Improvements of the ALI in vitro testing method for inhalable compounds

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Abstract

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.

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.

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.

• "All-in-one-plate"-workflow.

• Experimental setup.

• "Repeated-dose-exposures"-workflow.

• Exposure controls confirmed a good retainment of cellular viability.

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

• Aerosol and submerged exposures confirmed the toxicity ranking: CuSO₄ > SDS >> lactose > NaCl

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

• 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.

Experimental setup.