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Online Dose Determination For In Vitro Experiments With Nano Particles In The Karlsruhe Exposure System

Background

For the quantitative assessment of the toxicity of airborne nanoparticles the dose – response relationship is tested in in vitro test systems using bioassays of cell cultures as sensor. For the air-liquid interface exposure of cell cultures towards aerosols the Karlsruhe Exposure System (Mülhopt et al) (Fig. 1) was developed. After exposure the responses of the cells are analyzed to measure the biological responses such as viability, inflammatory or oxidative stress (Diabaté et al). For the determination of the dose response relationship accurate knowledge of the dose is an essential question (Teeguarden et al).

Aim

Online measurement of deposited particle dose in vitro exposure experiments for assessment of particle toxicity.

Methods for Dose-Determination

Two alternatives were developed and compared:

- 1. Fluorescein sodium dosimetry (FSD):** a highly sensitive method for a precise determination of the deposited mass exclusively on the membrane.

Fluorescein sodium aerosol is used as calibration particles (Fig. 3). After an exposure experiment the cell culture membrane is cut out of the Transwell® insert, washed in 10 ml ultra pure water and treated by sonication. The fluorescein sodium mass in the solution is analyzed by fluorescence spectroscopy.

- 2. Quartz crystal microbalance (QCM):** an online measurement of the deposited mass per cell surface unit and time providing a control on the exposure quality.

In the Karlsruhe Exposure System one of the 6 exposure chamber the membrane is replaced by the sensor crystal of a QCM. It monitors the deposited particle mass per area unit and time.

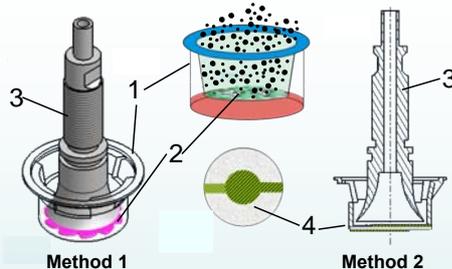


Fig. 2: Schemes of the measurement methods: in the Transwell® membrane insert (1) the membrane surface (2) is exposed to the aerosol flow (3). In the online dose determination the membrane (2) is replaced by the sensor crystal (4) of the QCM

Results

The FSD method and the QCM online monitoring are compared. Further experiments are required to improve the reproducibility of the measurement.

Deposited mass by spectroscopy [$\mu\text{g}/\text{cm}^2$] Average of 5 values	1,50±18%	1,67±13%
Deposited mass by microbalance [$\mu\text{g}/\text{cm}^2$] determined over 2 hours	1,74	2,37
Calibration factor ($m_{\text{QCM}}/m_{\text{FSD}}$)	1,16	1,42

References

- Diabaté, S., Mülhopt, S., Paur, H.-R., Krug, H.F. (2008) *Alternatives To Laboratory Animals*, 36, 285–298
- Mülhopt, S., Paur, H.-R., Diabaté, S., Krug, H.F. (2007) *Advanced Environmental Monitoring*, Y.J. Kim and U. Platt, eds. Springer Netherlands, pp. 402–414.
- Teeguarden, J.G., Hinderliter, P.M., Orr, G., Brian D. Thrall, B.D., Pounds, J. G. (2007) *Toxicological Sciences*, 95(2), 300–312

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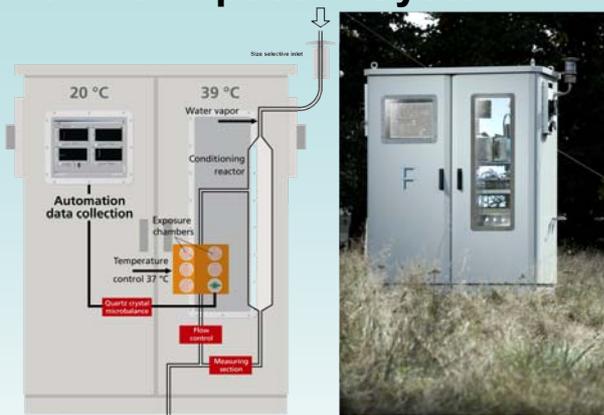


Fig. 1: Scheme (left) and image (right) of the Karlsruhe Exposure System for toxicity measurements of airborne nanoparticles. Passing a size selective inlet particles bigger than 1 μm are removed entering the exposure system. The aerosol humidity is adjusted to 85% by injection of steam. The human lung cell cultures are placed in VITROCELL® modules and subjected to a constant flow of the conditioned aerosol.

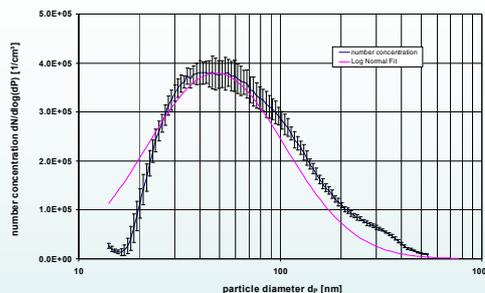


Fig. 3: Number size distribution of fluorescein sodium aerosol determined by SMPS 3071 (TSI)

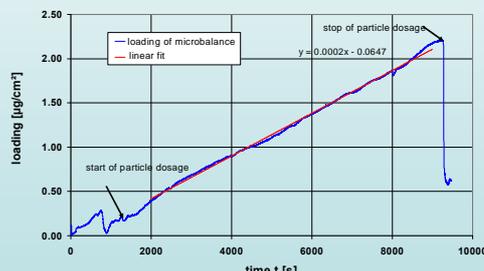


Fig. 4: Online measurement of deposited particle mass by QCM sensor