

Poster: strategies to reduce animal numbers for testing biologicals

## **Prevalidation study for testing the toxic effects of inhalable substances (gases) on human lung cells using an air/liquid culture technique**

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To date, studies of inhalation toxicity of air contaminants are restricted to animal experiments, mainly because of difficulties in exposing cell cultures directly to these substances. The increasing demand for assessing inhalation toxicity hazards calls for development of new testing strategies which comprise both in vitro and in vivo tests. For this purpose, we are evaluating a direct exposure strategy of cultured human lung cells to diverse inhalable substances at the air/liquid interface. In this approach, the well characterized alveolar cell line A-549 is grown on microporous membranes and exposed to test atmospheres. Appropriately designed systems for medium supply and gas support enable the nutrition and humidification and a direct contact of the exposed cells to the test gases at the same time.

Currently, four partners (Fraunhofer ITEM, ZEBET, UFZ and BAuA) are funded in part by the German Federal Ministry of Education and Research (BMBF) to assess in frames of prevalidation study the intra- and interlaboratory reproducibility and predictive capacity by testing the toxic effects of gases of well known in vivo toxicity (NO<sub>2</sub>, SO<sub>2</sub>, formaldehyde, ozone) after exposure of human lung cells at air/liquid interface. Particularly, at present time all four partners are working on optimisation and improvement of A-549 cell culturing on the microporous membranes, cell exposure to the test gases, as well as establishment and transfer of tests for the toxicological endpoints. The Neutral Red Uptake assay was chosen as a cytotoxic endpoint and Comet assay as a genotoxic endpoint. The aims of this prevalidation study are: 1) optimisation and refinement of experimental protocols; 2) generation of standard operating procedures (SOPs) according to GLP; 3) assessment of transferability and reproducibility within and between laboratories; 4) determination of the in vitro vs. in vivo dosimetry relations; 5) Development of a first prediction model that enables predicting in vivo toxicity from in vitro data.

In the presentation the concept of the prevalidation study as well as some preliminary data will be discussed.

*Keywords: inhalation toxicity, air/liquid culture technique, prevalidation study*