Journal of Hazardous Materials 429 (2022) 128319

Contents lists available at ScienceDirect

Journal of Hazardous Materials

journal homepage: www.elsevier.com/locate/jhazmat

Toxicity of micro and nano tire particles and leachate for model freshwater organisms

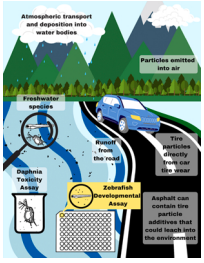
Brittany Cunningham [a], Bryan Harper [a], Susanne Brander [b], Stacey Harper [a,c,*]

[a] Department of Environmental and Molecular Toxicology, Oregon State University, Corvallis, OR, United States
[b] Coastal Oregon Marine Experiment Station, Department of Fisheries, Wildlife, and Conservation Sciences, Oregon State University, Newport, OR, United States
[c] School of Chemical, Biological and Environmental Engineering, Oregon State University, Corvallis, OR, United States

HIGHLIGHTS

- TP and leachate exposures caused mortality and developmental abnormalities.
- TP leachate was the main driver of toxicity for *D. magna* and larval zebrafish.
- Exposure to nano TPs enhanced toxicity in comparison to leachate alone.

GRAPHICAL ABSTRACT



ARTICLE INFO

Keywords:
Microplastics
Nanoplastic
Road-debris
Zebrafish
Daphnia
Development

ABSTRACT

Environmental sampling has documented a diversity of microplastics, including high levels of black rubber—generally identified as tire debris. Though organisms have been shown to ingest tire particles (TPs), past research focused on toxicity of leachate alone, overlooking potential effects of particles. To address these gaps, we assessed the toxicity of micro (1–20 μm) and nano (<1 μm) TPs for two model organisms, embryonic Zebrafish *Danio rerio* and the crustacean *Daphnia magna*. To assess effects on development, Zebrafish embryos were exposed to concentrations of TPs or leachate ranging from 0 to 3.0×10^9 particles/ml and 0–100% respectively (n = 4). Greater mortality and sublethal malformations were observed following nano TP and leachate exposures as compared to micro TPs. Unique abnormalities between the exposures indicates that there is both chemical and particle-specific toxicity. We also observed *D. magna* mortality following a 48 h exposure of neonate to TPs or leachate, ranging from 0 to 3.3×10^9 particles/ml and 0–100% respectively (n = 3). Though, particle-enhancement of toxicity was observed for both Zebrafish and *D. magna*, overall sensitivity to TPs differed. It is important to identify differential toxicities across species to achieve an understanding of the environmental impacts of TPs and the chemicals they leach.

1. Introduction

Pollution of micro- and nanoplastics degraded from larger plastic waste in the environment is a growing problem (Geyer et al., 2017). Though plastic pollution is often discussed as a single entity, the category of plastics actually encompasses many chemical compositions,

* Corresponding author at: Department of Environmental and Molecular Toxicology, Oregon State University, Corvallis, OR, United States.
E-mail address: Stacey.Harper@oregonstate.edu (S. Harper).

https://doi.org/10.1016/j.jhazmat.2022.128319
Received 16 September 2021; Received in revised form 12 January 2022; Accepted 18 January 2022
Available online 20 January 2022

colors, morphologies, additives, and sizes (Rochman et al., 2019). While much of the literature investigates the effects of plastics on freshwater organisms using only spherical polystyrene (PS) particles (Greven et al., 2016; Lu et al., 2016; Nasser and Lynch, 2016; Brun et al., 2017; Cui et al., 2017; Liu et al., 2018; Pitt et al., 2018b; Liu et al., 2019), microplastics of differing shape, size, and morphology confer differing toxicity (Lee et al., 2013; Frydkjær et al., 2017; Gray and Weinstein, 2017; Choi et al., 2020; Stienbarger et al., 2021). Therefore, to fully understand the effects of these plastic pollutants, it is important to conduct research with particles representative of those in the environment. Microplastic abundance and type can vary in differing media (Yonkos et al., 2014; Sutton et al., 2019; Wu et al., 2020). Additionally, rivers are major conveyors of plastics into other environments. For example, the Yangtze River in China has been reported to have 4137 plastic particles per cubic meter (Zhao et al., 2014). Overall, environmental sampling has shown fibers to be the most abundant microplastic morphology, with fragments as second most common (Sutton et al., 2019; Okoffo et al., 2021).

One of the most commonly found microplastics in environmental samples are black rubber, generally identified as tire debris or tire particles (TP) (Werbowski et al., 2021). In fact, some have asserted that tire wear particles and other tire-related debris is one of, if not the largest source of terrestrial microplastics in marine environments (Sherrington, 2016; Boucher and Friot, 2017). Such particles originate from friction of tires on roadways and flow into the environment from a variety of sources including wastewater (Sugiura et al., 2021), road/-stormwater runoff (Ziajahromi et al., 2020; Werbowski et al., 2021), and atmospheric deposition (Kukutschová et al., 2011). It has been estimated that 1524,740 metric tons of tire wear particles flow into the environment from the US each year (Kole et al., 2017). Estimated emission per capita for other countries (e.g. China, India, Japan, Norway, Sweden, and Denmark) range from 0.23 and 1.9 kg/yr (Kole et al., 2017). TPs are composed of a variety of materials including synthetic rubber (e.g. styrene butadiene styrene), filling agents, oils, vulcanization agents, and other additives (Eisentraut et al., 2018). Both the particles themselves, as well as chemicals they leach, may have detrimental effects on aquatic organisms they come in contact with (Wik and Dave, 2006; LaPlaca and van den Hurk, 2020).

The majority of tire-toxicity studies have focused on toxicity associated with the leachate component. It has been shown that exposure to TP leachate can cause mortality in freshwater organisms (Gualtieri et al., 2005); however, toxicity decreases when assessed with exposures of sediment elutriate (Marwood et al., 2011). One of the most commonly identified components of TP leachate is high levels of zinc (Zn) (Gualtieri et al., 2005), a heavy metal known to be toxic to aquatic organisms (Attar and Maly, 1982). In fact, it is estimated that up to 36% of Zn released into the atmosphere comes from TPs (Councell et al., 2004). TP leachate has also been shown to contain polycyclic aromatic hydrocarbons (PAHs) (LaPlaca and van den Hurk, 2020). Studies have found that fish take up elevated levels of PAHs released from TPs (Stephensen et al., 2003; LaPlaca and van den Hurk, 2020). Furthermore, components in tire leachate have been very recently linked to mortality in Coho salmon (Tian et al., 2021).

Though much research has focused on leachate, a few studies have investigated the toxicity of the TPs themselves. A variety of organisms will internalize micro TPs, including *Gammarus pulex* (Redondo-Hasselerharm et al., 2018), *Hyalella azteca* (Khan et al., 2019), *Mysid* sp. (Halle et al., 2020; Siddiqui et al., in review), *Menidia beryllina* [40], *Fundulus heteroclitus* (LaPlaca and van den Hurk, 2020), and *Pimephales promelas* (LaPlaca and van den Hurk, 2020). It should be noted that most of those exposures used micro TPs larger than the 20 μm maximum size used during this present study. Exposure to micro TPs increased mortality and affected reproduction in *H. Azteca* (Khan et al., 2019). Interestingly, Panko et al. (2013) found minimal effects to freshwater organisms, *Ceriodaphnia dubia*, *P. promelas*, *Chironomus dilutus*, and *H. azteca*, when TP exposures were done using spiked sediment. They hypothesized that the pH of the sediment decreased leaching, thereby decreasing observed toxicity as well (Panko et al., 2013). Overall, little research is available about the toxicity of TPs themselves and thus far, toxicity of nano TPs in freshwater model organisms has not been investigated or demonstrated.

Micro and nanoplastic research has primarily focused on marine environments (Blettler et al., 2018; Blettler and Wantzen, 2019); however, because freshwater environments are known to accumulate and convey tire wear particles (Wagner et al., 2018), it is important to consider how toxicity may differ for freshwater organisms. Model organisms, *Danio rerio* (Zebrafish) and *Daphnia magna* (crustacean) are ideal for this type of toxicity assessment. Guidelines from the EPA, as well as international standards exist for toxicity testing (Weber, 1991; OECD, 2004; Busquet et al., 2013). The variable toxicity demonstrated for TP leachate in the literature (Gualtieri et al., 2005) highlights the importance of assessing particle and leachate toxicity separately in more than one species. Additionally, investigation of effects on development of Zebrafish and mortality of *D. magna* using established assays allows for future comparisons to other toxicants, as well as ease of use in risk assessment.

In the present study, we assessed the toxicity of micro and nano-sized TPs, and additionally TP leachate to Zebrafish and daphnids using standard laboratory methods. It is important to evaluate toxicity using a range of particle sizes in addition to the leachate component, in an effort to separate attribution of toxicity to the chemical and physical constituents. Very little data exists on the actual quantity of TPs in the environment. Estimates for micro-sized TPs range from 0.03 to 59 mg/L in surface waters (Wik and Dave, 2009) and up to 12 mg/g dry weight in river sediments (Baensch-Baltruschat et al., 2020). However, these estimates are constrained by sampling technology or use of chemical markers, and likely underestimate the true quantity of TPs in the environment. Additionally, no method exists for measuring or estimating nano TPs, or nanoplastics of any kind, in the environment. Thus, environmentally relevant levels of nano TPs, or tire leachate derived chemicals, are impossible to know using current technology. As TPs continue to accumulate in aquatic environments, identifying levels at which these pollutants become toxic remains a valuable area of study with potential policy implications. Therefore, these model organisms were exposed to high concentrations of TPs and TP leachate in their early life stages to construct concentration-response curves and identify concentrations at which TPs and their leachate become toxic. This knowledge is crucial for the creation of risk assessments. This is some of the first research to investigate the effects of nano TPs on Zebrafish and *D. magna* and to evaluate the effect of TP size on toxicity.

2. Materials and methods

2.1. Tire particle and leachate preparation and characterization

A new, undriven, standard passenger car tire (Caldera Confidence C3 with DOT quality grades of Treadwear 480, Traction A and Temperature A) was used to generate the TPs and leachate for these experiments. This specific tire was picked to be as representative as possible; however, it should be mentioned that all tires differ from each other in composition and materials leached (Wik and Dave, 2006). Slices were taken from just the tread of the tires with a stainless steel blade. Slices were cut into 2–4 mm pieces. These were further reduced in size using liquid nitrogen cryomilling (Retsch CryoMill, Haan, Germany). After milling, 3.25 g of milled particles were suspended in 300 ml of liquid containing 50 mg/L of natural riverine organic matter (Suwanee River NOM, International Humic Susbstances Society) prepared in particle free water. This NOM was used throughout the experiments to minimize aggregation and keep the particles in suspension (Grillo et al., 2015). Glass beads were added to the TP suspensions and they were autoclaved, cooled and then rotated on a shaker table to further decrease the size of the particles. After 72 h, the glass beads were removed. The suspension was passed through a 20 μm sieve and then a 1 μm filter (Adantec mixed cellulose filter) to get a

suspension of TPs less than 1 μm. The 1 μm filters were then backflushed with fresh particle free water to get a solution of micro TPs between 1 and 20 μm. Particle counts were taken for the micro fraction using a flow cytometer (Accuri C6 Flow Cytometer, BD Biosciences, San Jose, CA) calibrated with size standards whereas the nano fraction was analyzed for particle hydrodynamic diameter and particle concentration using Nanoparticle Tracking Analysis (NTA version 3.4) on a NanoSight NS500 instrument (Malvern Instruments, Westborough, MA) equipped with a 405 nm laser. Dynamic light scattering (DLS) was used to further confirm particle hydrodynamic diameter and to measure the zeta potential for nano TPs using a Malvern Zetasizer Nano (Malvern Instruments Ltd., Worcestershire, UK) (Fig. S3 and S4). Measurements were done in triplicate. The particle counts of these stock solutions were used as the maximum exposure concentrations along with decreasing concentrations for dose-response creation from both Zebrafish and *D. magna* assays. The nano fraction was further filtered using a 0.02 μm filter (Whatman Anotop), to remove the nano TPs, leaving behind the leachate. This un-diluted leachate stock solution was used for the 100% leachate exposure.

