Effects of carbon black nanoparticles on human pulmonary cell lines and precision cut lung slices
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INTRODUCTION
Carbon Black nanoparticles (CBNPs) are among the most abundantly used nanomaterials and have been reported to cause adverse health effects after inhalative exposure. Pulmonary in vitro or ex vivo models are thus urgently needed to gain insights into potential mechanisms of toxicity. To this end, a joint research project is funded by the German Federal Ministry of Education and Research. In the course of this project, the effects of Printex® 90 and acetylene soot particles were compared in human pulmonary cell lines (16HBE14o⁻, Calu-3, A549) and precision cut lung slices (PCLS) of mice, rats and humans over a wide concentration range. Acetylene soot particles carry polycyclic aromatic hydrocarbons bound to the surface.

MATERIAL AND METHODS
Particle size distribution in the cell culture medium was determined by dynamic light scattering. Viability assays were LIVE/DEAD® staining and WST-1 assay for PCLS and WST-8 and neutral red assay in the case of cell lines. CBNP-induced formation of reactive oxygen species (ROS) was assessed in A549 and 16HBE14o⁻ cells by flow cytometry using the DCFH-DA assay. Furthermore, the effect of CBNP exposure on the transepithelial electrical resistance (TEER) was investigated in Calu-3 cells. With PCLS, the inflammatory response was assessed by measuring pro-inflammatory cytokines (i.e., IL-1α, TNF-α, IL-8).

RESULTS
Both CBNPs tested were nearly nontoxic in physiologically relevant concentrations. Statistically significant effects were observed in the WST-8 assay for both CBNPs i after 48h, whereas no effects were found in the neutral red assay. Increased ROS formation was observed with both CBNPs after 24 and 48 h. TEER values were measured after 24, 48 and 120h treatment with 10 and 50 μg/ml. Interestingly, acetylene soot particles caused significant TEER reduction at both dose levels and all time points tested whereas Printex® 90 reduced the TEER only after 120h and in the high dose. Neither Printex® 90 nor acetylene soot particles induced the secretion of proinflammators cytokines in mouse and rat PCLS.

CONCLUSION
Cytotoxic effects of CBNPs depend on their surface properties. Furthermore, this study demonstrates, that the combination of in vitro and ex vivo models provides a valuable tool to assess the acute effects of CBNPs on lung tissue.

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