## ANALYSIS OF IN VITRO CYTOTOXICITY AND GENOTIXICITY OF POLYSTYRENE NANOPARTICLES IN HUMAN HEPATOMA CELL LINE (HEPG2)

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Plastics are a major environmental concern as they can persist in the environment for hundreds of years. Plastic may be degraded into micro-particles < 5000 nm in diameter, and further into nanoparticles (NPs) < 100 nm in diameter. Among these, polystyrene nanoparticles (PS-NPs) are found to be the most represented NPs in the environment. In vivo and in vitro studies have suggested that PS-NPs may penetrate organisms through several routes i.e. skin, respiratory and digestive tracts, so it is essential to evaluate the safety and possible genotoxicity [1]. In this study, we evaluated the cytotoxicity and genotoxicity of PS-NPs in the human hepatoma cell line HepG2 in vitro. We comprehensively characterised the PS-NPs, assessed their cellular uptake, and measured the levels of reactive oxygen species by performing flow cytometry, evaluated cytotoxicity using AlamarBlue assay, and assessed genotoxicity through the alkaline comet assay [2, 3]. In this experiment, HepG2 cells were treated with PS-NPs at concentrations of 5-100  $\mu$ g/mL. Flow cytometry results showed an efficient uptake of the nanoparticles into the cells and that an increase of PS-NPs leads to a rise in levels of reactive oxygen species. Tested concentrations were not cytotoxic in HepG2 cells, but an increase in DNA damage was found to be statistically significant at most concentrations. Besides, a very strong direct relationship was detected between the percentage of DNA damage and NP concentrations. These findings suggest the effective uptake and genotoxic potential of polystyrene nanoparticles and raise concern about the safety of plastics.

<sup>[1]</sup> Kik, K., Bukowska, B., Sicińska, P. (2020). Polystyrene nanoparticles: Sources, occurrence in the environment, distribution in tissues, accumulation, and toxicity to various organisms. Environmental Pollution, 262, 114297.

<sup>[2]</sup> Longhin, E. M., El Yamani, N., Rundén-Pran, E., Dusinska, M. (2022). The Alamar blue assay in the context of safety testing of nanomaterials. Frontiers in Toxicology, 4, 981701. https://doi.org/10.3389/ftox.2022.981701

<sup>[3]</sup> Elespuru, R., et al. (2018). Genotoxicity assessment of nanomaterials: Recommendations on best practices, assays, and methods. Toxicological Sciences, 164(2), 391–416. https://doi.org/10.1093/toxsci/kfy100