TPs were further characterized using scanning electron microscopy (SEM) (Fig. 1). A drop of each size range sample was placed on a 5 × 5 mm silicon wafer (Ted Pella Inc. Prod No. 16008) mounted to an aluminum specimen mount (Ted Pella Inc. Prod No. 16111). Samples were then placed on a hot plate set at 35 °C for approximately 1 h to dry. All samples were then coated with a layer of gold-palladium using a Cressington 108auto sputter coater (Cressington Scientific Instruments, Watford, UK) to prevent sample charging and improve signal-to-noise ratio during imaging. Random images of the polydisperse microplastic particle samples were taken with a FEI Quanta 600 F environmental scanning electron microscope (FEI Company, Hillsboro, Oregon, USA) at 5 kV beam voltage. The FEI Quanta 600 F is housed in the Oregon State University Electron Microscopy Facility (Corvallis, Oregon, USA).

2.2. Zebrafish assay

TPs were prepared in 0.2 μm filtered fish water (FW) supplemented with 50 mg/L NOM and fractioned as described above. Oregon State University's Sinnhuber Aquatic Research Laboratory (SARL) maintains wild-type tropical 5D Zebrafish (*Danio rerio*) in a water flow-through system under standard laboratory conditions (Westerfield, 2000). Following a group spawn, embryos were collected and staged to ensure that they were all at the same stage of development. When they reach 8 h post-fertilization (hpf), the embryos were added to 200 μl of treatment solution in clear, untreated, flat bottom 96-well plates (Falcon 351172). These treatments consisted of suspensions of micro-sized (1–20 μm), nano-sized (<1 μm), or leachate from TP in FW and 50 mg/L NOM. Concentrations for micro TP exposures ranged from 1.0×10^4 - 1.29×10^6 particles / ml (approximately 0.63–81.18 mg/L) and concentrations for nano TP exposures ranged from 5.0×10^6 - 3.6×10^9 particles / ml (four plate replicates, n = 32 per concentration). The same initial weight of milled tire was suspended in FW for each replicate and the maximum concentrations of micro and nano TPs reflected the maximum number of particles in those size ranges created from the suspension of that initial mass of tire. For this reason, higher maximum concentrations of nano TP exposures were obtained than micro TP exposures. Exposures of TP leachate ranged from 10% to 100% (four plate replicates, n = 32 per concentration). Treatment solutions were made by diluting stock solutions of TP in FW.

Following addition of the embryos, the 96-well plates were covered and incubated at 26.9 °C under a 14:10 h light-dark cycle. The embryos, and later larval fish, were assessed at two time points (24 and 120 hpf) using a dissecting microscope. At 24 hpf, embryos were observed for mortality, developmental progression, and the presence of spontaneous movement. They were again observed at 120 hpf when they were assessed for a series of endpoints including: abnormalities of the body axis, brain, eye, caudal fin, pectoral fin, jaw, otic, pigment, snout, trunk, circulation and somites; as well as, edema of the yolk sack and pericardium, and changes in behavioral response to touch (Truong et al., 2011). All experiments were performed in compliance with national care and use guidelines and approved by the Institutional Animal Care and Use Committee (IACUC) at Oregon State University (protocol number #5114).

At the end of the exposure, a subsample of fish were preserved in paraformaldehyde for imaging. The larval Zebrafish were cleared using a protocol adapted for larval organisms with CUBIC™ clearing reagents (Susaki et al., 2015; Ohnuma et al., 2017). This clearing procedure removed dark pigmentation from the Zebrafish tissue, allowing us to clearly image micro-sized TPs within the fish. Samples were removed from the fixative and incubated first in phosphate-buffered saline (PBS) for 30 min, and then in CUBIC-L at 37 °C for seven days. They were then once again incubated in PBS for an additional 30 min and then in CUBIC-R + at 37 °C for an additional seven days to clear the remaining tissue. Cleared fish were imaged on an SZX10 Stereomicroscope (Olympus, Tokyo Japan).

2.3. Daphnia assay

TPs were prepared in 0.2 μm filtered Daphnia water supplemented with 50 mg/L NOM and fractioned as described above. *D. magna* (Ward's Science, Rochester, NY) were cultured in moderately hard water prepared with 192 mg/L $NaHCO_3$, 201 mg/L $CaSO_4$ * 2 H_2O, 120 mg/L $MgSO_4$, 8 mg/L KCl and 0.002 mg/L Na_2SeO_3 in reverse osmosis water and fed dried spirulina algae in addition to fermented Daphnia chow (Carolina Biologic Supply, Burlington, NC) and yeast under a 14/10

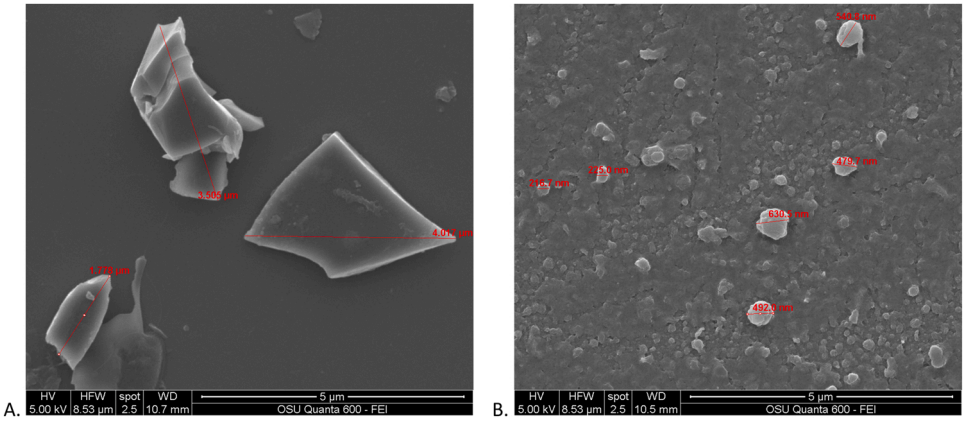


Fig. 1. Scanning Electron Microscopy (SEM) images of (A) micro (1–20 μm) and (B) nano (<1 μm) TWPs. Measurements verifying TWP size-ranges shown in red lettering.

light-dark cycle provided by full spectrum lights. The water quality parameters were measured to ensure that the Daphnia water was: 7.6–8.4 pH, 550–600 µS/cm conductivity, 70–100 mg/L alkalinity, and 160–180 mg/L hardness. Neonatal *D. magna* (<24 h) were collected and exposed to TP suspensions in both size classes across a range of particle concentrations (1.3×10^5 to 8.59×10^5 micro particles/ml, 8.18–54.05 mg/L, and 2.5×10^7 to 2.5×10^9 nano particles/ml). Exposures were conducted with individual neonate *D. magna* placed in 5 ml of exposure solution within a 10 ml glass beaker. Each exposure concentration had 5 replicate beakers, resulting in 5 individual neonate exposures per concentration. Three experimental replicates were conducted for an $n = 15$ for each exposure concentration. *D. magna* were exposed for 48 h and the number of immobile and surviving neonates at each concentration was recorded at 24 and 48 h (Weber, 1991; Guilhermino et al., 2000; OECD, 2004). At the end of the exposure, a subsample of *D. magna* were preserved in 2% paraformaldehyde for imaging on an SZX10 Stereomicroscope (Olympus, Tokyo Japan).

2.4. Data analysis

All statistical analyses were performed using RStudio Version 1.0.153 (RStudio, Boston, MA, USA). Fisher's exact test was used to compare specific developmental endpoints between treatment and controls in the embryonic Zebrafish assay. Due to the multiple comparisons of the Fisher's exact test, significant occurrence of a measured endpoint was based on a Bonferroni adjusted p-value < 0.0045. Analysis of variance (ANOVA) was used to evaluate differences in normal development for Zebrafish and was considered statistically significant at $p \leq 0.05$. All response curves were constructed, and EC_{50} and LC_{50} values were calculated using the DRC (Analysis of Dose-Response Curves) package (Ritz et al., 2015).

3. Results

3.1. Tire particle characterization

Size distributions for the micro and nano TPs show that on average the diameter of the particles were 1.8 ± 1 µm on the flow cytometer and 202 ± 77 nm on the NanoSight respectively (Fig. S1 and S2). DLS analysis of the nano TPs showed that the particles were highly stable with a zeta potential of -31.2 ± 6.11 mV (Fig. S3). Additionally, the size distribution of the nano TPs on the DLS, was 257.7 ± 143.9 nm with a low polydispersity index of 0.263 (Fig. S4). SEM was used to verify the size and characterize the morphology of TPs in both the micro and nano size ranges. Measurements showed that particle size ranges were as expected, with micro TPs being between 1 and 20 µm (Fig. 1A) and nano TPs being less than one micron (Fig. 1B). Though both micro and nano TPs are generated in the same process as fragments, the micro TPs tend to have more jagged edges, with the particles rounding out as they get down in the nano range.

3.2. Zebrafish toxicity

For both the particle and leachate exposures, increasing concentration significantly decreased the number of normal fish at 120 hpf (ANOVA, $p < 0.05$)(Fig. 2). The nano TP exposure had an $EC_{50} = 9.99 \times 10^8$ particles/ml and the leachate exposure had an EC_{50} of 88.65%. An EC_{50} value could not be calculated for the micro TP exposure because over 75% of the Zebrafish developed normally to the 120 hpf timepoint, even following the highest exposure of 1.29×10^6 particles/ml. The only abnormality that was shared by all three treatments (micro TP, nano TP and leachate) was a lack of spontaneous movement (SM) (Fig. 3). The number of fish not showing at 24 hpf increased in a dose-dependent manner along with the concentration of the TP or leachate (Fig. 4). EC_{50} values of 5.53×10^8 and 2.19×10^5 particles/ml and 68.19% were calculated for the nano TPs, micro TPs, and leachate, respectively. Additionally, a couple of similar malformations were observed in both the nano TP and the leachate exposures. A significant number of developing Zebrafish exposed to high concentrations of nano TPs and leachate showed a lack of touch response (TR) and the development of pericardial edema (PE) at 120 hpf. The occurrence of PE.

had EC_{50} values of 8.05×10^8 nano TP/ml and 84.1% leachate (Figs. 5F and 6C). EC_{50} values could not be calculated for the lack of TR, which did not follow a traditional sigmoidal curve (Figs. 5E and 6D). These abnormalities were not seen in those exposed to micro TPs (Fig. 3).

Zebrafish in TP leachate of 80% and above developed several unique abnormalities including malformed jaws, snouts, and eyes, as well as yolk sac edemas (Fig. 5). EC_{50} values for the malformed eye and yolk sac edema were 73.03% and 78.33% leachate respectively. The jaw and snout malformations did not follow sigmoidal curve and, consequently, EC_{50} values could not be calculated for them. At 100% leachate exposure, an average of 40% of the Zebrafish displayed malformed jaws and snouts. Interestingly, though leachate exposures showed the highest diversity of abnormalities and, unlike the nano TP exposures, neither significant hatching delays nor mortality were observed.

