APPARENT PERMEABILITY COEFFICIENTS OF CIPROFLOXACIN IN HUMAN LUNG EPITHELIAL CELLS APPROPRIATELY PREDICT BIOAVAILABILITY AFTER INHALATION EXPOSURE

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Fraunhofer ITEM

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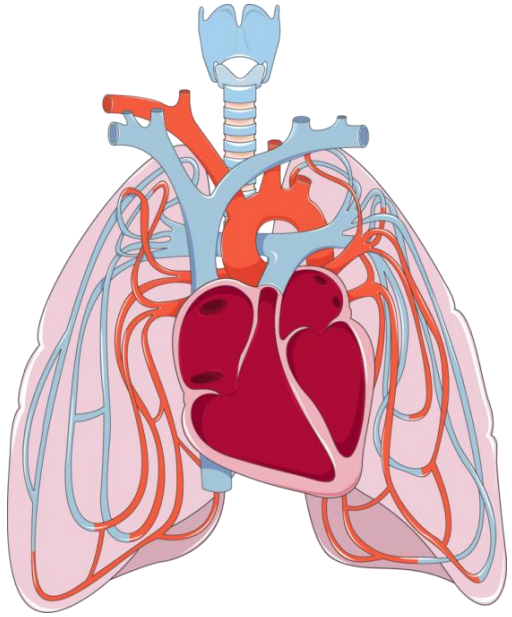

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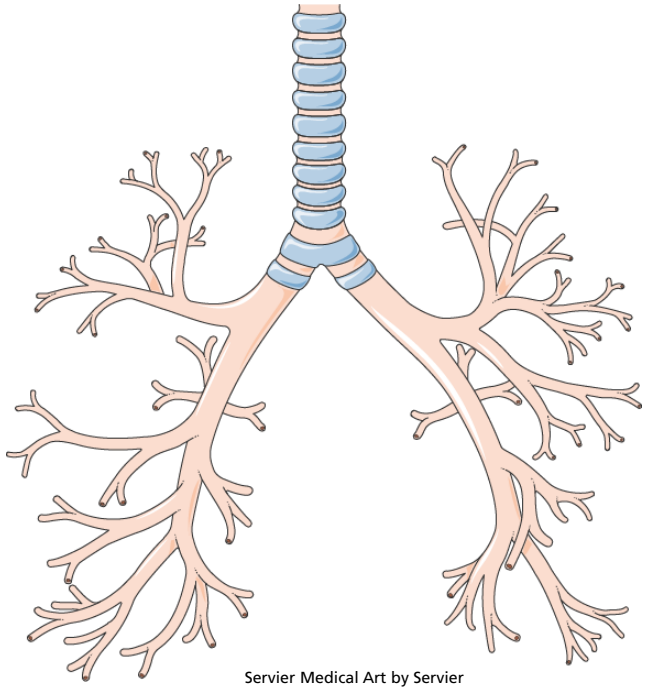

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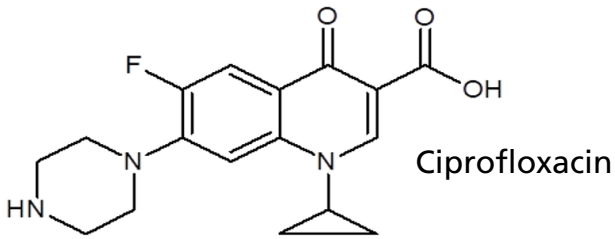
Why use inhalable antibiotics?

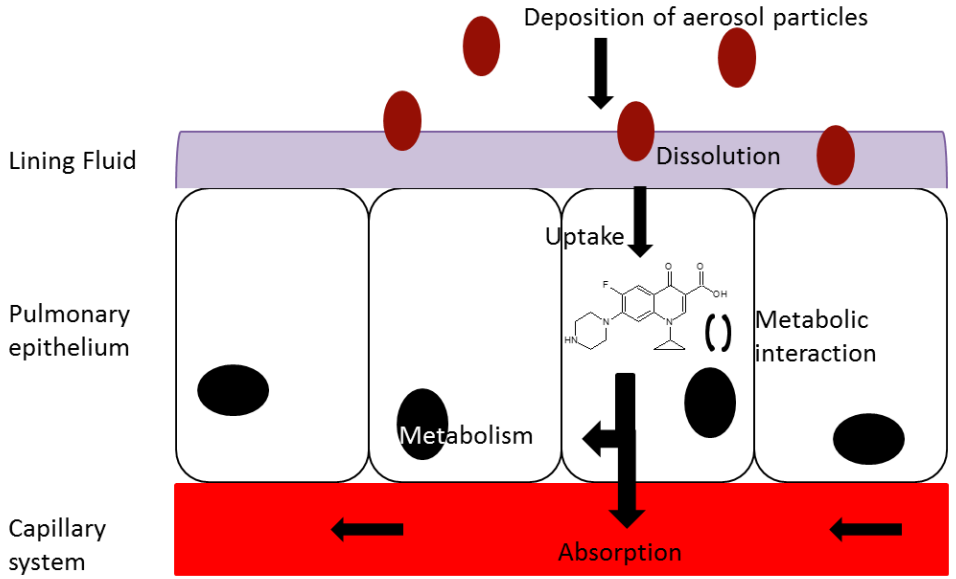
- Locally higher concentration achievable
- Fight problem germs
- Bypass the first pass metabolism
- Less side effects due to lower systemic availability



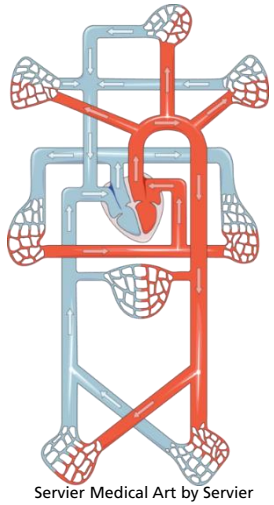
Ciprofloxacin

Systemic availability

- After local application → systemically available
- Systemic availability → essential for risk assessment
- Use of physiologically based pharmacokinetic (PBPK) modelling
- To test less on animals, PBPK and in vitro models can be used



Modified from Ong et al. (2013)



