Effect of (Co-) exposures of Printex90 and formaldehyde on a cell-based assay system under air-lifted Interphase (ALI) conditions

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Introduction

The increasing use of nanoparticles in a variety of products requires an intensive exploration of possible particle effects on humans. Usually, particle effects alone are focused rather than combined effects of particles and additional compounds.

Objectives

The BMBf project NanoCOLT aims at investigating long-term effects of modified carbon black nanoparticles on healthy and pre-damaged lungs using in vitro, ex vivo and in vivo approaches. Therefore, the effect of Printex90 particles (i) alone or after a (ii) previous, respectively a (iii) subsequent exposure to formaldehyde were investigated in a cell-based in vitro model using a human lung epithelial cell line.

Materials and Methods

Cell based model system: The human lung epithelial A549 cell line was purchased from a commercial supplier (ATCC; LGC Promochem). Cells were initially cultivated under their cell type specific conditions in 75cm² culture flasks using submerged conditions. Culture medium was changed every to 2-3 days. Before reaching 80% confluence, cells were subcultivated. During a cell passage an aliquot of the cells was then seeded on microporous membranes (Inserts, BD Falcon; 0.4µm pore size; growth area ~1cm²). Cells were further cultivated on the membranes for approximately 72hrs until they reached a confluent monolayer as inspected by light microscopy. Cultures were set to air-lifted conditions during a medium change approx. 24hrs before exposure. During the treatment, cells were nutrified by culture media from beneath the membrane solely while being exposed to the test substances from the top.

Test items: Formaldehyde Solution (F-8775) was obtained from Sigma-Aldrich. Printex®90 was purchased from Evonik Industries (Hanau, Germany) and characterized by the Engler-Bunte Institute (Karlsruhe, Germany).

Exposure: Cells were exposed to test and reference (clean air) substances under ALI-conditions using the P.R.I.T.®-ALI Technology. The system represents an exposure device, which has been developed by Fraunhofer ITEM and is routinely used in studies dealing with gases and airborne substances (Ritter & Knebel 2014). Cells were exposed solely or in combination with the gaseous and/or particulate test substance. Viability was recorded directly or 24hrs after exposure by use of WST-1 assay.

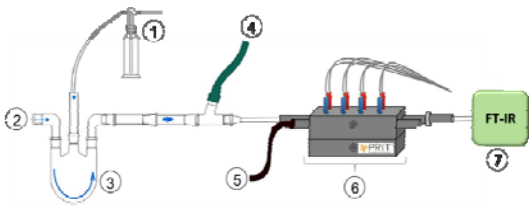

Figure 1: Scheme of the *experimental setup* for exposing human lung cells (A549) to gaseous test substances (formaldehyde) using air-liquid interface conditions. The system consists of three parts: A: Generation and transport of the test substance atmosphere (1-4) or the clean air (5) control respectively. B: The exposure unit for the target cells, grown under air-liquid interphase conditions (P.R.I.T.® ExpoCube® ;6). C: Analysing unit for testatmosphere (FT-IR Monitor); Position 4 represents the exhaust of excess testatmosphere.

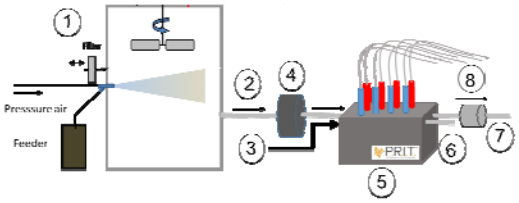

Figure 2: Scheme of the *experimental setup* for exposing human lung cells (A549) to airborne test substances (Printex 90) using air-liquid interface conditions. The setup consist of (1) an aerosolgeneration unit (2) sampling point for in vitro exposure (3) clean air supply for control situation, (4) scattering light photometer (5) P.R.I.T.® ExpoCube®; air-liquid interphase exposure of target cells, (6, 7) exhaust air flow, (8) particle filter for sampling purposes.

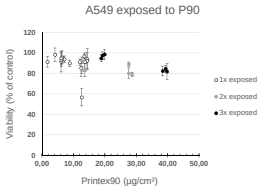

Figure 3. Exposure of A549 to Printex 90. Cells were exposed up to 3 times on consecutive days resulting in a calculated particle deposition of 1.7 up to 39.8 µg/cm².

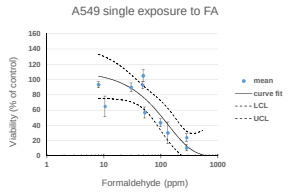

Figure 4. Exposure of A549 to increasing concentrations of formaldehyde. Cells were exposed to concentrations between 8ppm and 282ppm. The calculated EC50 value was 107ppm formaldehyde.

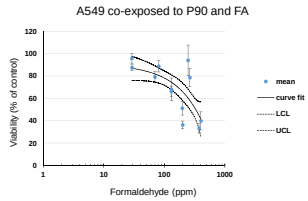

Figure 5. Co-exposure of A549 cells to Printex90 (about 12,7µg/cm²) followed by an exposure to increasing concentrations of formaldehyde. Cell viabilty was determined 24hr after exposure. The calculated EC50 value was 331ppm formaldehyde.

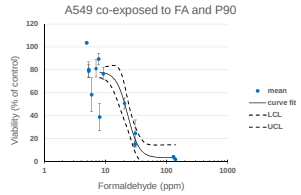

Figure 6. Co-exposure of A549 cells to increasing concentrations of formaldehyde followed by an exposure to Printex90 (about 12,7µg/cm²). Cell viabilty was determined 24hr after exposure. The calculated EC50 value was 21pmm formaldehyde.

Results

- Single and repeated exposures of A549 cells to Printex90 aerosols (1.7 to 39.8 µg/cm²) resulted in nearly unchanged viability (80 to 100 %age of air control).
- Exposure to formaldehyde (8ppm to 282 ppm) resulted in dose-dependent reduction of viability (EC_{50}(FA) = 107 ppm).
- Exposures to Printex90 before exposure to formaldehyde resulted in an increased EC_{50} value (331 ppm).
- Contrastingly to this, the opposed experimental situation (Printex90 following to formaldehyde exposure) resulted in a considerably reduced EC_{50} value (21 ppm).

Conclusions

The study shows that (i) complex exposure situations with combinations of particulate and gases can be realized in an in vitro model, and (ii) the resulting cellular effect is modulated as a function of the selected co-exposure situation.

References

Ritter, D., Knebel, J, "Investigations of the biological effects of airborne and inhalable substances by cell-based in vitro methods: fundamental improvements to the ALI concept", Advances in Toxicology Volume 2014, Article ID 185201,.

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