Human lung cells and Bariumsulfate nanoparticles from incineration processes - data from the project NanoEmission

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NANOEMISSION

Abstract

An increasing number of products used in our every-day life contains nanomaterials. Eventually such products end up in waste-incineration at the end of their life-cycle. The aim of the project NanoEmission, funded by the German Federal Ministry of Education and Research (BMBF), was to assess whether nanoparticles in the exhaust air emitted from waste-incineration plants pose a risk to humans and the environment. When comparing the cytotoxicities (MTT assay, Resazurin assay, LDH assay) of nano- and micro-scale BaSO4-particles before and after incineration in a technical center-scale incinerator and particles from the exhaust gas of combustion without added nanoparticles a significant difference between the effects of the various BaSO4-particles could not be detected. There were also no effects of the barium particles on Glutathion (GSH) level. Nevertheless particles from combustion without barium particles induced GSH in lung cells after 24 hours. In line with these findings fresh BaSO4 particles did not induce reactive oxygen species (ROS) in human lung cells. Also there were no significant differences between the cytotoxic effects of fresh BaSO4-nanoparticles and particles up to 200 nm from the exhaust gas of a large-scale waste incineration plant resulting from combustion of household waste with or without added BaSO4-nanoparticles. In line with these findings no significant differences were detected between the effects on cell cultures of fresh and annealed BaSO4-nanoparticles. Taken together these results suggest that thermal treatment does not have a significant effect on the toxicological profiles of BaSO4-nanoparticles. Ecotoxicity tests on Chlamydomonas reinhardtii showed no effects on growth, but induction of GSH up to 140 % (1 mg/ml). However this study is not a complete risk assessment and its results cannot be transferred directly to other nanomaterials. The mechanism behind the toxic effects of BaSO4-nanoparticles could not be resolved in this project completely. Nevertheless oxidative stress does not seem to be the major driving force behind it. For completion of the risk assessment of these particles further studies are necessary.

Fig. 1: Viability of NHBEC after 24 and 72 hours exposure to $BaSO_4$

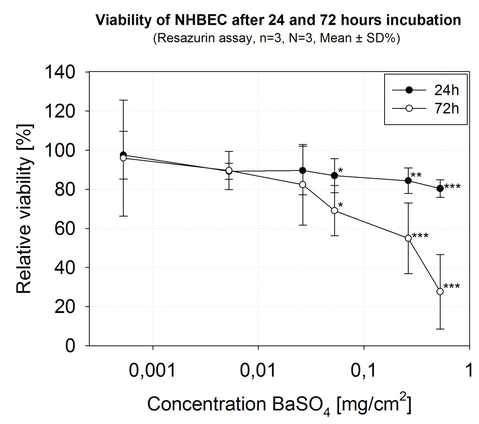


Fig. 2: Intracellular GSH content of NHBEC after 24 and 72 hours exposure to $BaSO_4$

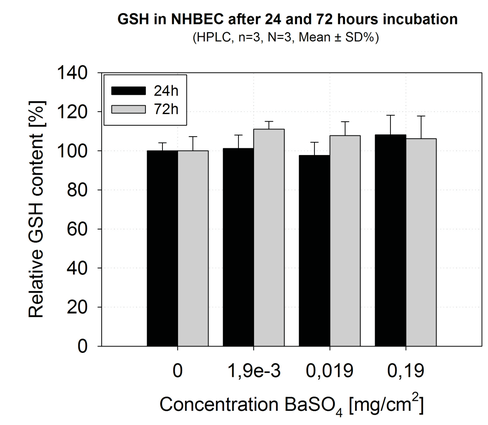


Fig. 3: Viability of NHBEC after 24 and 72 hours exposure to $BaSO_4$before and after incineration in technical center-scale incinerator

