Effects of Leachates From Tyre Wear Particles on Marine Copepods

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1. Introduction

There is an increased focus on TWP as part of microplastic pollution (Halle et al., 2021). Car tires consist of complex materials, containing a variety of chemical substances such as synthetic and natural rubbers along with black carbon, mineral oils, antioxidants and antiozonants, plasticizers and many more components (Turner and Rice, 2010). Compounds found in TWP leachates can be potentially toxic for aquatic organisms but are still not thoroughly investigated (Wagner et al., 2018). The main objective of this study was to investigate the effects of TWP leachates on different zooplankton species and evaluate whether the effects are modulated by their size and behaviour. Acute toxicity experiments were carried out by exposing adults of five copepod species (the cyclopoid *Oithona davisae*, the harpacticoid *Amonardia normanni* and the calanoids *Temora longicornis*, *Centropages hamatus* and *Acartia tonsa*) to different leachate concentrations to estimate the median lethal concentrations (LC_{50}). We hypothesized that the toxicity of TWP leachates on copepods will depend on 1) the concentration of TWP leachates, 2) the size and 3) feeding mode of copepods.

2. Methods

A new car tire (Imperial 145/70-13 71T- Snowdragon HP-Vinterdæk) was bought from a commercial store (thansen.dk). To generate leachates (Figure 1) for the acute toxicity tests and chemical characterization, a standardized protocol was adapted by Almeda et al. 2022.

