The human epithelial lung cell line “A549” as a suitable tool in *in vitro* inhalation toxicity testing

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Background

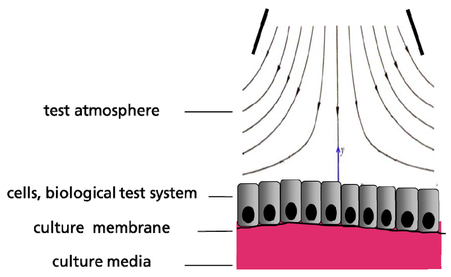
- Air-lifted interface (ALI) culture methods represent a state-of-the-art approach for investigations on biological effects of inhalable, gases, vapors or aerosols.
- Usually, such investigations aim at a high *in vivo* relevance by application of more complex cell models such as primary cells, 3D co-cultures or similar.
- Due to a demand for high numbers of cultures for routine testing especially in toxicity screening studies, “cheap and easy-to-use” cell culture models are urgently needed.

Objectives

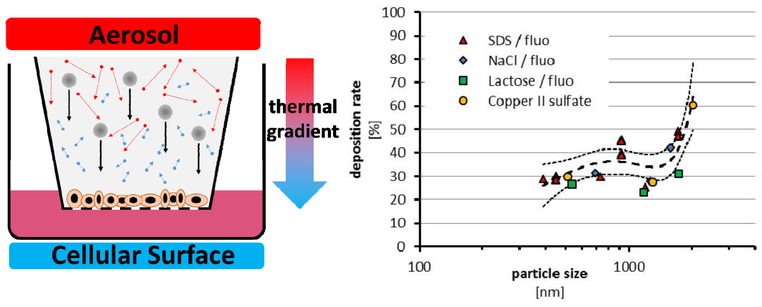
- The human alveolar epithelial type-II like cell line A549 represents a comprehensively characterized culture model (> 20.000 references)
- Although lacking tight-junctions and also with a carcinogenic origin, many studies have also confirmed primary characteristics (CYP-expression, surfactant biosynthesis, etc.)
- The objective of this overview is to highlight the qualities of A549 ALI cultures in inhalation toxicity testing *in vitro* with respect to applicability, in vivo relevance and reproducibility
- Summary of selected inhalation *in vitro* data from studies of the ITEMs lab during recent years

Inhalation toxicity testing *in vitro:* Methods

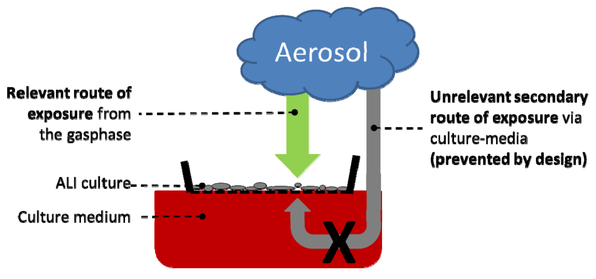
Efficient individual stagnation flow exposure of ALI cultures



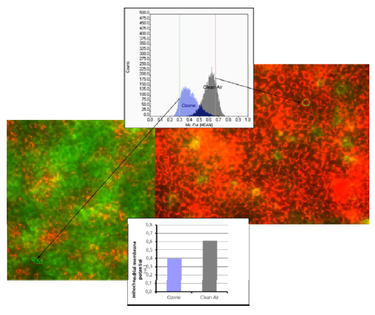
Thermophoresis leading to efficient particle exposure from aerosols



Prevention of irrelevant secondary route of exposure

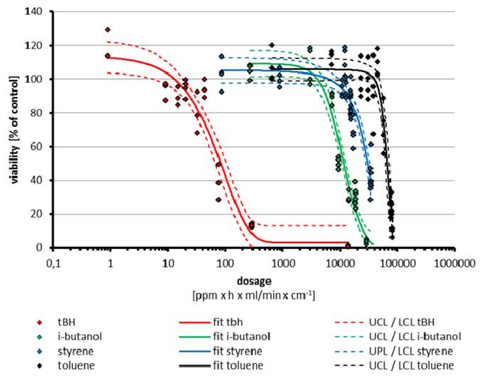


Observation of cells during exposure by fluorescence

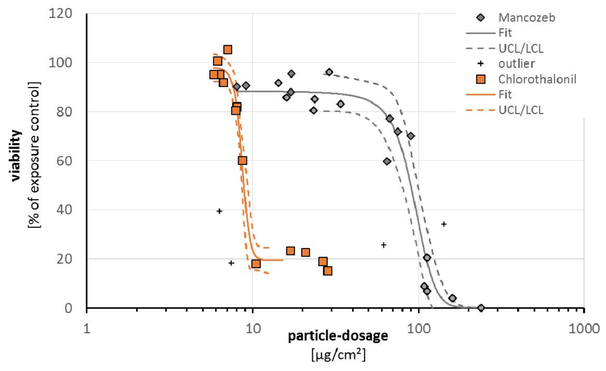


Applicability to gases, vapors and aerosols from test items, experimental or environmental atmospheres

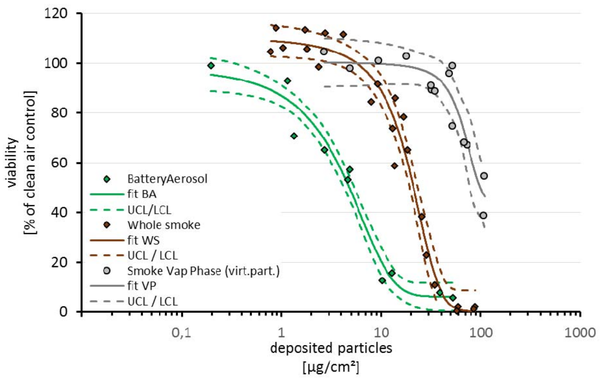
Gases / vapors
Testing of volatile organic compounds (VOCs)