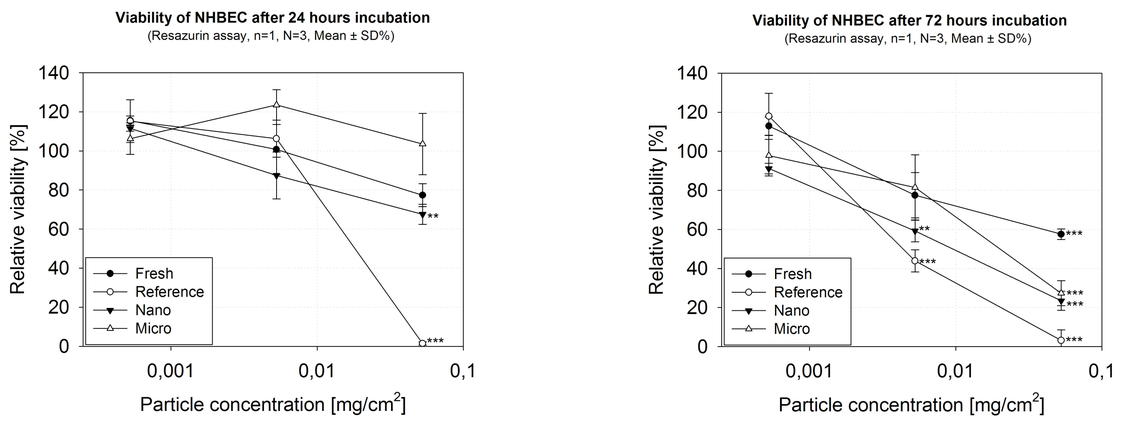


Fig. 4: Intracellular GSH content after 24 and 72 hours exposure to $BaSO_4$before and after incineration in technical center-scale incinerator

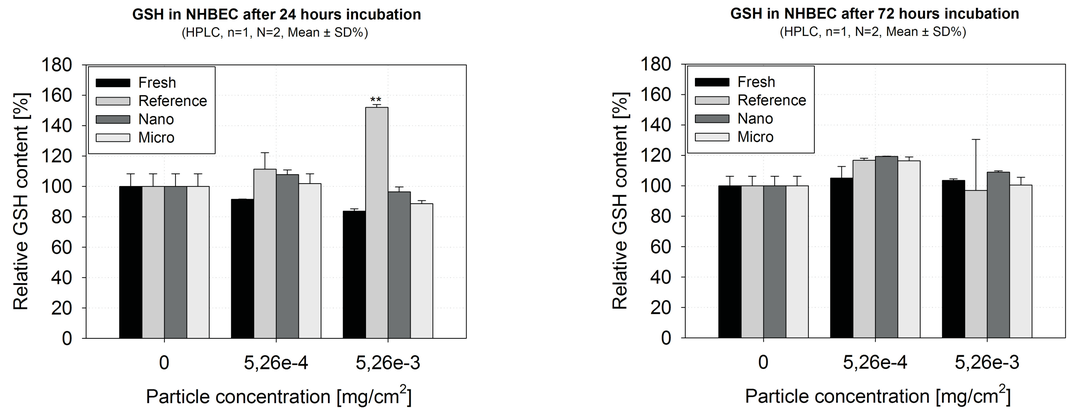


Fig. 5: Viability of NHBEC after 24 and 72 hours exposure to $BaSO_4$before and after incineration in large-scale waste incineration