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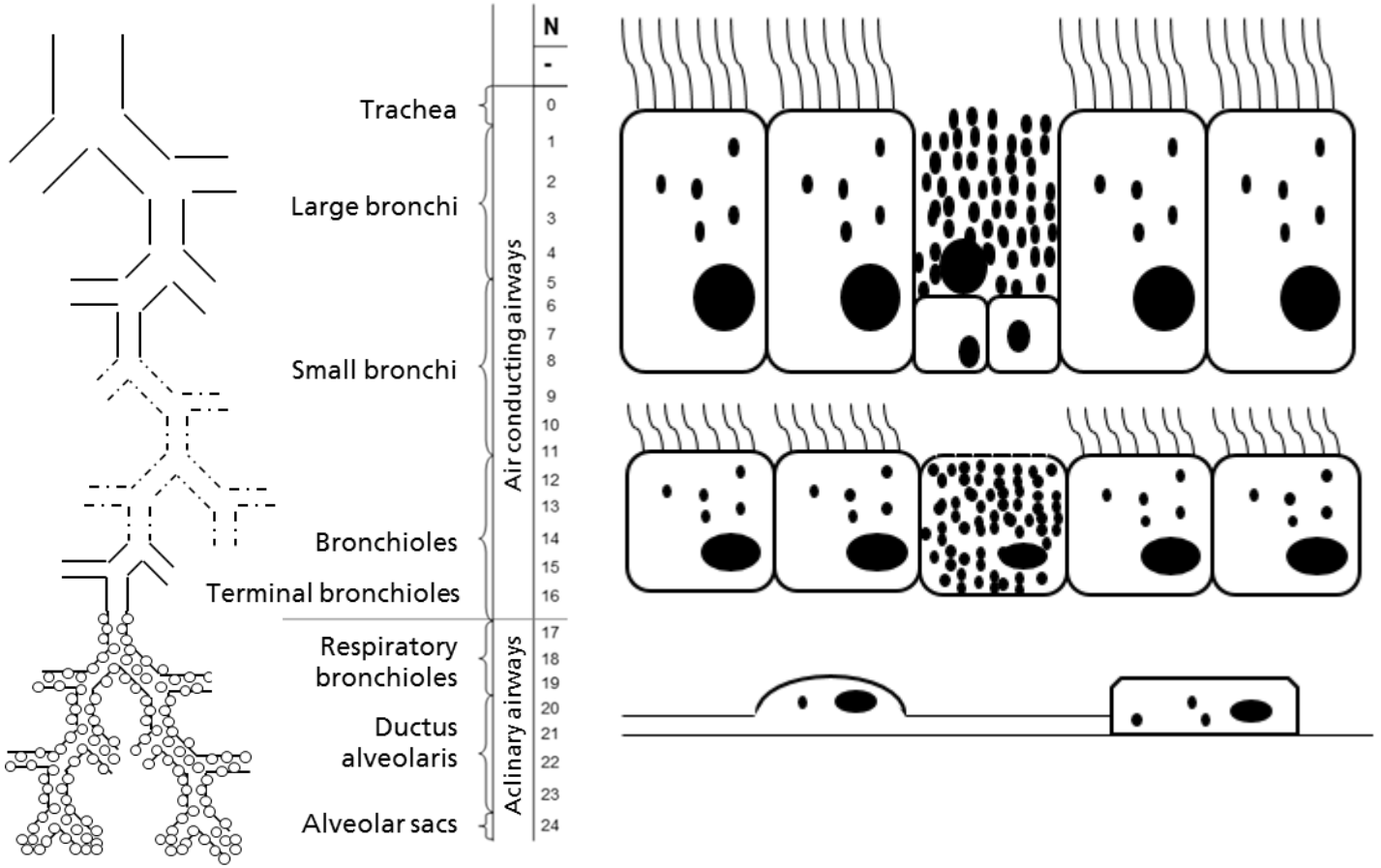
Lung structure

- Generation 0-7:
  - Upper tracheobronchial region (upp)
- Generation 7-16:
  - Lower tracheobronchial region (low)
- Generation 17-24:
  - alveolar region (alv)



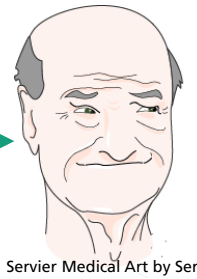
Modified from Schwarz (2012) and Ong et al. (2013)

Deposition Mechanism

Upper Respiratory Tract

Impaction
Diffusion

Particle Inhalation



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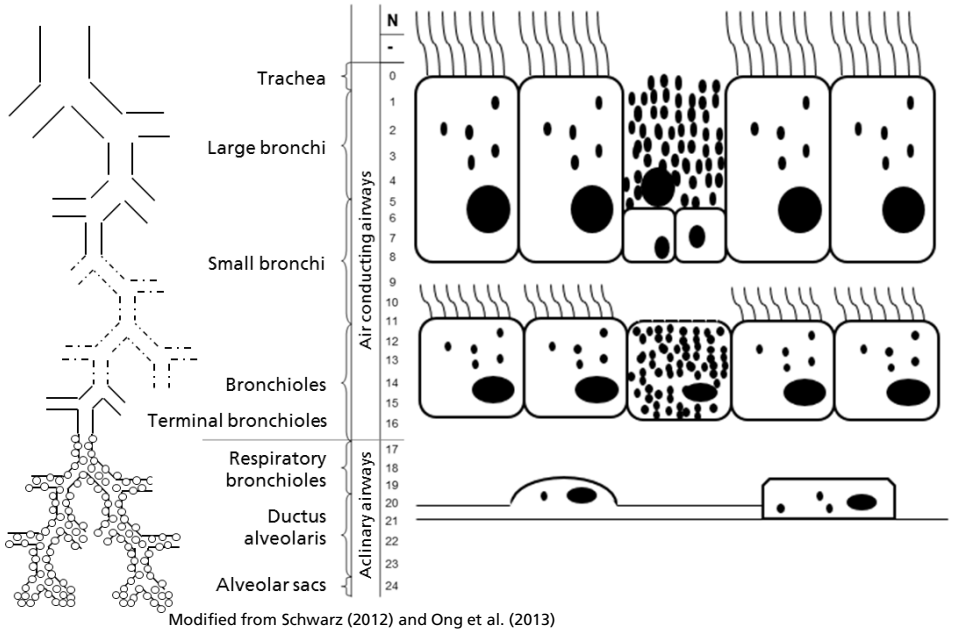
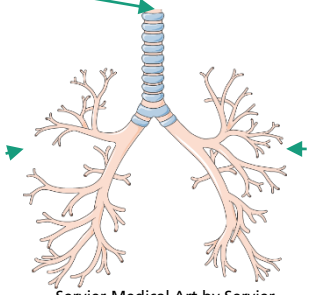


Modified from Schwarz (2012) and Ong et al. (2013)

