

Adverse effects of polystyrene nanoplastics on teleost fish: *in vitro* and *ex vivo* evidence

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Nanoplastics pollution in marine and freshwater environments poses a growing global risk to aquatic organisms. In this study, *in vitro* and *ex vivo* approaches were utilized to investigate the effects of 20 and 80 nm commercial polystyrene nanoplastics (PS-NPs) on teleost fish cell lines and primary gill, head kidney and spleen cell culture models. PS-NPs were characterized by transmission electron microscopy and by Dynamic Light Scattering. Subsequently, the severe cytotoxicity of 20 nm PS-NPs was demonstrated. The process of internalization was examined by employing fluorescent PS-NPs, which were localized within the nucleus as early as ½ hour post-incubation. Additionally, the cytoplasmic translocation and the adverse effect on cellular components were observed to be dependent on the size of the internalized nanoparticles. Piscine cells suffered structural damages dependent on both the size, dose and duration of exposure to PS-NPs. The noticeable alterations in cellular morphology, such as cell contraction and plasma membrane bleb formation were distinctive features of the execution phase of apoptosis, as confirmed by the Annexin V/propidium iodide and Tunel assays. The apoptotic responses were unrelated to intracellular ROS signaling. Transcriptional changes at sublethal and lethal PS-NPs doses were confirmed, with a more profound response to the latter consisting in a marked impairment of steroid biosynthesis, TGF-beta signaling pathway, focal adhesion and protein processing in endoplasmic reticulum. Our results clearly indicate that fish face a considerable danger from PS-NPs.

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