Particle specific effects were observed in the micro and nano TP exposures that were not seen in fish exposed solely to leachate. The abnormalities unique to Zebrafish exposed to nano TPs included axis malformations and hatching delay (Fig. 3). Additionally, there was a significant increase in total mortality. Though the average mortality of the highest exposure concentration was only 45%, a predicted LC_{50} of nano TPs is 4.72×10^{10} particles/ml. EC_{50} values of 9.35×10^8 and 1.02×10^9 particles/ml were calculated for hatch delay and axis

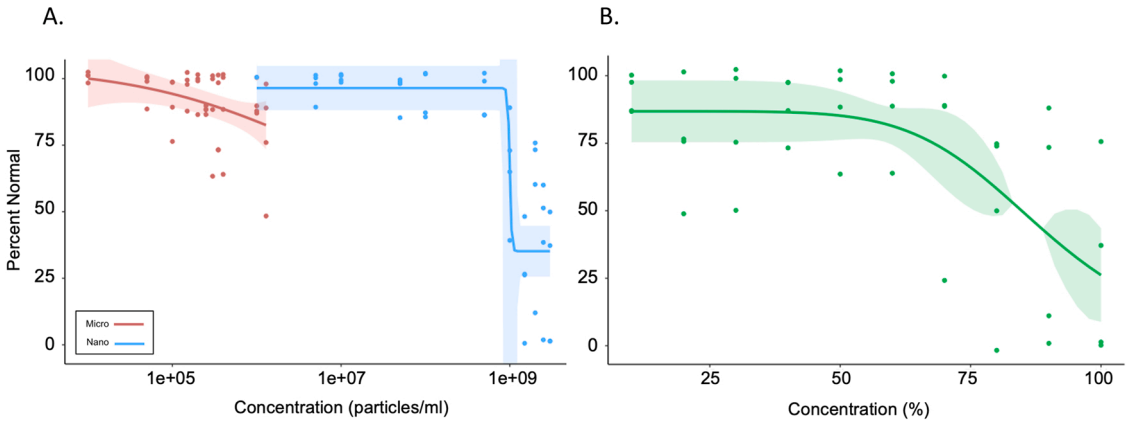


Fig. 2. Concentration-response curves for overall toxicity at 120 hpf following zebrafish exposure to (A) TWPs and (B) leachate. The shaded area is the 95% confidence interval for the fitted curve.

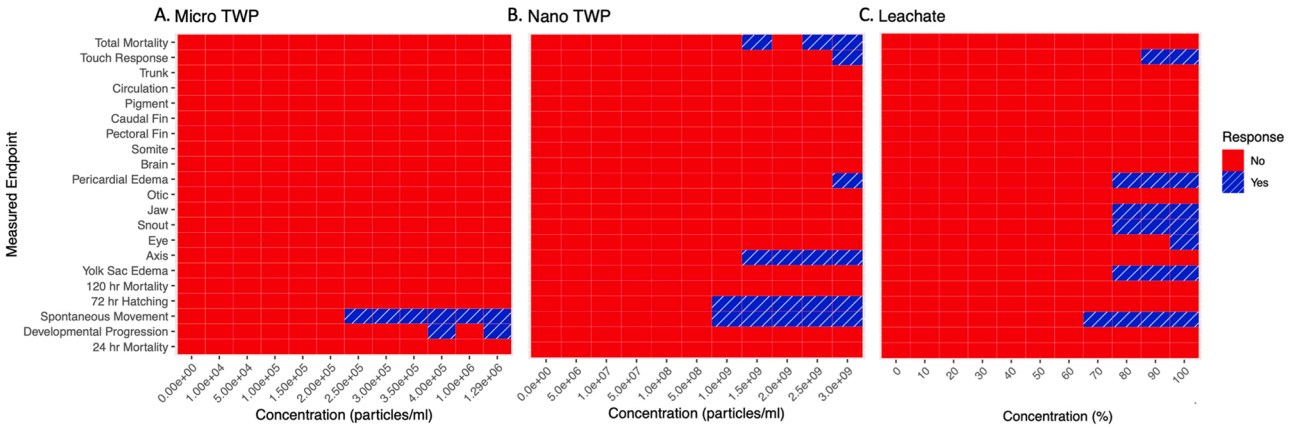


Fig. 3. Heatmaps detailing zebrafish exposure concentrations for (A) TWP micro-size, (B) nano-size and (C) leachate, at which a significantly larger amount (in hashed blue boxes) of zebrafish displayed that measured endpoint, compared to control zebrafish. Solid red boxes indicate no significant response. Significant occurrence of measured endpoint (Yes) based on Bonferroni adjusted p-value < 0.0045.

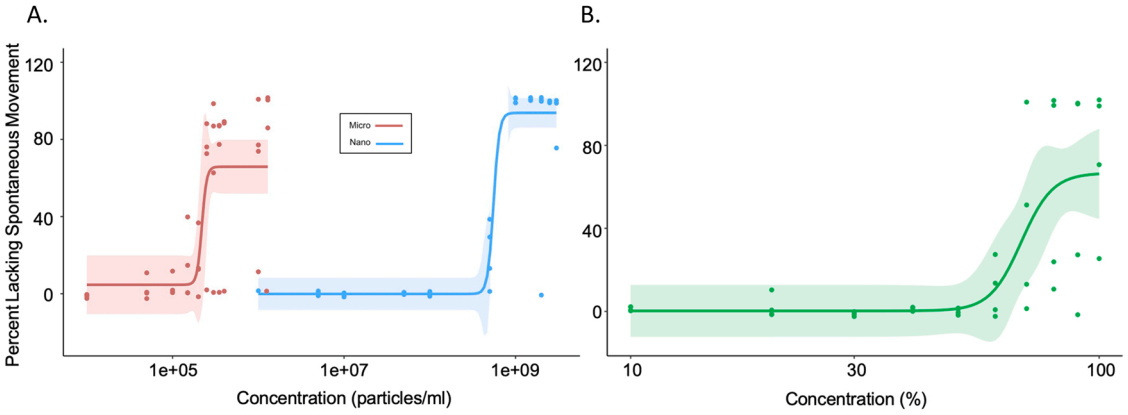


Fig. 4. Concentration-response curves for lack of spontaneous movement at 120 hpf following zebrafish exposure to (A) TWPs and (B) leachate. The shaded area is the 95% confidence interval for the fitted curve.

malformation, respectively (Fig. 6). Both delay, as well as the absence of hatching by 120 hpf, were common in the highest exposure of nano TPs. The least number of malformations were observed following exposure to the micro-sized TPs. In fact, significant abnormalities were only observed at 24 hpf. As mentioned above, Zebrafish exposed to 1.5×10^5 particles/ml and above lacked SM, and those exposed to 3.0×10^5 and 3.94×10^5 particles/ml showed significant delays in developmental progression. However, these delays were only observed in a maximum of 37.5% of fish (Fig. 7). This was the only exposure that showed delayed development at the 24 hpf time point, and by 120 hpf, the micro TP exposed Zebrafish had developed normally. Micro TP exposed Zebrafish internalized TPs (Fig. 8A,B). Internalization was sporadic, with Zebrafish containing no particles, 1–3 particles, or many (<5) particles (Fig. 8). This is likely because at this age the Zebrafish larvae are not actively feeding but simply engaging in mouth gaping which allows for incidental uptake.

3.3. *Daphnia* toxicity

All exposures, particles and leachate, caused mortality in the *D. magna* after 48 h (Fig. 9). LC_{50} values of $3.07x10^8$ and $4.76x10^5$ particles/ml and 20.50% were calculated for the nano TPs, micro TPs, and leachate respectively (Fig. 9). Using the calculated leachate equivalency in the nano TP exposures, calculated as the percent dilution of the nano TP stock solution for each of the nano exposure concentrations, we find an LC_{50} of 8.34%. A small, but insignificant amount of immobility was also observed at the 24 and 48 h time points. Unlike the Zebrafish, the micro TPs had a higher toxicity than the nano TPs. Additionally, it appears that the toxicity can be mainly attributed to released leachate and not a particle specific effect. The presence of the nano TPs may have enhanced the toxicity of the leachate. Images of the *D. magna* exposed to micro TPs showed that their guts were filled with TPs (Fig. 8C,D). Even at the lowest exposures, $1.3x10^5$ and $2.5x10^5$ particles/ml, the *D. magna* readily ingested the micro TPs. However, though they were seen to have a gut full of particles, these *D. magna* were alive and mobile after 48 h of exposure.

4. Discussion

4.1. *Impacts on movement*

4.1.1. *Spontaneous movement inhibition*

Both particle and leachate exposures had some concentration at which SM was impaired. SM is an involuntary coiling contraction that developing Zebrafish exhibit between 17 and 30 hpf (Kimmel et al., 1995; Saint-Amant and Drapeau, 1998). This effect has been linked to nervous system development and a lack of the behavior could indicate neurological problems (Jin et al., 2009). Decreased or missing SM has also been seen following exposure to high concentrations of propranolol (Fraysse et al., 2006), difenoconazole (Mu et al., 2013), bisphenol F (Mu et al., 2019), cyhalofop-butyl (Xia et al., 2017), phthalates (Qian et al., 2020), and sulfonamides (Lin et al., 2013). Alterations in swimming behavior has also been noted in larval and adult zebrafish exposed to other microplastics (Chen et al., 2017a; Mak et al., 2019; Qiang and

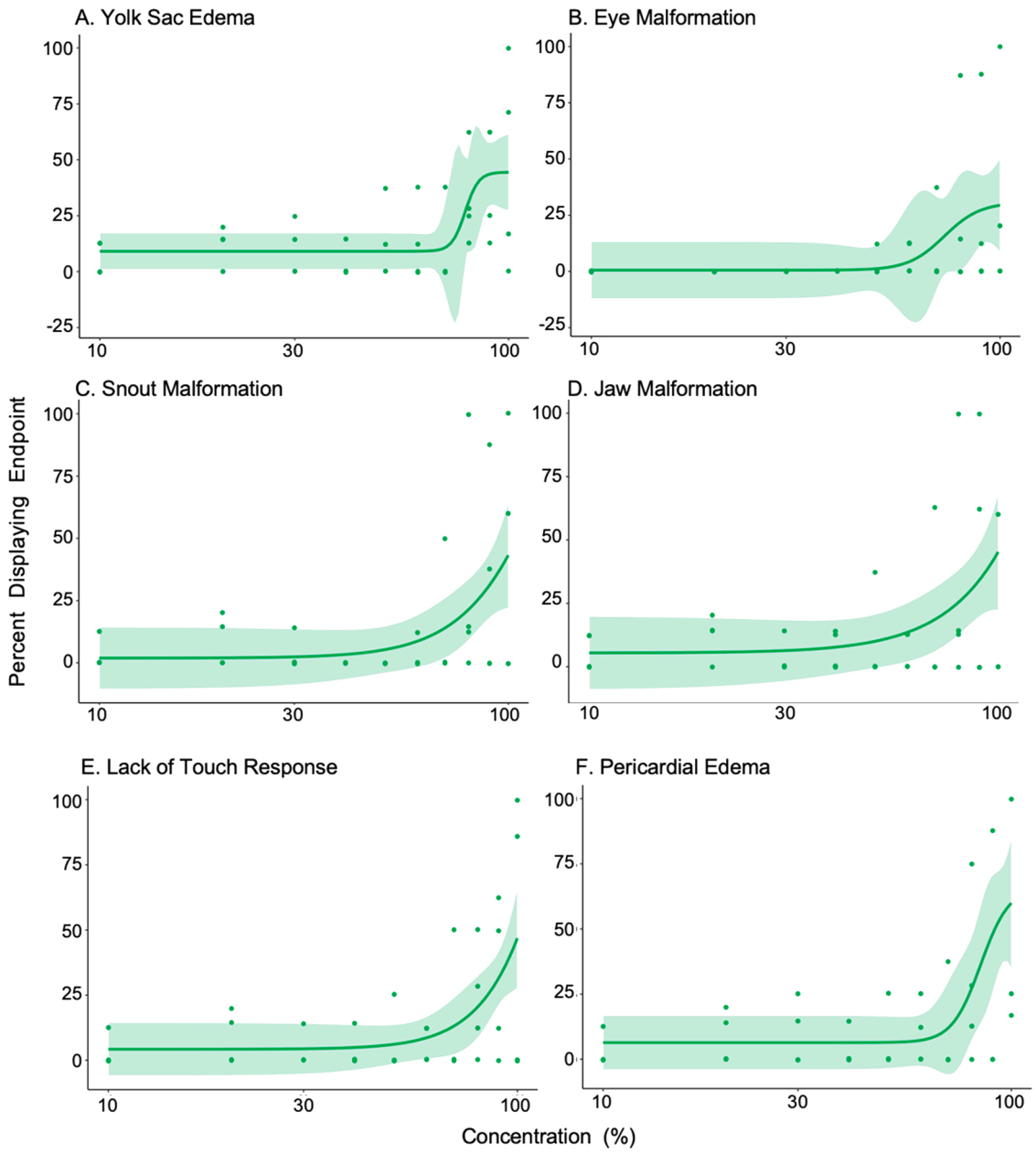


Fig. 5. Concentration-response curves for (A) yolk sac edema, (B) eye, (C) snout, (D) jaw, (E) touch response, and (F) pericardial edema at 120 hpf following zebrafish exposure to leachate. The shaded area is the 95% confidence interval for the fitted curve.