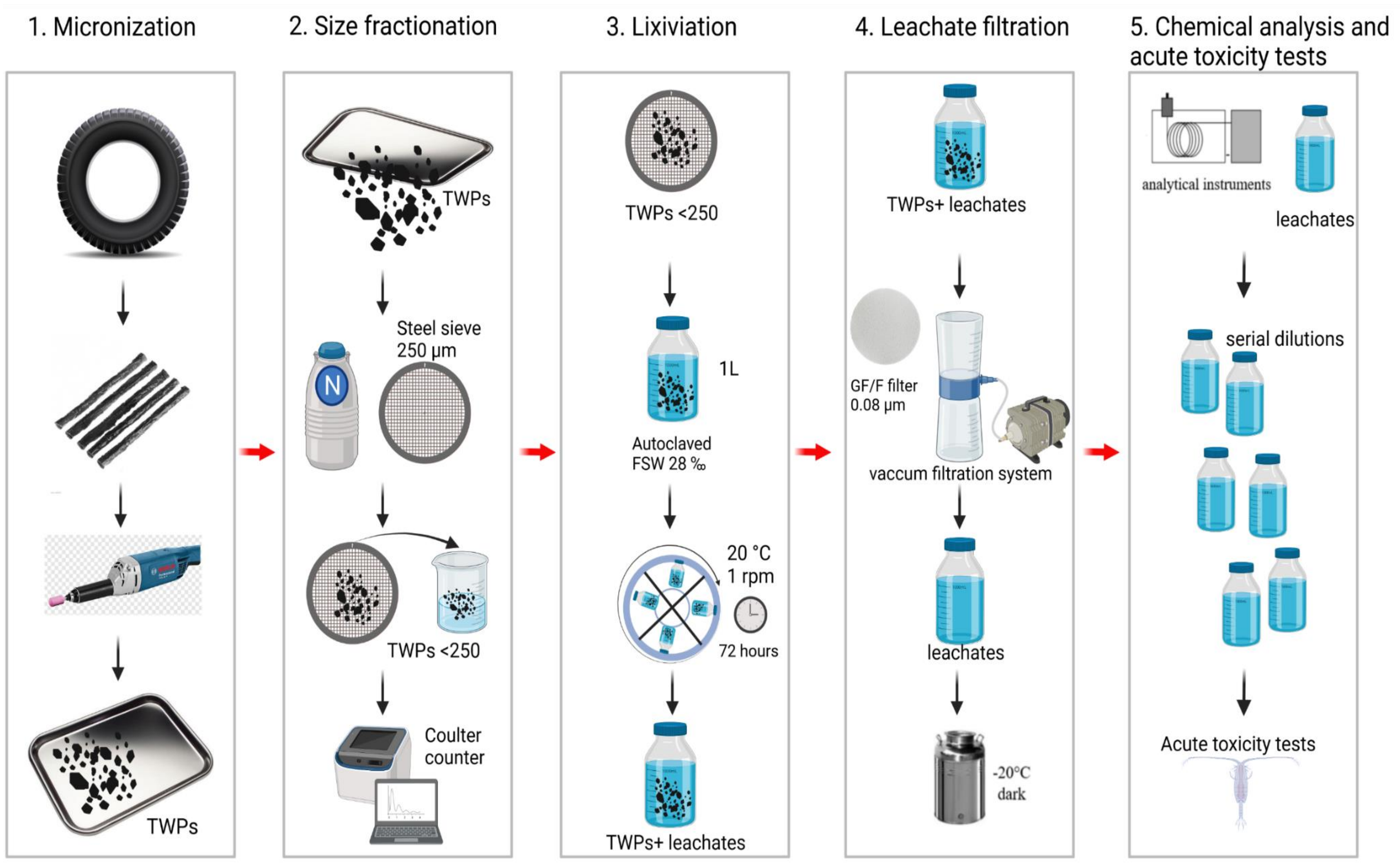

Figure 1: Overview of leachate generation in this study, adapted by Almeda et al. 2022.

Acute toxicity tests were executed by exposing the five copepod species to different leachate concentrations, in the absence of food, using mortality as an end point. All the experiments were conducted in triplicates with a range of TWP leachate concentrations (0.0025 (only for *T. longicornis*), 0.005, 0.05, 0.5, 1.25 , 2.5 and 5 g L^{-1}). The dilutions were made from dilution of the 100% stock solution corresponding to 5 g L^{-1}.

3. Results

Naphthalene and Tris (2-chloroisopropyl) phosphate were found in concentrations of 4.32 ± 0.23 and 0.11 ± 0.16 ng mL^{-1}, respectively. Pyrene was not detected in the leachates, even though the concentration in the TWP was relatively high. Zn and Sr were the metals with the highest concentration in the leachates. Metals like Mn, Ba. Cu etc. were found in concentrations < 30 ng mL^{-1} .

Table 1: Concentration of organic additives and metals in the TWP (µg g^{-1}) and in leachates (ng L^{-1}; mean ± SD). n.d. indicates not detected compounds.

Organic compounds	Concentration (µg g^{-1}; TWP)	Concentration (ng mL^{-1}; leachates)
Naphthalene (PAHs)	0.4	4.32 ± 0.23
Pyrene (PAHs)	11.0	n.d
Tris (2-chloroisopropyl)phosphate (OPFRs)	0.2	0.11 ± 0.16
Metals		
51 V	9	0.2 ± 0.1
52 Cr	49.3	4.5 ± 2.9
55 Mn	16.6	17.0 ± 9.2
56 Fe	1618.5	n.d
59 Co	4.1	1.2 ± 0.3
60 Ni	27.6	10.1 ± 2.7
63 Cu	13.7	0.5 ± 0.2
66 Zn	20057.1	1053.3 ± 74.0
75 As	1.5	1.0 ± 0.4
78 Se	1.9	n.d
88 Sr	8.4	4351.2 ± 36.1
95 Mo	1.4	11.4 ± 0.5
121 Sb	3.1	0.6 ± 0.1
137 Ba	15.1	29.1 ± 7.9
208 Pb	95.0	0.1 ± 0.1
238 U	0.1	2.8 ± 0.03

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Table 2: Concentration of metals in the TWP (ng g^{-1}) and in leachates (ng L^{-1}; mean ± SD). n.d. indicates not detected compounds.

