Comparison of cell viability assays for evaluating the cytotoxicity of HDPE microparticles in Tetrahymena thermophila (Protozoa, Ciliata)

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Abstract

The increasing presence of microscopic plastic debris in the aquatic environment has raised concerns about micro- and nanoplastic particles as emerging pollutants with specific properties that require adaptation of existing test procedures and harmonisation of experimental methods. High-density polyethylene (HDPE), widely used in various products and approved by the Food and Drug Administration for food contact, has been implicated in recent studies for potential environmental impacts, including effects on organism development and behaviour. In this study, the cytotoxicity of HDPE microparticles was investigated using the protozoan *Tetrahymena thermophila*, a model eukaryotic organism widely used in human health and environmental risk assessment, that combines the properties of single eukaryotic cells and whole organisms. Our aim was to compare various cytotoxicity assays commonly used for non-motile *in vitro* cell cultures to assess the potential risks of HDPE microparticles. The tested materials were extensively characterised in the test medium, and their cytotoxicity was compared to well-studied particulate controls (Ag and TiO2 nanoparticles). We included the cytotoxicity assays described in ISO standard 4988:2022 (ATP and MTT assays), and extended the evaluation with four additional assays targeting different mechanisms: resazurin assay, CyQuant and BrdU proliferation assay, and PI/Hoechst differential staining. After 24 hours of exposure, the surface of the organisms was examined using scanning electron microscopy, revealing no evidence of material adsorption. Light microscopy analysis confirmed the uptake of 5 μm particles into the *Tetrahymena thermophila* food vacuoles. Although the organisms internalised HDPE microparticles, the results of the six cytotoxicity assays showed no significant changes in metabolic activity, viability or proliferation of *Tetrahymena thermophila*. Our findings suggest that HDPE microparticles pose a low hazard potential. Our approach ensures compliance with quality criteria for particle effect studies and generates data in accordance with FAIR principles.

Keywords: Cell viability assays, Cytotoxicity, High density polyethylene (HDPE), Tetrahymena thermophila, Microplastics

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