Cheng, 2019; Santos et al., 2021). Similarly, Coho salmon exposed to 6PPD-quinone, derived from tire rubber, exhibited changes in swimming behavior (Tian et al., 2021). Fraysse et al. (2006) found that even at the highest exposure concentration, 108 μM propranolol, a small amount of fish still had SM. In contrast, this study's highest exposures to TPs or leachate showed SM was impaired in an average of 59% of the fish. Exposures to high levels of acetamiprid have also been shown to entirely eliminate SM (Ma et al., 2019). In the past, effects of compounds on SM has been attributed to interaction with ion channels [15], and inhibition of spinal (Qian et al., 2020) and motor neuron (Mu et al., 2019) development. For example, decreased SM following exposure to phthalates was linked to spinal defects (Qian et al., 2020). However, because only the nano TP-exposed embryos showed significant increases in axis malformations, the decrease in SM this study observed likely has a different mechanism. Furthermore, alterations in SM has also been linked to hatch delay (Mu et al., 2019), but again, the nano-TP exposures were the only ones to see significant hatching delays. This is similar to other studies which have noted that the SM endpoint is more sensitive than hatch delay (Lin et al., 2013; Qian et al., 2020). It is clear that TP and leachate exposure has impacts on the embryonic Zebrafish nervous system. Exposure of zebrafish to highway runoff containing heavy metals (e.g. zinc) and PAHs decreases SM (Wu et al., 2014). However, additional research is needed to identify other chemicals released from tires that may contribute to this effect. It is possible that chemicals in the leachate, which may also be released by the micro/nano TPs, limit the spread of action potential in the motor neurons. However, additional research is necessary to identify the exact cause of the SM inhibition.

4.1.2. Touch response inhibition

Another behavioral response shared by Zebrafish embryos exposed to both leachate and nano TPs, though not micro TPs, was a lack of response to touch at 120 hpf. One of the earliest behaviors that Zebrafish develop is movement in response to touch, starting around 21 hpf (Drapeau et al., 2002). Yet, following exposure to high concentrations of

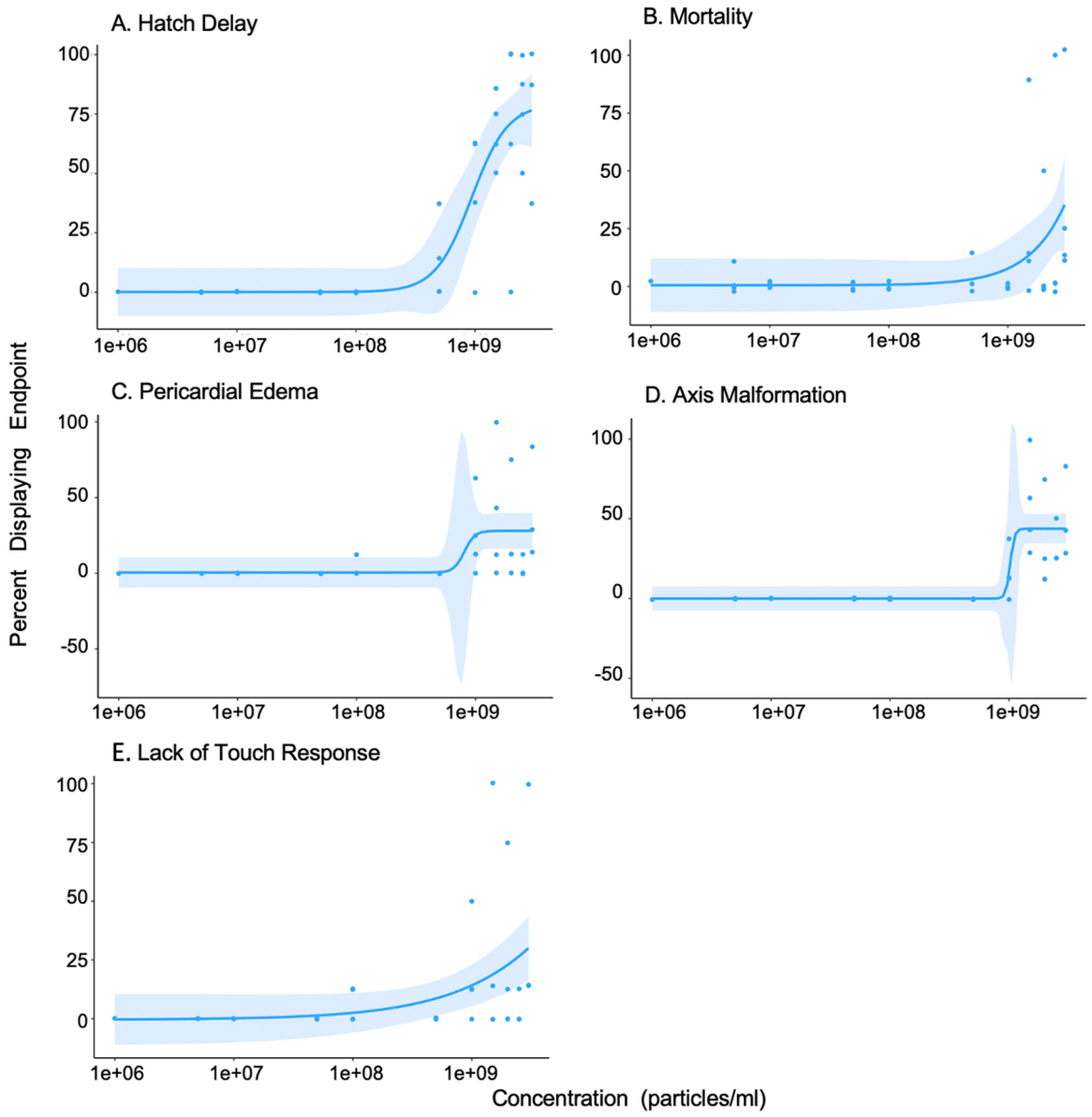


Fig. 6. Concentration-response curves for (A) hatch delay, (B) mortality, (C) pericardial edema, (D) axis, and (E) touch response at 120 hpf following zebrafish exposure to nano TWPs. The shaded area is the 95% confidence interval for the fitted curve.

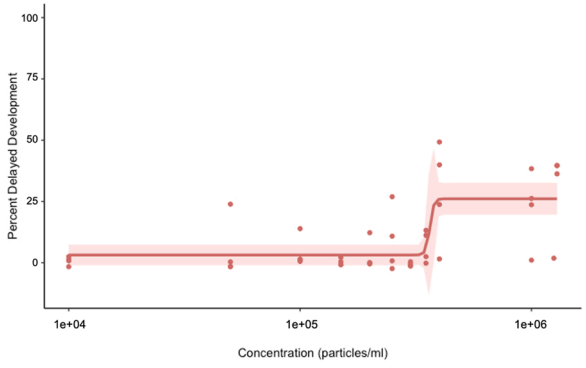


Fig. 7. Concentration-response curves for delayed development at 24 hpf following zebrafish exposure to micro TWPs. The shaded area is the 95% confidence interval for the fitted curve.

either the leachate or nano TPs, Zebrafish no longer exhibited this response. As with SM above, the disappearance of a response to touch likely indicates impairment of neurological development and the central nervous system (Zhao et al., 2019). Several genes have been identified that are associated with the reduced TR phenotype in Zebrafish (Drapeau et al., 2002). The TR is an early expression of the startle response seen in mature Zebrafish (Eaton et al., 2001). Therefore, the inhibition of this necessary response from exposure to TPs and leachate would be expected to decrease fitness. Ma et al. (2019) found that for exposure to acetamiprid TR was a less sensitive endpoint than SM. This makes sense within the context of this study's TP exposures as well. We expect the nano TP exposure to release similar amounts of chemicals to the leachate exposures; however, due to a lower surface area to volume ratio, we expect the micro TPs to release less. If this effect is in response to a chemical mechanism and not a physical effect from the particles, that would explain why we see the lack of SM in all exposures, but the lack of TR only in the nano TP and leachate exposures.

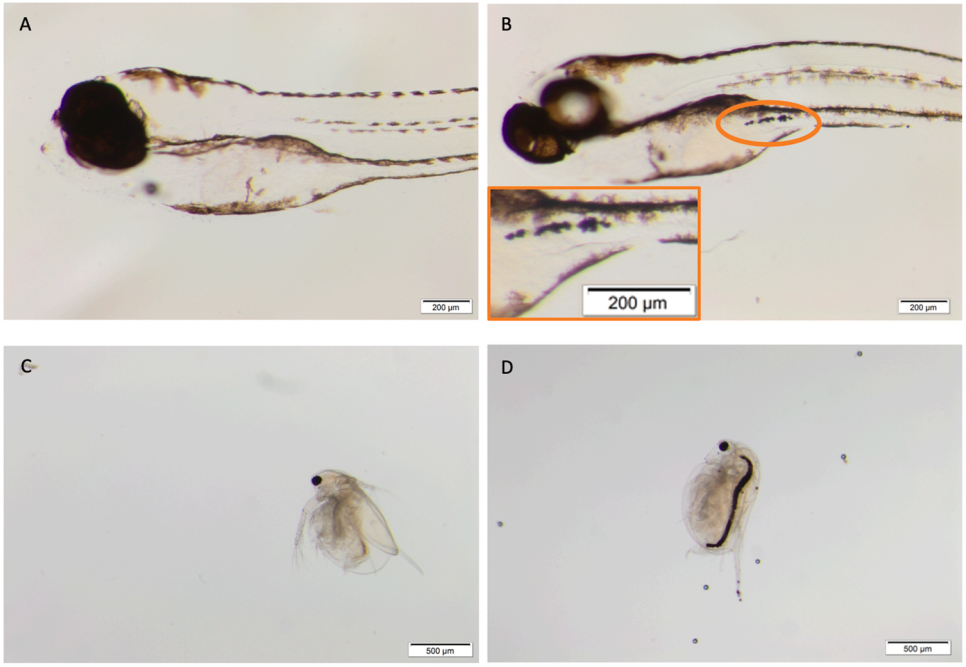


Fig. 8. Cleared Zebrafish (*D. rerio*) larvae image at 6.3X for (A) control and (B) exposures of 1.0 × 106 particles/ml micro TWP. Internalized TWPs are circled in orange. Daphnia (*D. magna*) image at 3.2X for (C) control and (D) exposures of 1.3 × 105 particles/ml micro TWP.

