Acute and long-term toxicity of micronized car tire wear particles to Hyalella azteca

Khan, Farhan; Halle, Louise Lynn; Palmqvist, Annemette

Published in:
Aquatic Toxicology

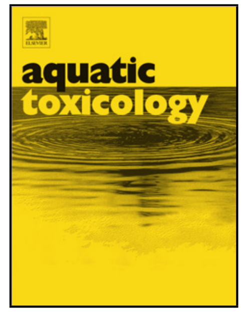
DOI:
10.1016/j.aquatox.2019.05.018

Publication date:
2019

Document Version
Peer reviewed version

Citation for published version (APA):
Khan, F., Halle, L. L., & Palmqvist, A. (2019). Acute and long-term toxicity of micronized car tire wear particles to *Hyalella azteca*. *Aquatic Toxicology*, *213*(31), Article 105216. https://doi.org/10.1016/j.aquatox.2019.05.018

Accepted Manuscript



Title: Acute and long-term toxicity of micronized car tire wear particles to *Hyalella azteca*

Authors: Farhan R. Khan, Louise Lynn Halle, Annemette Palmqvist

PII:	S0166-445X(19)30172-9
DOI:	https://doi.org/10.1016/j.aquatox.2019.05.018
Reference:	AQTOX 5216
To appear in:	*Aquatic Toxicology*
Received date:	27 February 2019
Revised date:	2 May 2019
Accepted date:	30 May 2019

Please cite this article as: Khan FR, Halle LL, Palmqvist A, Acute and long-term toxicity of micronized car tire wear particles to *Hyalella azteca*, *Aquatic Toxicology* (2019), https://doi.org/10.1016/j.aquatox.2019.05.018

Acute and long-term toxicity of micronized car tire wear particles to *Hyalella azteca*

Farhan R. Khan*, Louise Lynn Halle, Annemette Palmqvist

Department of Science and Environment, Roskilde University, Universitetsvej 1, PO Box 260, 4000 Roskilde, Denmark

*Corresponding author: frkhan@ruc.dk; farhan.khan@gmx.com (F. R. Khan).

Highlights

- *Hyallela azteca* indescriminately ingest tire wear particles (TWP).
- TWP and tire leachate acute exposure have distinct profiles.
- TWP exposure impacts long-term mortality, reproduction and growth.

Abstract

Ecotoxicological studies relating to tire wear particles (TWP) have focussed, up until very recently, almost entirely on the released leachate. Little is known about the toxicology effects of TWP dispersed in freshwater. In the present small-scale study we exposed *Hyallela azteca* to TWP dispersed in water with the aim of (i) determining the potential acute and chronic impacts of TWP exposure (ii) challenging the prevailing idea that tire leachate is the primary causative agent of tire-related toxicity. *H. azteca* were shown to indescriminately ingest TWP with a gut retention time of 24-48 h. Acute (48 h) TWP exposure followed an expected concentration-response curve from which an LC_{50} of 3426±172 particles mL^{-1} was determined, but leachate exposure did not conform to a sigmoidal concentration-response pattern and therefore an LC_{50} was not derivable. However, toxicity profiles of TWP and leachate appeared to be sufficiently different as to suggest a dissimilar mechanism of toxicity. Mortality, reproductive output (neonate production) and net growth were all significantly impacted at the higher exposure concentrations (500-2000 particles mL^{-1}) following 21 days exposure. Our study demonstrates that TWP exposure elicits short-term and longer-term toxicity on a key freshwater organism.

Keywords: TWP (tire wear particles), tread particles, tire rubber, microrubber, tire leachate; freshwater

A recent IUCN (International Union for the Conservation of Nature) report suggested that tire wear particles (TWP) contribute a significant proportion of the ocean's microplastics (Boucher and Friot, 2017). Further study into tire emissions calculated the global environmental release of rubber from road vehicles as being close to 6 million tonnes per year equivalent to 0.81 kg per person per year (Kole et al., 2017). Although environmental release into the aquatic environment has been established, the potential ecotoxicological impact of TWP remains largely unknown, but has been recognised as an information gap that needs to be urgently addressed in order understand the risk related to TWP (Wagner et al., 2018)

