Investigating tire tread particle toxicity to fish using Rainbow Trout cell lines

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Tire and road wear particles (TRWP) are generated during the abrasion of tires while driving, and are composed of rubber and tire additives, but also road minerals, bitumen and brake particles embedded during generation. Recently, questions were raised about their potential contribution to microplastics released into the aquatic environment and their potential toxicological impacts. Our study aimed to assess the toxicity of TRWP and associated chemicals to fish using two Rainbow Trout (*Oncorhynchus mykiss*) cell lines representing the gill (RTgill-W1) and the intestinal (RTgutGC) epithelium. The toxicity of cryogenically milled tire tread (CMTT) particles, used as a proxy for TRWP, was assessed considering several potential exposure pathways: 1) exposure to CMTT with direct contact in order to quantify a combined particle/leachate effect, 2) exposure to CMTT leachate only, to assess the toxicity of the leaching chemicals, 3) exposure to CMTT digestate, to investigate if fish gastro-intestinal conditions could result in a different chemical profile and change the toxicity and 4) exposure to thermooxidised CMTT, to determine the effect of aging on the induced toxicity. In accordance with OECD TG249, cell viability was assessed after 24 hours acute exposure using a multiple-endpoint assay indicative of cell metabolic activity, membrane integrity and lysosome integrity. Chemical composition of the exposure medium was analyzed to assess which chemicals could be responsible for the observed acute effects. The determined fish cell line in vitro EC50 values were 2.02 g/L and 4.65 g/L for RTgill-W1 and RTgutGC cell lines, respectively, and in the same range as what was previously reported *in vivo*, and far above tire and road wear particle environmental concentrations reported in river water (4 mg/L). However, road runoff concentrations were found to exceed 100 mg/L and our results showed that the non-toxic concentration was in the range of 40 to 130 mg/L, hence more research into long-term effects of such high exposure is required. Moreover, our results showed that the presence of the particles was greatly contributing to the overall observed toxicity, as the leachate alone induced lower toxicity. Aging of the particles also resulted in a lower toxicity, due to less chemicals leaching out in the medium. On the opposite, *in vitro* digestion of the CMTT resulted in a higher toxicity in comparison to water leachates, due to higher concentrations of metals and organic compounds leaching in the digestive fluids, among which were Zn, 2-mercaptobenzothiazole, 1,3-Diphenylguanidine (DPG), and N-(1,3-dimethylbutyl)-N'-phenyl-p-phenylenediamine (6PPD). Although traces of 6PPD-quinone were also measured, further cytotoxicity assays using RTgill-W1 and RTgutGC cell lines showed no toxicity of this compound up to 3 mg/L, while several studies reported toxicity *in vivo* at far lower concentrations. This lack of toxicity when exposing the gill and intestinal cell lines could be due to a specific mode of action of this chemical compound, such as neurotoxicity, which we will investigate further using a Rainbow Trout brain cell line.