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Effects of environmental weathering on the acute toxicity of tire wear particle eluate to the mysid shrimp, Americamysis bahia

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By

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A Thesis
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Abstract

This research is based on the the impacts of microplastic to marine environments. The primary objective of this research was to quantify the toxicity of environmentally aged tire particles, weathered in a marine environment, on marine organisms through acute toxicity testing using mysid shrimp, *Americamysis bahia*. Seven tire groups (six used-tire groups and one new-tire group) of the same brand and model tire spanning manufacture year 2013 to 2018 were used. Tire particles were artificially created from all tire groups and baseline toxicity was measured using the eluate from unweathered tire particle groups through 96-hour acute toxicity tests using *A. bahia*. These results were then compared to toxicity results from a subset of the same tire groups that were deployed in a marine environment for weathering. Toxicity of unweathered tire particle groups had an LC50 range of 1.97 to 3.51 g/L and the toxicity of weathered tire groups had an LC50 range of 3.67 to 12.09 g/L. These toxicities were found to span four distinct toxicity categories based on ratio tests of the LC50 values. Eluate from each test treatment was analyzed for metals by ICP-MS. Cu and Ni were the only metals found to be significantly lower after weathering. The concentrations of Cu, Ni, and Zn at the LC50s were correlated with their respective LC50s based on the tire wear particle concentrations. Cu and Ni had strong positive correlations showing an inverse relationship with toxicity, indicating that these metals likely do not contribute to toxicity but instead that the tires are the source of the metals. Overall, Zn concentrations showed no correlation and were at the approximate LC50 for Zn alone, indicating that it may be contributing to toxicity.

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Table of Contents

List of Tables

List of Figures:

1.0 Introduction

This project focused on quantifying the toxicity of tire particles to a marine organism and assessing how toxicity changes after tire particles were weathered in a marine environment. Toxicity was measured through 96-hour acute toxicity testing using mysid shrimp, *Americamysis bahia,* following the United States Environmental Protection Agency (EPA) Ecological Effects Test Guideline OSCPP 850.1035: Mysid Acute Toxicity Tests (EPA, 2016). This research is based on the emerging body of work regarding the occurrence and impacts of microplastics, specifically, tire particle accumulation in marine environments. The following sections of this thesis will explain the significance of this work, frame the issue in a larger context, and justify the merit of the study in advancing the science regarding the impacts of microplastics on the environment.

1.1 Microplastics and tire particles

While no internationally agreed definition of a microplastic exists, many researchers use a definition of particles in the size range of 1 µm to 5 mm (GESAMP, 2015), and this same size range was adopted in 2020 by the California State Water Resource Control Board. Microplastics are found globally, in all environmental compartments (air, water, sediments, soil); are highly persistent in the environment; and are widely considered to be the most abundant contaminants in marine ecosystems (Auta et al., 2017; Leads and Weinstein, 2019; Unice et al., 2013). These anthropogenic particles are deposited into the marine environment through terrestrial activities, and as point and non-point source runoff (Auta et al., 2017; Wik and Dave, 2009). Historically, microplastics were recognized as primarily composed of polyvinyl chloride (PVC), nylons, polyethylene terephthalate (PET), polyethylene (PE), polypropylene (PP), and polystyrene (PS). To date, these plastics have dominated this field of study. In recent years micronized rubber (MR) particles, particles < 1mm in size that have elastomeric or rubber like properties meaning they can return to their original shape following deformation (Halle et al. 2019), have been gaining the attention of the scientific community due to the recognition of their abundance in the environment (Hüffer et al., 2019). These particles, most notably attributed to automobile tires, are being grouped into the category of microplastics based on their largely synthetic composition (Halle et al., 2020; Auta et al., 2017; Wagner et al., 2018; Hüffer et al., 2019). Hüffer et al. (2019) estimates that these tire-derived particles account for up to 60% of all microplastics found in the environment. For this study, tire particles will be considered a type of microplastic.

Tire wear particles (TWP) are secondary microplastics, produced through the abrasion of tire treads from contact with road surfaces (Wagner et al., 2018). Studies indicate that TWP are generated in a variety of sizes from approximately 6 nm to several 100 µm (Dahl et al., 2006; Kreider et al., 2010; Mathissen et al., 2011). Most TWP are deposited onto road surfaces and tend to accumulate near their source of generation. These particles are transported to the environment by natural processes such as atmospheric transport and runoff during rain events (Wik and Dave, 2009). Kole et al. (2017) determined that urban areas with high amounts of city driving (stop and go versus highway driving) tend to have the greatest accumulations of TWP. Given the diffuse nature of generation, the range of physical sizes, and the potential to be transported across environmental compartments, TWP are released to the environment where they can leach toxicants to marine and freshwater environments (Leads and Weinstein, 2019; Peter et al., 2018; Siegfried et al., 2017; Wik and Dave, 2009).

Tires are a complex mixture of natural and synthetic rubbers, metals, and chemical compounds added to promote durability and for different performance attributes. The primary components of tire tread are natural rubber, synthetic polymers such as styrene-butadiene copolymer, carbon black, extender oils, metals (primarily zinc oxide), and sulfur compounds used in the vulcanization process (Edil et al., 2008). However, as documented in the Vanderbilt Rubber Handbook the exact composition can vary widely (Benko et al., 2010); Wagner et al. (2018) and Kreider et al. (2010) have documented tire treads often contain several types of both natural and synthetic rubber, several types of carbon black, pigments, oils, waxes, silica, chemical compounds such as sulfur, activators, reinforcing agents, elastomers, and protective agents such as 6-PPD. The specific formulation of tires varies based on different grades of tires and tires suited for specific temperatures and environments.

Tire particles generated during the normal use and wear of tires are known to sorb environmental chemicals, such as toluene and xylene (Alamo-Nole et al., 2010) and other organic compounds such as n-hexane, cyclohexane, benzene, chlorobenzene, di-n-propylether (Hüffer et al., 2020). They also accumulate physical substances from the environment such as road dust from road surfaces, which is a complex mixture of chemicals associated with automobile fluids, brake pads, and chemicals and materials from the wear of the physical road surface (Sommer et al., 2018; Wagner et al., 2018; Kreider et al., 2010; Halle et al., 2020). The sorption and accumulation of materials are shown to impact the physical properties of TWP including the density (Kreider et al., 2010; Unice et al., 2019) as well as potentially altering the toxicity of the particles once they are generated and released into the environment (Day et al., 1993; Kreider et al., 2010).

1.2 Prevalence

Tires follow a similar history and growth of production as conventional plastics. The first rubber tires were created in 1846, the first fully synthetic rubber tire was produced in Germany in 1947, and radial tires became commercially available in the 1950s (Halle et al., 2020; Ramirez-Hernandez and Conde-Acevedo, 2013). In 1964 it was estimated that US tire consumption was 142 million units per year (Thompson et al., 1966). Since that time, tire use and consumption have grown exponentially worldwide. Global tire production is estimated at around 2.5 billion units annually, with light-duty trucks and passenger vehicles making up the greatest proportion of the tire industry (Smithers, 2019).

Thompson et al. (1966) published the first study identifying the existence of rubber-related particles in road dust and linked those particles to tire wear using styrene-butadiene as a chemical marker. Since that study, TWP have slowly gained the interest of the scientific community. It is estimated that approximately 1 kg/year of TWP are generated per capita in Europe and as much as 4.7 kg/year per capita are generated in the United States (Kole et al., 2017; Unice et al., 2019). Kole et al. (2017) used national estimates from governmental records on the amount of tires and the number of miles driven per year from 13 countries (i.e. The Netherlands, Norway, Sweden, Denmark, Germany, United Kingdom, Italy, Japan, China, India, Australia, United States, and Brazil) and showed passenger or light vehicles contributed between 24-81% of total generated TWP. These researchers estimated that in the United States light truck and passenger vehicles contribute approximately 34% of all TWP. A study conducted by the Dutch government on the amount, occurrence, and sources of different types of microplastics in the environment determined the relative contributions of TWP to various environmental compartments. They concluded: 11% enter surface waters either directly or through sewers, 5% emitted to air, 36% were retained in soils near roadways, 43% is retained on roadways, and the remaining portions are deposited in sludge (Verschoor et al., 2016). Particles retained on roadways, in soils, and wastewater sludges degrade slowly (Cadle and Williams, 1980) and can be a continuing source of both physical TWP and the chemicals associated with those particles to receiving waters (Järlskog et al., 2020).

1.3 Tire wear particles in the environment

The primary routes of TWP dispersal into the environment are through atmospheric transport and runoff during rain events. The spread and mode of transport of TWP are dependent on the size of particles generated and physical properties such as the density of these particles. A study by Rhodes et al. (2012) determined that the density of recycled tire crumb rubber ranged from 1.13 to 1.16 g/cm^3, and the density of field collected TWP containing roadway dust and surface materials from asphalt was slightly more variable with a density range from 1.18 to 1.8 g/cm^3 (Unice et al., 2019).

The majority of TWP generated are large particles, greater than 10 µm in diameter, and are deposited on or near roadways where they can be transported to the environment through uncontrolled or inadequately controlled runoff (Leads and Weinstein, 2019; Verschoor et al., 2016). Only a small fraction of TWP, 1-10% by mass, are generated at or below the 10 µm size range and can be transported long distances, by wind, from the point of generation (Grigoratos and Martini, 2015; Panko et al., 2013a; Verschoor et al., 2016). Particles larger than 10 µm can also be dispersed by wind, yet due to the Clean Air Act regulatory human health benchmarks of 2.5 and 10 µm, this size range has been a focus of studies regarding the atmospheric dispersal of these particles.

