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In Vitro Cytotoxicity and Genotoxicity Assessment of Novel Cellulose Nanomaterials using intestinal cells

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Cellulose nanomaterials (CNMs) have been investigated for several applications, including in food and food packaging (e.g. as candidates for zero-calorie filler/thickener/stabilizers; as substitutes of petroleum-based food packaging materials). The widening of these applications will lead to human exposure via oral route, and potentially, to adverse health outcomes. To contribute to the CNMs safety evaluation, the aim of this study was to analyse the in vitro cytotoxicity and genotoxicity of two new micro/nanofibrillated celluloses (CMF/CNFs), using the HT29-MTX-E12 human intestinal cell model. CNMs were synthetized from industrial Eucalyptus globulus kraft and their physicochemical properties were characterized. Upon cells exposure to 3.1 - 200 µg/mL of CNMs during 24 h, the cytotoxicity was evaluated by the MTT and clonogenic assays, and the genotoxicity by the cytokinesis block micronucleus (CBMN) and comet assays.

None of the CNMs was cytotoxic in the concentration-range tested. Concerning genotoxicity assessment, CMF induced a significant level of DNA damage (comet assay) in cells exposed for 3h to 25, 50 and 100 µg/mL and for 24h, to 50 µg/mL, compared with controls. No increases were observed with the FPG-modified comet assay compared with negative control. Cells treatment with the CNF for 3h significantly increased DNA damage at 14.3, 25, 50 µg/mL while a 24h treatment produced significant damage at 50 µg/mL, compared with control. For the latter concentration, induction of oxidative DNA damage was observed for both time points. In contrast, no increase in chromosomal damage was observed using the CBMN assay upon 52h of exposure. To our knowledge, this is the first study in which CNMs were evaluated for their genotoxic effects using the HT29MTX-E12 cell model, relevant for their potential ingestion. Our findings show that cytotoxicity, the endpoint generally used to assess their biocompatibility, is not sufficient to assess their safety to humans. Ongoing studies including the in vitro simulation of human digestion will allow a more comprehensive assessment of CNMs safety. This should be done at an early stage of their development, to ensure their sustainable and innovative application in food technology.

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