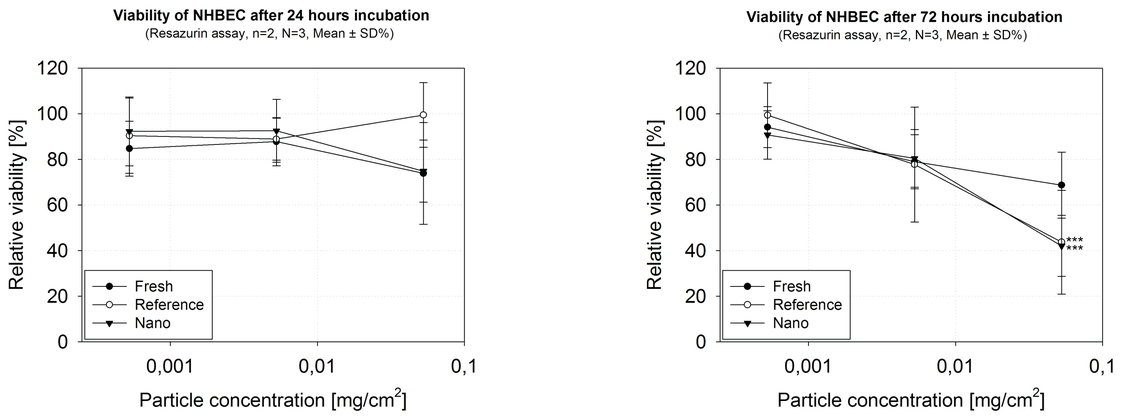


Fig. 6: Intracellular GSH content after 24 and 72 hours exposure to $BaSO_4$ before and after incineration in large-scale waste incineration

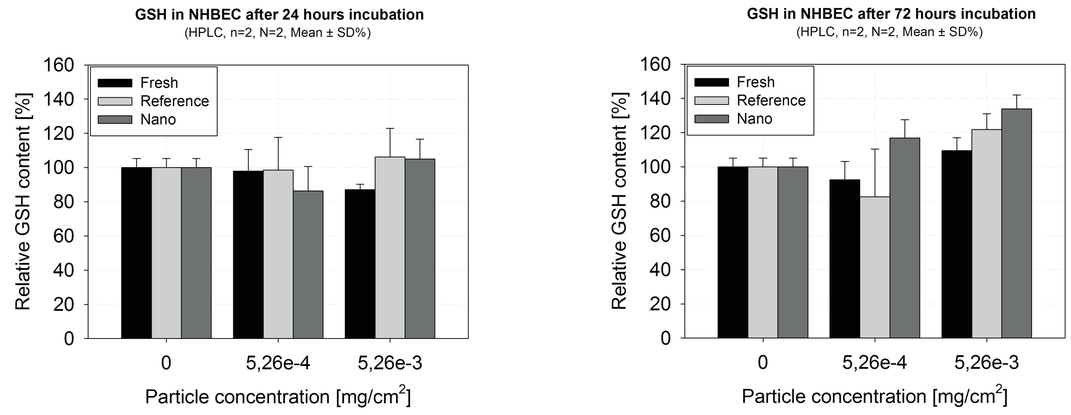


Fig. 7: Growth rate C. reinhardtii after 72 hours exposure to $BaSO_4$

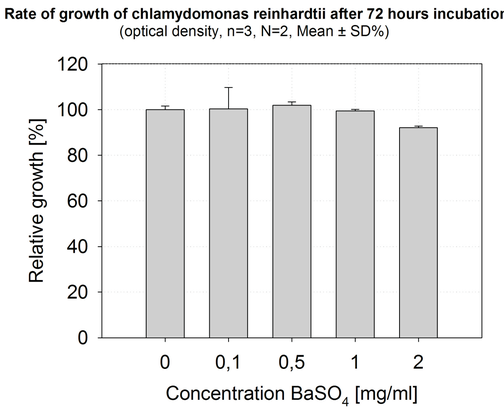
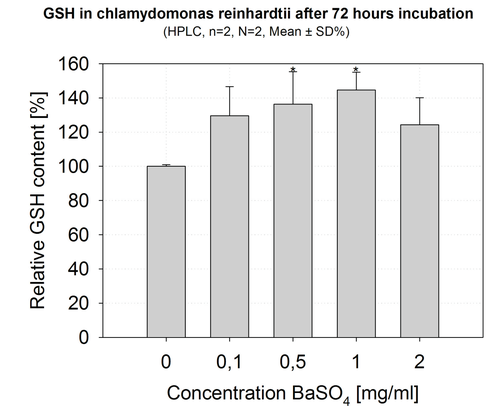


Fig. 8: GSH content of C. reinhardtii after 72 hours exposure to $BaSO_4$



Methods:

