
Secondary PVC microplastics are more toxic than primary PVC microplastics to *Oryzias melastigma* embryos

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Abstract

Although irregular-shaped secondary microplastics (SMP) account for most MPs in marine environments, little is known about their effects on marine organisms. We investigated the embryotoxicity of polyvinyl chloride SMP and primary microplastics (PMP) to the marine medaka *Oryzias melastigma* using mortality, heart rate, hatching rates, hatching time and malformation as endpoints. This study aimed to determine the physical impacts of MPs and elucidate underlying mechanisms of physical MP toxicity through O₂ flow determination and measuring the gene expression involved in embryonic development. PMP were purchased in powder form, while SMP were prepared by milling a pristine PVC plastic water pipe; both were sieved to a size range of 53–106 μm . For the 25 day exposure assay, fertilized *O. melastigma* eggs were randomly assigned to 6-well plates containing 15 embryos/well. Seawater leachates generated from the PMP and SMP were also studied to account for chemical toxicity. Exposure of marine medaka embryos to PMP and SMP caused a range of negative effects, including changes in heart rate, morphological abnormalities and malformation types. At high exposure concentrations, SMP shortened hatching time and induced more significant teratogenic effects on larvae relative to the PMP. Physical damage from SMP to the chorion surface appears to be the main toxicity mechanism, caused by their irregular shape and reduced aggregation relative to PMP. In contrast, real-time changes in oxygen demonstrated that hypoxia caused by greater PMP adsorption to the chorion surface contributes to their toxicity relative to SMP. Modulation of genes involved in hypoxia-response, cardiac development and hatching confirmed the toxicity mechanisms of PMP and SMP. The chemical contribution to observed toxicity was negligible. Our findings indicate that although PMP exposure can lead to measurable toxic effects, their use underestimates the threat of SMP and they do not necessarily represent the toxic mechanisms observed for SMP.

Keywords: physical impacts, secondary MP, primary MP, adhesion, oxygen exchange, damage, chorion

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