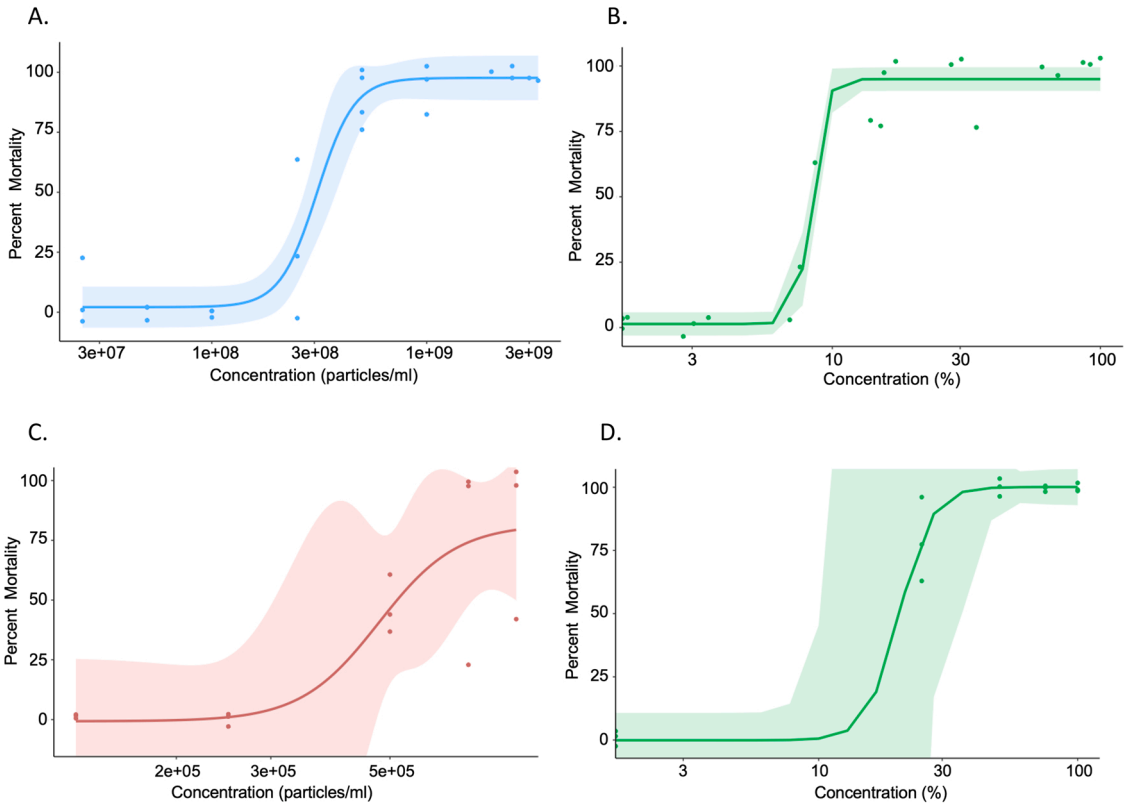


Fig. 9. Concentration-response curves for *Daphnia magna* 48 h mortality after exposure to A) nano TWPs, B) leachate from nano TWPs matched to A, C) micro TWPs, and D) leachate. The shaded area is the 95% confidence interval for the fitted curve.

4.2. Pericardial edema

The second significant abnormality shared by embryonic Zebrafish exposed to either nano TPs or leachate was PE, or fluid accumulating in the pericardial space. This too is likely attributed to chemicals in the leachate fraction of the exposure. PE is a common abnormality in fish exposed to PAHs (Incardona et al., 2004), a compound found in the leachate of TPs and other tire-debris (LaPlaca and van den Hurk, 2020). Additionally, the tissue of fish exposed to TP leachate has been found to have elevated levels of PAHs (Stephensen et al., 2003; LaPlaca and van

den Hurk, 2020). Furthermore, PE has been observed in Zebrafish exposed to untreated road runoff (McIntyre et al., 2014), likely containing tire debris. The toxicity of urban runoff is generally attributed to the PAHs it contains (McIntyre et al., 2014; Wu et al., 2014). Exposure to PAHs causes heart defects in other fish as well (Fallahtafti et al., 2012; Incardona et al., 2012). It is unknown if PAHs are the sole contributor to the emergence of PE in the TP leachate exposed Zebrafish. For example, metals (Fraysse et al., 2006; Kim et al., 2019) and nano metals including ZnO (Bai et al., 2010) and Ag (Asharani et al., 2008; Cunningham et al., 2021) have also been shown to cause PE. Other nano and microplastics cause PE (Malafaia et al., 2020; Sökmen et al., 2020) as well, and nanoplastics have been found to enter the pericardium (Veneman et al., 2017; Pitt et al., 2018a). Therefore, though less likely, the nano TPs themselves could also play a role in triggering PE.

4.3. Leachate specific effects

TP Leachate was found to be toxic to developing Zebrafish. This is in line with the literature which shows that leachate and individual chemicals found in leachate are toxic to fish (Day et al., 1993; Chibwe et al., 2021; Tian et al., 2021). Embryonic Zebrafish exposed only to TP leachate displayed developmental abnormalities unique from those of the particle exposures including malformed eyes, snout, and jaw, as well as yolk sac edema. When Chibwe et al. (2021) exposed Fathead Minnows to TP leachate they also observed malformed jaws and eyes. The toxicity in their leachate was mainly associated with the benzothiazoles and aryl-amines in the mixture. Furthermore, Zebrafish exposed to untreated road runoff were found to develop unusually small eyes (McIntyre et al., 2014). Certain responses such as hatch delay (Chibwe et al., 2021) and increased mortality (Day et al., 1993) have been documented in other studies, but were not observed in this study's leachate-only exposures. The likely reason for this is that commercial tire, and therefore leachate, composition varies greatly. Additionally, the conditions during the leachate extraction (e.g. pH and temperature) have been shown to alter leachate composition and toxicity (Gualtieri et al., 2005; Marwood et al., 2011). Because of this, the differences in toxicity is not surprising. A full understanding of every component of a study's leachate would be necessary to explain the observed differences in toxicity.

Similar abnormalities to those observed in our leachate exposures have been reported in Zebrafish exposed to a variety of chemicals. For example, ethanol exposure results in shortened snouts, abnormal eyes, and yolk sac edema (Reimers et al., 2006). Dioxins have also been shown to cause yolk sac edema in developing Zebrafish and this has been attributed to the alteration of the permeability of water barriers (Hill et al., 2004). Similarly, atractylodin and β-eudesmol not only induced yolk sac edema, but jaw malformations as well (Tshering et al., 2021). Additionally, it is known that PAHs, a common component of TP leachate and road runoff, result in Zebrafish with yolk sac edema (Philibert et al., 2016), and smaller jaws (Incardona et al., 2012) and eyes (Incardona et al., 2004). Incardona et al. (2004) found that the craniofacial defects that accompanied PAH exposure were downstream effects of heart failure. Therefore, these defects that emerged following TP leachate exposure may also be related to the significant occurrence of PE, discussed above.

4.4. Nano TP specific effects

High concentrations of nano TPs resulted in toxic effects that were not observed in the leachate and micro TP exposures. These included mortality, hatching delay, and axis malformations. Mortality and delayed hatching have both been observed in fish exposed to TP leachate (Day et al., 1993; Chibwe et al., 2021) and road runoff (McIntyre et al., 2014). Though these effects were not observed in our leachate-only exposures, others have found that the presence of TPs enhances toxicity in comparison to leachate alone (Halle et al., 2020; Chibwe et al., 2021). This may be why the nano TP exposure, which includes exposure to leachate that the nano particles release throughout the experiment, was the only one to have significant mortality. Though no other data has been published on exposure of Zebrafish to TPs themselves, and not leachate, TPs have been found to induce mortality in freshwater amphipod *Hyalella azteca* (Khan et al., 2019; Halle et al., 2021). Untreated highway runoff, which likely contains TPs, causes mortality in Zebrafish (McIntyre et al., 2014; Young et al., 2018). Exposure to PAHs, a common tire component, can also be lethal to developing Zebrafish (Incardona et al., 2004). Nanoplastics have also been shown to facilitate the uptake of PAHs into embryonic Zebrafish (Zhang and Goss, 2020). Other materials found in tires that have been shown to cause mortality in Zebrafish include carbon black (Kim et al., 2019), copper (Zhang et al., 2012), and cadmium (Al-Sawafi et al., 2017).

The inhibition of SM, as was observed in nano TP-exposed Zebrafish, has previously been linked to hatching delays (Lin et al., 2013; Xia et al., 2017; Qian et al., 2020). Nanoplastics have been shown to enter the Zebrafish chorion (Lee et al., 2019), which could contribute to hatching delay. Additionally, Zn, a common component in tire material, is known to interfere with the enzymes involved in fish hatching (Jezierska et al., 2009). The hatch delay in nano-exposed fish may also be linked to the significant increase in axis malformations, as these malformations are often seen together (Asharani et al., 2011; Saili et al., 2012; Huang et al., 2021). The physical constrain of the chorion on the developing fish could cause axis curvature. Additionally, chemicals leached from the nano TPs during treatment likely play a role in the defects observed. Spinal defects are common in Zebrafish exposed to phthalates (Zhou et al., 2019; Qian et al., 2020) through the alteration of spine-development gene expression (Qian et al., 2020). Exposure to perfluorooctanesulphonicacid (PFOS) (Huang et al., 2010) and bisphenol F (Mu et al., 2019) have both been shown to cause hatching inhibition and spinal deformities. However, physical aspects of the TP themselves likely also contribute to the toxicity. Nanoplastics have been shown to accumulate in the spinal cord (Lee et al., 2019), and may affect the development of that area. Additionally, exposure to nano- and microplastics can cause axis curvature as well (Malafaia et al., 2020; Sökmen et al., 2020).

4.5. Micro TP specific effects

The only impact exclusively observed in micro TP exposures was a delay in development at 24 hpf. The pores of the Zebrafish chorion are 0.5–0.7 μm in diameter (Rawson et al., 2000) and microplastics have been shown to accumulate on the chorion (Qiang and Cheng, 2019; Duan et al., 2020). The micro TPs in our experiment were between 1 and 20 μm and much too large to pass through the chorion pores. Because the chorion pores are necessary for transport of oxygen, nutrients, and waste (Cheng et al., 2007), blockage of them could limit oxygen and ionic exchange, or damage the chorion. However, this would be expected to result in premature hatching. Zebrafish exposed to polyethylene microplastics, which were seen to coat the chorion, induced early hatching (Malafaia et al., 2020). Additionally, fish eggs in low oxygen conditions hatch prematurely (Alderdice et al., 1958). Early hatching was not observed in this experiment, thus blockage of the chorion pores is unlikely to have caused the developmental delays. Further research is necessary to understand the mechanism through which micro TPs delayed Zebrafish development.

By the 120 hpf time point, Zebrafish exposed to micro TPs had hatched and developed normally. There are many aspects of the micro TPs that likely contribute to their lower toxicity. Though a lower particle count was used for micro TP exposures, the mass of tire in the micro exposures was larger than that in the nano exposures. However, due to their larger size, and therefore smaller surface-area-to-volume ratio, the micro particles likely leached less into the exposure water than the nano TPs. A lower level of accompanying leachate would explain the minimal

toxicity observed. For example, research has shown that in soil, TPs release less leachate, due to lower pH, and the resulting elucidate is less toxic (Marwood et al., 2011; Panko et al., 2013). Additionally, though they were internalized by the hatched Zebrafish, it appeared the micro TPs were quickly passed through the gut and easily removed from the body. In contrast, nanoplastics are more likely to remain in an organism for longer period of time (Yoo et al., 2021) and have the potential to translocate to other tissues and organs (Pitt et al., 2018a). This may be why we saw particle specific effects from the nano TPs that were not found in the micro, or even leachate, exposures.