Tracheobronchial region

Impaction and diffusion (particles 3-10µm)



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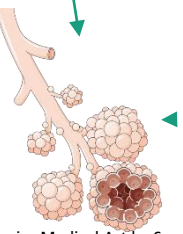
Impaction
Diffusion

Alveolar region

Sedimentation and diffusion (particles < 3µm)



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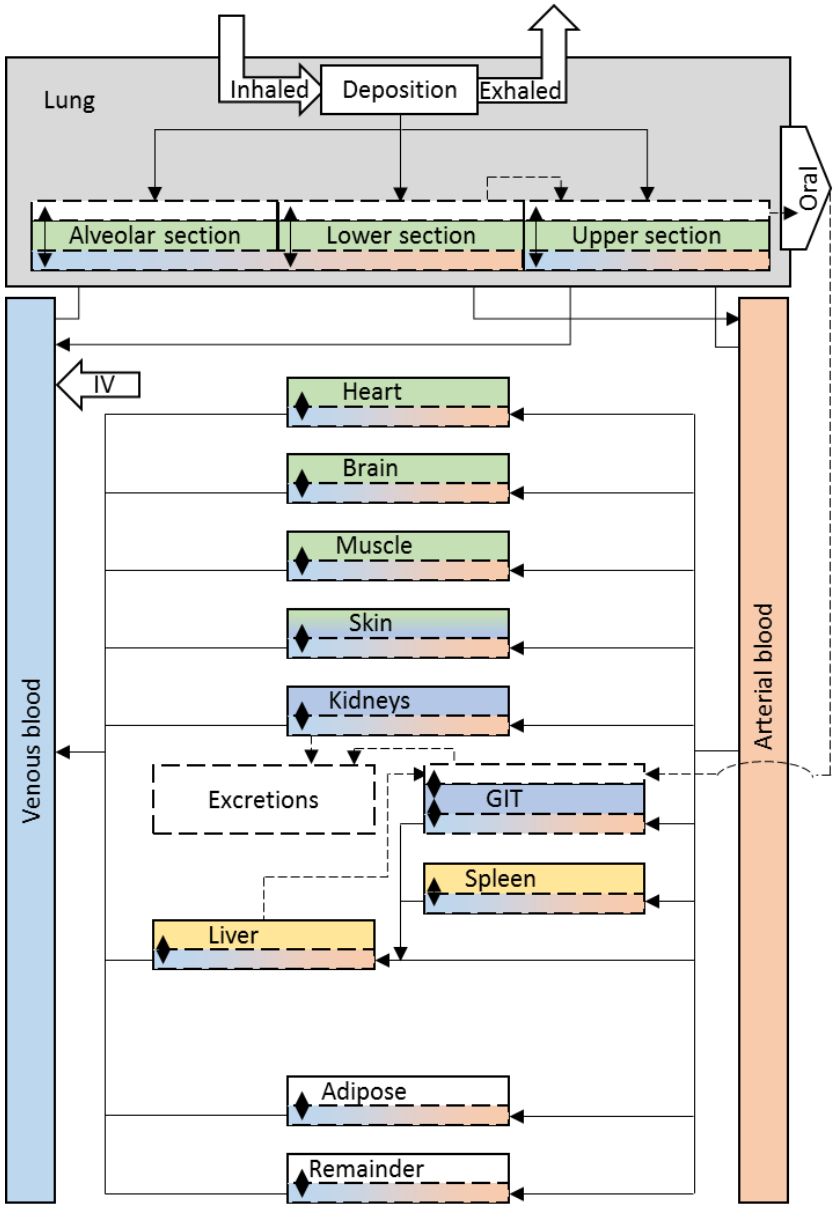
Sedimentation
Diffusion

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

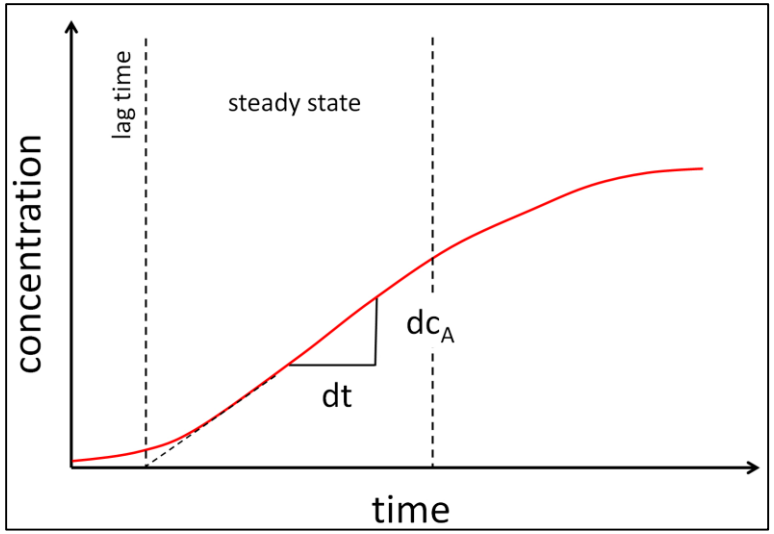


Fraunhofer ITEM

Apparent permeability coefficient (P_{app})

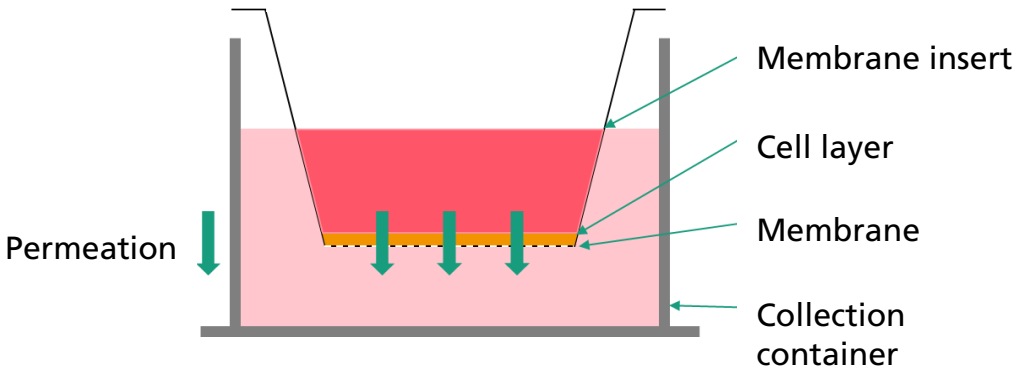
- A coefficient specific to a substance permeating through a specific phase or interface