Early research into the potential toxicity of tires was conducted by immersing whole tires into water and then exposing aquatic organisms to the leached chemicals (Day, 1993). Although this was not the first study on tire-related toxicity, it confirmed the thinking that the toxicity of tire rubber is attributed solely to the leachate which was subsequently made the only topic of ecotoxicological interest in tire rubber (Stephensen et al., 2003; Wik and Dave., 2006; Wik et al., 2009; Turner and Rice, 2010; Marwood et al., 2011). Tire leachate has been shown to contain a suite of toxic substances predominantly trace metals, polycyclic aromatic hydrocarbons and assorted volatile organics used in vulcanization and as antioxidants which are released into the aqueous environment (Marwood et al., 2011). However, to ascribe the toxicity of the tire solely to the leachate negates any potential role of the rubber particle itself.

In light of the "microplastic vector effect" which describes how plastic particles can transport sorbed toxicants into organisms (Syberg et al., 2015), some studies have started to emerge describing the direct impacts of TWP. The exposure of freshwater benthic invertebrates to TWP showed overall negligible effects on the tested species (Panko et al., 2013; Redondo-Hasselerharm et al., 2018), but these studies were conducted in sediments. The choice of this matrix is understandable given that sediments act as a sink for TWP (Wagner et al., 2018), but this does not take into account the transit from road surface through the water column and the likelihood of encountering aquatic biota. Thus, in the present small-scale study we exposed *Hyallela azteca*, an established freshwater model organism, to water dispersed TWP with the aim of (i) determining the potential acute and chronic impacts of TWP exposure (ii) challenging the prevailing idea that tire leachate is the primary causative agent of tire-related toxicity.

In this study, TWP was produced from a road worn tire (Continental Premium Contact 5 50175/65 R14 82, approx. 10000 km under Danish driving conditions) by grinding the tire surface against a course grind stone (size: 200 mm x 25 mm) rotating at 4000 rpm. Tire rubber was dry sieved through a 500 μm metal sieve prior to use in experiments. Thus we consider this TWP to be laboratory produced by surface abrasion rather than road-wear generated TWP which may also include non-rubber road particles (Camatini et al., 2001, Kreider et al., 2010). To accurately add TWP to the exposures, particles were dispersed in water at a concentration of 0.1 g mL^{-1} with use of a surfactant (0. 1% TWEEN80, Cospheric LLC) and then a ratio of TWP weight to particle number was determined by counting the number of particles in a set volume of dispersion (Khan et al., 2015). A weight of 0.29 g was equivalent to 1.0 x 10^6 particles. In all exposures, TWP was sieved through a 1µm nylon mesh and thoroughly rinsed in deionised water before dispersion in freshwater media ensuring that the surfactant was not carried into the exposure (Khan et al., 2015). *H. Azteca* were from an established laboratory culture and maintained in artificial freshwater medium at 25°C with a 16:8 light:dark photoperiod. *H. azteca* were fed with rabbit chow (Chrisco, Køge, Denmark) twice a week (Pedersen et al., 2013).

To determine whether *H. azteca* ingested TWP, individuals were fed *ad libitum* for 1 day and then depurated for 2 days in clean medium. The transit of TWP through digestive tract was photographed with a camera mounted on a stereomicroscope. Acute toxicity tests (48 h mortality) were conducted with 7-9 day old juvenile *H. azteca* exposed to TWP dispersion or the corresponding leachate (i.e. released chemicals but no particles present). Acute exposures were conducted in '*Daphnia magna* multi-well plates' (Daphtox kit, MicroBioTests Inc., Belgium) with each well an exposure containing 10 mL of exposure solution and 5 individuals. Each concentration was performed on 1 row (4 wells) of the Daphtox plates and averaged, and then performed on 3 separate plates (i.e. n=3). TWP tests were prepared by dispersing the calculated particle weight in 50 mL medium (i.e. one solution per plate). Leachate experiments were made by first dispersing particles in medium for a period of 48 h at 25°C and then by filtering the particles from solution using a 1 µM mesh. The collected leachate was used in the exposures. It is worth noting that studies involving tire leachate exposure often employ elevated temperature, agitation, and/or solvent extraction to facilitate leaching from the tire rubber (Wagner et al., 2018). By not using these methods our leachate likely better reflects the release of chemicals from TWP in an environmentally relevant scenario. The concentration range for TWP exposures was 0-15000 particles mL^{-1} and leachate exposure range was corresponded to the leached chemicals from 0-125000 particles mL^{-1}.