1.3.1 Runoff

In urban areas, runoff from roadways and other impervious surfaces is the leading source of chemical deposition in aquatic environments (Scholz and McIntyre, 2016). Most TWP are deposited in the environment near their point of generation. However, significant amounts of the deposited TWP are transported by runoff and enter water bodies through both point source and non-point source discharges. Non-point sources of pollution, primarily in the form of land-based runoff, are diffuse and can include atmospheric inputs directly to water bodies (Auta et al., 2017; Siegfried et al., 2017). As described by Scholz and McIntyre (2016) the difference between point and non-point sources, especially regarding stormwater, can be difficult to differentiate, as stormwater is frequently channeled through municipal stormwater conveyance systems designed to collect or sequester contaminants in surface runoff from urban landscapes (diffuse sources), and then discharge that runoff to a river or a lake or other water body at a discrete location through a pipe (point source).

The fate and transport of TWP to the marine environment are gaining increasing attention. A study conducted by Leads and Weinstein (2019) on the Charleston Harbor in South Carolina found that TWP account for approximately 18% of all microplastics in intertidal and subtidal sediments and approximately 18% in the sea surface microlayer (the top 1 millimeter of seawater). Studies such as the Leads and Weinstein analysis with direct environmental measurements are scarce; as a result, most studies rely on models to estimate the relative deposition of tire particles to various environmental compartments. Unice et al. (2019) used a detailed mass balance approach, with the DUFLOW model, and estimated that only 2% of TWP generated on road surfaces are transported to marine environments with the majority retained in land-based treatment systems such as swales and catchment systems, soils, river and lake sediments, and municipal wastewater treatment plants. Combining these estimates[1], approximately 30,500 metric tons of TWP are released to marine environments annually in the United States. This figure is likely biased low due to the possibility of resuspension of TWP from road surfaces and roadside soils, uses of sludge and wastewater treatment biosolids (containing microplastics), and through alternative end-of-life tire disposal programs (Alimi et al., 2018; Järlskog et al., 2020).

As governments across the world grapple with vast quantities of end-of-life tires, alternative uses for these waste tires to divert them from landfills are increasingly being encouraged. The US Tire Manufacturer Association (2017) estimated that 25% of end-of-life tires in the US are used in ground rubber markets such as turf fields, in playgrounds, landscaping, mulch, and as asphalt additives; another 8% are used in civil engineering projects such as septic system drain fields, landfill caps, and in erosion prevention. The impact from these applications and the amount of tire particles that are ultimately transported to marine systems from these uses has yet to be quantified and should be considered as another source of TWP to the environment.

1.4 Impacts of TWP

Impacts from TWP to the environment can be caused by physical damage from the particles and from chemical toxicity as the chemicals associated with the TWP leach into the environment. As discussed, TWP generated through the normal use of tires consist of a mixture of chemicals added at manufacture and chemicals that accumulate from road surfaces (Kole et al., 2017; Kreider et al., 2010;

[1] 1,524,740 tones tire particles generated per year in the US (Kole et al. 2017) multiplied by 2% direct deposition to marine environments (Unice et al. 2019)

Siegfried et al., 2017; Unice et al., 2019). Consequently, TWP transport these chemical substances to aquatic and marine systems (Kole et al., 2017).

1.4.1 Weathering of microplastics

Once in the environment, microplastics are subject to further degradation through natural processes resulting in fragmentation into ever smaller particles. Multiple processes such as UV exposure, mechanical abrasion, and biological degradation can promote changes to particle size, impact their ability to be transported across environmental compartments, and alter their chemical composition (Alimi et al., 2018). This fragmentation increases the likelihood of chemical sorption and desorption as the particle surface area increases (Alimi et al., 2018; Wagner et al., 2018).

In the marine environment, microplastics can act as both a source and sink for organic and inorganic compounds which may alter the toxicity of the microplastic when compared to unweathered or virgin microplastics. Sorption of organic compounds to microplastics can occur through several mechanisms such as hydrophobic interactions, electrostatic interactions, pore-filling, hydrogen bonding and other intermolecular forces (Torres et al., 2021), with hydrophobic interactions likely being the dominant mechanism (Wang et al., 2020). Wang et al. (2020) explain that microplastics tend to have large hydrophobic surface areas that have a high tendency to sorb planar, hydrophobic organic compounds. Rochman et al. (2012) demonstrated that common microplastics sorbed organic compounds while in marine environments, and similar findings have resulted from the Pellet Watch Program (Ogata et al., 2009). Holmes et al. (2011) found that aged, beached microplastics (microplastics that have undergone erosion, abrasion, and fragmentation) have a greater tendency to accumulate metals in marine systems than in their unweathered form. The salinity of the aqueous solution surrounding a microplastic can also impact the sorption of organic compounds where the solubility of hydrophobic compounds decreases as salinity increases (Alimi et al., 2018), favoring the aggregation of hydrophobic compounds on the microplastics. TWP have a more complex interaction with organic compounds than conventional microplastics due to their chemical composition and have been shown to both absorb compounds into the polymer matrix and also adsorb organic compound through mechanisms such as hydrophobic interactions (Hüffer et al., 2020). These researchers showed organic compounds generally absorb to styrene butadiene rubber and adsorb to the carbon black. This distinction illustrates the complexity introduced by the mixtures inherent in tire composition and the associated implications to the understanding of the environmental interactions of microplastics.

1.5 Eluate testing

Leachate testing is the leading method for assessing the toxicity of materials in aquatic environments under specific conditions (EPA, 2019). The EPA defines the aqueous solution resulting from a laboratory leachate test as eluate (EPA, 2019). Laboratory leachate testing is conducted where some media is placed in an eluant (contacting water or other aqueous solution), and the transfer of chemicals and substances occurs through gradients from the pore-phase of the test media to the contacting eluant through mass transport until equilibrium is achieved (EPA, 2019). The ability of a material to leach substances is dependent on factors such as particle size (surface area exposed to contacting eluant), the characteristics of the chemicals of concern (solubility), and water quality parameters of the contacting eluant (pH, ionic strength, temperature). The particle size determines the rate of leaching, and the extent of leaching is controlled through chemical equilibrium (EPA, 2019). The eluant is then filtered to remove test media and the resulting solution, the eluate, is used in toxicological testing (EPA, 2019). Researchers often create eluate under increased temperatures combined with mechanical mixing to increase the rate of partitioning from the test media to the contacting liquid (Halle et al., 2020).

Early tests such as those conducted by Day et al. (1993) and Kellough (1991) used eluate from whole tires or cut-up tires. These tests conducted on freshwater organisms found that sensitivity to tire eluate varied and that rainbow trout, *Oncorhynchus mykiss,* appeared to be most affected in acute toxicity tests when compared to *Daphnia magna* and *Pimephales promelas*. Additionally, Day et al. (1993) demonstrated that eluate solutions from used tires exhibited a greater toxic effect than new tires and that tires weathered in the marine environment for long periods (from a submerged tire reef) exhibited no toxicity to freshwater organisms. In recent years, impacts from tire particles have become the primary focus of this field of study as it is recognized that the particles themselves are more environmentally relevant than whole or shredded tires and can potentially exhibit different toxicities (Halle et al., 2019).

1.5.1 Freshwater toxicity

One of the first studies to assess the toxicity of tire particle eluate, conducted by Wik and Dave (2006), investigated the effects of eluate from 25 different used tires on *D. magna.* These researchers artificially created TWP (using a rasp to simulate road generated particles), and used those particles to create eluate at 44°C which they then used in 48-hour acute toxicity tests with percent immobilization of *D. magna* neonates as the endpoint. Toxicity was found to vary widely across the various tires with EC_{50} values ranging between 0.4 to greater than 10 g/L (Wik and Dave, 2006).

Panko et al. (2013b) conducted a study to determine toxicity of simulated tire road wear particles (particles generated using a simulated asphalt road and driving conditions and collected using a vacuum system installed behind the tire) to both sediment and water dwelling organisms. These researchers artificially created tire road wear particles in a road wear simulator and performed chronic toxicity tests on *Ceriodaphnia dubia, P. promelas, Chironomus dilutus*, and *Hyalella azteca* using either sediment spiked with 10 g/kg TWP or elutriate from the spiked sediment. These researchers showed no significant adverse effects for the benthic invertebrates *H. azteca* after a 42-day exposure or *C. dilutus* after a 35-day exposure to TWP-spiked sediments. They also conducted 7-day chronic toxicity tests on *C. dubia*, and 32-day chronic toxicity test on *P. promelas* using elutriate generated from the 10 g/kg TWP spiked sediments. The researchers determined that toxicity from chronic exposures to the TWP-spiked sediment for *C. dilutus* and *H. azteca* or from chronic exposure to elutriate from the TWP-spiked sediment to *C. dubia* and *P. promelas* were low (effects were determined to not be statistically significant for any of the test organisms when compared to the respective control treatments).

A similar test was conducted by Redondo-Hasselerharm et al. (2018) who included a 28-day chronic toxicity test on the effects of the ingestion of TWP from multiple different used tires, using freshwater species (*Gammarus pulex, Asselus aquaticus, and Tubifex spp, Lumbriculus variegatus*). They mixed TWP with sediments and found no adverse effects on the survival and growth of these organisms at 10 g/kg TWP in sediment dry weight.