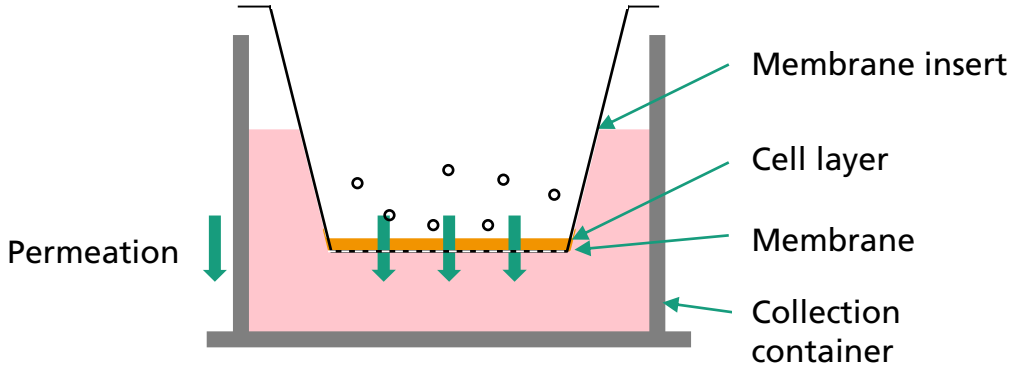
$$P_{app} = \frac{\Delta M}{\Delta t * A * c_0}$$



Hsu et al. (2018)

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



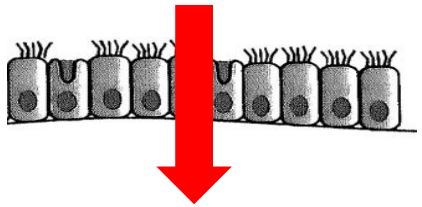


Fraunhofer ITEM

Goal

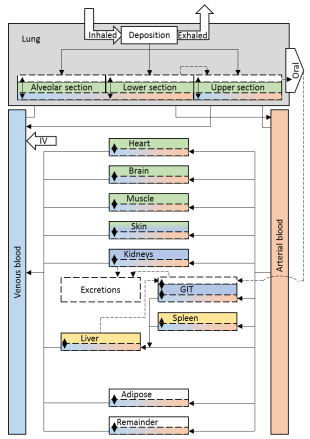
- Investigate the transport of an inhalable version of the antibiotic ciprofloxacin HCl monohydrate (CHM)
  - Pulmonary barrier models (airway and alveolar)
  - Calculate P_{app} coefficients

inhalable ciprofloxacin



ciprofloxacin analytics (LC-MS/MS)

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



P_{app}

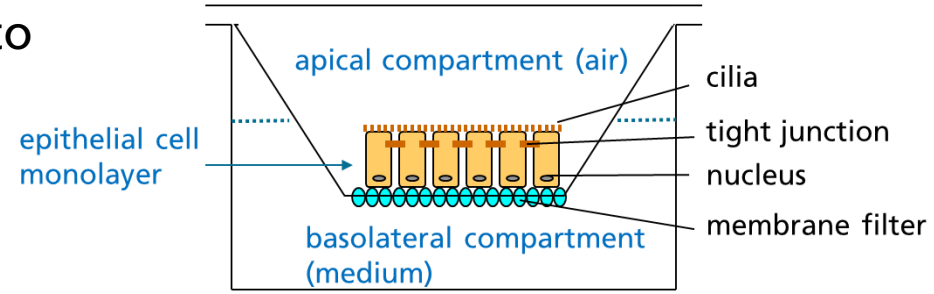
- Calculation of blood levels and comparison with existing human data