Long-term tests were performed with TWP only and were conducted as 21 static renewal tests with 21-23 d old *H. azteca* with mortality, reproduction and growth as the determined endpoints. The exposure range for the chronic exposure was 0-2000 particles mL^{-1}. Exposures to determine mortality and reproduction were performed in 250 mL beakers each containing 150 mL medium and 10 organisms (n=5). Growth was assessed in concurrent exposures containing only 1 individual thus allowing the growth of that individual to be monitored (n=10). In all chronic exposures the medium was renewed once a week, at which time feeding and counting offspring was completed. Post exposure stereomicroscope pictures were taken weekly of the individually exposed *H. azteca* to determine growth.

H. azteca were shown to indescriminately ingest TWP with a gut retention time of 24-48 h. (Figure 1). Starting at 1 h TWP is seen clearly at the top of GI tract. By 24 h the majority of the GI tract is filled with rubber particles. Following depuration in clean water, TWP was not visible in the gastrointestinal tract after 48 h, suggesting a gut residence time of between 24 and 48. The acute toxicities of the TWP and leachate are found in Figure 2. TWP exposures fit an expected concentration-response curve from which an LC_{50} 3426±172 particles mL^{-1} (equivalent to approximately 1 mg mL^{-1} or 1 g L^{-1}) could be derived (Figure 2). Conversely, leachate toxicity did not conform to a sigmoidal concentration-response pattern and therefore an LC_{50} was not derivable. Comparison of the two datasets suggests the acute toxicity of the

TWP is distinct from that of its leached chemicals. At low concentrations leachate appears more toxic, but at higher concentrations the particle appears to exert great toxic effect. Although such results require verification it could suggest an important role for the particle in delivery of chemicals into the organisms upon ingestion rather than leaching them into the ambient environment. If this were the case, then the concentration of chemicals leached *in vivo* would be greater than the leachate concentration *ex vivo*; the rubber particle, in effect, acting as a 'Trojan Horse'. Conversely it could also suggest a physical impact of TWP in the GI tract following ingestion. Therefore the mechanism of toxicity requires further elucidation, as does understanding the digestive physiology of the test organism, particularly in relation to gut retention time, which is likely to play an important role in determining adverse effects.

Mortality, reproductive output (neonate production) and net growth were all significantly impacted at the higher exposure concentrations following 21 day exposure (Figure 3). After 21 d exposure to 2000 particle mL^{-1} (0.58 g L^{-1}) 92.5±6.9 % of the *H. azteca* died. Neonate production was reduced from an average of 18.9±5.3 offspring per beaker in control exposures (i.e. no added TWP) to 2.4±4.3 offspring per beaker at an exposure concentration of 1000 particle mL^{-1} (0.29 g L^{-1}) ($p<0.05$ One-way ANOVA with post hoc Tukey's HSD). Effects on *H. azteca* growth were found at a concentration of 500 particles mL^{-1} (0.145 g L^{-1}) with net growth over the exposure period reducing from 18.0±5.4 % in control treatments to 6.7±2.5% ($p<0.05$ One-way ANOVA with post hoc Tukey's HSD). Long-term studies were not designed to delineate leachate effects from particle effects and thus it is not possible to determine which fraction of the TWP is responsible for the reduction in fitness measures. However, the three chosen endpoints are vital to population health and therefore population level impacts of TWP should be investigated.

The lack of ecotoxicological data regarding the TWP has been identified as a key knowledge gap in assessing potential risk. Moreover, the direct impacts of TWP and effects in aquatic environments rather than sediment matrices have been specifically highlighted (Wagner et al., 2018). It is likely that the highest TWP concentrations used in our study exceed environmental realism, however our study provides primary evidence that acute toxicity related to TWP does not only originate from the leachate and that the particle itself can cause adverse effects. Overall, our study demonstrates that TWP exposure elicits short-term and longer-term toxicity on a key freshwater species.