A recent study by Tian et al. (2021) linked a specific chemical compound ubiquitously associated with automotive tires to a phenomenon known as urban runoff mortality syndrome (URMS). URMS has been observed with Coho salmon, *O. kisutch*, in urban creeks of the Pacific Northwest (Scholz et al., 2011). McIntyre et al. (2021) were able to replicate URMS, in toxicity tests using eluate from both new and used tires at a concentration of 250 mg/L, producing complete mortality in juvenile *O. kisutch* within a period of 5 hours. These findings led to the development of analytical methods using UPLC-HRMS accompanied by rigorous toxicological and database searches to identify a single chemical compound, 6PPD-quinone ($C_{18}H_{22}N_2O_2$), a degradant of 6PPD which is widely used as an antioxidant and antiozonant in tires, as the causal agent in URMS (Tian et al., 2021). This substance was tested using an industrial grade and synthetic sample of 6PPD-quinone which produced matching toxicity results.

1.5.2 Saltwater toxicity

Only a few studies have demonstrated the potential toxicity of tire-derived or tire-related particles in marine settings, yet each adds to the understanding of how these particles are acting in marine settings. Turner and Rice (2010) demonstrated toxicity to a marine alga, *Ulva lactuca*, using eluate from artificially created particles of several end-of-life tires using test concentrations from 25 to 500 mg TWP/L. The researchers found reduced ability of the alga to photochemically convert energy at eluate concentrations as low as 25 mg/L. These researchers also found that exposures to zinc concentrations equivalent to what was measured in the eluate did not account for all toxicity observed, suggesting that other components of the TWP eluate are responsible for the overall toxicity to the alga.

Hartwell et al. (1998) performed a study using eluate generated from shredded tires, with an approximate size of 1 cm^3. These researchers created several 50 g/L eluate solutions which they used to assess toxicity on sheepshead minnows (*Cyprinidon variegatus)* and daggerblade grass shrimp, (*Palaemonetes pugio)*. They found that mortality was the highest at the lowest salinity test condition (5 parts per thousand (ppt)) and decreased to non-significant levels in the highest salinity test condition (25 ppt) for both test organisms. Hartwell et al. (1998) also measured the highest metals concentrations in the 5 ppt salinity test solutions and the lowest metals concentrations in the 25 ppt salinity solution. In a later study, Hartwell et al. (2000) found that salinity altered the toxicity of eluate solutions to *Vibrio fischeri* (currently *Aliivibrio fischeri)* and found toxicity decreased as the salinity of the test solution increased from 0-15%.

In another study, Halsband et al. (2020) conducted toxicity tests using eluate from several types of tire crumb rubber (rubber granulate to be used in turf playfields). Testing was performed using several eluate concentrations from 0.01 g/L to 100 g/L crumb rubber. Eluate solutions were created in seawater at 34-35 psu, and toxicity tests were carried out over a 14 and 17-day period on two species of marine copepods (*Acartia and Calanus sp.*), with mortality as the endpoint. The results of these tests suggest copepod toxicity to tire crumb rubber eluate occurs at concentrations as low as 5 g/L

The research in this study attempted to model the aquatic toxicity of TWP exposures to mysid shrimp using a natural seawater contacting solution. The goals were to generate dose response relationships of artificially generated particles from a new tire and several groups of used tires to a marine organism and compare those toxicity estimates to test estimates of the same particles aged in a marine environment. Toxicity testing was conducted in strict adherence to best laboratory practices and in accordance with EPA test guidelines. This study is the first to show the toxicity of tire generated particles to mysid shrimp and to compare how the toxicity changes following the aging of these particles in a marine setting.

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2.0 Methods

2.1 Experimental design

Used passenger car tires were collected from local tire distributors. Tires from five cars were collected, creating six used tire treatment groups by year (years 2013-2018); all tires were the same brand and model and only differed by size (tire diameter) and manufacture/production year. Tires were obtained from five separate cars and composited by unique car and year combinations (defined here as a tire group), and included tires manufactured in: 2013 (four tires from the same vehicle); 2014 (four tires from the same vehicle); 2015 (three tires) and 2018 (one tire) from the same vehicle which were maintained as two separate tire groups; 2016 (two tires from the same vehicle); and 2017 (two tires from the same vehicle). A new tire manufactured in 2018 of the same brand and model was purchased for the new tire group. All collected tires were recently removed from their respective vehicles and stored in a garage (not exposed to wind, rain, sunlight, or temperature extremes) until tire particle generation occurred. The general experimental design is included in Figure 1. For clarity, tire "group" refers to the TWP that were composited by year and car, or the new tire (7 total groups), and tire "treatment" is used to describe if tires were weathered or remained unweathered (2 treatments).

Tire particles were artificially created, using an angle grinder (Milwaukee Model # 6142-31S) with a special tire shaping disc (Miller Tire part # 46MCM90). Only the tread of tires was used to generate tire particles. Tire particles were collected using a shop vacuum connected to the dust shroud on the angle-grinder. Particles from each tire group were collected in separate shop vacuum bags. Before any grinding activities, tires were briefly cleaned with a stiff-bristled scrub brush to remove gross contamination (dirt/mud), and all foreign objects (objects stuck in the tire tread such as rocks, nails, etc.) were removed from the tire tread. The angle grinder, dust shroud, shaping disc, and shop vacuum inlet and outlet were cleaned of tire particles between tire groups. Only tire particles retained in shop

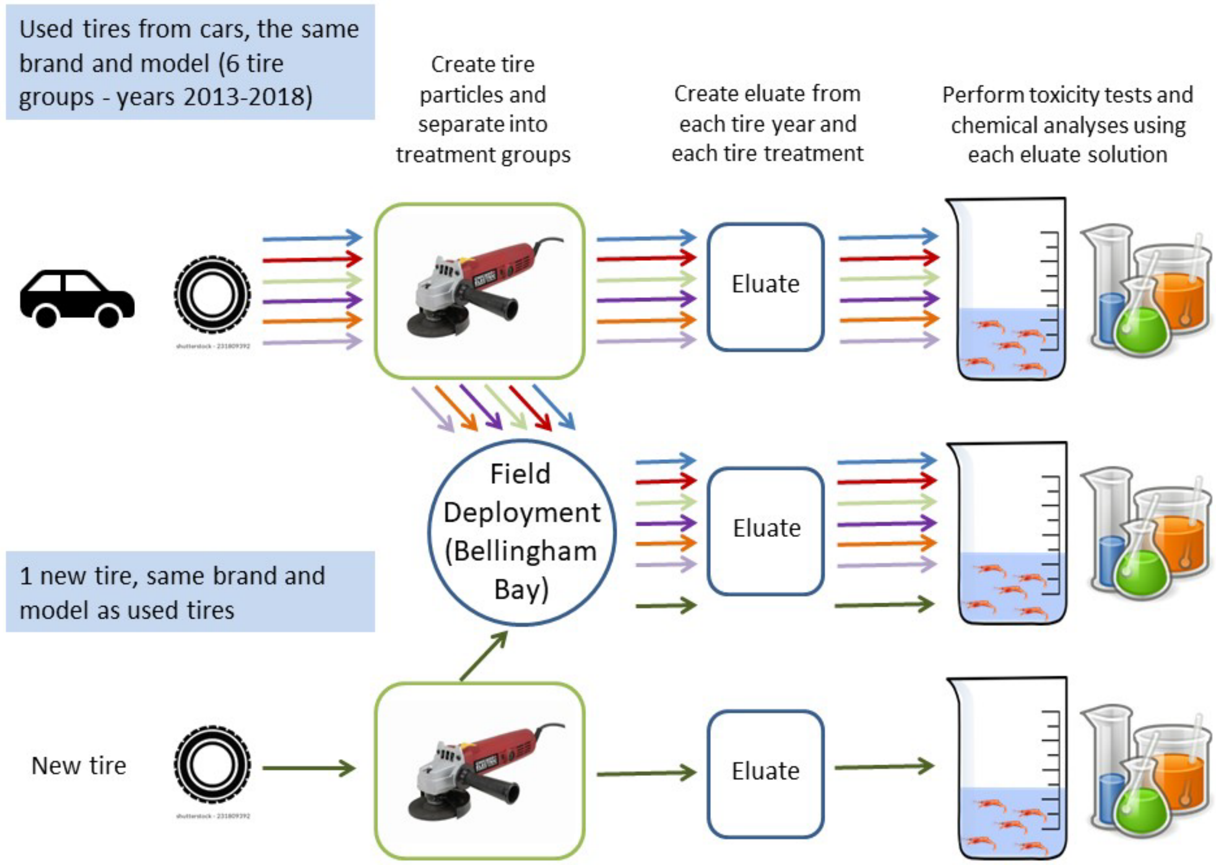

Figure 1. Experimental design: Tire particles were artificially created using an angle grinder. Tire particle groups were separated into two treatments: one for the generation of unweathered eluate and one for the generation of the weathered (field deployed TWP) eluate. All eluates were used in 96-hour acute toxicity tests using mysid shrimp, *A. bahia*.

vacuum bags were used in this experiment. This method was adapted from a procedure from the McIntyre lab from WSU Puyallup (McIntyre et al., 2021).

Following the grinding, tire particles were dry sieved using sieve sizes 1000 µm, 500 µm, and 0.64 µm to determine the relative size distribution of the particles generated. 93-95% of particles generated, by weight, were retained on the 500 µm sieve, and the remaining (smaller) particles were retained on the 0.64 µm sieve. All particles larger than 1,000 µm were segregated and not used for toxicity testing. The tire particle groups were divided in half by weight; one treatment was retained in the lab at -20°C (e.g. the unweathered treatment) and one was weathered in the marine environment. All tire years and treatments were used to create eluate test solutions and these solutions were used in both toxicity testing and chemical analyses.

