

Title: Tire wear particles effects on the model species *Danio rerio*: influence of ageing

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Abstract

Tire wear particles (TWP) are formed when friction occurs between tires and the road surface. Aside physical damage to organism, they may cause parallel toxicity exerted by compounds on the TWP composition that are released over time have already been reported. Thus, the study evaluated the toxicity of a TWP suspension (0.5 and 512 mg TWP/L) and ageing stages [immediately, 7, and 30 days after preparation (0dA, 7dA), and 30dA, respectively]], using *Danio rerio* as a model species. The 96h assays followed the OECD guideline 236 (OECD, 2013) using both embryos and post-hatching larvae to determine potential different routes of uptake. Mortality and malformations in addition to the determination of the heartbeat rate (HBR), total and interocular length (TL and IL, respectively), swim bladder area (SBA), and behaviour (total swim distance and time) were registered. No significant mortality and malformations were registered in all assays of embryos and larvae (>10%). In embryos, HBR presented a pattern of tachycardia between concentrations of 0.5 and 8 mg TWP/L, whilst a pattern of bradycardia at higher concentrations (<32 mg TWP/L), for all ageing stages. In the post-hatching larvae, SBA decreased significantly ($p < 0.05$) at all tested concentrations (except 0.5 mg TWP/L) compared to the control_0dA. The activity time of larvae from the embryo assay was close to zero during and understanding of the impact that this type of particulate may have on ecosystems. The results obtained highlight that further studies are needed to increase knowledge on TWP effects on ecosystems and, ultimately, human health.

Keywords: Emerging contaminant; environmental effect; surface waters; freshwater fish