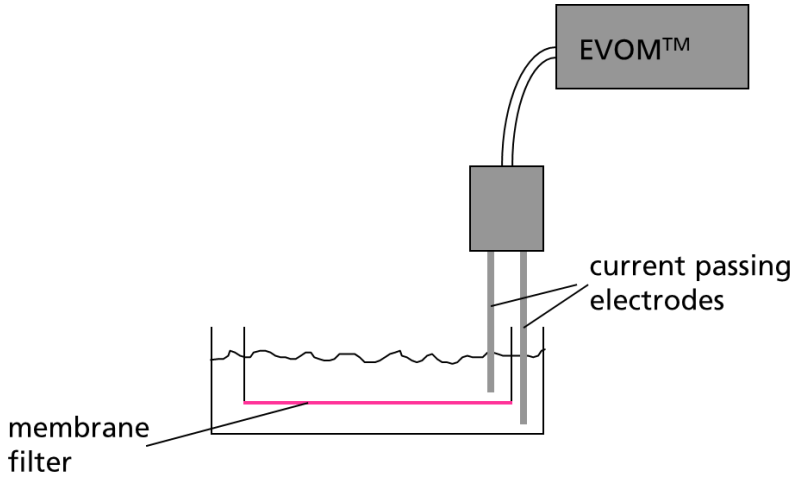
Calculation of blood levels

Experimental steps for P_{app} determination

1. Pre-culture of Calu-3 (airway) or hAELVi (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

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





Fraunhofer ITEM

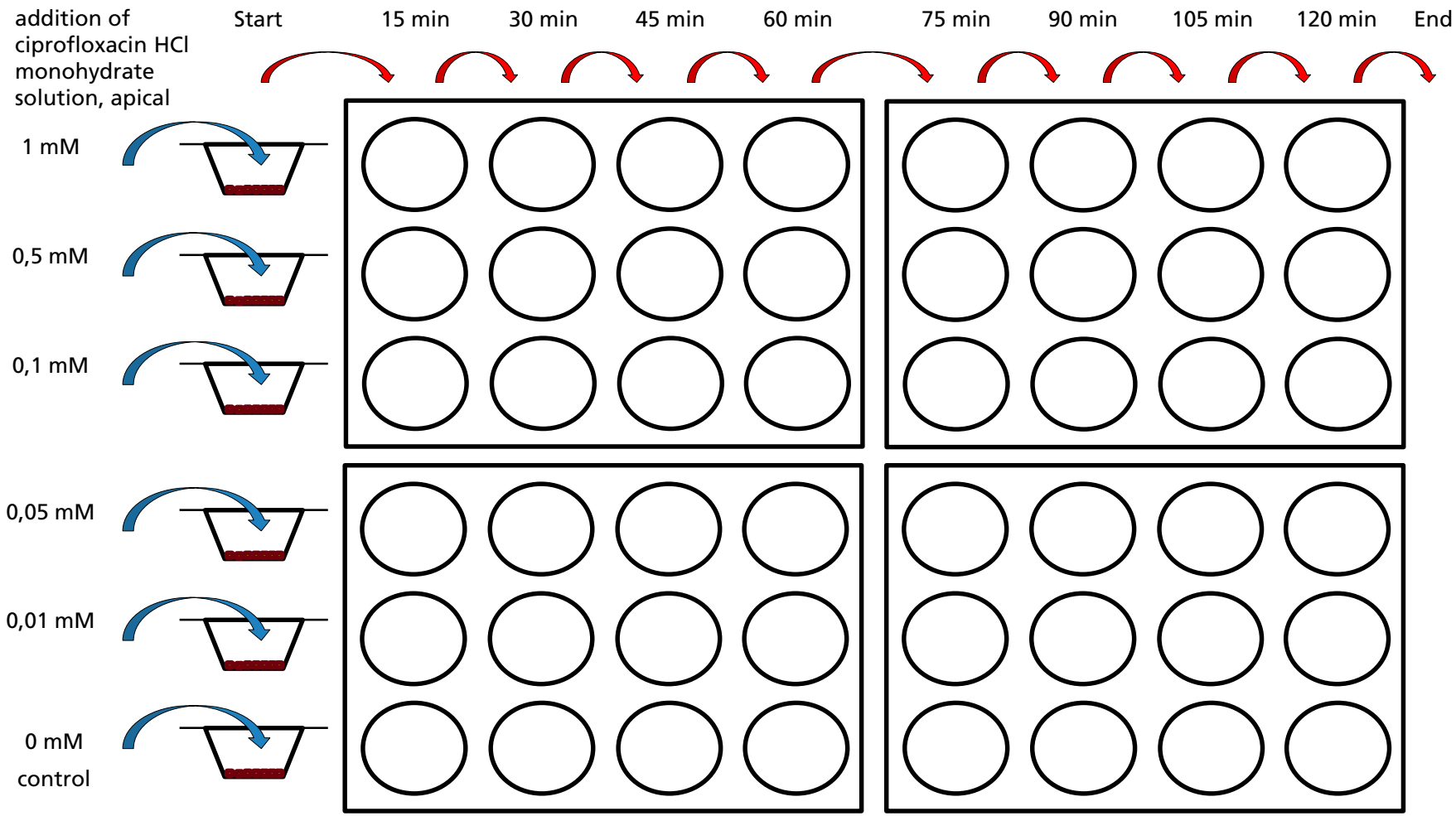
Structure of the transport experiments

Calu-3 cells:

- At least 8 days in culture
- TEER value > 400 $\Omega * cm^2$
- Cells in monolayer

hAELVi cells:

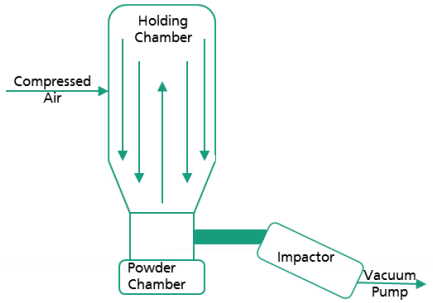
- At least 14 days in culture
- TEER value > 400 $\Omega * cm^2$
- Cells in monolayer



Fraunhofer ITEM

Exposure of cells under ALI conditions

PreciseInhale™ System



Modified from http://www.preciseinhale.com/explore/

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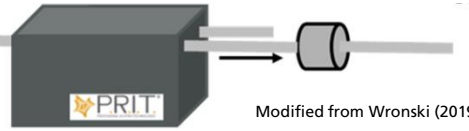
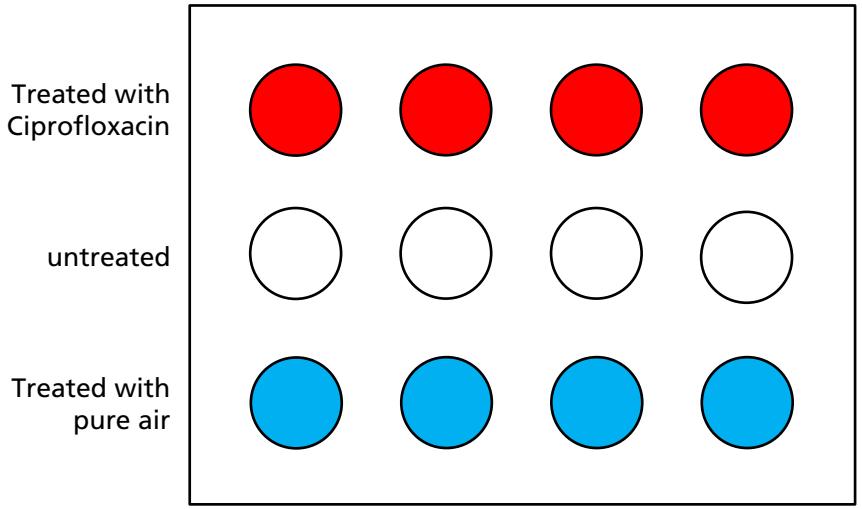
Exposure of cells in transwell membranes



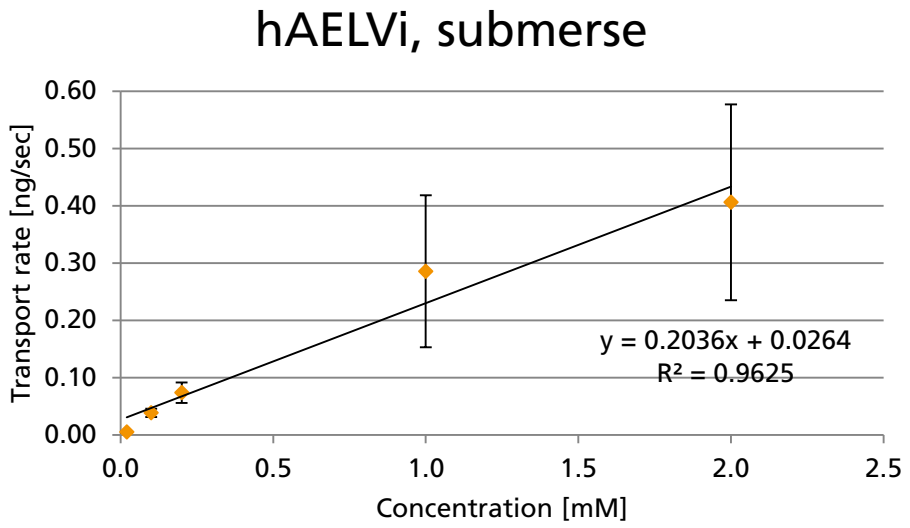
Modified from Wronski (2019)