2.1.1 Test organism

All toxicity tests were conducted using mysid shrimp, *A. bahia* as the test organism, with mortality as the endpoint. Mysid shrimp are a marine invertebrate widely accepted for use by the U.S. EPA for both acute toxicity testing and in Whole Effluent Toxicity (WET) testing under the National Pollution Discharge and Elimination System (NPDES) permitting program. Mysid shrimp are model test organisms for their ecological importance, their ability to be cultured in the laboratory setting, their life-cycle length, and their sensitivity to toxicants (Nimmo and Hamaker, 1982). Limit and range-finding toxicity tests were carried out using mysid shrimp that were 24-hours old from an in-house culture. Definitive testing, reference toxicant tests, and the sediment test were performed on mysid shrimp, *A. bahia*, 24-48 hours obtained from Aquatic Biosystems in Fort Collins, CO.

2.2 Weathering

Weathering of tire particles consisted of a field deployment to Bellingham Bay for a period of 82-days (9/23/20-12/14/20). This duration is comparable to previous work conducted in the Sofield lab using traditional microplastics (Allie Johnson, MS thesis, unpublished). The weather during this period is characterized as the Pacific Northwest autumn and marks the transition from summer to winter. This period encompasses the seasonal return of precipitation to the Pacific Northwest following the regionally dry summer months. In urban areas, precipitation and stormwater runoff are intrinsically linked and it has been shown that stormwater from the earliest parts of a storm event (first flush events) often contain the greatest concentration of potential toxicants (Kayhanian et al., 2012). In urban areas, stormwater runoff is often channeled directly into receiving waters (rivers, lakes, marine systems) and acts as a transport mechanism for chemicals and particulates accumulated on roadways and other impervious surfaces (Scholz and McIntyre, 2016).

During the weathering period the average air temperatures decreased from 13.8 - 4.4°C, with 48 rain events totaling 13.11 inches of rain (NOAA National Center for Environmental Information [NCEI], 2021). Bellingham, WA atmospheric data (Station ID: USC00450587) is available in Appendix A. The average tide level increased from 4.9 feet above the mean lower low water level in September to 5.2 feet above the mean lower low water level in December (NOAA Center for Operational Oceanographic Products and Services [CO-OPS], 2021). Tidal data (Station: 9449211 Bellingham, WA) is available in Appendix B.

For weathering, tire particles were placed into 25-micron nylon mesh bags. Each tire group was placed in a separate mesh bag that was sewn shut. Each bag was fitted with strong tethers of various colors to indicate the specific TWP year. The mesh bags were secured to an anchor located at an established site in Bellingham, WA. The anchor was located at approximately 2.5 feet below mean sea level and was intended to remain submerged most of the year. There were 10 instances over the course of weathering where anchored tire treatments could have contacted bed sediments. Additionally, the soft-bottom sediments at the site of weathering may have produced turbidity during storm events and other local disturbances that could have interacted with the deployed tire groups during the weathering process.

In effort to separate sediment from the weathered TWP following retrieval, all weathered TWP were sonicated for 30 minutes in their respective nylon mesh deployment bags, in water collected from the deployment site (collected at the time weathered TWP were retrieved). Weathered TWP treatments were air-dried in the laboratory and stored in a freezer at -20^{o} C before creation of eluate. Grab samples of both sediment and water from the deployment location were collected during retrieval. The sediment was air-dried in the laboratory and both the sediment and water were stored in a freezer at -20°C before analysis or use in toxicity testing. The sediment samples were used to establish whether any residual sediment in the TWP was contributing to toxicity and the water samples were used as blanks for LC/MS-QTOF chemical analysis (LC/MS-QTOF results not reported here).

2.3 Eluate

The source of seawater for this research is from WWU's Shannon Point Marine Center (SPMC) located in Anacortes, WA. At SPMC, natural seawater is pumped directly from the channel outside the laboratory facility (Guemes channel), at approximately 25 ft. below mean sea level, and circulated throughout the marine lab. Natural seawater was filtered at SPMC in two stages, first utilizing a 5 µm filter cartridge followed by a 0.2 µm filter cartridge. Filtered seawater was transported in carboys back to the Bellingham lab for use in mysid culturing, toxicity testing, and chemical analyses. The SPMC water quality data can be found in Appendix C. All seawater used for toxicity testing was also filtered in the laboratory before use in toxicity tests. Filtration was performed in two steps; first, the seawater was filtered through a 25 µm stainless steel screen and then a 10 µm nylon mesh to mimic eluate preparation procedures. This procedure was utilized for all eluate, reference toxicant, and control test chambers.

Eluate was created by thawing tire treatments, weighing exact quantities of tire particles, and transferring those particles to a prelabeled, muffle furnaced glass container. Tire particles were mixed with 21°C filtered natural seawater at a salinity of 25 ppt. Containers were covered with Parafilm® and placed in an environmental chamber set to 21°C, in the dark, on a rotary shaker table set to 100 rpm, for a period of 48-hours.

After 48-hours, the eluate solutions were removed from the environmental chamber and filtered to 10 µm, as mentioned above, to separate tire particles from the seawater contacting solution. These eluate solutions were used for toxicity testing within four hours of filtering. The eluate generated for each test was a concentrated solution and was created at the highest test concentration of each toxicity test. The concentrated eluate solutions will be termed "eluate stock" when discussed later. The various test concentrations were created by diluting eluate stock solutions using 25°C, filtered natural seawater from SPMC at a salinity of 25 ppt.

2.4 Toxicity testing

Acute toxicity tests were performed to determine the median lethal concentration (LC50) for all tire years and treatments utilizing the EPA Ecological Effects Test Guideline OSCPP 850.1035: Mysid Acute Toxicity Tests (EPA, 2016). Toxicity testing consisted of a combination of limit, range-finding, and definitive tests, and was conducted on the 7 weathered tire treatments and 7 unweathered tire treatments. All toxicity tests were performed at 25°C, using filtered natural seawater from SPMC at a salinity of 25 ppt. Eluate stock test solutions were created as discussed in Section 2.2.

Water quality parameters that included pH (Oakton pHTester 10), DO (YSI Pro20 Dissolved Oxygen Meter), and salinity (Vista A366ATC Portable Salinity Refractometer) were measured and recorded for all eluate stock solutions before testing and in all test chambers at the conclusion of each toxicity test. All toxicity tests were carried out using an environmental chamber set to 25°C, with a 16:8 light-dark cycle. 400 ml glass beaker test chambers were covered with acid-washed Petri dishes while in the environmental chamber. All test chambers were fed at 12-hour intervals with *Artemia* nauplii hatched daily and counts for mysid mortality were conducted at 24-hour intervals.

2.4.1 Limit and range testing

A limit test was performed to establish the appropriate test concentrations for definitive testing at 10 g/L (g tire particles per L filtered natural seawater), from a composite of tire groups. The 10 g/L concentration was chosen based on literature where 10 g/L was used as an upper test limit in several studies (e.g., Wik and Dave, 2006; Panko et al., 2013b; Redondo-Hasselerharm et al., 2018). Based on these results, range finding tests were conducted for each tire treatment at 10 g/L, 1 g/L, and 0.1 g/L.

The weathered tire treatments for the 2018 and 2016 tires showed lower toxicity (the weathered 2018 tire had 50 % mortality at 10 g/L and the weathered 2016 tire had no mortality at 10 g/L), which resulted in a higher concentration series in definitive testing for these two tire groups.

2.4.2 Definitive testing

Definitive tests were conducted using a control (natural seawater) five eluate concentrations and three replicates with 10 organisms per test chamber. Test concentrations for all unweathered tire treatments and all but two weathered tire treatments were 15 g/L, 7.5 g/L, 3.75 g/L, 1.875 g/L, and 0.94 g/L. Weathered tire treatments for the used 2018 tire and the 2016 tire treatment had test concentrations of 25 g/L, 15 g/L, 9 g/L, 5.4 g/L, and 3.24 g/L. Pre-labeled, muffle furnaced test chambers were filled to 350 mL with the corresponding test concentration. Following the creation of test chambers, chambers were placed in the environmental chamber set to 25°C for 1 hour to increase the temperature to 25°C before the addition of mysid shrimp (acclimated to 25°C and 25 ppt salinity).

In-house cultures could not produce adequate brood required for definitive testing. *A. bahia* <24-hours were ordered from Aquatic Biosystems in Fort Collins, CO and shipped overnight to our testing facility. At Western Washington University, mysids were acclimatized to the test temperature (25°C) using filtered natural seawater from WWU's SPMC. Mysids were between 24 and 48 hours old when toxicity tests started. This is not the standard age used in the EPA (2016) method, but a search of the Ecotox database (EPA 2021) resulted in 32% of the entries for "mysid" with an average age of 48 hours old or older.

2.5 Quality control

Quality control was performed using reference toxicant tests and negative seawater controls. Cadmium chloride reference toxicant tests were utilized to verify the fitness and sensitivity of the mysid shrimp and method precision during definitive testing (EPA 2016b). Reference toxicant test chambers were prepared before the preparation of each definitive test and stored in the environmental chamber at 25°C before the addition of test organisms. Reference toxicant tests consisted of preparing five nominal cadmium chloride concentrations (200 µg/L, 100 µg/L, 50 µg/L, 25 µg/L, 12.5 µg/L) and two replicates. Reference toxicant test chambers were prepared using filtered natural seawater from WWU's SPMC at 25°C at a salinity of 25 ppt. Test chambers were prepared by adding measured quantities of a cadmium chloride stock solution to filtered seawater (350 mL total volume) in muffle furnaced beakers. Negative seawater controls were also utilized and consisted of using filtered natural seawater from WWU's SMPC at 25°C at a salinity of 25 ppt. Reference toxicant and control test chambers were populated with the same <48-hour old mysid shrimp simultaneously with the definitive test chambers. All test chambers were randomly distributed in the environmental chamber throughout the duration of the toxicity testing.