1. Primary culture of normal human bronchial epithelial cells (NHBEC)
Bronchial tissue was obtained from lung resections of cancer patients. Normal tissue as used in our cell cultures was obtained in proximity of the tumor. Before transport the tissue material was transferred to cold, sterile Leibovitz L15-buffer (Biochrom, Berlin, Germany) as soon as possible. The tissue was dissected and cut into pieces of approx. 0.2-0.5 cm². These pieces were transferred onto culture dishes. After five minutes serum-free medium (AECG-Medium, Promo Cell, Heidelberg, Germany) was added. Medium was changed every two days. The first subconfluent monolayer was obtained after 2-3 weeks. Then the pieces could be transferred to new culture dishes and the monolayer could be split and seeded onto new culture dishes ($10x\ 10^3$ cells/ cm^2).
2. Characterisation of the nanoparticles
The pure nanoparticles were characterised by SEM-EDX images in different magnifications (50 - 200000fold) by our project partners TEER RWTH Aachen and LFG Erlangen.
3. Establishing and characterisation of the suspension
The characterisation was done by dynamic light scattering with a Malvern Zetasizer Nano ZS after the stock solution was sonicated for 2 minutes with an ultrasonic probe. The concentration of the stock solution was 10 mg/ml and it was diluted appropriately prior to measurement.
4. Determination of cell viability
Resazurin-assay: Cells were incubated with Resazurin-solution (11 mg/l in PBS) for 30 min at 37°C. In viable cells Resazurin is reduced to Resorufin by mitochondrial enzymes. The concentration of Resorufin was determined by fluorescence spectrometry (ex: 530 nm; em: 590 nm).
5. Determination of intracellular glutathione (GSH) content by HPLC
Cells were harvested using trypsin, resuspended in 0.1 N HCl and frozen at -80°C. After reduction with DTT and subsequent derivatisation with Monobromo bimane the total amount of cellular GSH was determined by HPLC with fluorescence detection (ex: 380 nm; em: 480 nm). Conditions for chromatography were: stationary phase: Chromolith® Performance RP-18 endcapped 4,6×100 mm; mobile phase: solvent A: 2% methanol / 98% water / 0,25% acetic acid (pH 4,3); solvent B: 90% methanol / 10% water / 0,25% acetic acid (pH 3,9). Injection volume: 30 µl.
6. Growthrate
Effekts of the test particles on the growth of the alga *Chlamydomonas reinhardtii* were determined according to OECD guideline 201. Algae were exposed to various concentrations of the particles for 72 hours during exponential growth-phase and relative growth was calculated in relation to un-treated controls.
7. Statistics
One-Way-Anova, * $p < 0,05$, ** $p < 0,01$; *** $p < 0,001$ Significance to control
Incineration, precipitation and characterisation of the nano particles
Were conducted by our project partners

This project was funded by the German Ministry of Education and Research (BMBF).
FKZ: 03X3578D

Scientific Stakeholder Meeting on Nanomaterials in the Environment, UBA, Dessau, October 10th and 11th, 2017

Further Results:

- Before the incineration tests we compared two $BaSO_4$-nanoparticles from different producers (Solvay, Huntsman). The impact of the nano particles was determined in NHBEC and peripheral lung cells (PLC).
- We examined: viability (Resazurin assay, LDH assay, MTT assay), modulation of intracellular GSH content, induction of ROS, induction of CYP1A1, induction of apoptosis, Uptake of $BaSO_4$ and release of Ba^+ -ions in to cell culture medium.
- There were only few differences between both the materials and cell types.
- After the first comparative tests we continued tests with $BaSO_4$ from Huntsman and NHBEC and performed eco-toxicological tests with the algae *Chlamydomonas reinhardtii*, bioluminescent bacteria *Vibrio fischeri* and water flea *Daphnia magna*. It was shown that *Vibrio fischeri* is the most sensitive model organism in our eco-toxicological tests.
- In addition to the results for particles from incineration shown here we also determined cytotoxicity by LDH assay, expression of CYP1A1 and release of cytokines
- Finally we compared the impact of untreated $BaSO_4$ and thermally treated (900 °C) $BaSO_4$. In all assays we could show, that thermal treatment has no significant effect on toxic properties of the particles studied. Nevertheless these findings cannot be transferred onto other nano materials without testing.