

Genotoxicity testing of nanomaterials: adequacy of the standardized *in vitro* mammalian cell micronucleus test

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Common toxicity tests might not be fully adequate for evaluating nanomaterials since their unique features are also responsible for unexpected interactions with assay components or detection systems. The *in vitro* cytokinesis-block micronucleus (CBMN) test is recommended by OECD (Test Guideline No.487) for genotoxicity assessment. Nevertheless, cytochalasin-B (Cyt-B) used to inhibit cytokinesis may affect nanoparticle (NP) uptake, leading to inaccurate results. This study evaluated whether the presence of Cyt-B influences cellular uptake and MN production by TiO₂ NP in SH-SY5Y cells. Following OECD recommendations, two options to prevent interference were applied: (1) post-treatment (application of NP for 3-24h, removal of the NP, and addition of Cyt-B), and (2) delayed co-treatment (application of NP for 3–24h, addition of Cyt-B 3-6 h later, and further incubation for 24h). These options were compared to the traditional co-treatment. TiO₂ NP were significantly internalized by the cells after 3-24h treatments, but no differences were observed between the presence or absence of Cyt-B. CBMN test showed progressive increases in the MN frequencies after 6 or 24h treatments in the three treatment options; however, no differences were obtained in the comparisons between treatment options. Despite previous studies highlighted the possible interference of Cyt-B on NP cellular uptake and, consequently, on MN detection by CBMN test, current data do not support that hypothesis. Additional experiments are necessary to define the most suitable protocol of CBMN test for assessing genotoxicity of nanomaterials.

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