2.6 Chemical analysis (metals)

Multiple-element metals analysis, for each TWP eluate at 10 g tire particles/L filtered seawater, was carried out with an Agilent 7500ce ICP-MS. Following the analytical method EPA Method 200.8 (EPA 1994), samples were acidified to 3.5% (v/v) trace metal grade nitric acid solution and analyzed for Cd, Cr, Co, Cu, Fe, Mn, Ni, Pb, Sb, and Zn, which is similar to the metals analyzed by Halsband et al. (2020) and Redondo-Hassleharm et al. (2018).

2.7 Sediment analysis

It could not be confirmed that all sediment was removed from the weathered tire particles before toxicity testing, therefore the toxicity of sediment was determined. Potential sediment concentrations in eluate were determined from sediment concentrations measured in four weathered TWP eluate solutions (years 2018-new, 2017, 2016, and 2014) prepared at 10 g/L. These eluate solutions were prepared using the 48-hour eluate method described in Section 2.2. After preparation, each solution was filtered using a 1.2 μm Whatman filter (Whatman model # 1822-047) to capture sediments remaining in each eluate solution. Horowitz (1991) explains that 1.2 μm filters can capture sediments defined as particles of grain size 2 μm - 2,000 μm, consisting of course clay, silts, and sand particles. Filters were air-dried in the lab. The difference in weight of each filter before and after eluate filtration was used to calculate the amount of sediment in each eluate solution. This quantity was then averaged across the four filters. The amount of sediment that was potentially included in the tire leachate was estimated to be 8.38E-4 g sediment /g weathered TWP (equivalent to 5.03E-3 g and 1.26 E-2 sediment in the 10 and 25g TWP/L, respectively). The sediment toxicity analysis consisted of creating eluate solutions of 1 and 5 g sediment/L following the eluate procedure in Section 2.2. Toxicity testing was carried out using the two concentrations and following the same procedures as definitive testing (described in Section 2.3.2), with the exception that only the two concentrations were used with 10 organisms in each of five test replicates for each sediment.

2.8 Statistical Analyses

2.8.1 Definitive testing

Dose response modeling and statistical analyses were conducted in R (R Core Team, 2020) and figures were produced using the package drc (Ritz et al., 2009). Model selection was determined using the 'mselect' feature in the drc package. The fit of several models was evaluated using summary statistics generated by the drc package. The drc package produces the LC50 estimate, the upper and lower confidence intervals, and the standard error of the estimate. These numbers are specific to the model chosen. Four models (LL.2, LL.3, W1.2, and W2.2) were evaluated to model the definitive test data. The LL.2 model showed the best overall fit for the evaluation of LC50 values from definitive tests. Ritz et al. (2015) explains that the LL.2 model (a log-logistic 2-parameter model with the lower limit fixed at 0 and upper limit fixed at 1) is well suited for modeling quantal or binomial data. This model is best suited for data that is symmetric about the inflection point (LC50).

Toxicity categories for the definitive testing results were determined using a ratio test of the measured LC50 values as described by Ritz et al. (2009). Testing of the ratio-based comparisons against confidence interval overlap showed that the ratio-based method had lower type I error rates and showed an increase in power of 20-30% (Wheeler et al. 2016). The ratio test as described by Wheeler et al. (2006) was performed using the compParm function in the package drc in R and consists of pairwise comparisons of all LC50 estimates (Ritz et al., 2009). The results of this ratio test are the basis for the toxicity categories presented in later sections of this report. Ratio test output data is available in Appendix G.

2.8.2 Reference testing

The reference toxicant analyses were also conducted in R (R Core Team, 2020) using the package drc (Ritz et al., 2009). Summary statistics from the drc output indicated that model LL.2 is well suited to model this data. This model was used to create Figure 3 depicting the dose response curves for these data. The LC50 results from the reference toxicant test were modeled using a control chart (Figure 4). As explained by Environment Canada (Canada, 2005), control charts are a visual method of determining if the variation of successive reference toxicant results are satisfactory. Environment Canada (2005) explains that the logarithmic results are plotting against the upper and lower warning limit (plus and minus two standard deviations of the logarithmic mean). Satisfactory reference tests would show LC50 results falling within the warning limits.

2.8.3 Chemical analyses (metals)

Paired t-tests were utilized to compare metal results by analyte as a function of treatment (unweathered and weathered). Paired t-tests were performed to determine if treatment results were significantly different. Statistical analyses were conducted in R (R Core Team, 2020) using the base ‘stats’ package and the function ‘t.test.’ There are three assumptions that must be tested for these results to be considered robust. As explained in STHDA (2021) the first assumption is whether the samples are paired. The ICP data consists of two treatments of the same analyte and satisfies the first assumption. The second assumption is a large sample size ($n > 30$). The ICP metal results have only seven measurements per treatment and therefore fails this assumption. The final assumption is that the differences of the pairs is normally distributed. A Shapiro-Wilks test was performed to assess the normality (at $\alpha = 0.05$) of the paired data from each analyte.

3.0 Results

3.1 Toxicity testing

Definitive testing resulted in four toxicity categories based on the LC50 values measured and results from a ratio test of the median lethal concentration values. Categories were assigned to curves or groups of curves that could not be separated statistically from every other tire in that category; it is possible that a tire in a category could be different from another tire within these categories. Category 1 consists of all unweathered tire treatments (i.e. 2018 new tire, 2018, 2017, 2016, 2015, 2014, and 2013) and weathered tire treatments 2014 and 2013; Category 2 consists of weathered tire treatments 2017 and 2015; Category 3 consists of weathered tire treatment 2018 new tire; and Category 4 consists of weathered tire treatments 2018 and 2016. Figure 2 shows all concentration response curves generated during definitive testing with the four categories highlighted. The LC50 for *A. bahia* in Category 1 ranged from 1.97 to 4.17 g/L, for Category 2 the LC50 ranged from 5.19 to 5.59, for Category 3 the LC50 was

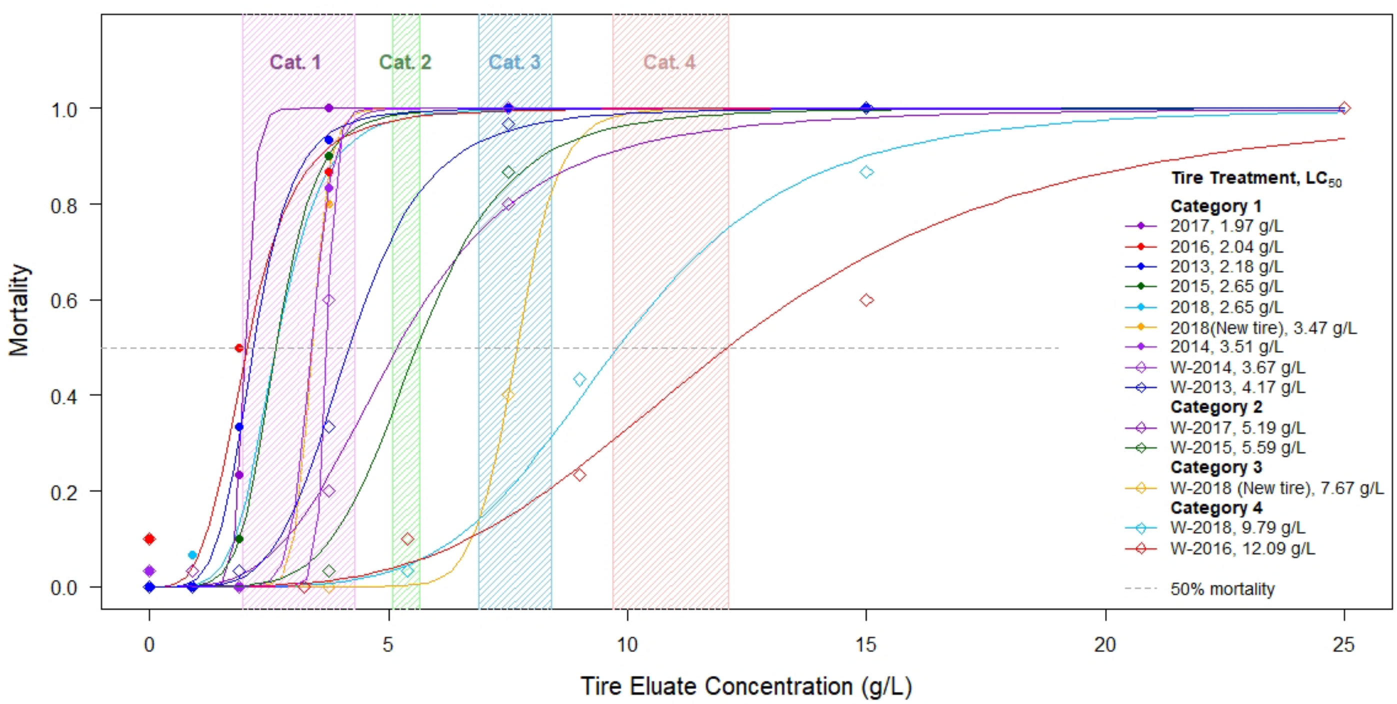

Figure 2. Definitive testing concentration response modeling. The points show the average mortality at the specific test concentration. The legend order presents the concentration response curves as shown on the figure from left to right. The "W" in the key indicates a weathered tire treatment. Closed circles represent unweathered tire treatments, and the open diamonds represent weathered tire treatments. The horizontal dashed line on the plot indicates 50% mortality. Data are the mean of 3 replicates for each concentration.