4.6. Daphnia toxicity

D. magna are a pelagic freshwater invertebrate that have limbs to find and manipulate food particles (Fryer, 1991). They are primarily filter feeders grazing on phytoplankton (Miner et al., 2012), but have also been shown to lift material, such as detritus, accumulated on the benthos (Fryer, 1991). After 48 h, *D. magna* mortality was observed in high concentrations of both particle and leachate exposures. For the exposure to leachate, mortality was observed even at the lowest leachate concentration of 25%. Tatarazako et al. (2007) report and EC_{20} value of 16% leachate for *D. magna.* In contrast, Gualtieri et al. (2005) noted no mortality of *D. magna* until exposure to 100% TP leachate, with their next lowest exposure being 50%. The literature documents a wide range of EC_{50} and LC_{50} values for exposure of *D. magna* to leachate (Wik and Dave, 2006; Wik, 2007; Wik and Dave, 2009; Lu et al., 2021). As discussed above (Section 4.4), the composition and toxicity of tire leachate varies greatly based on the conditions under which it was created (Wik and Dave, 2005; Rhodes et al., 2012). For example, exposure of leachate containing TPs to UV increases toxicity (Wik, 2007; Lu et al., 2021). Additionally, variation in rubber formulation of different tires can result in a 20-fold deviation in toxicity (Wik, 2007). Therefore, it is not surprising that we observed mortality of *D. magna* at different leachate concentrations than others. However, this continues to highlight the importance of documentation in all aspects of leachate creation as well as procedures that closely mimic environmental conditions. Wik (2007) assessed the toxicity of leachates made from tires containing different additives to identify the most toxic components. They found that leachates containing antidegradents were the most toxic (Wik, 2007). Moreover, the toxicity of leachate has been attributed to non-polar organic compounds, such as PAHs (Wik and Dave, 2006), as well as metals such as Zn (Rhodes et al., 2012).

Much less information exists on the effects of exposure to TPs themselves, without the leachate. This current study found a dose-dependent response for both the micro and nano sized TP exposures, where mortality increased along with TP concentration. Additionally, the micro TPs were readily ingested and could be seen in the gut starting at 130,000 particles/ml. Though the particle number was lower in the micro TP exposures, the mass of tires was higher. This could explain the lower LC_{50} value for the micro TP exposure in comparison to the nano TP exposure. Likely the physical presence of the larger particles and their accumulation in the gut has negative impacts. Wik and Dave (2005) exposed *D. Magna* to grated tires, with clumps several millimeters in length. They used twelve different tires and found that 48-hr EC_{50} values ranged from 0.0625 to 2.41 g/L TPs. The effects of micro TPs have been assessed for other freshwater organisms. Similar to what we observed in *D. magna*, Khan et al. (2019) found *H. azteca* indiscriminately take up TPs. However, they observed higher toxicity with a 48-hr LC_{50} of 3426 $\pm$ 172 particles/ml This could be attributed to differences in the study organisms, including differences in where in the water column they reside (benthic vs. pelagic), but is more likely due to the tire selected, and method of particle creation. No other studies were found on the toxicity of nano TPs. This is the first data to show that TPs of this size are toxic.

The majority of the toxicity observed in all of the *D. magna* exposures is likely due to the leachate fraction. Wik (2007) assessed the toxicity of leachates made from tires containing different additives to identify the most toxic components. They found that leachates containing antidegradents were the most toxic (Wik, 2007). Generally, the toxicity of leachate to *D. magna* has been attributed to organic compounds such as PAHs (Wik and Dave, 2006), as well as Zn (Rhodes et al., 2012) (summarized in Wik and Dave, 2009). However, there may be particle contributions to the toxicity as well. As mentioned above, the micro TPs may cause physical obstructions. Additionally, comparing the leachate LC_{50} (20.5%) and the LC_{50} calculated for the equivalent leachate that would be in the nano TP exposures (8.34%), we find that the value is slightly lower for the nano TPs. This is in line with particle-enhanced toxicity that others have observed (Halle et al., 2020; Chibwe et al., 2021). It is not fully understood how the presence of the particles enhances the toxicity of the leachate. It is possible that the internalized TPs facilitate uptake of chemicals into additional areas of the organism's tissues, as demonstrated with other plastics(Chen et al., 2017b, Zhang and Goss, 2020).

5. Conclusion

Recently, the pervasiveness of TPs in the environment, and especially in water bodies, has been realized. The leachate produced from tires is toxic under certain conditions; however, the particles have long been ignored as potential toxicants themselves. This research demonstrates the importance of investigating both the particle and leachate components of the toxicity. Though the leachate was responsible for some portion of the effects observed in both the Zebrafish and *D. magna* exposures, we noted particle-specific effects that were not present in leachate only exposures. Therefore, the TPs alone are not benign. This is some of the first research to demonstrate that the toxicity of tires has particle and size specific effects. Particularly, the nano TPs, which are expected to release more leachate due to their high surface-area-to-volume ratio, exhibited particle-enhanced toxicity. That is, greater mortality and more severe effects were documented when nanoparticles were present with leachate for both the *D. magna* and Zebrafish. Overall, leachate is still one of the greatest contributors to the toxicity, which is likely why we saw fewer effects in Zebrafish exposed to micro TPs alone. As we hypothesized, the sensitivity of the different organisms to TPs and leachate differed with both particles and leachate causing mortality in *D. magna*, but only the nano TPs causing mortality in Zebrafish. More research is needed on the mechanisms through which TPs result in mortality and developmental abnormalities. Additionally, components in the leachate need to be identified and investigated for their contribution to the mixtures toxicity. This knowledge can be used to inform policy and educate both the public and decision makers.

Funding Sources

This research was funded by the National Science Foundation Growing Convergence Research Big Idea, USA (to SH, SMB), 1935028.

Ethics Statement

All animal studies were conducted under the Oregon State University Institutional Animal Care and Use Committee authorized protocol (ACUP #5114).

CRediT authorship contribution statement

Susanne Brander, Stacey Harper, Bryan Harper: Conceptualization. **Brittany Cunningham**: Investigation, Analysis, Manuscript writing. **Brittany Cunningham, Susanne Brander, Bryan Harper, Stacey Harper**: Methodology, Review, Editing. **Stacey Harper**: Supervision, Project administration, Funding acquisition. All authors have approved the published version of the manuscript.

Statement of Novelty

To our knowledge no paper has been published detailing the effects of nano tire particles (TPs) on organisms. We are the first to show that both micro and nano TPs have unique effects on the development of a vertebrate model organisms, Zebrafish. Additionally, we generated concentration-response curves for each of the endpoints observed. These are necessary for the creation of accurate risk assessment for both TPs and micro/nano plastics in general.

TPs and their leachate should be considered hazardous material based on established physical and chemical dangers they pose to organisms as well as their persistence in the environment.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

Authors would like to thank Jared Stein and the OSU electron microscopy facility for assisting with SEM image generation for TP characterization, and John Dickens for his instruction on clearing fish tissue.