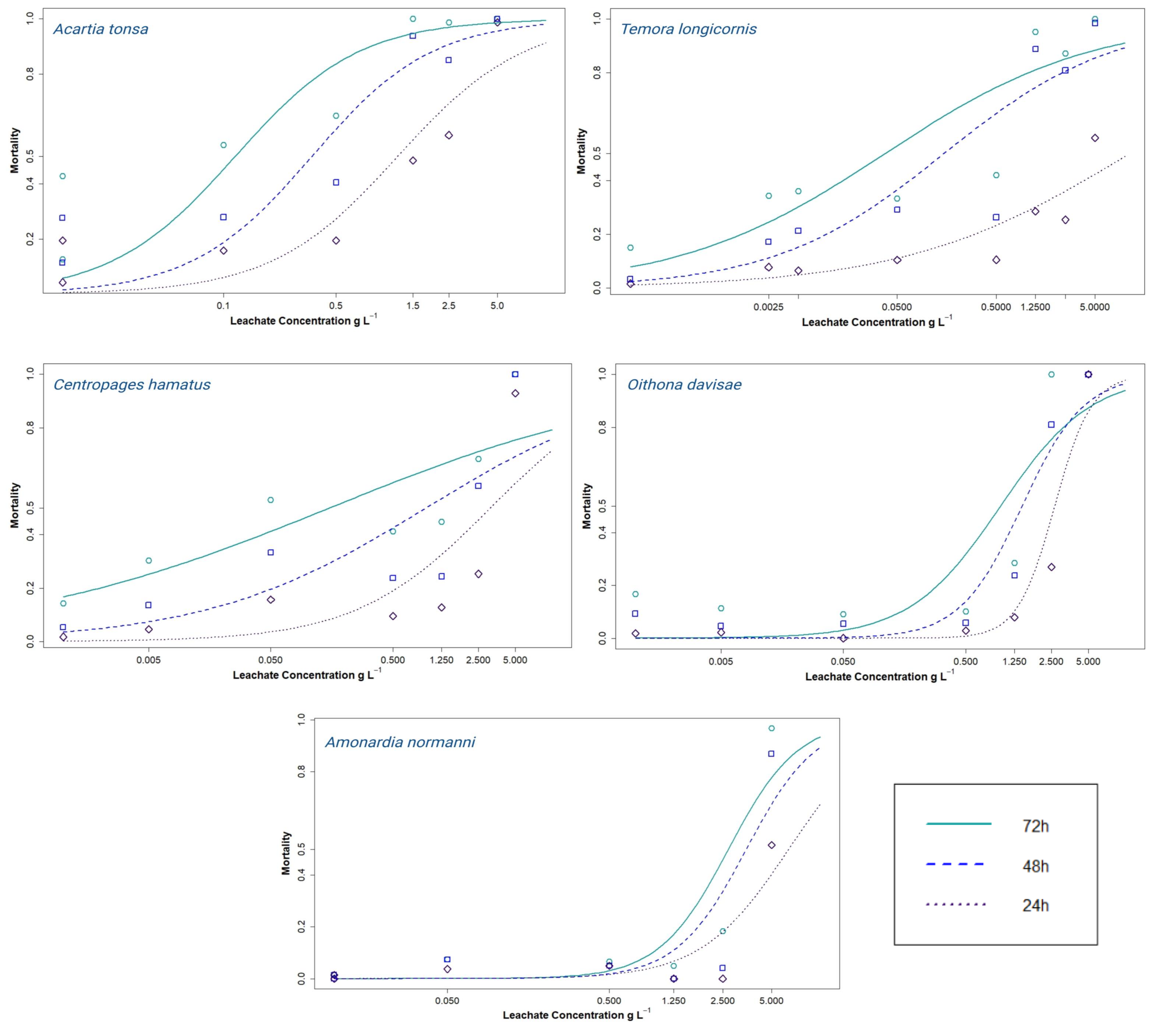

Figure 2: Mortality (proportion; mean ± SD) of *A.tonsa, T.longicornis, C.hamatus, O.davisae and A.normanni* after 24, 48 and 72h of exposure to a range of leachate concentrations (g L^{-1}), including control.

Table 2: LC_{50} (g L^{-1}) after 24, 48 and 72 h for all investigated species (mean ± SE).

Species	LC_{50} 24 h	LC_{50} 48 h	LC_{50} 72 h
A.tonsa	1.03 ± 0.26	0.26 ± 0.07	0.08 ± 0.03
T.longicornis	11.09 ± 8.41	0.14 ± 0.06	0.04 ± 0.02
C.hamatus	3.13 ± 0.93	0.93 ± 0.36	0.15 ± 0.10
O.davisae	2.66 ± 0.29	1.44 ± 0.22	0.96 ± 0.20
A.normanni	6.38 ± 1.70	3.50 ± 0.49	2.71 ± 0.36

LC_{50} **varied among species** and generally **decreased with exposure time** (Table 2). In general, zooplankton mortality increased with higher leachate concentrations and longer exposure time with the **calanoids** being the **most sensitive species** (Figure 2).

4. Conclusions

Toxicity was concentration and time dependent. Furthermore, species-specific differences were detected, based on taxonomy, **not copepod size. The sensitivity of the species:**

T. longicornis > A. tonsa > C. hamatus > O.davisae > A. normanni

…discussion

Species-specific response could be explained by:

- Swimming/feeding behavior
- Increased sensitivity under starvation stress
- Oral intake (by "drinking" water)
- Defense mechanisms

…and future perspectives

- Establish protocols for leachate production and chemical characterization
- Quantify TWP concentration in the ocean
- TWP leachate toxicity should be investigated along with other environmental stressors
- Long-term experiments
- Defense mechanisms

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