Dry Particle aerosols
Testing of fungicide aerosols



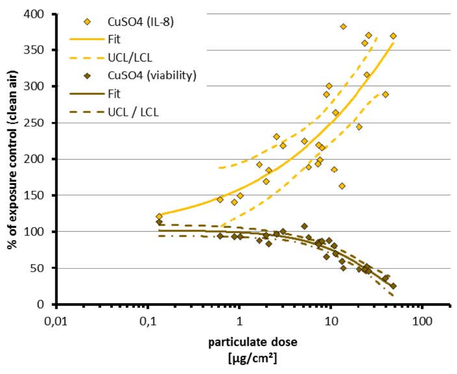
Experimental or environmental aerosols and atmospheres
Testing of combustion products: battery failure or tobacco smoke

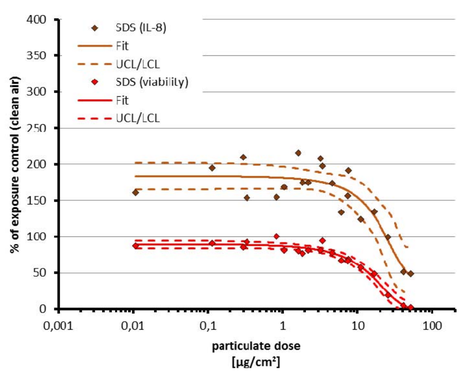


In vivo relevance by *in vitro* ↔ *in vivo* correlations

Approach a) …by referencing to relevant positive controls

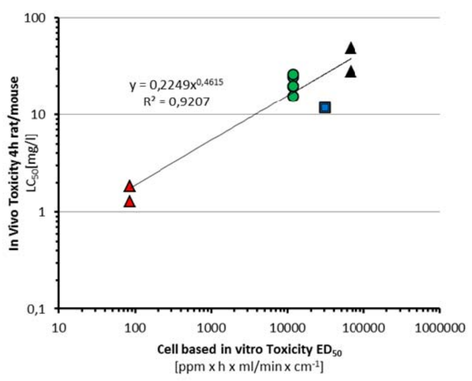
Human inhalation irritants
cupper-II-sulfate or sodium dodecyl sulfate



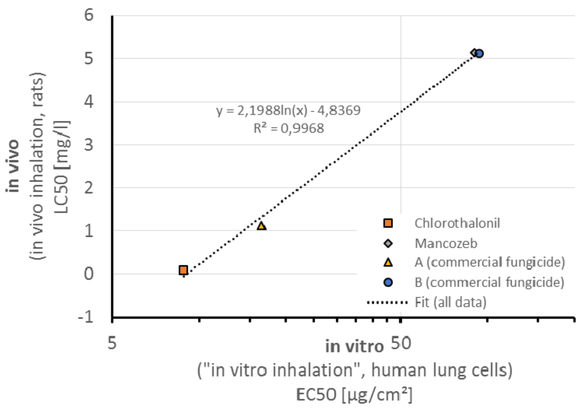


Approach b) …by referencing to in vivo inhalation data

Gas/vapor inhalation
in vivo / in vitro data

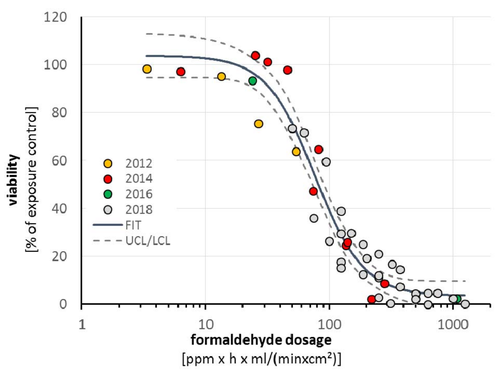


Dry particle aerosol inhalation
in vivo / in vitro data

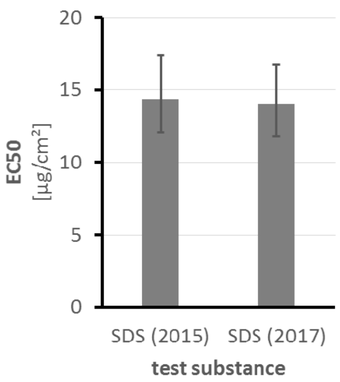


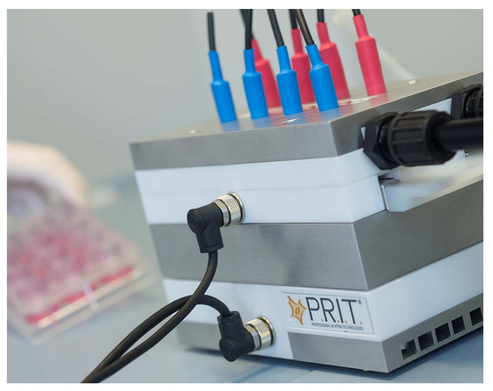
Reproducibility of *in vitro* inhalation toxicity data over years

Gas/vapor exposure dose-response data



Dry particle aerosol exposure EC_{50} data





Conclusions

- A549 human lung ALI cultures represent a relatively easy-to-use biological model that can be made available in the lab in large culture numbers (e.g. > 100/week) at moderate costs.
- Inhalation toxicity studies using A549 human lung ALI cultures have been shown to lead to a relevant prediction of *in vivo* toxicity.
- If applied under appropriate conditions, considerable reproducibility can be achieved, necessary for establishment of *in vitro* toxicity databanks and historical lab controls.
- These characteristics qualify A549 ALI cultures as biological models in first stages of tiered *in vitro* inhalation approaches, mechanistic studies may follow applying more complex cell models.

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