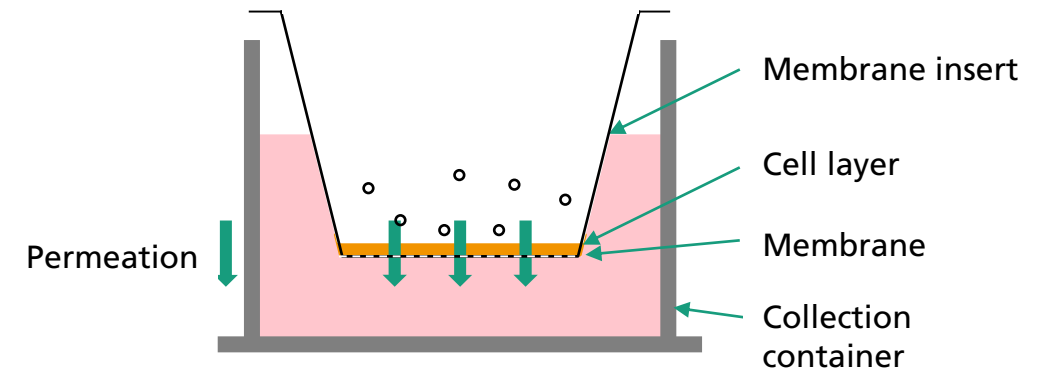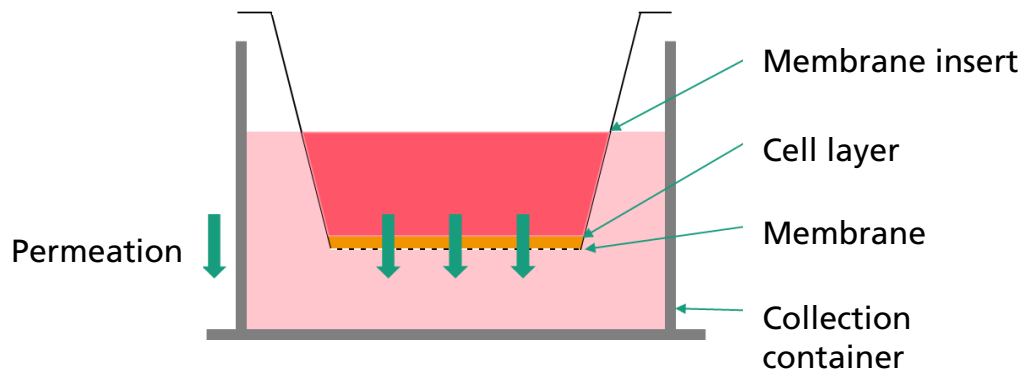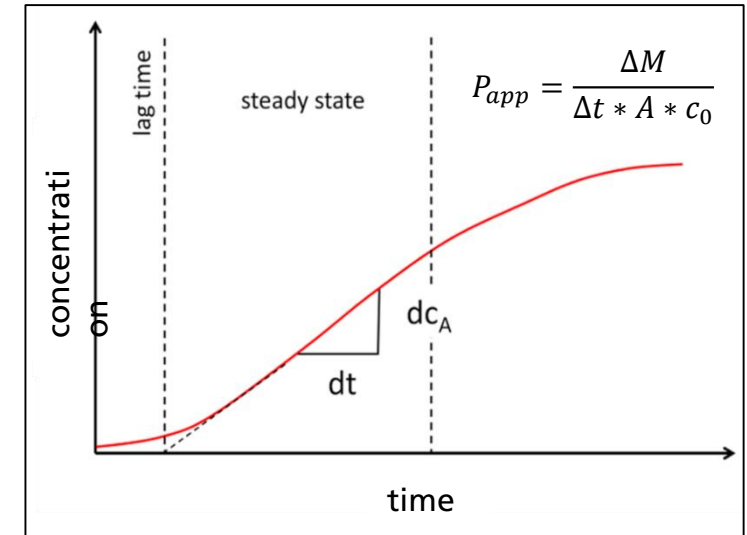
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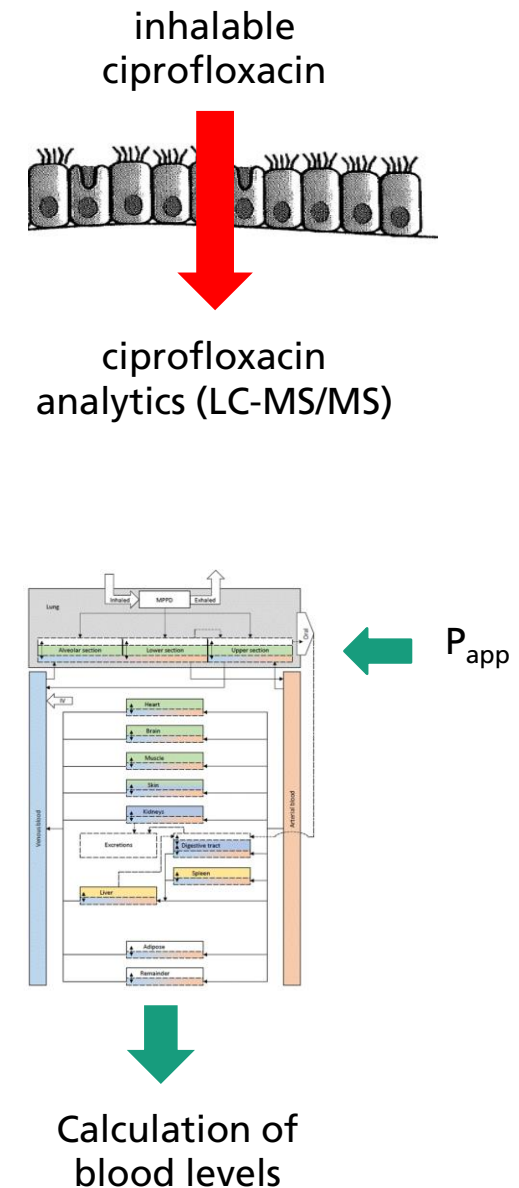
Lung structure

