

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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The increasing demand for assessing inhalation toxicity hazards calls for new testing strategies comprising both in vitro and in vivo assays. For this purpose, we are currently evaluating a direct exposure strategy, in which cells are exposed to toxic gases at the air/liquid interface. The human carcinoma alveolar epithelial cell line A549, grown on microporous membranes, is exposed to test atmospheres in a system enabling at the same time steady state nutrification, humidification and direct gas exposure. Under coordination of the Fraunhofer Institute, we are assessing the intra- and interlaboratory reproducibility and predictive capacity of the method by characterizing the toxicity of four gases, i.e. NO₂, SO₂, formaldehyde, and ozone. The aims of this study are: optimisation and refinement of experimental protocols; generation of standard operating procedures; assess

ment of reproducibility within and between laboratories; establishment of test acceptance criteria; determination of the in vitro vs. in vivo dose-response relationships. After transfer of the method, optimization of protocols and experimental procedures the four partners started definite testing of the gases. Each gas, together with an online analytical monitoring system, is passed from one lab to the next after six weeks of experimentation. The test design comprised one hour gas exposure followed by direct determination of cytotoxicity (electrical current exclusion method, CASY®, Innovatis) and genotoxicity (COMET assay). So far, the project has proven satisfying transferability of the test system, depending on the laboratory being practiced in this complex methodology.