References

Al-Sawafi, A., Wang, L., Yan, Y., 2017. Cadmium accumulation and its histological effect on brain and skeletal muscle of zebrafish. J. Heavy Met. Toxic. Dis. 2, 2.
Alderdice, D., Wickett, W., Brett, J., 1958. Some effects of temporary exposure to low dissolved oxygen levels on Pacific salmon eggs. J. Fish. Board Can. 15, 229–250.
Asharani, P., Lianwu, Y., Gong, Z., Valiyaveettil, S., 2011. Comparison of the toxicity of silver, gold and platinum nanoparticles in developing zebrafish embryos. Nanotoxicology 5, 43–54.
Asharani, P., Wu, Y.L., Gong, Z., Valiyaveettil, S., 2008. Toxicity of silver nanoparticles in zebrafish models. Nanotechnology 19, 255102.
Attar, E.N., Maly, E.J., 1982. Acute toxicity of cadmium, zinc, and cadmium-zinc mixtures to Daphnia magna. Arch. Environ. Contam. Toxicol. 11, 291–296.
Baensch-Baltruschat, B., Kocher, B., Stock, F., Reifferscheid, G., 2020. Tyre and road wear particles (TRWP)-A review of generation, properties, emissions, human health risk, ecotoxicity, and fate in the environment. Sci. Total Environ. 733, 137823.
Bai, W., Zhang, Z., Tian, W., He, X., Ma, Y., Zhao, Y., Chai, Z., 2010. Toxicity of zinc oxide nanoparticles to zebrafish embryo: a physicochemical study of toxicity mechanism. J. Nanopart. Res. 12, 1645–1654.
Blettler, M.C., Abrial, E., Khan, F.R., Sivri, N., Espinola, L.A., 2018. Freshwater plastic pollution: Recognizing research biases and identifying knowledge gaps. Water Res. 143, 416–424.
Blettler, M.C., Wantzen, K.M., 2019. Threats underestimated in freshwater plastic pollution: mini-review. Water, Air, Soil Pollut. 230, 1–11.
Boucher, J., Friot, D., 2017. Primary Microplastics in the Oceans: A Global Evaluation of Sources. Iucn Gland,, Switzerland.
Brun, N.R., Beenakker, M.M., Hunting, E.R., Ebert, D., Vijver, M.G., 2017. Brood pouch-mediated polystyrene nanoparticle uptake during Daphnia magna embryogenesis. Nanotoxicology 11, 1059–1069.
Busquet, F., B. Halder, T. Braunbeck, L.A. Gourmelon, A. Kleensang, S. Belanger, G. Carr, and S. Walter-rohde. 2013. OECD guidelines for the testing of chemicals 236-fish embryo acute toxicity (FET) test. OECD Obs.
Chen, Q., Gundlach, M., Yang, S., Jiang, J., Velki, M., Yin, D., Hollert, H., 2017a. Quantitative investigation of the mechanisms of microplastics and nanoplastics toward zebrafish larvae locomotor activity. Sci. Total Environ. 584, 1022–1031.
Chen, Q., Yin, D., Jia, Y., Schiwy, S., Legradi, J., Yang, S., Hollert, H., 2017b. Enhanced uptake of BPA in the presence of nanoplastics can lead to neurotoxic effects in adult zebrafish. Sci. Total Environ. 609, 1312–1321.
Cheng, J., Flahaut, E., Cheng, S.H., 2007. Effect of carbon nanotubes on developing zebrafish (Danio rerio) embryos. Environ. Toxicol. Chem.: Int. J. 26, 708–716.
Chibwe, L., Parrott, J.L., Shires, K., Khan, H., Clarence, S., Lavalle, C., Sullivan, C., O'Brien, A., De Silva, A.O., Muir, D.C., 2021. A Deep Dive into the Complex Chemical Mixture and Toxicity of Tire Wear Particle Leachate in Fathead Minnow. Environ. Toxicol. Chem.
Choi, J.S., Hong, S.H., Park, J.-W., 2020. Evaluation of microplastic toxicity in accordance with different sizes and exposure times in the marine copepod Tigriopus japonicus. Mar. Environ. Res. 153, 104838.
Councell, T.B., Duckenfield, K.U., Landa, E.R., Callender, E., 2004. Tire-wear particles as a source of zinc to the environment. Environ. Sci. Technol. 38, 4206–4214.
Cui, R., Kim, S.W., An, Y.-J., 2017. Polystyrene nanoplastics inhibit reproduction and induce abnormal embryonic development in the freshwater crustacean Daphnia galeata. Sci. Rep. 7, 12095.
Cunningham, B., Engstrom, A.E., Harper, B.J., Harper, S.L., Mackiewicz, M.R., 2021. Silver Nanoparticles Stable to Oxidation and Silver Ion Release Show Size-Dependent Toxicity In Vivo. Nanomaterials 11, 1516.
Day, K., Holtze, K., Metcalfe-Smith, J., Bishop, C., Dutka, B., 1993. Toxicity of leachate from automobile tires to aquatic biota. Chemosphere 27, 665–675.
Drapeau, P., Saint-Amant, L., Buss, R.R., Chong, M., McDearmid, J.R., Brustein, E., 2002. Development of the locomotor network in zebrafish. Prog. Neurobiol. 68, 85–111.
Duan, Z., Duan, X., Zhao, S., Wang, X., Wang, J., Liu, Y., Peng, Y., Gong, Z., Wang, L., 2020. Barrier function of zebrafish embryonic chorions against microplastics and nanoplastics and its impact on embryo development. J. Hazard. Mater. 395, 122621.
Eaton, R., Lee, R., Foreman, M., 2001. The Mauthner cell and other identified neurons of the brainstem escape network of fish. Prog. Neurobiol. 63, 467–485.
Eisentraut, P., Dümichen, E., Ruhl, A.S., Jekel, M., Albrecht, M., Gehde, M., Braun, U., 2018. Two birds with one stone—fast and simultaneous analysis of microplastics: microparticles derived from thermoplastics and tire wear. Environ. Sci. Technol. Lett. 5, 608–613.
Fallahtafti, S., Rantanen, T., Brown, R.S., Snieckus, V., Hodson, P.V., 2012. Toxicity of hydroxylated alkyl-phenanthrenes to the early life stages of Japanese medaka (Oryzias latipes). Aquat. Toxicol. 106, 56–64.
Fraysse, B., Mons, R., Garric, J., 2006. Development of a zebrafish 4-day embryo-larval bioassay to assess toxicity of chemicals. Ecotoxicol. Environ. Saf. 63, 253–267.
Frydkjær, C.K., Iversen, N., Roslev, P., 2017. Ingestion and egestion of microplastics by the cladoceran Daphnia magna: effects of regular and irregular shaped plastic and sorbed phenanthrene. Bull. Environ. Contam. Toxicol. 99, 655–661.
Fryer, G., 1991. Functional morphology and the adaptive radiation of the Daphniidae (Branchiopoda: Anomopoda). Philos. Trans. R. Soc. Lond. Ser. B: Biol. Sci. 331, 1–99.
Geyer, R., Jambeck, J.R., Law, K.L., 2017. Production, use, and fate of all plastics ever made. Sci. Adv. 3, e1700782.
Gray, A.D., Weinstein, J.E., 2017. Size-and shape-dependent effects of microplastic particles on adult daggerblade grass shrimp (Palaemonetes pugio). Environ. Toxicol. Chem. 36, 3074–3080.
Greven, A.C., Merk, T., Karagöz, F., Mohr, K., Klapper, M., Jovanović, B., Palić, D., 2016. Polycarbonate and polystyrene nanoplastic particles act as stressors to the innate immune system of fathead minnow (Pimephales promelas). Environ. Toxicol. Chem. 35, 3093–3100.
Grillo, R., Rosa, A.H., Fraceto, L.F., 2015. Engineered nanoparticles and organic matter: a review of the state-of-the-art. Chemosphere 119, 608–619.
Gualtieri, M., Andrioletti, M., Vismara, C., Milani, M., Camatini, M., 2005. Toxicity of tire debris leachates. Environ. Int. 31, 723–730.
Guilhermino, L., Diamantino, T., Silva, M.C., Soares, A., 2000. Acute toxicity test with Daphnia magna: an alternative to mammals in the prescreening of chemical toxicity? Ecotoxicol. Environ. Saf. 46, 357–362.
Halle, L.L., Palmqvist, A., Kampmann, K., Jensen, A., Hansen, T., Khan, F.R., 2021. Tire wear particle and leachate exposures from a pristine and road-worn tire to Hyalella azteca: Comparison of chemical content and biological effects. Aquat. Toxicol. 232, 105769.
Halle, L.L., A. Palmqvist, K. Kampmann, and F.R. Khan. 2020. Ecotoxicology of micronized tire rubber: Past, present and future considerations. Science of the Total Environment **706**:135694.
Hill, A.J., Bello, S.M., Prasch, A.L., Peterson, R.E., Heideman, W., 2004. Water permeability and TCDD-induced edema in zebrafish early-life stages. Toxicol. Sci. 78, 78–87.
Huang, H., Huang, C., Wang, L., Ye, X., Bai, C., Simonich, M.T., Tanguay, R.L., Dong, Q., 2010. Toxicity, uptake kinetics and behavior assessment in zebrafish embryos following exposure to perfluorooctanesulphonicacid (PFOS). Aquat. Toxicol. 98, 139–147.
Huang, J., Sun, L., Mennigen, J.A., Liu, Y., Liu, S., Zhang, M., Wang, Q., Tu, W., 2021. Developmental toxicity of the novel PFOS alternative OBS in developing zebrafish: An emphasis on cilia disruption. J. Hazard. Mater. 409, 124491.
Incardona, J.P., Collier, T.K., Scholz, N.L., 2004. Defects in cardiac function precede morphological abnormalities in fish embryos exposed to polycyclic aromatic hydrocarbons. Toxicol. Appl. Pharmacol. 196, 191–205.
Incardona, J.P., C.A. Vines, B.F. Anulacion, D.H. Baldwin, H.L. Day, B.L. French, J.S. Labenia, T.L. Linbo, M.S. Myers, and O.P. Olson. 2012. Unexpectedly high mortality in Pacific herring embryos exposed to the 2007 Cosco Busan oil spill in San Francisco Bay. Proceedings of the National Academy of Sciences **109**:E51-E58.
Jezierska, B., Ługowska, K., Witeska, M., 2009. The effects of heavy metals on embryonic development of fish (a review). Fish. Physiol. Biochem. 35, 625–640.
Jin, M., Zhang, X., Wang, L., Huang, C., Zhang, Y., Zhao, M., 2009. Developmental toxicity of bifenthrin in embryo-larval stages of zebrafish. Aquat. Toxicol. 95, 347–354.
Khan, F.R., Halle, L.L., Palmqvist, A., 2019. Acute and long-term toxicity of micronized car tire wear particles to Hyalella azteca. Aquat. Toxicol. 213, 105216.
Kim, K., Wang, C.-H., Ok, Y.S., Lee, S.-E., 2019. Heart developmental toxicity by carbon black waste generated from oil refinery on zebrafish embryos (Danio rerio): combined toxicity on heart function by nickel and vanadium. J. Hazard. Mater. 363, 127–137.
Kimmel, C.B., Ballard, W.W., Kimmel, S.R., Ullmann, B., Schilling, T.F., 1995. Stages of embryonic development of the zebrafish. Dev. Dyn. 203, 253–310.
Kole, P.J., Löhr, A.J., Van Belleghem, F.G., Ragas, A.M., 2017. Wear and tear of tyres: a stealthy source of microplastics in the environment. Int. J. Environ. Res. Public Health 14, 1265.
Kukutschová, J., Moravec, P., Tomášek, V., Matějka, V., Smolík, J., Schwarz, J., Seidlerová, J., Šafářová, K., Filip, P., 2011. On airborne nano/micro-sized wear particles released from low-metallic automotive brakes. Environ. Pollut. 159, 998–1006.