7.67 g/L, and for Category 4 the LC50 ranged from 9.79 to 12.09 g/L. The definitive test concentration-response summary data is presented in Table 1 and documents the LC50 values estimated for each tire treatment, the standard error of the estimate (LC50), the confidence intervals, and the drc model used to generate the test statistics.

Table 1. Unweathered and weathered definitive testing concentration response summary statistics.

Categories	Treatment	Year	Estimate (LC50)	Std.Error	Lower CI	Upper CI
1	Unweathered	2017	1.97	0.46	1.08	2.87
	Unweathered	2016	2.04	0.16	1.73	2.35
	Unweathered	2013	2.18	0.14	1.89	2.46
	Unweathered	2015	2.65	0.18	2.30	3.00
	Unweathered	2018	2.65	0.18	2.29	3.01
	Unweathered	2018 (New)	3.47	1.42	0.70	6.25
	Unweathered	2014	3.51	1.00	1.55	5.48
	Weathered	2014	3.67	0.70	2.30	5.05
	Weathered	2013	4.18	0.28	3.64	4.71
2	Weathered	2017	5.19	0.41	4.37	6.00
	Weathered	2015	5.59	0.38	4.85	6.34
3	Weathered	2018 (New)	7.67	0.87	5.96	9.38
4	Weathered	2018	9.79	0.56	8.69	10.89
	Weathered	2016	12.09	0.83	10.46	13.72

The testing guidance EPA OSCPP 850.1035 (EPA 2016) states that during testing control mortality must be under 10%, temperature is to remain constant (+/-) 1°C, pH should be between 7.5 and 8.5 and vary less than 1 pH unit, salinity should not vary more than (+/-) 2 ppt, and that D.O. should be between 60-100% saturation. Data quality objectives established were met in all tests during definitive testing for control treatment mortality, temperature, and pH. Data quality objectives were not always met for salinity and D.O.; there was a 3 ppt exceedance (28 ppt) in salinity during testing observed in one weathered test (weathered 2015 definitive test), and low DO (below 60% saturation) was observed in several test chambers.

Temperature was independently verified using a non-mercury thermometer and remained steady in the environmental chamber. The pH never varied more than 0.8 pH units across test treatments; salinity in test treatments ranged between 25 - 28 ppt; D.O. was the most variable with a maximum range in controls 34 - 82.1%, a range of 20.3 - 83.9% in the weathered tire test treatments and had a range of 35.6 - 92.3% in the unweathered tire test treatments. Low D.O. levels did not contribute to mortality as they occurred most frequently in the lowest test concentrations where organism mortality was rarely observed. Table 2 shows the range of water quality parameter values at the conclusion of testing and of the eluate stock solutions measured prior to test initiation. The raw definitive test data is presented in Appendix D. Ratio testing raw data is presented in Appendix E.

Table 2. Definitive testing water quality parameters.

Water quality parameters prior to testing

Treatments	Temperature	pH range	Salinity range (ppt)	D.O. range			
				(mg/L)	Median	(%)	median
Controls	25°C	8.1 - 8.7	25 - 26	6.79 - 8.8	7.71	80 - 98.4	89.7
Weathered	25°C	7 - 7.8	25 - 26	6.62 - 7.36	6.67	74.4 - 83.7	78.7
Unweathered	25°C	7.4 - 7.8	25 - 26	6.83 - 8.24	7.16	79.5 - 93.8	83

Water quality parameters following testing

Treatments	Temperature	pH range	Salinity range (ppt)	D.O. range			
				(mg/L)	Median	(%)	median
Controls	25°C	7.4 - 8.1	25 - 27	3.41 - 6.96	6.24	38.4 - 82.1	73.5
Weathered	25°C	7.3 - 8.1	25 - 28	1.76 - 7.03	5.88	20.3 - 83.9	68.5
Unweathered	25°C	7.3 - 8.1	25 - 27	3.02 - 7.78	6.08	35.6 - 92.3	72.4

3.2 Reference Toxicant Testing

A cadmium chloride reference toxicant test was performed concurrently with each definitive test. Reference toxicant tests were prepared using nominal concentrations of cadmium chloride as discussed in Section 2.5. Dose response modeling results are included as Figure 3. The LC50 for the reference toxicant tests ranged from 36.51 – 55.48 µg/L cadmium chloride across the seven tests. Reference toxicant dose response summary data is presented in Table 3. This table shows the model generated LC50 measured for each tire treatment, the drc model, the standard error of the estimate, and the associated confidence intervals generated by the LL.2 model. The results from the reference toxicant tests were compared using a control chart (Canada 2005). The control chart shows that all LC50 reference toxicant values fall within the +/- two standard deviation control limits, indicating that the

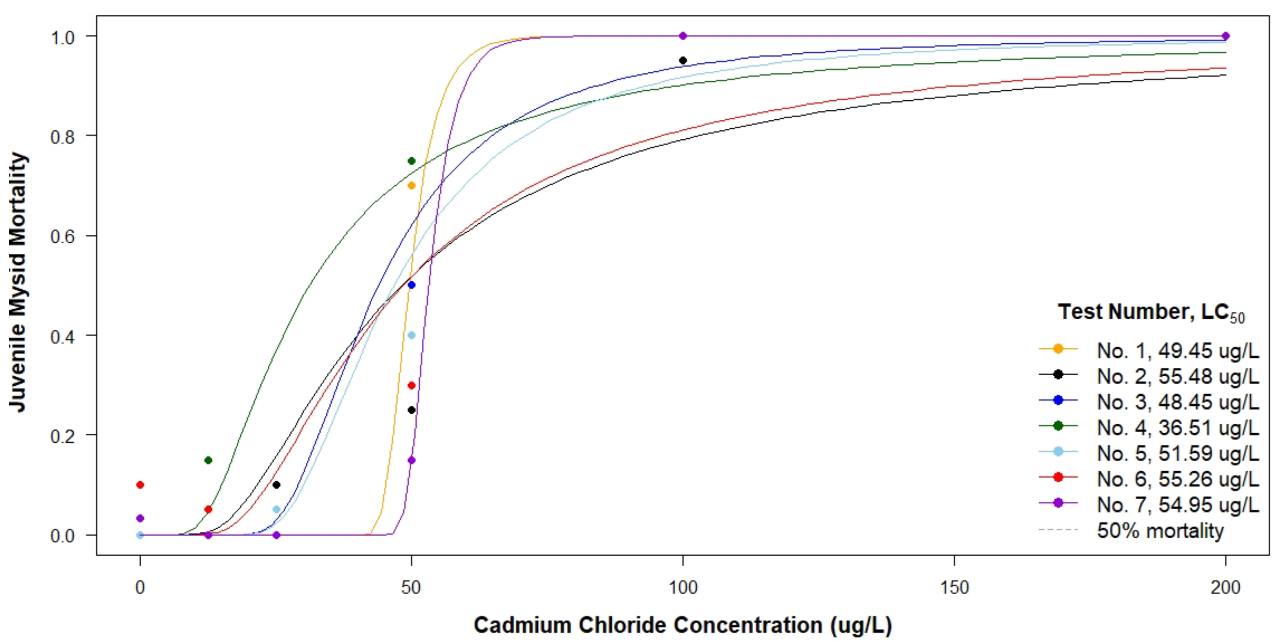

Figure 3. Reference toxicant concentration response modeling. The closed circles indicate the average value at each concentration. The legend shows the LC_{50} values for each reference test. A dashed line is shown on the plot indicating the 50% mortality level.

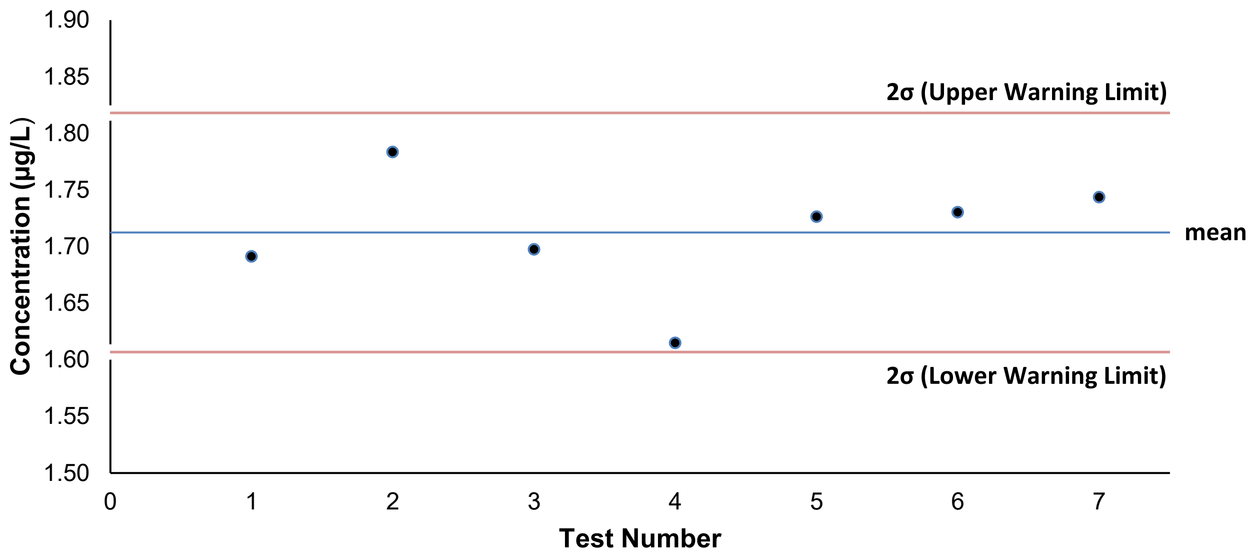

Figure 4. Reference toxicant control chart. Reference toxicant cadmium chloride LC50 concentrations were log transformed and plotted to determine if concurrent tests are satisfactory (fall within control limits of +/- 2 standard deviations) (Canada 2005).

reference toxicant results are acceptable (Figure 4) (Canada 2005). Reference toxicant raw data is presented in Appendix F.