References

Boucher, J., Friot, D., 2017. Primary microplastics in the oceans: A global evaluation of sources; IUCN International Union for Conservation of Nature.

Camatini, M., Crosta, G. F., Dolukhanyan, T., Sung, C., Giuliani, G., Corbetta, G. M., Cencetti, S., Regazzoni, C., 2001. Microcharacterization and identification of tire debris in heterogeneous laboratory and environmental specimens. Materials characterization, 46, 271-283. https://doi.org/10.1016/S1044-5803(00)00098-X

Day, K. E., Holtze, K. E., Metcalfe-Smith, J. L., Bishop, C. T., Dutka, B. J., 1993. Toxicity of leachate from automobile tires to aquatic biota. Chemosphere 27, 665-675. https://doi.org/10.1016/0045-6535(93)90100-J.

Khan, F. R., Syberg, K., Shashoua, Y., Bury, N. R., 2015. Influence of polyethylene microplastic beads on the uptake and localization of silver in zebrafish (Danio rerio). Environ. Pollut. 206: 73-79. https://doi: 10.1016/j.envpol.2015.06.009

Kole, P. J., Löhr, A. J., Van Belleghem, F. G. A. J., Ragas, A. M. J., 2017. Wear and tear of tyres: a stealthy source of microplastics in the environment. Int. J. Environ. Res. Publ. Health 14, 1265-1296. https://doi.org/10.3390/ijerph14101265.

Kreider, M. L., Panko, J. M., McAtee, B. L., Sweet, L. I., Finley, B. L., 2010. Physical and chemical characterization of tire-related particles: Comparison of particles generated using different methodologies. Sci. Total Environ. 408, 652−659. https://doi.org/10.1016/j.scitotenv.2009.10.016

Marwood, C., McAtee, B., Kreider, M., Ogle, R. S., Finley, B., Sweet, L., Panko, J., 2011. Acute aquatic toxicity of tire and road wear particles to alga, daphnid, and fish. Ecotoxicology 20, 2079-2089. https://doi.org/10.1007/s10646-011-0750-x.

Panko, J.M., Kreider, M.L., McAtee, B.L., Marwood, C., 2013. Chronic toxicity of tire and road wear particles to water- and sediment-dwelling organisms. Ecotoxicology 22, 13-21. https://doi.org/10.1007/s10646-012-0998-9.

Pedersen, S., Palmqvist, A., Thorbek, P., Hamer, M., Forbes, V., 2013. Pairing behavior and reproduction in *Hyalella azteca* as sensitive endpoints for detecting long-term consequences of pesticide pulses. Aquat. Toxicol, 144, 59-65. https://doi.org/10.1016/j.aquatox.2013.09.027

Redondo-Hasselerharm, P. E., de Ruijter, V. N., Mintenig, S. M., Verschoor, A., Koelmans, A. A., 2018. Ingestion and Chronic Effects of Car Tire Tread Particles on Freshwater Benthic Macroinvertebrates. Environ. Sci. Technol. 52, 13986-13994. https://doi.org/10.1021/acs.est.8b05035

Stephensen, E., Adolfsson-Erici, M., Celander, M., Hulander, M., Parkkonen, J., Hegelund, T., Sturve, J., Hasselberg, L., Bengtsson, M., Förlin, L., 2003. Biomarker responses and chemical analyses in fish indicate leakage of polycyclic aromatic hydrocarbons and other compounds from car tire rubber. Environ. Toxicol. Chem. 22, 2926-2931. https://doi.org/10.1897/02-444.

Syberg, K., Khan, F. R., Selck, H., Palmqvist, A., Banta, G., Daley, J., Sano, L., Duhaime, M., 2015. Microplastics: Addressing Ecological Risk Through Lessons Learned. Environ. Toxicol. Chem. 34: 945-953. https://doi: 10.1002/etc.2914

Turner, A., Rice, L., 2010. Toxicity of tire wear particle leachate to the marine macroalga, *Ulva lactuca*. Environ. Pollut. 158, 3650-3654. https://doi.org/10.1016/j.envpol.2010.08.001.