LaPlaca, S.B., van den Hurk, P., 2020. Toxicological effects of micronized tire crumb rubber on mummichog (Fundulus heteroclitus) and fathead minnow (Pimephales promelas). Ecotoxicology 29, 524–534.
Lee, K.-W., Shim, W.J., Kwon, O.Y., Kang, J.-H., 2013. Size-dependent effects of micro polystyrene particles in the marine copepod Tigriopus japonicus. Environ. Sci. Technol. 47, 11278–11283.
Lee, W.S., Cho, H.-J., Kim, E., Huh, Y.H., Kim, H.-J., Kim, B., Kang, T., Lee, J.-S., Jeong, J., 2019. Bioaccumulation of polystyrene nanoplastics and their effect on the toxicity of Au ions in zebrafish embryos. Nanoscale 11, 3173–3185.
Lin, T., Chen, Y., Chen, W., 2013. Impact of toxicological properties of sulfonamides on the growth of zebrafish embryos in the water. Environ. Toxicol. Pharmacol. 36, 1068–1076.
Liu, Z., Cai, M., Yu, P., Chen, M., Wu, D., Zhang, M., Zhao, Y., 2018. Age-dependent survival, stress defense, and AMPK in Daphnia pulex after short-term exposure to a polystyrene nanoplastic. Aquat. Toxicol. 204, 1–8.
Liu, Z., Yu, P., Cai, M., Wu, D., Zhang, M., Huang, Y., Zhao, Y., 2019. Polystyrene nanoplastic exposure induces immobilization, reproduction, and stress defense in the freshwater cladoceran Daphnia pulex. Chemosphere 215, 74–81.
Lu, F., Su, Y., Ji, Y., Ji, R., 2021. Release of zinc and polycyclic aromatic hydrocarbons from tire crumb rubber and toxicity of leachate to daphnia magna: effects of tire source and photoaging. Bull. Environ. Contam. Toxicol. 107, 1–6.
Lu, Y., Zhang, Y., Deng, Y., Jiang, W., Zhao, Y., Geng, J., Ding, L., Ren, H., 2016. Uptake and accumulation of polystyrene microplastics in zebrafish (Danio rerio) and toxic effects in liver. Environ. Sci. Technol. 50, 4054–4060.
Ma, X., Li, H., Xiong, J., Mehler, W.T., You, J., 2019. Developmental toxicity of a neonicotinoid insecticide, acetamiprid to zebrafish embryos. J. Agric. Food Chem. 67, 2429–2436.
Mak, C.W., Yeung, K.C.-F., Chan, K.M., 2019. Acute toxic effects of polyethylene microplastic on adult zebrafish. Ecotoxicol. Environ. Saf. 182, 109442.
Malafaia, G., de Souza, A.M., Pereira, A.C., Gonçalves, S., da Costa Araújo, A.P., Ribeiro, R.X., Rocha, T.L., 2020. Developmental toxicity in zebrafish exposed to polyethylene microplastics under static and semi-static aquatic systems. Sci. Total Environ. 700, 134867.
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.
McIntyre, J., Davis, J., Incardona, J., Stark, J.D., Anulacion, B., Scholz, N., 2014. Zebrafish and clean water technology: Assessing soil bioretention as a protective treatment for toxic urban runoff. Sci. Total Environ. 500, 173–180.
Miner, B.E., L. De Meester, M.E. Pfrender, W. Lampert, and N.G. Hairston Jr. 2012. Linking genes to communities and ecosystems: Daphnia as an ecogenomic model. Proceedings of the Royal Society B: Biological Sciences **279**:1873–1882.
Mu, X., Liu, J., Yuan, L., Yang, K., Huang, Y., Wang, C., Yang, W., Shen, G., Li, Y., 2019. The mechanisms underlying the developmental effects of bisphenol F on zebrafish. Sci. Total Environ. 687, 877–884.
Mu, X., Pang, S., Sun, X., Gao, J., Chen, J., Chen, X., Li, X., Wang, C., 2013. Evaluation of acute and developmental effects of difenoconazole via multiple stage zebrafish assays. Environ. Pollut. 175, 147–157.
Nasser, F., Lynch, I., 2016. Secreted protein eco-corona mediates uptake and impacts of polystyrene nanoparticles on Daphnia magna. J. Proteom. 137, 45–51.
OECD. 2004. Test No. 202: Daphnia sp. Acute Immobilisation Test.
Ohnuma, S., Katagiri, T., Maita, M., Endo, M., Futami, K., 2017. Application of tissue clearing techniques to 3D study of infectious disease pathology in fish. Fish. Pathol. 52, 96–99.
Okoffo, E.D., O'Brien, S., Ribeiro, F., Burrows, S.D., Toapanta, T., Rauert, C., O'Brien, J. W., Tscharke, B.J., Wang, X., Thomas, K.V., 2021. Plastic particles in soil: state of the knowledge on sources, occurrence and distribution, analytical methods and ecological impacts. Environ. Sci.: Process. Impacts 23, 240–274.
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.
Philibert, D.A., Philibert, C.P., Lewis, C., Tierney, K.B., 2016. Comparison of diluted bitumen (dilbit) and conventional crude oil toxicity to developing zebrafish. Environ. Sci. Technol. 50, 6091–6098.
Pitt, J.A., Kozal, J.S., Jayasundara, N., Massarsky, A., Trevisan, R., Geitner, N., Wiesner, M., Levin, E.D., Di Giulio, R.T., 2018a. Uptake, tissue distribution, and toxicity of polystyrene nanoparticles in developing zebrafish (Danio rerio). Aquat. Toxicol. 194, 185–194.
Pitt, J.A., Trevisan, R., Massarsky, A., Kozal, J.S., Levin, E.D., Di Giulio, R.T., 2018b. Maternal transfer of nanoplastics to offspring in zebrafish (Danio rerio): A case study with nanopolystyrene. Sci. Total Environ. 643, 324–334.
Qian, L., Liu, J., Lin, Z., Chen, X., Yuan, L., Shen, G., Yang, W., Wang, D., Huang, Y., Pang, S., 2020. Evaluation of the spinal effects of phthalates in a zebrafish embryo assay. Chemosphere 249, 126144.
Qiang, L., Cheng, J., 2019. Exposure to microplastics decreases swimming competence in larval zebrafish (Danio rerio). Ecotoxicol. Environ. Saf. 176, 226–233.
Rawson, D.M., Zhang, T., Kalicharan, D., Jongebloed, W.L., 2000. Field emission scanning electron microscopy and transmission electron microscopy studies of the chorion, plasma membrane and syncytial layers of the gastrula-stage embryo of the zebrafish BrachyDanio rerio: a consideration of the structural and functional relationships with respect to cryoprotectant penetration. Aquac. Res. 31, 325–336.
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.
Reimers, M.J., La Du, J.K., Periera, C.B., Giovanini, J., Tanguay, R.L., 2006. Ethanol-dependent toxicity in zebrafish is partially attenuated by antioxidants. Neurotoxicol. Teratol. 28, 497–508.
Rhodes, E.P., Ren, Z., Mays, D.C., 2012. Zinc leaching from tire crumb rubber. Environ. Sci. Technol. 46, 12856–12863.
Ritz, C., Baty, F., Streibig, J.C., Gerhard, D., 2015. Dose-response analysis using R. PloS One 10, e0146021.
Rochman, C.M., Brookson, C., Bikker, J., Djuric, N., Earn, A., Bucci, K., Athey, S., Huntington, A., McIlwraith, H., Munno, K., 2019. Rethinking microplastics as a diverse contaminant suite. Environ. Toxicol. Chem. 38, 703–711.
Saili, K.S., Corvi, M.M., Weber, D.N., Patel, A.U., Das, S.R., Przybyla, J., Anderson, K.A., Tanguay, R.L., 2012. Neurodevelopmental low-dose bisphenol A exposure leads to early life-stage hyperactivity and learning deficits in adult zebrafish. Toxicology 291, 83–92.
Saint-Amant, L., Drapeau, P., 1998. Time course of the development of motor behaviors in the zebrafish embryo. J. Neurobiol. 37, 622–632.
Santos, D., Luzio, A., Matos, C., Bellas, J., Monteiro, S.M., Félix, L., 2021. Microplastics alone or co-exposed with copper induce neurotoxicity and behavioral alterations on zebrafish larvae after a subchronic exposure. Aquat. Toxicol. 235, 105814.
Sherrington, C., 2016. Plastics in the marine environment. Eunomia Res. Consult. Ltd 13.
Siddiqui, S., J. Dickens, B. Cunningham, S. Hutton, E. Pedersen, B.J. Harper, S.L. Harper, and S. Brander. in preparation. The internalization and sublethal effects of tire wear micro and nanoparticles in two estuarine indicator species. Submitted to Environmental Pollution.
Sökmen, T.Ö., Sulukan, E., Türkoğlu, M., Baran, A., Özkaraca, M., Ceyhun, S.B., 2020. Polystyrene nanoplastics (20 nm) are able to bioaccumulate and cause oxidative DNA damages in the brain tissue of zebrafish embryo (Danio rerio). Neurotoxicology 77, 51–59.
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.: Int. J. 22, 2926–2931.
Stienbarger, C.D., Joseph, J., Athey, S.N., Monteleone, B., Andrady, A.L., Watanabe, W. O., Seaton, P., Taylor, A.R., Brander, S.M., 2021. Direct ingestion, trophic transfer, and physiological effects of microplastics in the early life stages of Centropristis striata, a commercially and recreationally valuable fishery species. Environ. Pollut. 285, 117653.
Sugiura, M., Takada, H., Takada, N., Mizukawa, K., Tsuyuki, S., Furumai, H., 2021. Microplastics in urban wastewater and estuarine water: Importance of street runoff. Environ. Monit. Contam. Res. 1, 54–65.
Susaki, E.A., Tainaka, K., Perrin, D., Yukinaga, H., Kuno, A., Ueda, H.R., 2015. Advanced CUBIC protocols for whole-brain and whole-body clearing and imaging. Nat. Protoc. 10, 1709–1727.
Sutton, R., A. Franz, A. Gilbreath, D. Lin, L. Miller, M. Sedlak, A. Wong, C. Box, R. Holleman, and K. Munno. 2019. Understanding microplastic levels, pathways, and transport in the San Francisco Bay region. San Fransisco Estuary Institute, SFEI-ASC Publication# 950. October 2019.
Tatarazako, N., M. Katoh, and K. Kadokami. 2007. Evaluation of environmental impact of tire chips by bioassay. Page 109 *in* Scrap Tire Derived Geomaterials-Opportunities and Challenges: Proceedings of the International Workshop IW-TDGM 2007 (Yokosuka, Japan, 23–24 March 2007). CRC Press.
Tian, Z., Zhao, H., Peter, K.T., Gonzalez, M., Wetzel, J., Wu, C., Hu, X., Prat, J., Mudrock, E., Hettinger, R., 2021. A ubiquitous tire rubber–derived chemical induces acute mortality in coho salmon. Science 371, 185–189.
Truong, L., Harper, S.L., Tanguay, R.L., 2011. Evaluation of embryotoxicity using the zebrafish model. Pages 271-279 Drug safety evaluation. Springer,.
Tshering, G., Plengsuriyakarn, T., Na-Bangchang, K., Pimtong, W., 2021. Embryotoxicity evaluation of atractylodin and β-eudesmol using the zebrafish model. Comp. Biochem. Physiol. Part C: Toxicol. Pharmacol. 239, 108869.
Veneman, W.J., Spaink, H.P., Brun, N.R., Bosker, T., Vijver, M.G., 2017. Pathway analysis of systemic transcriptome responses to injected polystyrene particles in zebrafish larvae. Aquat. Toxicol. 190, 112–120.
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.
Weber, C.I., 1991. Methods for measuring the acute toxicity of effluents and receiving waters to freshwater and marine organisms. Environmental Monitoring Systems Laboratory, Office of Research and ….
Werbowski, L.M., Gilbreath, A.N., Munno, K., Zhu, X., Grbic, J., Wu, T., Sutton, R., Sedlak, M.D., Deshpande, A.D., Rochman, C.M., 2021. Urban Stormwater Runoff: A Major Pathway for Anthropogenic Particles, Black Rubbery Fragments, and Other Types of Microplastics to Urban Receiving Waters. ACS ES&T Water,.
Westerfield, M., 2000, The zebrafish book: a guide for the laboratory use of zebrafish. ⟨http://zfin.org/zf_info/zfbook/zfbk.html⟩.
Wik, A., 2007. Toxic components leaching from tire rubber. Bull. Environ. Contam. Toxicol. 79, 114–119.
Wik, A., Dave, G., 2005. Environmental labeling of car tires—toxicity to Daphnia magna can be used as a screening method. Chemosphere 58, 645–651.
Wik, A., Dave, G., 2006. Acute toxicity of leachates of tire wear material to Daphnia magna—Variability and toxic components. Chemosphere 64, 1777–1784.
Wik, A., Dave, G., 2009. Occurrence and effects of tire wear particles in the environment–A critical review and an initial risk assessment. Environ. Pollut. 157, 1–11.

Wu, L., Jiang, Y., Zhang, L., Chen, L., Zhang, H., 2014. Toxicity of urban highway runoff in Shanghai to zebrafish (Danio rerio) embryos and luminous bacteria (Vibrio qinghaiensis. Q67). Environ. Sci. Pollut. Res. 21, 2663–2676.
Wu, M., Yang, C., Du, C., Liu, H., 2020. Microplastics in waters and soils: Occurrence, analytical methods and ecotoxicological effects. Ecotoxicol. Environ. Saf. 202, 110910.
Xia, L., Zheng, L., Zhou, J.L., 2017. Effects of ibuprofen, diclofenac and paracetamol on hatch and motor behavior in developing zebrafish (Danio rerio). Chemosphere 182, 416–425.
Yonkos, L.T., Friedel, E.A., Perez-Reyes, A.C., Ghosal, S., Arthur, C.D., 2014. Microplastics in four estuarine rivers in the Chesapeake Bay, USA. Environ. Sci. Technol. 48, 14195–14202.
Yoo, J.-W., Cho, H., Jeon, M., Jeong, C.-B., Jung, J.-H., Lee, Y.-M., 2021. Effects of polystyrene in the brackish water flea Diaphanosoma celebensis: Size-dependent acute toxicity, ingestion, egestion, and antioxidant response. Aquat. Toxicol. 235, 105821.
Young, A., Kochenkov, V., McIntyre, J.K., Stark, J.D., Coffin, A.B., 2018. Urban stormwater runoff negatively impacts lateral line development in larval zebrafish and salmon embryos. Sci. Rep. 8, 1–14.
Zhang, W., Sun, X., Chen, L., Lin, K.F., Dong, Q.X., Huang, C.J., Fu, R.B., Zhu, J., 2012. Toxicological effect of joint cadmium selenium quantum dots and copper ion exposure on zebrafish. Environ. Toxicol. Chem. 31, 2117–2123.
Zhang, Y., Goss, G.G., 2020. Potentiation of polycyclic aromatic hydrocarbon uptake in zebrafish embryos by nanoplastics. Environ. Sci.: Nano 7, 1730–1741.
Zhao, G., Wang, Z., Xu, L., Xia, C.-X., Liu, J.-X., 2019. Silver nanoparticles induce abnormal touch responses by damaging neural circuits in zebrafish embryos. Chemosphere 229, 169–180.
Zhao, S., Zhu, L., Wang, T., Li, D., 2014. Suspended microplastics in the surface water of the Yangtze Estuary System, China: first observations on occurrence, distribution. In: Marine Pollution Bulletin, 86, pp. 562–568.
Zhou, Y., Chen, X., Teng, M., Zhang, J., Wang, C., 2019. Toxicity effects of captan on different life stages of zebrafish (Danio rerio). Environ. Toxicol. Pharmacol. 69, 80–85.
Ziajahromi, S., Drapper, D., Hornbuckle, A., Rintoul, L., Leusch, F.D., 2020. Microplastic pollution in a stormwater floating treatment wetland: Detection of tyre particles in sediment. Sci. Total Environ. 713, 136356.