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# Applicability of rat precision-cut lung slices for nanomaterial toxicity testing

*Dr. Ursula G. Sauer, Scientific Consultancy – Animal Welfare, Neubiberg*

*and Sandra Vogel, Alexandra Aumann, Annemarie Hess, Susanne N. Kolle, Lan Ma-Hock, Wendel Wohlleben, Martina Dammann, Volker Strauss, Silke Treumann, Sibylle Gröters, Karin Wiench, Bennard van Ravenzwaay, Robert Landsiedel, BASF SE, Ludwigshafen*

The applicability of rat precision-cut lung slices (PCLuS) to predict nanomaterial respiratory toxicity was investigated. Sixteen OECD reference nanomaterials (NMs) (TiO2, ZnO, CeO2, SiO2, Ag, multi-walled carbon nanotubes (MWCNT)) were evaluated thereby covering dissolving and non-dissolving materials just as fibrous NMs. The addressed in vitro effects reflect the spectrum of early events that, to date, have been recognized for NM toxicity: total protein, reduction in mitochondrial activity, caspase-3/-7 activation, glutathione depletion/increase, cytokine induction. Additionally, the lung slices were submitted to histopathological evaluation. Ion shedding NMs (ZnO and Ag) induced severe tissue destruction detected by loss of total protein. Two anatase TiO2 NMs, the CeO2 NMs, and two MWCNTs caused significant (determined by trend analysis) cytotoxicity in the WST-1 assay. At non-cytotoxic concentrations, different TiO2 NMs and one MWCNT increased GSH levels, presumably a defence response to reactive oxygen species, and these substances further induced a variety of cytokines. One of the SiO2 NMs increased caspase-3/-7 activities at non-cytotoxic levels, and one rutile TiO2 only induced cytokines. Hence, PCLuS can detect different early effects of NM toxicity. Investigating these effects is, however, not sufficient to predict apical effects found in vivo. Reproducibility of test substance measurements was not fully satisfactory, especially in the GSH and cytokine assays. Effects were frequently observed in negative controls pointing to tissue slice vulnerability even though these prepared and handled with utmost care. Qualitative comparisons of in vivo to in vitro effects reveal some concordances for the metal oxide NM, but less so for the MWCNT. The highest effective dosages, however, exceeded those reported for in vivo rat short-term inhalation studies. For NM testing, the PCLuS system requires test protocol optimization, and study results should be considered preliminary.