- Generation 0-7:
  - Upper tracheobronchial region (upp)
- Generation 7-16:
  - Lower tracheobronchial region (low)
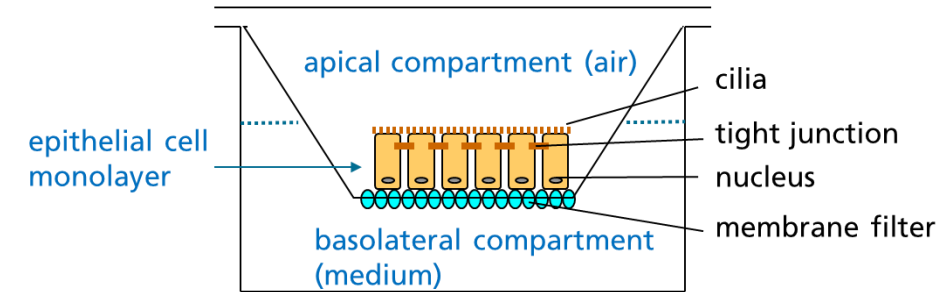- Generation 17-24:
  - alveolar region (alv)
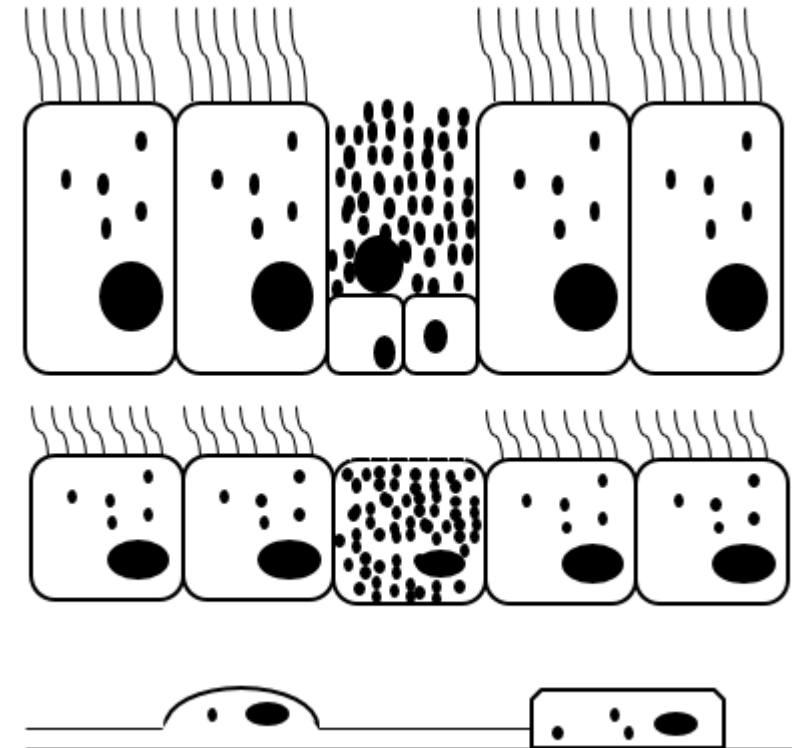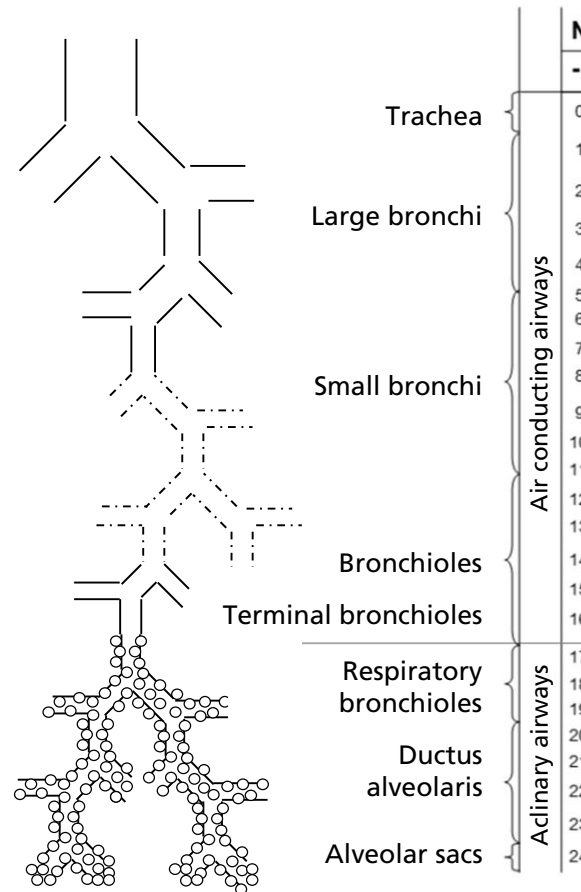
Apparent permeability coefficient (P_{app})