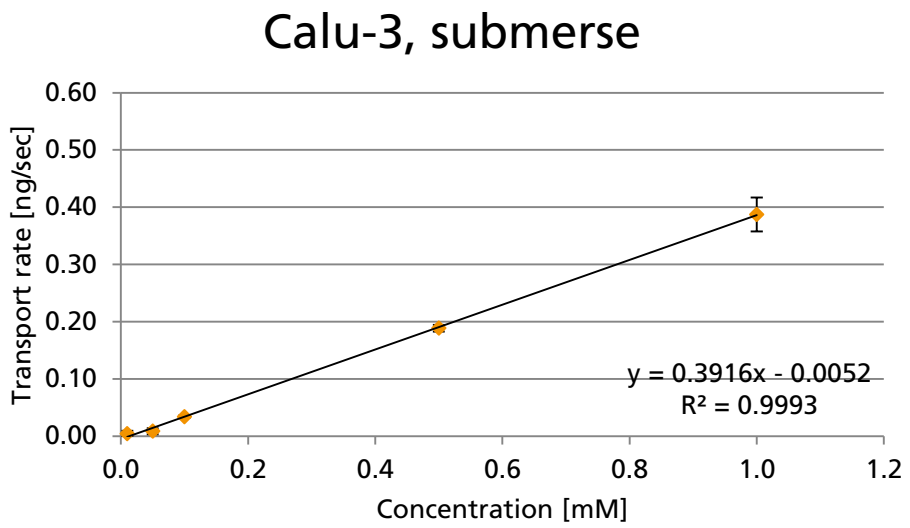


Particle size distribution

- Measurment of size distribution
  - For each substance and condition
- Impactor
  - How many particles in size from … to …

Experimentally determined transport rates





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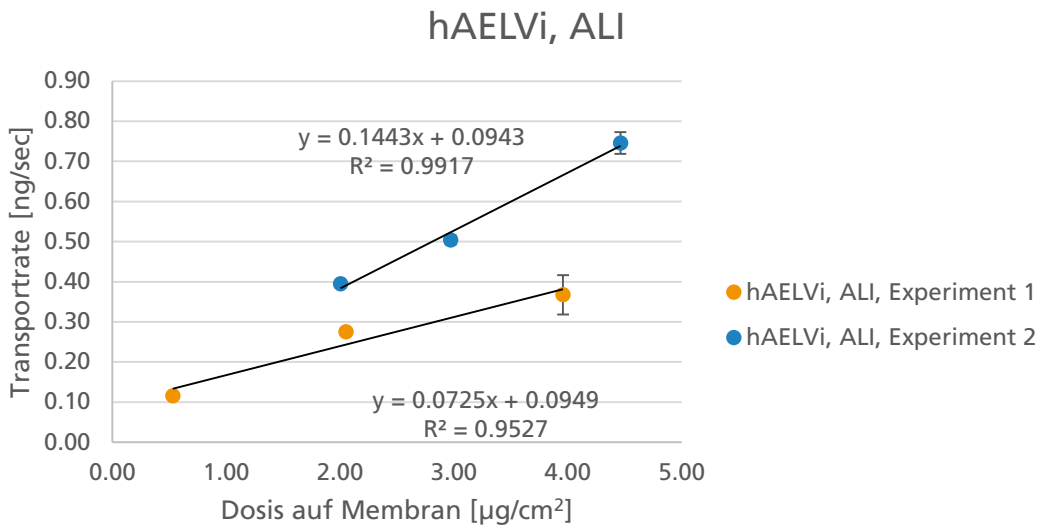
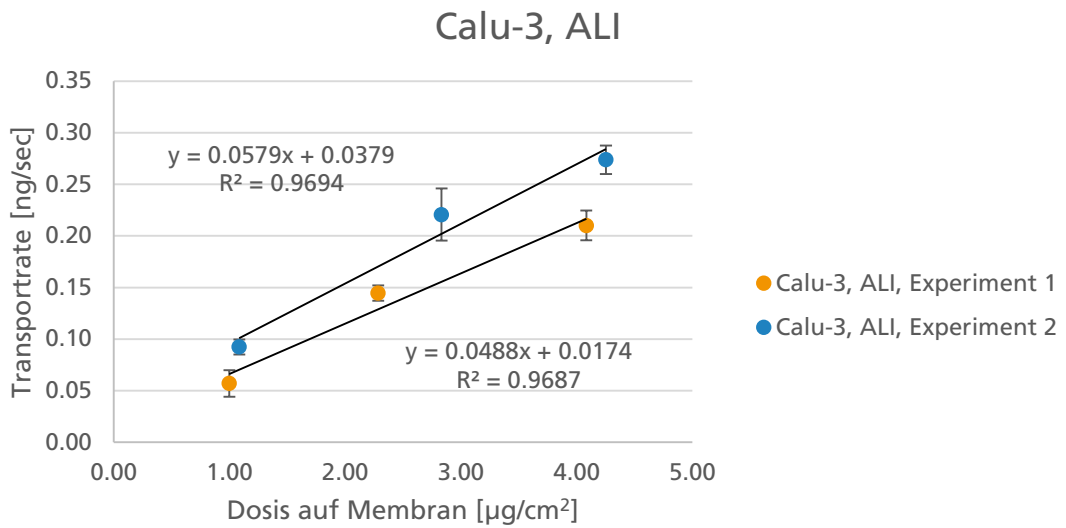
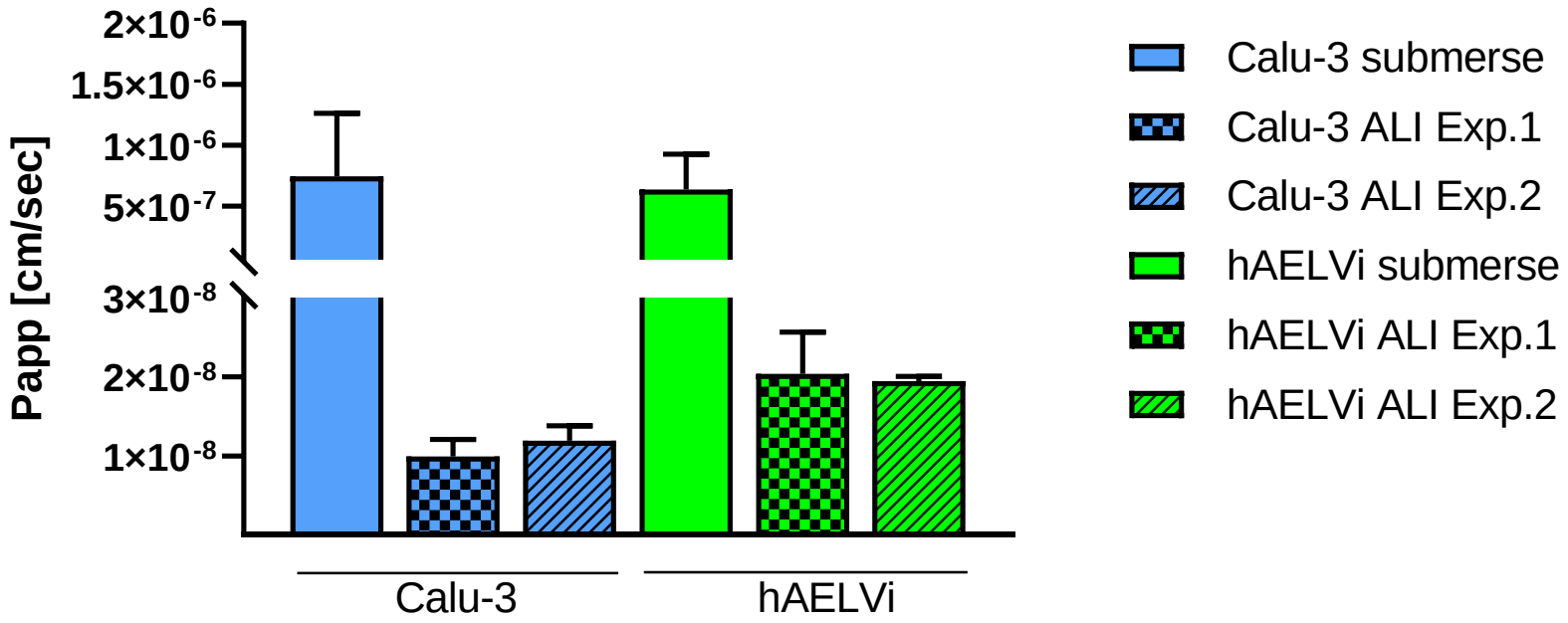
Experimentally determined transport rates