Table 3. Reference toxicant test data, model summary statistics.

Test Number	Estimate (LC50)	Std. Error	Lower CI	Upper CI
1	49.45	3.12	43.29	55.53
2	55.48	5.77	44.18	66.78
3	48.45	3.71	41.19	55.73
4	36.51	3.87	28.93	44.09
5	51.59	3.95	43.85	59.33
6	55.26	4.79	45.88	64.64
7	54.95	21.03	13.73	96.17

3.3 Metal analysis

Metals analysis in 10 g/L eluates resulted in detectable aluminum (Al), cobalt (Co), copper (Cu), manganese (Mn), nickel (Ni), and zinc (Zn) from all tires. At the LC50 concentration for eluate from all tire groups (both unweathered and weathered) there were exceedances for both the Criterion Minimum Concentration (CMC) for acute exposure and the Criterion Continuous Concentration (CCC) for marine chronic exposure for Cu and Zn of the EPA Aquatic Life Criteria (EPA 2016c). Ni exceeded the CCC in all tire groups and treatments and exceeded the CMC for weathered tire treatment 2018- new tire; both unweathered and weathered for tire treatment 2018; weathered tire treatment 2017; weathered tire treatment 2016; both unweathered and weathered tire treatments 2015. The metal concentrations at the LC50 are presented in Table 4.

Table 4. Metal (ICP-MS) results normalized to LC50 concentrations. Analytes Al, Ni, Cu, Zn, and the EPA Aquatic Life Criteria are presented in mg/L. Analytes Cr, Co, Mn, Sb, Pb are presented in µg/L. Bold values indicate exceedances of both the Aquatic Life Criteria for CMC (Acute) and CCC (Chronic) exposures. Italicized values exceed the Aquatic Life Criteria for CCC (Chronic) exposures only. ND = non-detect.

Tire Group	Treatment	Al	Cr	Mn	Co	Ni	Cu	Zn	Cd	Sb	Pb
2018-New	Unweathered	0.078	ND	0.592	0.198	*0.063*	**0.032**	**0.954**	ND	ND	ND
	Weathered	0.172	ND	1.610	0.346	**0.071**	**0.063**	**0.557**	ND	ND	ND
2018	Unweathered	0.024	ND	0.574	0.189	**0.074**	**0.031**	**0.667**	ND	0.234	ND
	Weathered	0.123	ND	2.890	0.569	**0.183**	**0.101**	**0.640**	ND	ND	ND
2017	Unweathered	0.027	ND	2.282	0.633	*0.049*	**0.021**	**0.737**	ND	0.478	ND
	Weathered	0.037	ND	0.952	0.817	**0.085**	**0.052**	**0.596**	ND	ND	ND
2016	Unweathered	0.024	ND	0.591	0.099	*0.061*	**0.024**	**0.135**	ND	ND	0.519
	Weathered	0.169	1.904	2.104	0.649	**0.221**	**0.135**	**0.639**	ND	ND	ND
2015	Unweathered	0.029	ND	0.621	0.206	**0.081**	**0.032**	**0.681**	ND	ND	ND
	Weathered	0.049	ND	0.829	0.311	**0.107**	**0.055**	**0.637**	ND	ND	ND
2014	Unweathered	0.027	ND	0.231	0.145	*0.073*	**0.034**	**0.166**	ND	ND	ND
	Weathered	0.054	ND	0.843	0.243	*0.066*	**0.035**	**0.736**	ND	ND	ND
2013	Unweathered	0.036	0.291	1.130	0.374	*0.064*	**0.026**	**0.812**	ND	ND	ND
	Weathered	0.042	ND	0.104	0.446	*0.069*	**0.040**	**0.797**	ND	ND	ND
EPA Aquatic Life Criteria	CMC (Acute)		1.100			0.074	0.005	0.090	0.033		0.210
EPA Aquatic Life Criteria	CCC (Chronic)		0.050			0.008	0.003	0.081	0.008		0.008

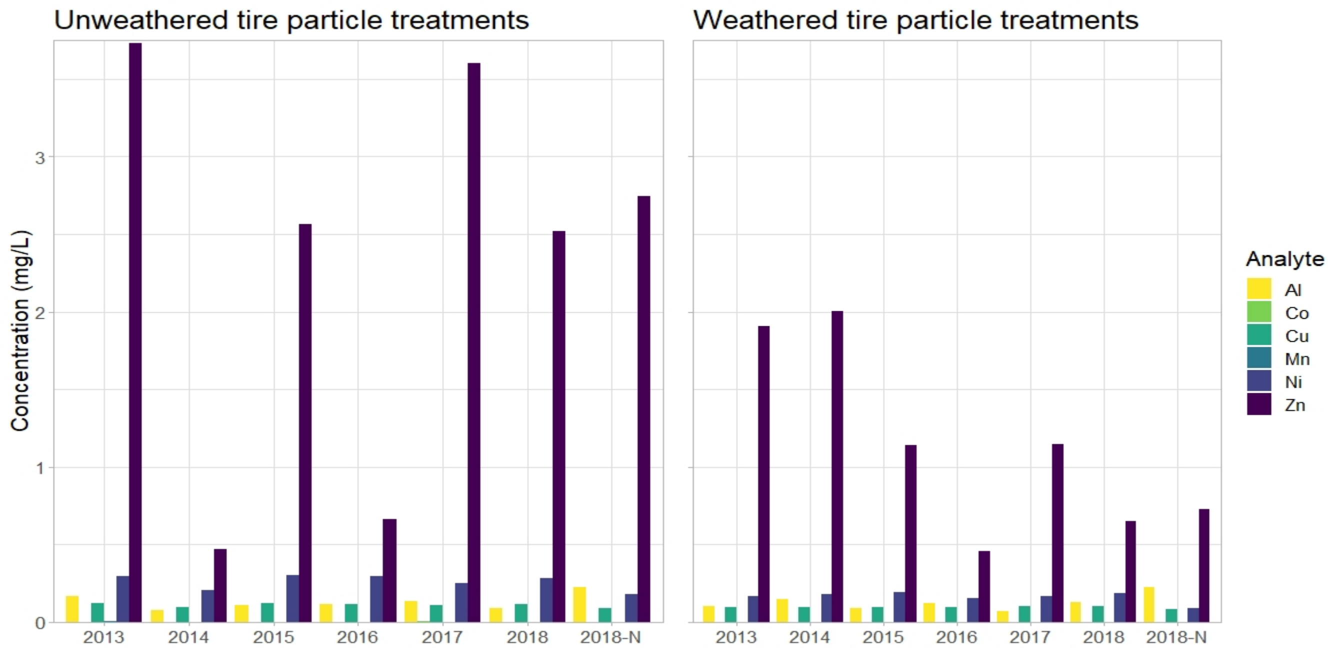

Figure 5. Metal concentrations in unweathered and weathered tire eluate at 10 g/L test concentrations. Tire groups are listed by year from oldest group to newest. The 2018-N represents the new tire group.

A plot of the analyte concentrations before and after weathering is presented in Figure 5. This figure shows that most analytes were detected in very low concentrations relative to Zn, which is 1 to 3 orders of magnitude greater than the other metals, on average. All weathered tire groups showed a net decrease in metal concentrations with the exception of Zn in the weathered 2014 tire group as shown in Figure 5. Table 5 shows the analyte detection ranges for both the weathered and unweathered tire groups including a paired t-test results for each analyte as a function of treatment (unweathered versus weathered). After testing the assumptions as defined in methods section 2.6.3, all metals showed that the distribution of the differences between unweathered and weathered treatments, for all tire years are not significantly different from the normal distribution. Paired t-test results show that there are significant differences between unweathered and weathered treatments for Ni and Cu only as shown in Table 5. Metals analysis raw data is presented in Appendix G.

Table 5. ICP analyte concentrations. The range of the metal concentrations in eluate from unweathered and weathered tire particles. P-values generated through paired t-tests at α = 0.05.

Analyte	Unweathered	Weathered	P-Value
Al	0.077 - 0.225 mg/L	0.071 - 0.225 mg/L	0.726
Mn	0.657 - 5.197 µg/L	0.250 - 2.950 µg/L	0.430
Co	0.414 - 3.204 µg/L	0.444 - 1.575 µg/L	0.171
Ni	0.181 - 0.304 mg/L	0.093 - 0.191 mg/L	0.001
Cu	0.093 - 0.121 mg/L	0.082 - 0.103 mg/L	0.004
Zn	0.473 - 3.729 mg/L	0.455 - 2.004 mg/L	0.065

The concentrations of Cu, Ni and Zn, the three metals with both measurable results and exceedances of water quality criteria values, were converted to the concentration at the LC50 and plotted against the LC50 (Figure 6). Zn showed relatively consistent concentrations across the dose response measurements with a clustering of the highest concentrations associated with the most toxic (low LC50) values. Additionally, Zn had no correlation to the measured LC50 values. Cu and Ni showed very high correlation to the LC50 values and showed an inverse relationship with toxicity where the highest concentrations strongly correlated to the highest measured LC50 values (lowest toxicity).

