Effects of tire particles on the reproduction of earthworms (*Eisenia fetida*) and bioaccumulation potential of tire-related chemicals

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1. Introduction

Tire and Road Wear Particles (TRWP) are produced during the wear of tire rubber on the road pavement. Thus, road pavement and road debris have been shown to be embedded in the rubber to form a heterogeneous material composed of rubber polymer, minerals, bitumen and various chemicals originating from the road environment or from the rubber itself. Most of the TRWP released are expected to be deposited on the road side[1] and be transferred into the nearby soil from which a fraction could eventually enter water streams[2]. Field measurements suggest that concentrations of TRWP could reach 0.1 - 100 g kg^{-1} on the road side and the soil adjacent to the road [3]. Thus, soil dwelling organisms could be exposed to significant levels of TRWP, with potential toxic effects to be determined. This study investigated the effects of tire particles at environmentally relevant soil concentrations on survival, growth and reproductive output of an earthworm species, *Eisenia fetida*. The bioaccumulation potential of several commonly used tire-related chemicals was established and their bioaccumulation kinetics were determined.

2. Materials and methods

Cryogenically Milled Tire Tread (CMTT) particles were used as a surrogate for environmental TRWP. To generate CMTT particles, the upper layer of the tread from 3 tires of different brands were cut into small pieces of 1 cm^3 and cryogenically milled using a model A Hammer Mill (Pulva®, references available upon request). Experimental setup followed the OECD 222 (reproduction test) and 317 (bioaccumulation test) test guidelines (TG) with slight modifications. Briefly, CMTT were mixed with water (100 g/L) for 24 h, dried at 40°C for 24 h and mixed with natural soil (LUFA 2.0) to obtain two final mixtures containing 0.05% and 5% (w/w) of CMTT, respectively. In the bioaccumulation test, 4 treatments were tested: Control soil (no CMTT), low contaminated soil (0.05% CMTT), high contaminated soil (5% CMTT) and a soil contaminated with the previously prepared CMTT leachate only (100 g/L). Three pre-aclimated adult earthworms were introduced to each beaker containing the soil moistened with the CMTT leachate (100 g/L) except in the control treatment. Sampling of the worms followed the OECD TGs. The worms were freeze-dried, extracted with acetone/dichloromethane, and analysed by GC-MS/MS for polycyclic aromatic hydrocarbons (PAHs) and LC-MS/MS for Benzothiazole (BT), 2-(Methylthio)benzothiazole (MTBT), 1,3-Diphenylguanidine (DPG), N-(1,3-dimethylbutyl)-N'-phenyl-p-phenylenediamine (6PPD) and 6PPD-quinone (6PPD-Q). In the reproduction test, 3 treatments were tested: Control soil (no CMTT), low contaminated soil (0.05% CMTT) and high contaminated soil (5% CMTT). Ten adults worms were introduced to the beakers and were removed after 4 weeks. Juveniles were retrieved after 8 weeks, counted and weighed.

3. Results and discussion

3.1. *Bioaccumulation of tire-related chemicals in earthworms*

The tissue concentrations of 4 PAHs and 5 other tire-related chemicals in the worms exposed to the treatment with CMTT leachate only, or to 0.05% CMTT, did not differ from the control. Worms exposed to 5% CMTT exhibited much higher tissue chemical concentrations than the control. Our results show that the tire-

related chemicals were taken up by the earthworms during the uptake phase but also rapidly eliminated during the depuration phase (Figure 1). The Biota-Soil Accumulation Factor (BSAF) determined for the organisms exposed to the highly contaminated soil were <1, except for the apolar phenanthrene, fluoranthene and pyrene, where the BSAF ranged from 1 to 2. This indicates that the tire-related chemicals exhibit a low bioaccumulation potential in *Eisenia Fetida*.

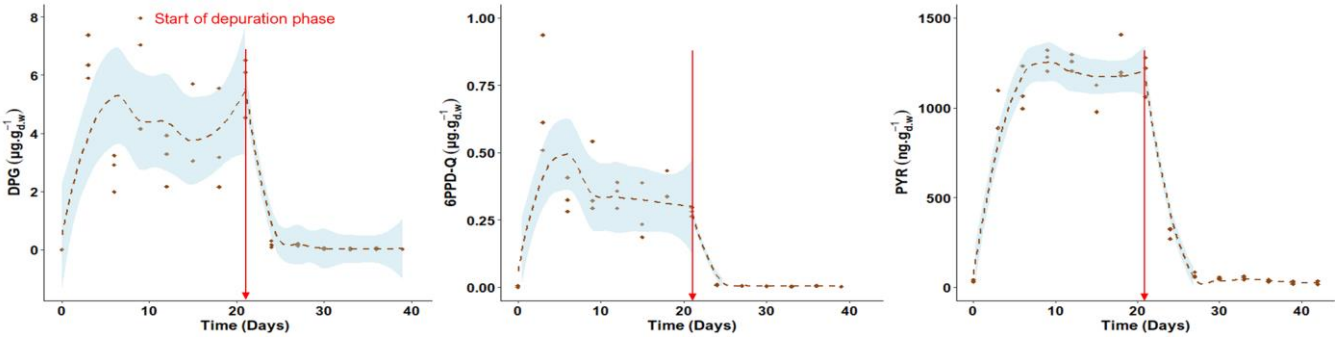

Figure 1: Bioaccumulation kinetics of DPG, 6PPD-Q and Pyrene (PYR) in the tissues of earthworms (Eisenia Fetida) exposed to a natural soil contaminated with 5% of CMTT. The red arrow represents the transfer of the worms into a non-contaminated soil (start of depuration phase). The brown dashed line represents the fit of a loess regression and the shaded blue area represent the 95% confidence interval.

3.2. *Effects of CMTT exposure on reproductive output*

No mortality or impact on growth in the adult worm population was observed in all treatments tested (control, 0.05% CMTT and 5% CMTT) after 4 weeks of exposure. A slightly positive trend (not statistically significant) between number of juveniles retrieved after 8 weeks and exposure to increasing CMTT concentrations was observed. Moreover, the mean individual weight of the worms exposed to 0.05% and 5% CMTT was lower than the weight of the worms exposed to the control soil (9.7 mg versus 12.8 mg, p-value = 0.067 and 8.9 mg versus 12.8 mg, p-value = 0.054, respectively). An increase of the reproduction rate along with exposure to CMTT could be due to a hormetic response where the reproductive output of organisms is boosted by exposure to sub-lethal chemical stress as was observed for *Eisenia andrei* exposed to pesticides metabolites[4]. Consequently, the higher energy cost for adult organisms to increase reproduction rate could lead to lower individual weight of juveniles, as we observed. An alternative hypothesis is that the cocoons or juveniles were affected by the exposure to CMTT which prevented normal development or growth compared to the control treatment.

4. Conclusions

Overall, our results indicate that tire-related chemicals in natural soil were bioavailable to earthworms but only led to a significant chemical increase in the organisms exposed to a highly contaminated soil (5% CMTT). The chemicals investigated did not show strong bioaccumulation potential and were rapidly eliminated during the depuration phase. A slight decrease in weight of the juveniles exposed to CMTT was observed in the reproduction test. More research is needed to confirm these results and reveal the underlying physiological mechanisms involved.

5. References

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Acknowledgement – The authors would like to thank Mathieu Renaud, Janine Wong and Gilda Dell'Ambrogio for their help in the preparation and follow-up of the bioaccumulation and reproduction tests.