- A coefficient specific to a substance permeating through a specific phase or interface

- Cell lines (human)
  - Immortalized cells from the epithelium of the lung
  - Calu-3: tracheobronchial region
  - AT-1: alveolar region (functionally immortalized)

$$P_{app} = \frac{\Delta M}{\Delta t * A * c_0}$$

concentration

lag time

steady state

dc_A

dt

time

Membrane insert

Cell layer

Membrane

Collection container

Permeation

Membrane insert

Cell layer

Membrane

Collection container
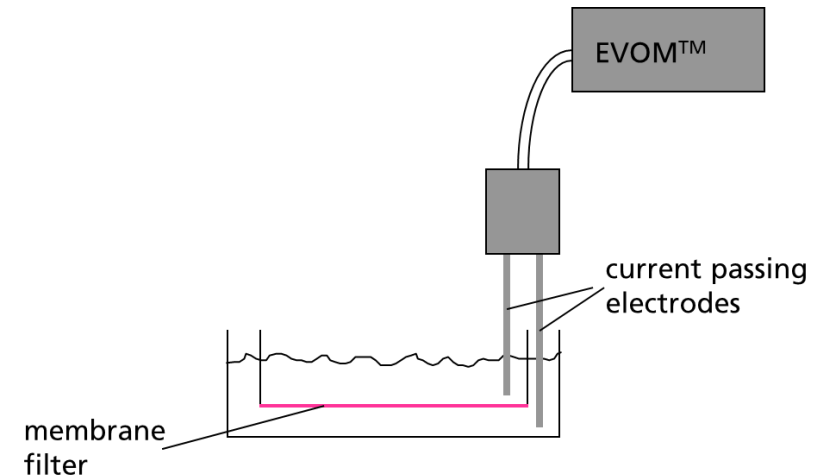
Permeation

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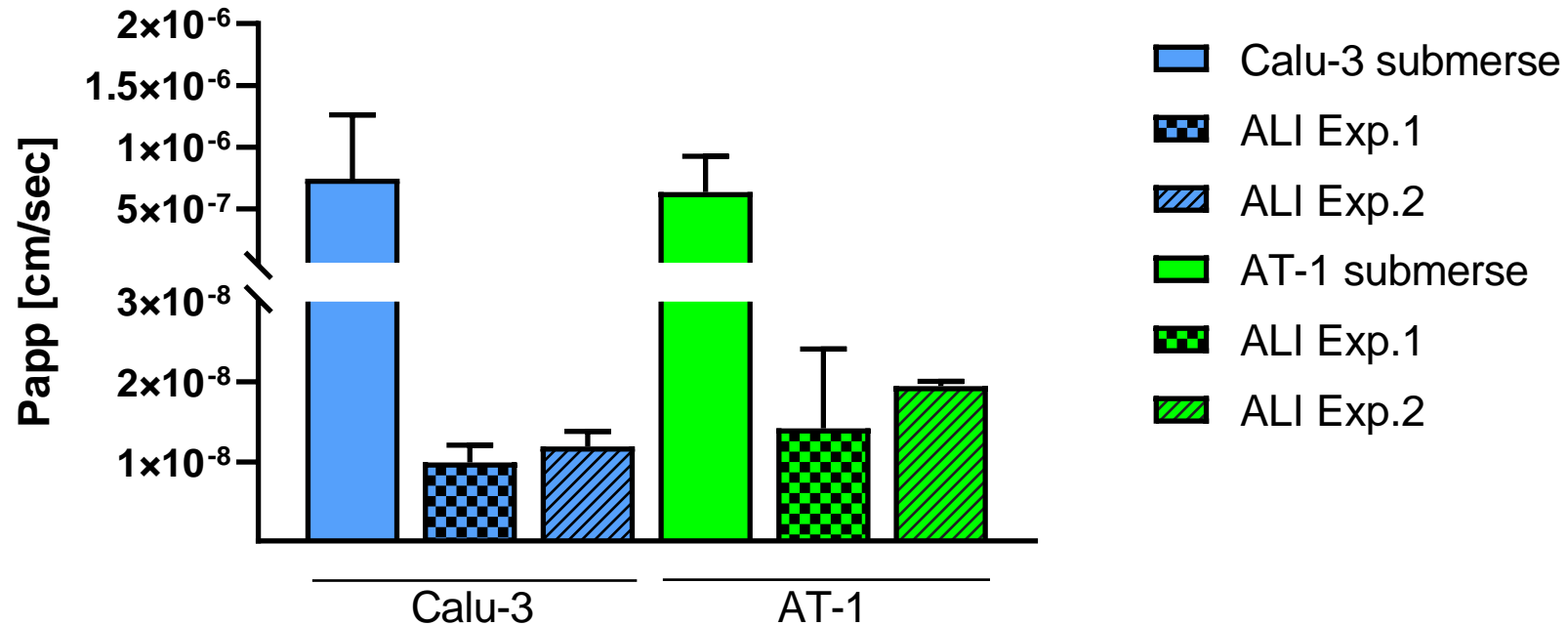# Experimental steps for P_{app} determination

1. Pre-culture of Calu-3 (airway) or AT-1 (alveolar region) cells to ensure cellular differentiation and formation of tight monolayers.

2. Cellular barrier integrity assessment (TEER)

3. Permeability (absorption) assessment:

   - Apical exposure to ciprofloxacin

   - Ciprofloxacin analytics in the basolateral media compartment (LC-MS/MS)

   - Calculation of the Papp coefficient

$$\mathrm{P}app = \frac{\Delta Q}{\Delta t \cdot 60 \cdot \mathrm{A} \cdot C0}\left(\frac{cm}{s}\right)$$

apical compartment (air)

epithelial cell monolayer

basolateral compartment (medium)

cilia

tight junction

nucleus

membrane filter

EVOM™

current passing electrodes

membrane filter

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# Apparent permeability coefficient – comparison
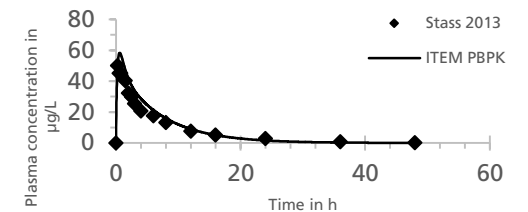
ITEM - PBPK modelling