Fraunhofer ITEM

Apparent permeability coefficient – comparison



Calu-3: Ciprofloxacin

Submerse:

$7{,}43*10^{-7}$ cm/sec

ALI:

Exp.1 = $9{,}91*10^{-9}$ cm/sec;
Exp.2 = $1{,}19*10^{-8}$ cm/sec

hAELVi: Ciprofloxacin

Submerse:

$6{,}34*10^{-7}$ cm/sec

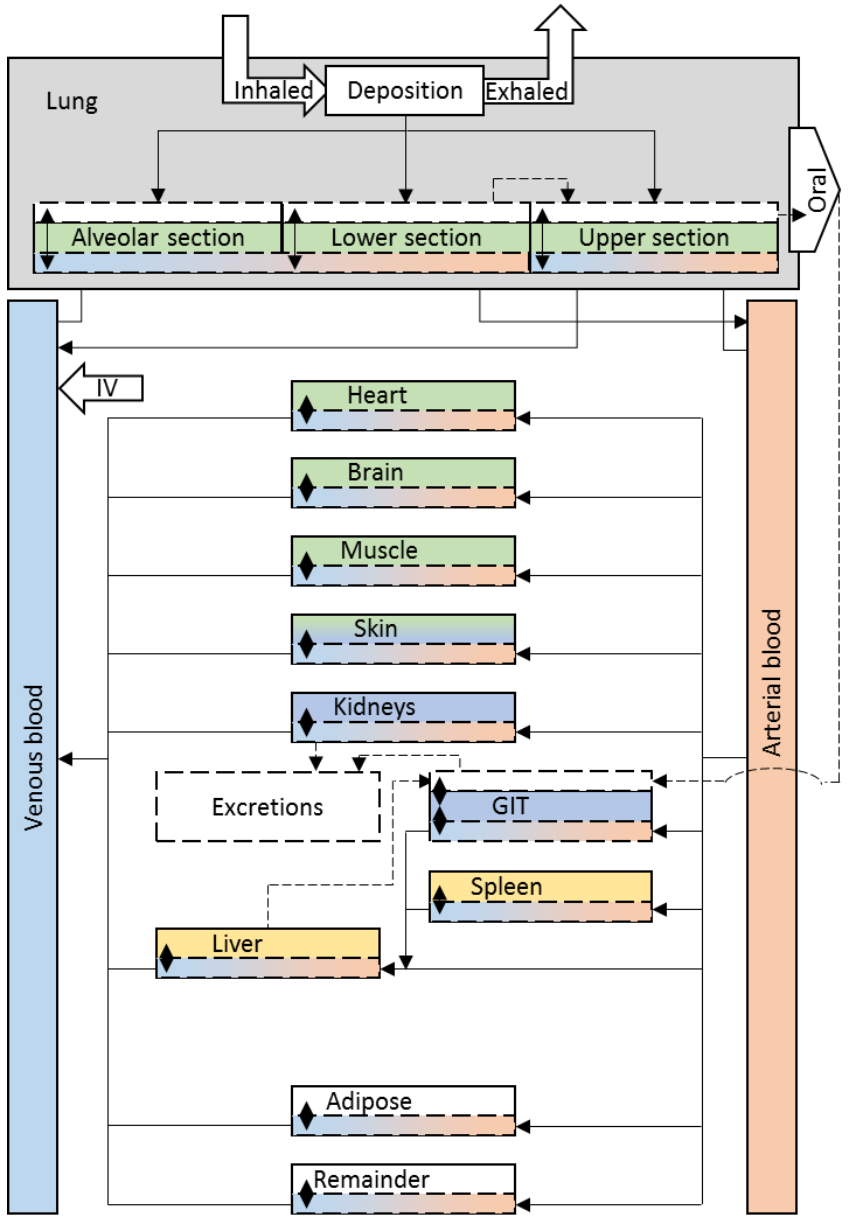
ALI:

Exp.1 = $2{,}03*10^{-8}$ cm/sec;
Exp.2 = $1{,}95*10^{-8}$ cm/sec

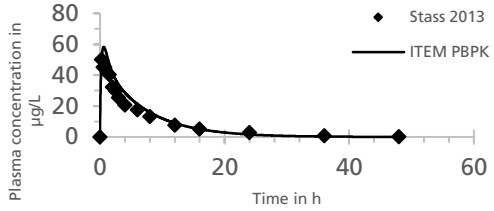
The P_{app} coefficients were calculated while assuming a 2µm thick Lining Fluid on the cells under ALI conditions.

