Human hazard evaluation of multicomponent nanomaterials

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Introduction

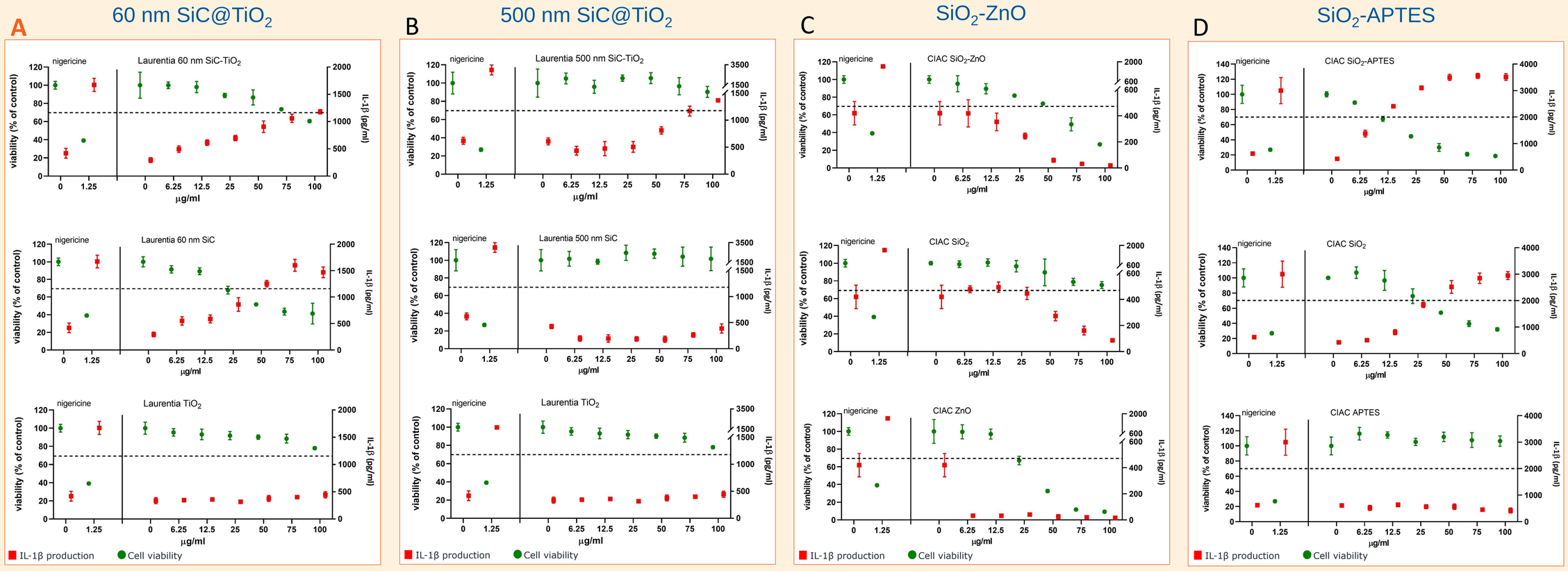
The exponential rise of new materials, including multicomponent nanomaterials (MCNMs), represent a challenge for toxicological testing. Within SUNSHINE, dose-response relationships for potential human hazard endpoints are being evaluated for four industrially relevant MCNMs using a tiered testing approach. Tier1 tests are simple in vitro assays, while Tier2 are more complex *in vitro* model, including 3D and co-culture models. Three different endpoints were evaluated: cytotoxicity, genotoxicity and inflammasome activation, with comparisons between MCNMs and their individual components.