- Pharmacokinetics based on data from Sadiq et al. 2017

- Aerosol dissolved in Lining Fluid (LF, white beam)

- Through cell layer (green beam) into blood

- Usual blood flow through "body"

- Excretion of Ciprofloxacin

  - fu = 65%

  - Clearance rate 120mL/min

  - 45% via Kidneys

  - 55% via liver model / digestive tract

  - Lung data (lit., IV) exchanged with experimental data

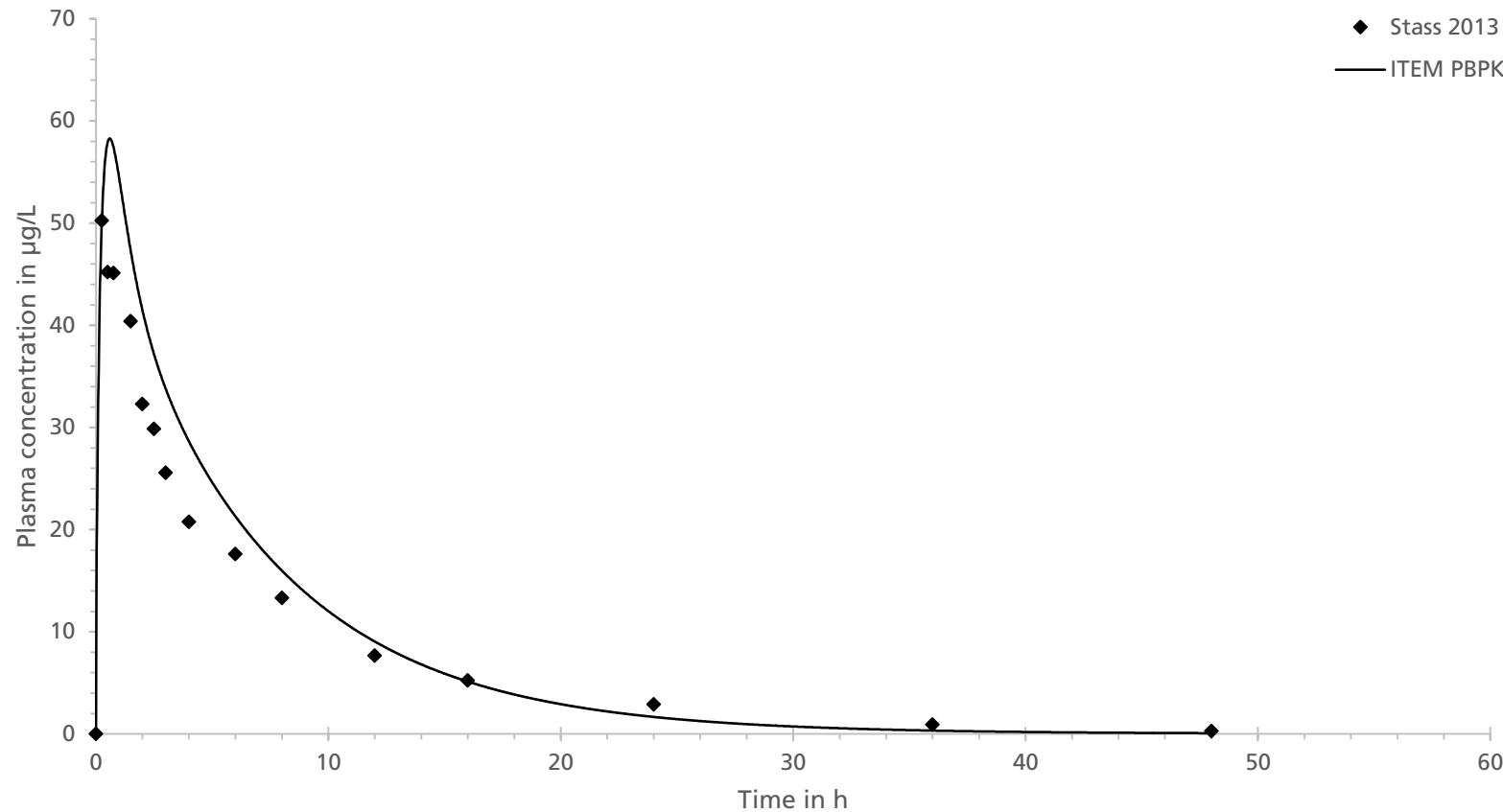- PBPK model: coding and mathematical system provided by Norman Nowak

