Genotoxicity of amorphous silicon dioxide nanomaterials

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Introduction

Nanosized silica is among the most commonly used nanomaterials. However, few studies are available on the genotoxicity of these materials. Here, we applied the cytokinesis-block micronucleus assay in human bronchial epithelial BEAS 2B cells to investigate the genotoxicity of four types of synthetic nanosized amorphous silicon dioxides used in food and rubber products.

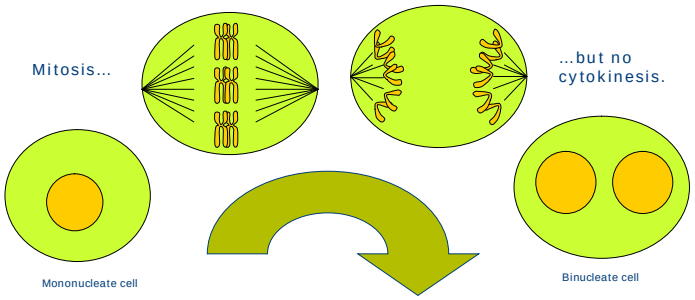
Materials and methods

The SiO2 nanoparticles studied derived from EU Joint Research Centre Nanomaterials Repository and had been produced by precipitation (NM-200, NM-201) or thermally (NM-202, NM-203). The materials differed from each other with respect to physical characteristics such as shape, specific surface area (160-230 m2/g), and primary particle size (8-20 nm).

Human bronchial epithelial (BEAS 2B) cells were exposed to the nanoparticles for 48 h. Cytotoxicity was assessed by cell counting using Trypan blue. Five doses of each material, showing ≤55±5 % toxicity, were chosen for further testing.

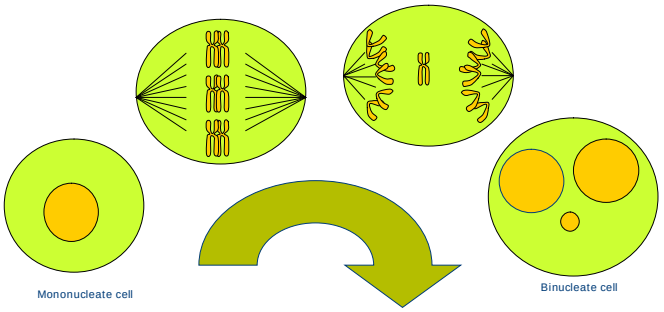
Genotoxicity was studied by cytokinesis-block micronucleus assay. The BEAS 2B cells were again exposed for 48 h and cytochalasin B was added to all cultures 6 h after the start of the treatment. Treated cells were stained with acridine orange and 4',6-diamidino-2-phenylindole (DAPI). Two independent scorers evaluated the frequency of micronuclei in binucleate cells. Mitomycin C was used as a positive chemical control. Negative control cultures received only medium.

Principles of cytokinesis-block micronucleus assay

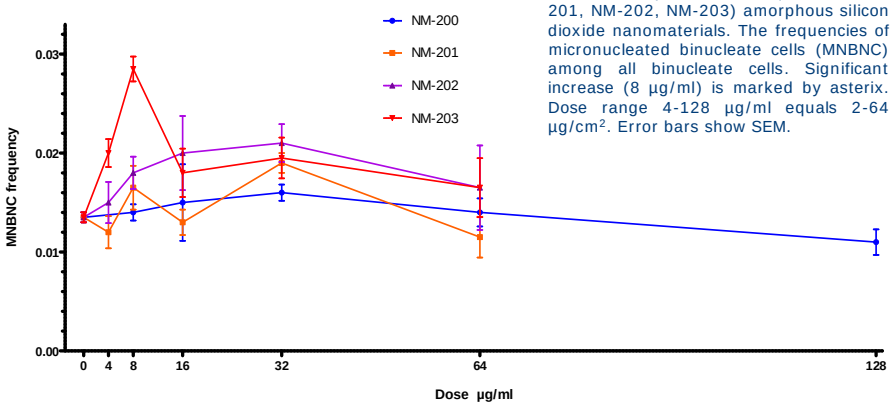


Cytochalasin B inhibits cytoplasmic division by blocking the formation of contractile microfilaments. Cytokinesis-block method makes it possile to limit micronucleus analysis to cells that have divided once after the exposure.

Micronuclei are formed when chromosomal fragments or entire chromatids or chromosomes are left behind in anaphase. This can be due to an effect on mitotic apparatus or double-strand breaks caused by errors in repair of DNA damage.

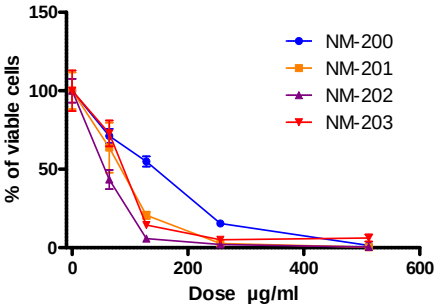


Micronucleus assay results



Micronucleus induction in BEAS 2B cells after 48-h exposure to four (NM-200, NM-201, NM-202, NM-203) amorphous silicon dioxide nanomaterials. The frequencies of micronucleated binucleate cells (MNBNC) among all binucleate cells. Significant increase (8 µg/ml) is marked by asterix. Dose range 4-128 µg/ml equals 2-64 µg/cm^2. Error bars show SEM.

Cytotoxicity



Viability of BEAS 2B cells after 48-h exposure to four silicon dioxide nanomaterials (NM-200, NM-201, NM-202, NM-203). Cytotoxicity was tested untill 512 µg/ml. Dose range 64-512 µg/ml equals 32-256 µg/cm^2. Error bars show SEM.

Conclusions

- Synthetic nanosized amorphous silicon dioxides were not efficient inducers of chromosome damage as measured by the micronucleus assay
- Nano-SiO_2 manufactured by sedimentation did not induce micronuclei
- Pyrogenic nano-SiO_2 showed marginal induction of micronuclei

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