An alternative model for testing of acute respiratory local toxic and physiological effects based on in vitro and isolated perfused lung (IPL) technologies

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Background and objective

Due to the demand for further developments of predictive, robust alternative models in inhalation toxicology, a combined in vitro / ex vivo model was set up to cover both local acute toxic effects and respiratory physiological effects from inhalable powders.

Experimental design

The in vitro and ex vivo approaches are based on an air-lifted interface (ALI) cell culture model and an isolated perfused rat lung (IPL) model including exposure to highly concentrated aerosols generated from small amounts of micronized powders. The in vitro inhalation model included a human lung alveolar epithelial cell model (A549) in concentrated aerosols generated from small amounts of micronized powders. Due to the demand for further developments of predictive, robust alternative models in inhalation toxicology, a combined in vitro / ex vivo model was set up to cover both local acute toxic effects and respiratory physiological effects from inhalable powders.

Results

- Five commercial fungicides, sodium dodecyl sulphate (SDS) and n-dodecane were used as test items and positive/negative controls for acute local lung toxicity, respectively.
- Small amounts of testing materials of less than 500 mg only were needed in each model to establish dose-response curves (in vitro) or testing results (ex vivo).
- ED50-values from in vitro testing were correlated to LD50-values from acute rat inhalation in vivo testing and showed promising predictivity.
- Similarly, exposure to harmful test items as well as to the positive control SDS resulted in formation of oedema and for SDS also in acute decrease of lung function parameters in the IPL model as a measure for acute respiratory toxicity.
- Hence, the inhalation effects from toxic fungicides could primarily be assigned to cell toxicity.

Conclusions

In summary, the complementary in vitro / ex vivo model appeared very promising to investigate both acute local lung toxicity and respiratory physiological effects from inhalation of bulk powder materials using only small amounts of material with short study times and meaningful predictivity:
- In vitro model: promising “quantitative” prediction of respiratory/inhalation-related toxicity; calculation of median in vivo inner lung surface load indicates relevance of in vitro dosage range.
- Ex vivo model: “qualitative” toxicity of the substances, in addition detection of impairment of breathing mechanics.
- Possible perspective: in vitro -> ex vivo -> in vivo tiered approach for acute inhalation toxicity testing for aiming at replacement, reduction and refinement of animal experimentation (“3R principles”).