ITEM - PBPK modelling

- Pharmacokinetics between blood and organs based on:
  - Sadiq et al. (2017)
  - Lung data (lit., IV) exchanged with experimental data



- P_{app}: inhalable drug
  - Experimental data
  - Calu-3 and hAELVi



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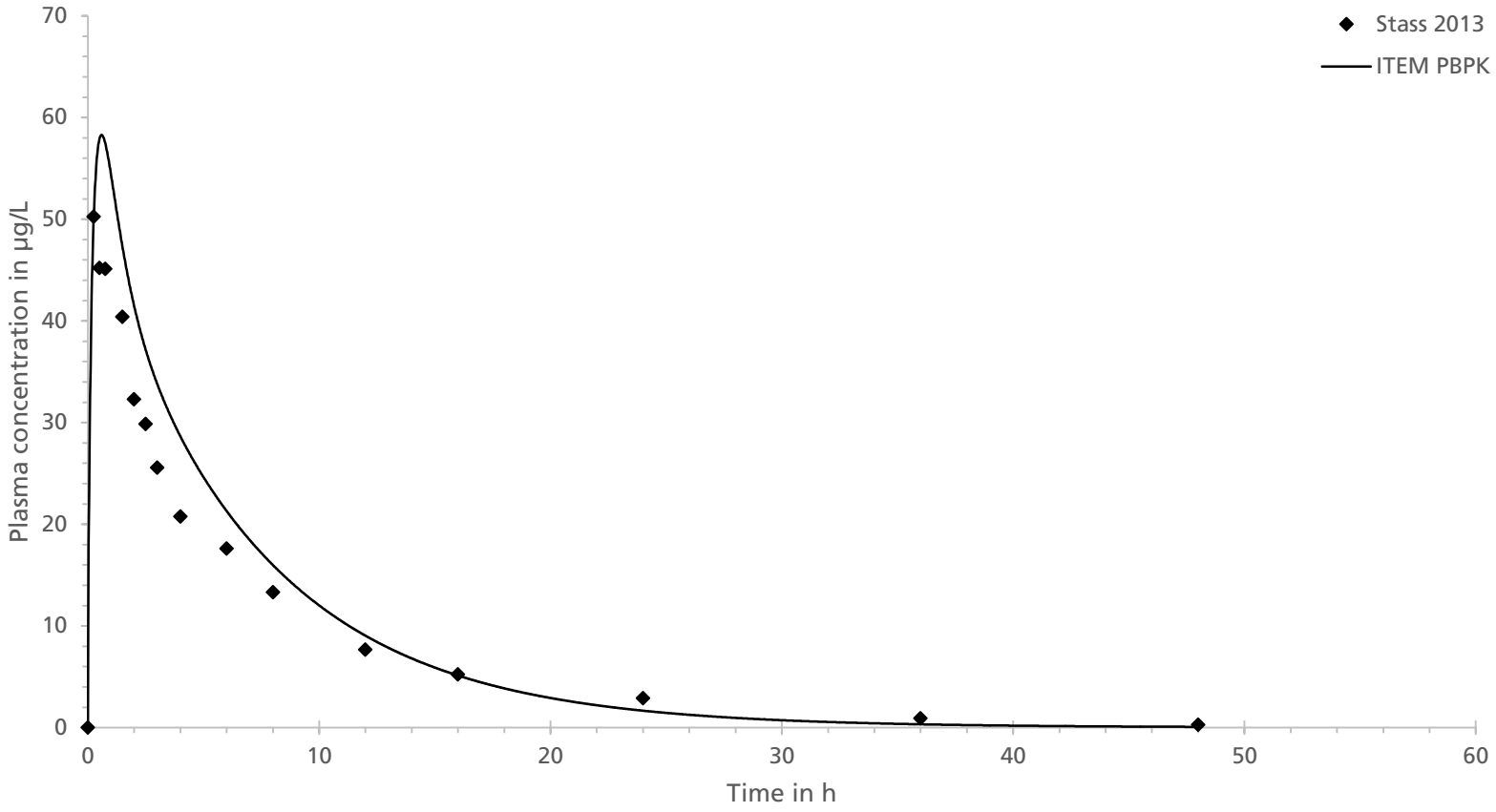
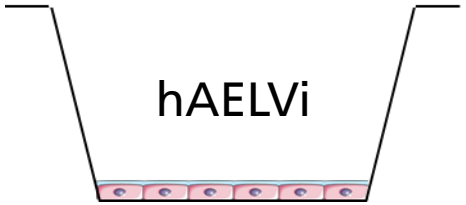
PBPK – comparison with human data of healthy volunteers



- Dose Ciprofloxacin:
  - 32.5mg (inhalable)
- in vivo data
  - Stass et al. (2013)
  - Healthy human volunteers
- In vitro data
  - Experimental data
  - Human cell models (Calu-3, hAELVi)

Conclusion/outlook

- Successful determination of P_{app} values



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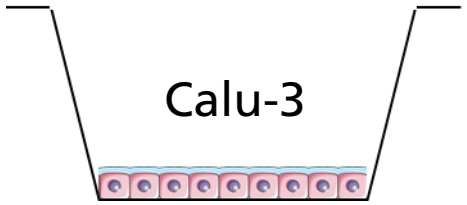

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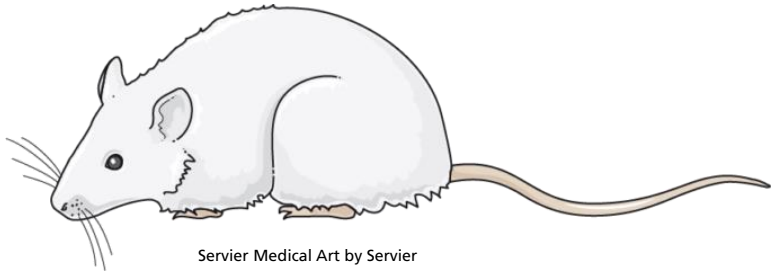
- PBPK model:
  - Experimental data are a near perfect fit to literature data (human)

- Reduction in the number of animal experiments
  - Cannot be completely replaced
  - Acquisition of new data, improvement of models
  - ➢ Reduce the number of animal experiments considerably



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Acknowledgement

Thank you very much for your attention!

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