In-vitro digestion of tire and road wear particles: bioavailability of metals and polycyclic aromatic hydrocarbons

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1. Introduction

Tire and Road Wear Particles (TRWP) are generated at the road interface while driving as a result of the friction of the tire wear on the pavement. TRWP have been suspected to represent a large part of the microplastic particles release in the environment. Yet, its fate in the different environmental compartments and potential toxic impacts on the environment remains largely under investigated. Nonetheless, the potential impact of TRWP on aquatic organisms has recently gained attention since the occurrence of TRWP in the aquatic environment has been demonstrated in surface water and sediments of numerous regions. Moreover, the ingestion of TRWP by aquatic species, including fish, has recently been demonstrated. However, data regarding the bioavailability and toxicity of contaminants associated with these particles are still lacking. Several metals enter in the composition of tires including Zinc, used as a catalyst during the vulcanization process of rubber. Several metals from the pavement are also embedded in the tire matrix during the generation of TRWP. Despite the regulation of its use as extender oils in the rubber industry in 2014, Polycyclic Aromatic Hydrocarbons (PAHs) are still found in substantial amounts in the formulation of most tires. Furthermore, during the manufacture of tires, several compounds used as antioxidants and vulcanization accelerants might present a threat to wildlife. Data on the bio-accessibility of TRWP-bound compounds remains poor and this issue needs to be addressed. Due to the complex elemental composition of TRWP, it is critical to investigate the solubilization potential of a wide array of compounds from these particles into the guts of aquatic organisms. Therefore, our study aimed (i) to investigate the solubilization kinetics of metals and PAHs from TRWP into simulated gastric and intestinal fluids of a fish model species: the rainbow trout and (ii) to assess the impact of influencing factors such as co-ingestion of natural food organic matter on the compounds solubilization.

2. Materials and methods

Tire particles and Tire and Road Wear Particles (TRWP) were produced by Cardno Chemrisk (Pittsbourg, United States). TRWP were generated on a road simulator consisting of a drum coated with road pavement composed of a mixture of minerals and bituminous binder representative of a typical pavement from European roads. In-vitro digestion experiments were performed with both Simulated Gastric Fluid (SGF) and Simulated Intestinal Fluid (SIF) in order to mimic the gut content of fish species. SGF was mainly composed of an acidic luminal buffer (pH = 2) with pepsin and SIF included natural bile porcine extract and pancreatin (pH = 7,4). The solubilization kinetics of metals and PAHs were assessed over a 26h incubation period at 20°C in SGF and SIF successively to better represent gut transit time of most fish species. Furthermore, to investigate the effect of co-ingestion of food organic matter on the bio-accessible fraction of metals and PAHs, an in-vitro digestion with *Gammarus pulex* as a surrogate for natural food items of our model fish species was performed. All experiments were performed in triplicates along with control experiments where SGF and SIF were replaced with Phosphate Buffer Saline (PBS). Analyses of metals were performed with ICP-OES (Shimadzu® ICPE-9000) for Zn and Fe and with ICP-MS/MS (Agilent® 8900) for metals detected in low concentrations (Mn, Co, Cu, Fe, Ni, Ba, Pb). Samples of SGF and SIF were also liquid/liquid extracted with dichloromethane, purified on activated silica column and analysed with GC-MS/MS for quantification of the 16 regulated PAHs.

3. Results and discussion

3.1. Solubilization Kinetics of Zn from TRWP

The solubilization of Zn during the in-vitro digestion was fast and enhanced compared to the control with PBS. Equilibrium was rapidly reached after 30 minutes of digestion with SGF leading to 14,5 % of total zinc solubilized. The replacement of SGF by SIF enabled to rekindle the solubilization of Zn to reach 23 % of the total Zn solubilized into the gastrointestinal fluids at the end of the experiment (Figure 1). In the control experiment with PBS, Zn concentrations were below the LOQ (500µg.L^{-1}) for all sampling times.

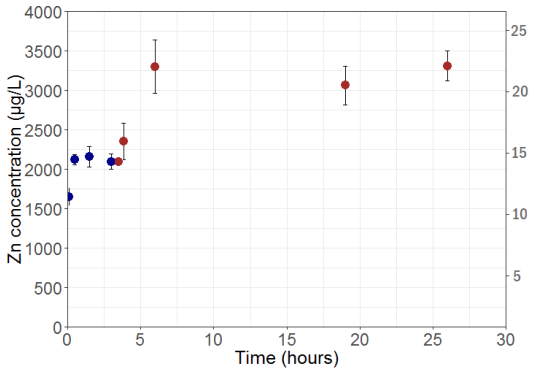

Figure 1: Solubilization kinetic of Zn in SGF (blue) and SIF (red) during the 26-h in-vitro digestion experiment.

These results highlight the importance of pH, bile surfactants and proteins in the solubilization process of metals in gastrointestinal fluids. As a low pH has been previously identified to facilitate the solubilization of metals from tire particles into aqueous solutions[1], mechanisms involving proteins or surfactants forming complexes with metals are also suspected[2]. Solubilization of Mn, Co, Cu, Fe, Ni, Ba, Pb was also observed in the gastrointestinal fluids and the results will be presented.

3.2. PAHs solubilization in gastrointestinal fluids

Fourteen PAHs were detected in tire particles and five of them were detected in quantifiable amounts in gastrointestinal fluids after a 24-h in-vitro digestion. Our results show that PAHs were not solubilized in SGF and in the control (all concentrations were below the LOQ) after an incubation time of 3-h suggesting a poor effect of pH and pepsin on their solubilization.

	Fluoranthene	Phenanthrene	Pyrene	Benzo(e)pyrene	Benzo(g,h,i) perylene
Concentration in SIF (ng.L^{-1})	727	450	3092	42	145
% of total PAH in TRWP	1,4	1,4	1,5	0,5	0,4

Table 1: Concentration and percentage of the total PAHs concentration in tire particles solubilized in SIF after a 24h digestion

However, five PAHs were quantified in SIF after an incubation time of 24-h and represented 0,4 – 1,5 % of the total concentration measured in tire particles (table 1). The greater solubilization of PAHs in SIF compared to the control showed the importance of micelles (characterized in SIF by dynamic laser scattering and contact angle measurements) and of the hydrophilic properties of this fluid in the solubilization process of HOCs[3].

4. Conclusions

Our results demonstrate the importance of accounting for the ingestion of TRWP as an important route of exposure to metals and PAHs for aquatic organisms. As the solubilization of potential toxic compounds was greatly enhanced by the gut fluids and leads to bioavailability of these compounds, further studies investigating the uptake by the epithelial cells and transfer throughout the whole organism will be performed.

5. References

(1) Selbes, M.; Yilmaz, O.; Khan, A. A.; Karanfil, T. Leaching of DOC, DN, and Inorganic Constituents from Scrap Tires. *Chemosphere* **2015**, *139*, 617–623.

(2) Mayer, L. M.; Weston, D. P.; Bock, M. J. Benzo [a] Pyrene and Zinc Solubilization by Digestive Fluids of Benthic Invertebrates—a Cross-phyletic Study. *Environ. Toxicol. Chem. Int. J.* **2001**, *20* (9), 1890–1900.

(3) Mohamed Nor, N. H.; Koelmans, A. A. Transfer of PCBs from Microplastics under Simulated Gut Fluid Conditions Is Biphasic and Reversible. *Environ. Sci. Technol.* **2019**, *53* (4), 1874–1883.