Fraunhofer ITEM

Improvements of the ALI *in vitro* testing method for inhalable compounds

Abstract 1050

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Situation

Actually, the **experimental approach to investigate inhalable aerosols *in vitro* can be difficult** because of several important factors:

- effects of non-solvable particles can be hard to analyse quantitatively,
- important setup specific parameters like particle deposition rates are missing,
- particle doses necessary to induce a biological effect are not reached (low deposition efficiency),
- no established positive/negative controls for testing inhalable aerosols *in vitro*,
- complex generation of defined control aerosols (particle sizes and concentrations),
- exposure setups are not sufficiently characterised and verified .

Objectives

Establishment of a simple and robust experimental concept.

- Water soluble substances,
- dry particle aerosols by nebulisation of salt solutions,
- simple and reproducible setup of particle size and concentration,
- quantitative dose determination by a tracer or substance specific analysis and
- characterisation and verification of
  - the exposure procedure,
  - the biological test system and toxic effect and
  - the complete experimental design

  in one experimental setup.

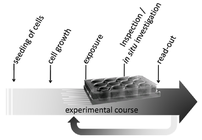
Approach

Application of the concept with a first set of four model aerosols and A549 human lung alveolar cell line.

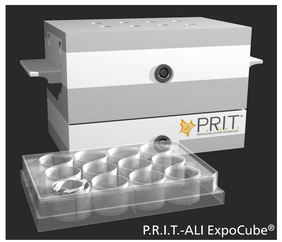
- Simple and efficient aerosol exposure using the **P.R.I.T.-ALI ExpoCube®.**
  - Application of **standard multiwell plates** throughout the whole testing procedure („all-in-one-plate"- and „repeated-dose-exposures"- workflow).
  - **Thermophoresis** for improved particle deposition from aerosols on the exposed cells.
- Quantification of exposure doses by measurement of deposited matter (fluorescein tracer or Cu(II) assay), aerosol photometer and filter analysis.

Approach (cont'd)

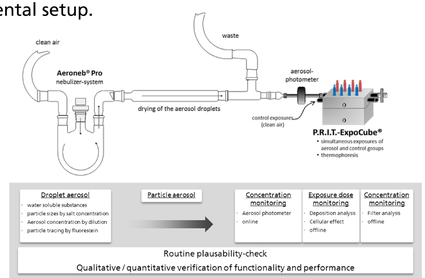
- "All-in-one-plate"-workflow.



- "Repeated-dose-exposures"-workflow.

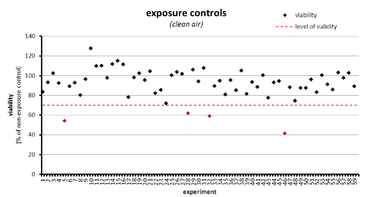


- Experimental setup.



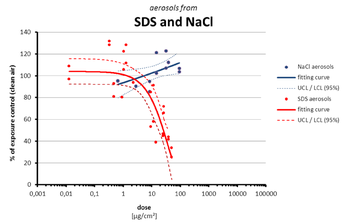
Results

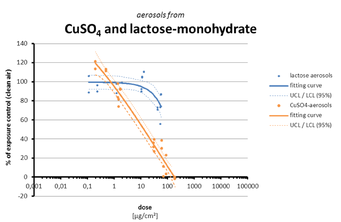
- Exposure controls confirmed a good retainment of cellular viability.



Results (cont'd)

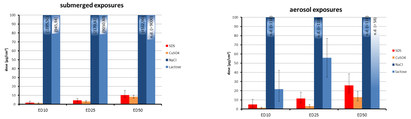
- Dose-response curves dependent on the particle dose were established.



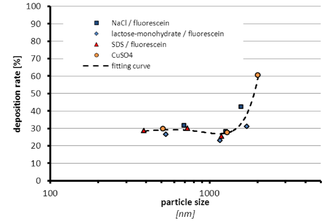


- Aerosol and submerged exposures confirmed the toxicity ranking:

  $CuSO_4$ > SDS >> lactose > NaCl



- Results from deposition and aerosol analysis resulted in deposition rates dependent on particle sizes.



Conclusion

- Verification of aerosol control exposure by compare of ALI aerosol toxicity rankings to submerged exposures.
- **High deposition efficiencies** of 25 to 30 % were found experimentally for particle sizes down to 389 nm using the P.R.I.T.-ALI ExpoCube® exposure conditions.
  (For comparison: common ALI conditions based on particle diffusion/sedimentation mechanisms enable deposition rates in the range of 1 to 2 % for these particle sizes.)
- Simple and **effective concept** which can also be applied to **dermal toxicity**.
- Easy-to-generate positive/negative aerosols for **routine testing of particulate inhalable compounds *in vitro.***
- **Simultaneous biological and physical characterisation/verification** in one experimental design.