# Preliminary data on the cytotoxic effects of polyethylene terephthalate and polystyrene nanoplastics on Huh-7 hepatocytes

M. Morataya-Reyes[1], A. Villacorta[1,2], A. Hernández[1] and R. Marcos[1]

[1] *Departament de Genètica i de Microbiologia, Facultat de Biociències, Universitat Autònoma de Barcelona, Cerdanyola del Vallès (Barcelona), Spain*
[2] *Facultad de Recursos Naturales Renovables, Universidad Arturo Prat, Chile*
*E-mail: herlem.morataya@uab.cat*

Increasing demand for plastic products and inadequate waste management have resulted in their accumulation in the environment, where different physicochemical/biological mechanisms cause them a steady fragmentation to reach micro and nanoplastics (MNPLs) size, with structural differences and with potential changes in their ways of action. Although there is evidence of human exposure to MNPLs, their effects on secondary organs after trespassing dermal, respiratory, and gastrointestinal barriers are still not understood. One of these potential target organs is the liver.

To address this gap in knowledge, we are studying the effect of nanoplastics coming from two of the most used plastics, polyethylene terephthalate (PET) and polystyrene (PS), in Huh-7 cells as an *in vitro* model of human hepatocytes. In our experimental approach cells were exposed to 50 µg/mL of PET (100 nm), pristine PS (50 nm), and carboxylate PS (PS-COOH, 50 and 100 nm) for exposures lasting 1, 6, 24, 48, and 72 h. Our results indicate an increase in their internalization with time for PS, 100 nm PS-COOH, and PET. It is interesting to point out the high internalization of 50 nm PS-COOH, which was present in more than 99% of the cells from the first hour of exposure. We confirmed the NPLs internalization by using confocal microscopy, showing all the plastics to be inside the cellular membrane and also into the nucleus.

Furthermore, after exposing cells to 25, 50, 100, and 200 µg/mL of the selected NPLs, we observed a dose-dependent decrease in Huh-7 viability, although in all cases it was above 70%. Finally, we have detected the generation of intracellular reactive oxygen species (ROS) in the exposed cells showing a time- and size-dependent relationship.

After these preliminary results, we plan to follow the hazard assessment of the selected NPLs by determining genotoxic damage, changes in the cytokine profiles, and immunologic response associated with gene expression changes. This data will be used to propose biomarkers to extend our acute experiments to long-term exposure scenarios and to provide relevant information for regulatory authorities.

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