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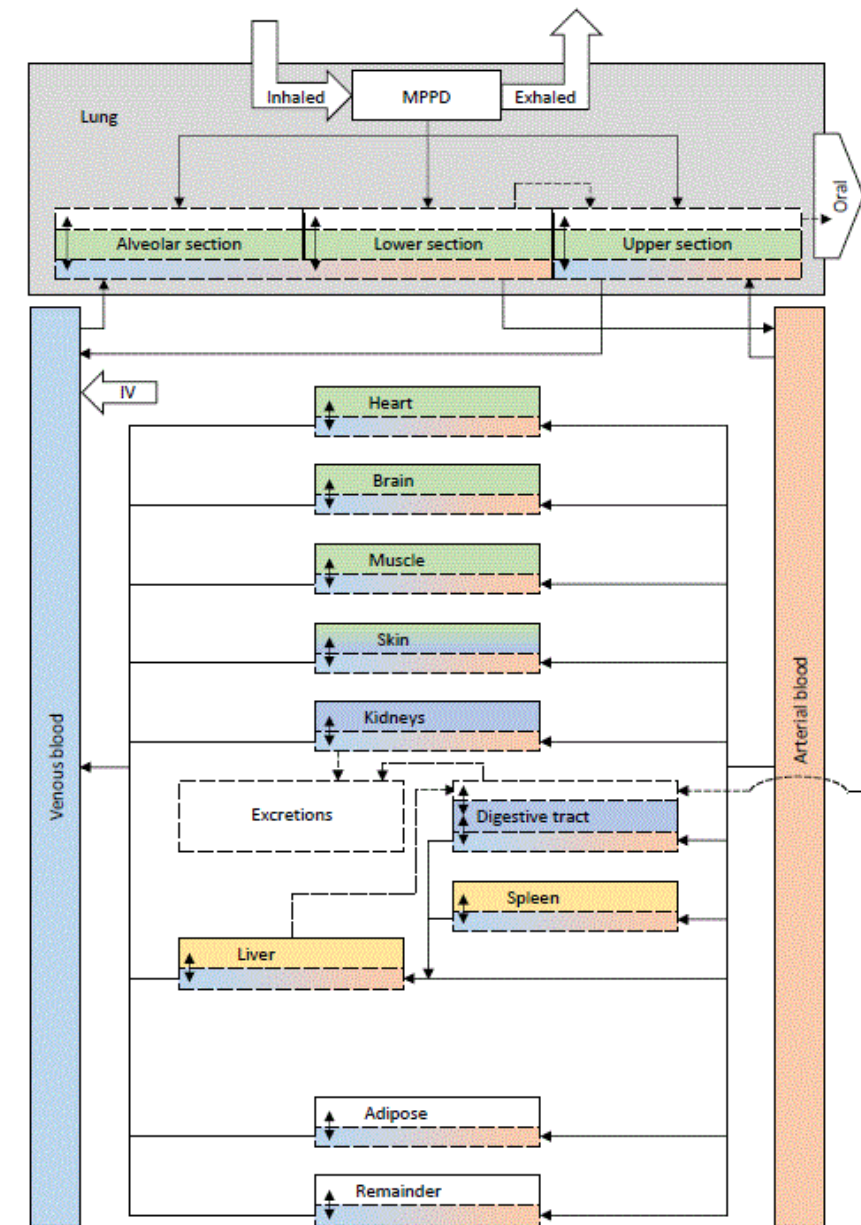
ITEM - PBPK modelling
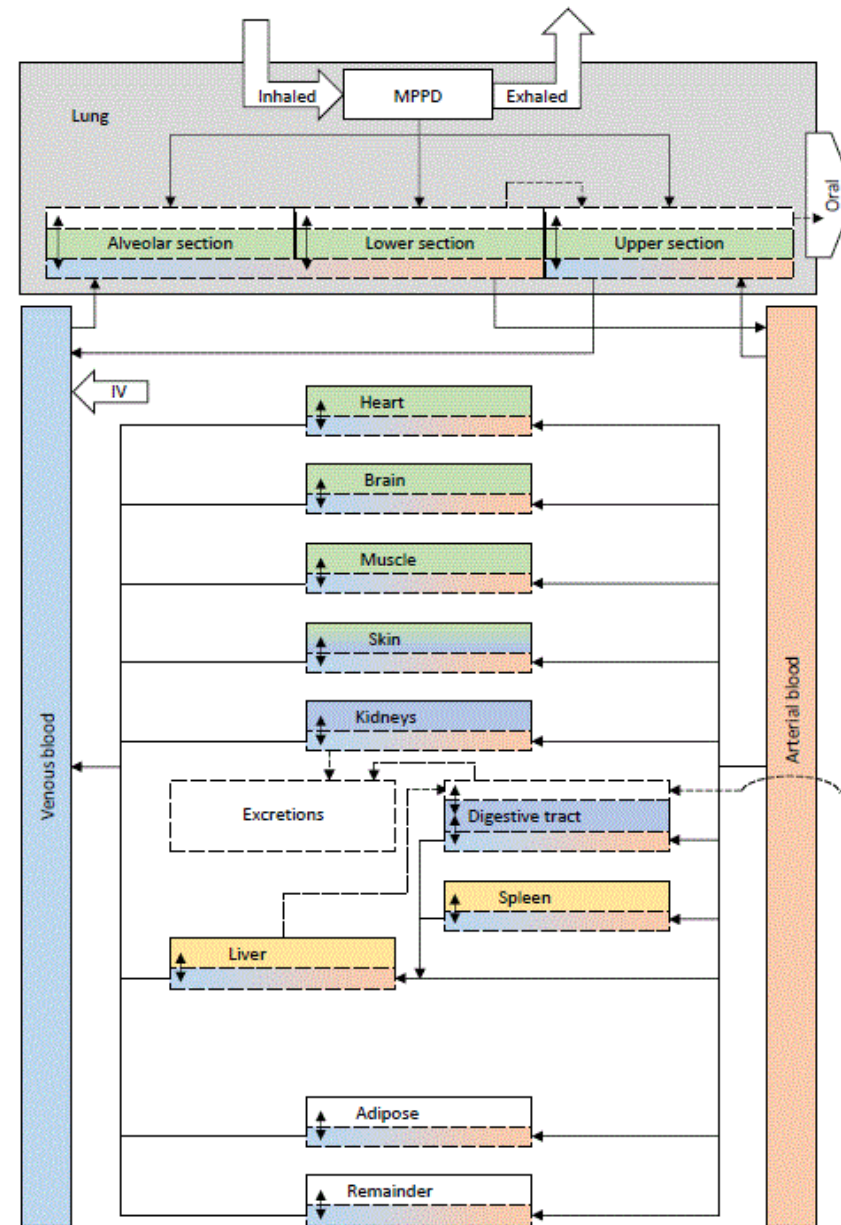
P$_{app}$: inhalable drug
- Experimental data
- Calu-3 and At-1

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PBPK – comparison with human data of healthy volunteers

Legend:
- ◆ Stass 2013
- —— ITEM PBPK

X-axis: Time in h
Y-axis: Plasma concentration in µg/L

- **Dose Ciprofloxacin:**
  - 32.5mg (inhalable)
- **in vivo data**
  - Stass 2013
  - Healthy human volunteers
- **In vitro data**
  - Experimental data ALI
  - Human cell models (Calu-3, AT-1)

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Summary and conclusion Part 2

- Successful determination of P_{app} values

- P_{app} values were comparable in Calu-3 and AT-1 cells

- PBPK model: Simulated blood levels using P_{app} values obtained at ALI conditions are a near perfect fit to human literature data

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Conclusion

- In vitro data are able to predict acute lung toxicity

- Good correlation to rat OECD 403 in vivo data

- Systemic absorption can be estimated based on PBPK modelling

- Reduction in the number of animal experiments

Please do not hesitate to contact us

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