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## The relationship between the biological effects of Titanium Dioxide nanofibers and their aspect ratio

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A strict relationship between the toxicity of fiber-like nanomaterials and their aspect ratio emerges from the fiber paradigm. As a consequence, fiber shortening is expected to reduce material toxicity. Titanium dioxide nanofibers (TiO2NF) are a novel fibrous nanomaterial, used in several industrial applications but still requiring complete toxicological characterization. We evaluated the toxicity of commercial TiO2NF (length, 0.2-30um; thickness, 0.2 to 0.6um; aspect ratio 1:28, consisting of primary TiO2 nanoparticles), before and after ball-milling, which lowered their aspect ratio to 1:8. The evaluated endpoints were cell viability, inflammatory markers, and trans-epithelial electrical resistance (TEER), an indicator of the epithelial barrier competence. TiO2 NF exhibited cell specific cytotoxicity, markedly decreasing viability in A549 epithelial cells but not in Raw 264.7 macrophages. A dose- and time-dependent TEER decrease in CaLu-3 cell monolayers was also detected. Ball-milling significantly mitigated these effects but, conversely, enhanced the expression of inflammatory markers in macrophages.

This study indicates that TiO2NF exert significant toxic effects including cytotoxicity, macrophage activation and epithelial barrier impairment. While aspect ratio reduction mitigates TiO2NF effects on cell viability and epithelial barriers, it enhances the inflammogenic activity of the nanomaterial, indicating that different structural determinants are implied in the biological effects of fiber-like nanomaterials.

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