Methods

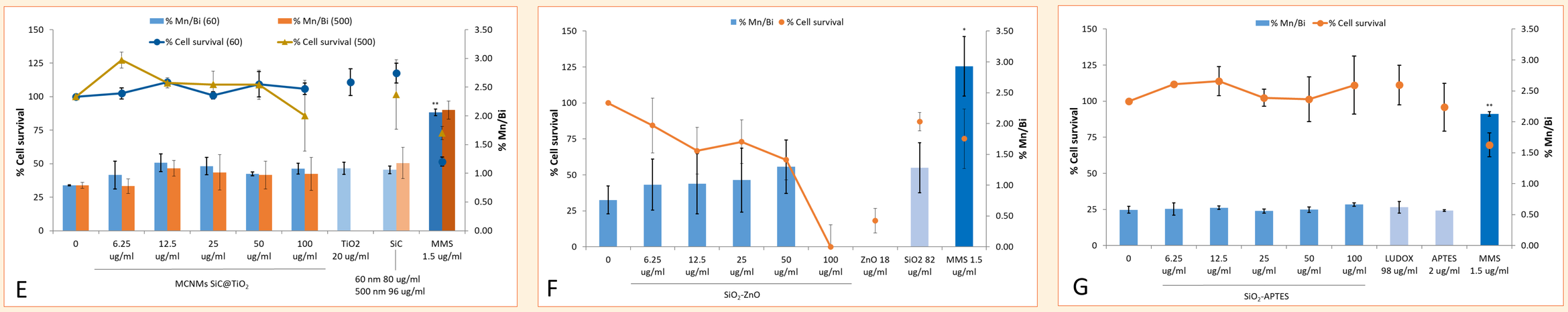
MCNM dispersion: Nanogenotox protocol, using 0.05% BSA water as a dispersant
Cytotoxicity: Relative Population Doubling (RPD) represents the increase in the number of population doublings in cells exposed to MCNMs versus levels in untreated control, and was expressed as a percentage
Genotoxicity: Cytokinesis Block Micronucleus assay (CBMN) was performed on the human lymphoblastoid TK6 cell line. MCNM exposure was for 24h, followed by incubation with Cytochalasin B to block cytokinesis for 1.5 cell cycles
Inflammasome activation: evaluated based on a combination of cytotoxicity and IL-1beta release, measured via WST-1 and ELISA, respectively

Results

Inflammasome activation



Cytotoxicity and genotoxicity



60 and 500 nm SiC@TiO_2
Both MCNMs trigger inflammasome activation (A, B), although only 60 nm SiC follows the same behaviour. Regarding cytotoxicity and genotoxicity, there is no significant increase in cell death or DNA damage for the samples tested (E).

SiO_2-ZnO
This MCNM does not elicit inflammasome activation (C). However, in both SU and RIVM testing, SiO_2-ZnO reveals a dose-related cytotoxicity (C, E). For this reason, it was not possible to assess the genotoxicity response at the MCNM's highest concentration (100 µg/ml) and for ZnO alone.

SiO_2-APTES
Both MCNM and its SiO_2 component induce IL-1ß release, activating the inflammasome (D). No genotoxicity was induced by this material. Whilst there was no reduction in cell survival (by RPD, G), WST-1 testing indicated a reduction of cell viability over the dose range applied (D).

Conclusion and further work

The two materials provided by Laurentia (60 and 500 nm SiC@TiO_2) activated inflammasome without compromising cyto- and genotoxicity. Similar behaviour was seen for CIAC's SiO_2-APTES; however, the two cytotoxicity methods used (WST-1 and RPD) show differing results, as they look at different mechanisms of cell death in different cell types. Most likely, pyroptosis is involved in inflammasome activation and not in the RPD measurement. SiO_2-ZnO from CIAC did not elicit inflammasome activation, but showed a concentration-dependent decrease in cell viability for all concentration tested. It is worth noting that the MCNM was more toxic than its single components. A possible mechanism of action for SiO_2-ZnO toxicity could be the leaching of Zn ions.
On the basis of these Tier1 assay results, selected materials will undergo Tier2 testing with assays suitable to their relevant life-cycle exposure. The more complex *in vitro* models that will be applied involve the use of lung coculture systems coupled to MCNM aerosolisation exposure scenarios.

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