Wagner, S.; Hüffer, T.; Klöckner, P.; Wehrhahn, M.; Hofmann, T.; Reemtsma, T., 2018. Tire wear particles in the aquatic environment - a review on generation, analysis, occurrence, fate and effects. Water Res. 139, 83–100. https://doi.org/10.1016/j.watres.2018.03.051

Wik, A., Dave, G., 2006. Acute toxicity of leachates of tire wear material to *Daphnia magna* - Variability and toxic components. Chemosphere 64, 1777-1784. https:// doi.org/10.1016/j.chemosphere.2005.12.045.

Wik, A., Nilsson, E., Källqvist, T., Tobiesen, A., Dave, G., 2009. Toxicity assessment of sequential leachates of tire powder using a battery of toxicity tests and toxicity identification evaluations. Chemosphere 77, 922-927. https://doi.org/10.1016/j.chemosphere.2009.08.034.

Figure Legends

Figure 1. Time series of *Hyalella azteca* ingesting TWP over 24 h followed by 48 h depuration (dep). All scale bars show 1 mm. The stereomicroscope images show *H. azteca* indiscriminately ingest the tire rubber and retain it within the gastrointestinal tract for at least 1-2 days post-exposure.

Figure 2. Acute toxicity (48h) of TWP and leachate to juvenile *H. azteca*. Data shows mean values of % mortality (n=3) ± standard deviation. The data describes the acute toxicity of the particles (A) as being distinct from the leachate (B) collected from the same particle number.

The particulate LC_{50} was 3426±172 particles mL^{-1}, but the LC_{50} of the leachate exposure could not be determined as the data did not fit a sigmoidal concentration-response pattern.

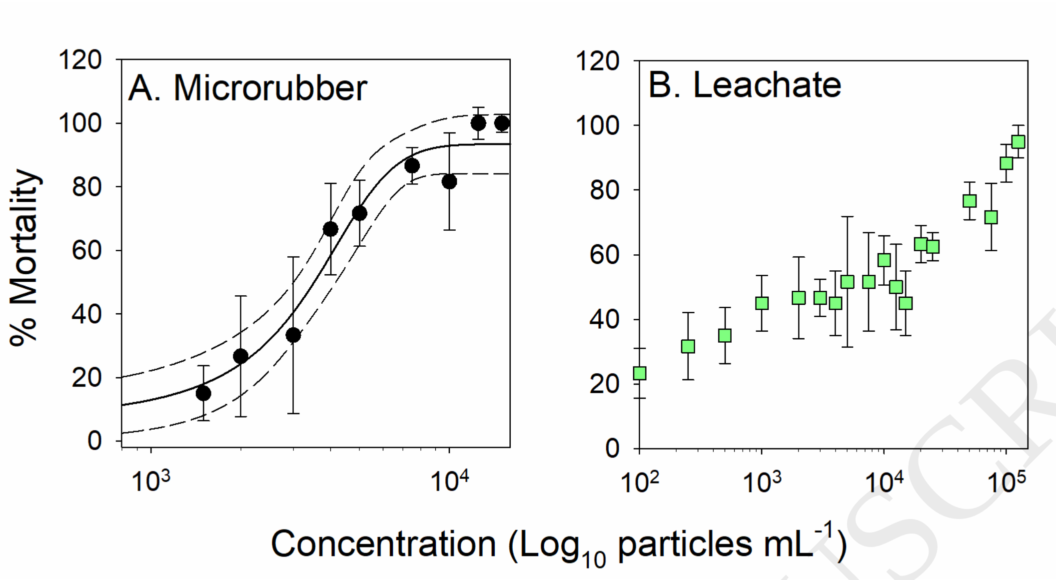


Figure 3. Chronic (21 day) exposure of *H. azteca* to TWP. Mortality (A), reproductive output (neonate production, B) and net growth (C) were significantly impacted at the higher exposure concentrations. Data shows mean values ± standard deviation. Significant difference from the control is denoted by asterisks ($p<0.05$, one-way ANOVA with post-hoc Tukey test).