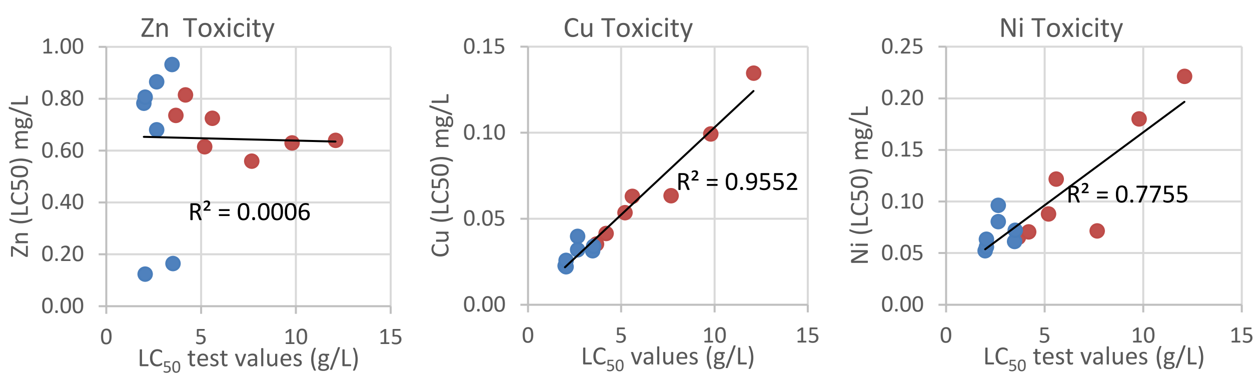

Figure 6. Concentrations of Cu, Ni, and Zn calculated to be in the solution at the LC50 plotted against their respective tire wear particle LC50 values. Blue represents the unweathered tire groups and orange represents the weathered tire groups. The EPA Aquatic Life CMC (acute exposure) values for Zn is 0.09 mg/L.; for Cu is 0.005 mg/L; for Ni is 0.074 mg/L. The EPA Aquatic Life CCC (chronic exposure)

3.4 Sediment Analysis:

Sediment toxicity analyses show that residual sediment associated with the weathered tire particles is not likely to contribute to the observed toxicity from weathered tires. Two sediment concentrations were tested (1 and 5 g/L) using five replicates. For the 1 g/L treatment only one treatment showed any mortality (one mysid out of fifty). For the 5 g/L treatment *A. bahia* mortality was low (8%) across the entire test. Sediment testing data is shown in in Figure 7. Sediment testing raw data is presented in Appendix H.

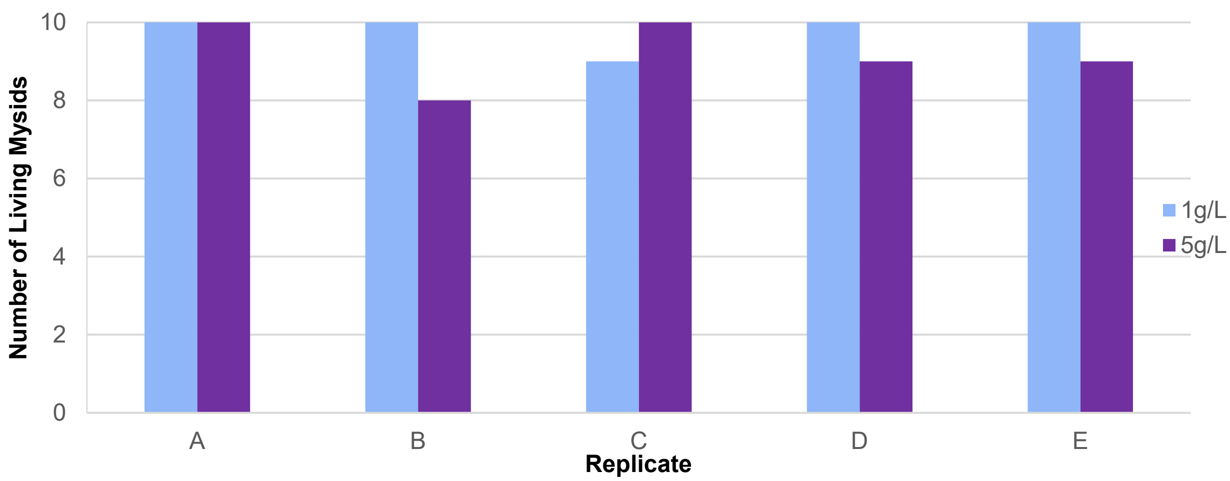

Figure 7: Sediment test data. The number of surviving mysids at the conclusion of a 96-hour acute toxicity test using eluate from sediment collected from the site when weathered tire particles were retrieved. Tests were performed at 1 and 5 g/L with five replicates of each concentration with 10 organisms per replicate were used for this test.

4.0 Discussion

Over the course this study the toxicological impact of seven tire groups to marine systems using *A.bahia* were explored. The focus of this work was to first establish a baseline of toxicity from the tire groups that were not weathered in the marine environment to *A. bahia* and then explore how these measurements changed when tire treatments were aged in the marine environment. To interpret these results, the impact that marine sediments, accumulated during weathering, might have on the toxicity measurements obtained from weathered tire groups were also explored. Finally, to explain the mechanism of toxicity all tire treatments were analyzed for inorganic elements and organic compounds (results pending).

4.1 Toxicity testing

Definitive testing produced four toxicity categories across tire particle groups in this study. Category 1 consists of all unweathered tire treatments (2018-new tire, 2018, 2017, 2016, 2015, 2014, 2013) and weathered tire treatments 2014 and 2013. Category 2 consists of weathered tire treatments 2017 and 2015. Category 3 consists of weathered tire treatment 2018-new tire. Category 4 consists of weathered tire treatments 2018 and 2016. Toxicity for unweathered tire groups ranged from 1.97 to 3.51 g/L and the toxicity of weathered tire groups ranged from 3.67 to 12.09 g/L. A study by Halsband (2020) showed eluate from tire crumb rubber resulted in LC50s of less than 5 g/L and 35 g/L at 48-hours in marine copepods, *Acartia* and *Calanus spp*, respectively. Their tire crumb rubber granulate was collected from a sports field and exposed to natural seawater (salinity 34-35 psu[2] at 20^{o}C) for 14 days. The length of time for eluate creation (14 days compared to 2 days in my study) indicates there was more time for chemicals to leach, but also more time for chemical volatilization and degradation. This research using marine copepods is the most comparable study to this study. Similar to mysid shrimp,

[2] Practical salinity unit, 1 psu = 1 ppt (Reid, 2011).

copepods, especially *Acartia*, are model organisms that have been used to assess toxicant impacts on marine systems and food webs (Gorbi et al., 2012).

The design for this study allowed for several comparisons on the toxicity of tire eluate in marine environments, specifically: 1) toxicity of new tires compared to used tires, 2) toxicity of different ages of used tires from different cars and from the same car, and 3) the toxicity of unweathered TWP versus TWP weathered in a marine environment. The first comparison was designed to address whether toxicity could be attributed to the chemical components of tires or to chemicals that associate with the tires during use. The second comparison was designed to address whether different manufacture years and the amount of time a tire was used on roadways[3] affected the toxicity and whether toxicity can be characterized based on the car the tires were acquired from or the year the tire was manufactured. The final comparison was designed to address the effect of environmental aging on TWP and assess how toxicity is impacted by exposure to marine environments.

4.1.1 Toxicity of new tires compared to used tires

An early study assessed the toxicity of eluate from a new and used tire in freshwater acute toxicity tests (Day et al. 1993). The researchers created leachate by submerging whole tires in large aquariums for a period of 40 days. Eluate samples were removed from the aquariums at several intervals (5, 10, 20 and 40 days) and acute toxicity tests were performed with each eluate solution. After several acute tests on three test organisms (*O. mykiss, D. magna,* and *P. promelas)* these researchers concluded that used tires produced greater toxicity (LC50 range of 3.19 - 5.21 g/L) than new tires (LC50 range of 15.98 - 24.66 g/L) for *O. mykiss*.

In this study, there was no difference in toxicity for the new tire compared to used tires in the unweathered tire tests. The tire groups in this study consisted of one new tire manufactured in 2018

[3] Because all tires were collected from a tire distributor that recently took the tires off a car, it was assumed that the older tires had been in use longer, although the number of miles driven on a tire could not be determined.

and six used tire groups from manufacture years 2013-2018 all from the same brand and model tire. The best comparison for new and used tires is between the new tire and the used 2018 tire group as this eliminates potentially confounding difference between years of manufacture and any differences in tires use over the various tire groups. While toxicity was statistically the same for the 2018 new tire (LC50 = 3.47 g/L) and 2018 used tire (LC50 = 2.65 g/L) in unweathered treatments (which is most comparable to the Day et al. 1993 study) the results were more variable in the weathered treatments with the 2018 new tire having a more toxic effect (LC50 = 7.67 g/L) than the used 2018 tire group (LC50 = 9.79 g/L). Based on LC50 ratio tests, as discussed in Section 3.1, the measured toxicity of the weathered 2018 new tire was statistically different from that of the weathered 2018 used tire and these groups were classified into separate toxicity categories (categories 3 and 4). Based on these results, the toxicity of new and used tires may differ, but weathering attenuated the toxicity producing statistically separate categories. These results should be confirmed with more samples.

4.1.2 Toxicity of different ages of used tires

No statistical difference in toxicity was found within the unweathered tire groups as confirmed by ratio tests of the LC50 concentrations. Unweathered tire groups showed no correlation between age of tire and the estimated LC50 as shown in Figure 8 where these tire groups had an R^2 = 0.03. Weathered tires showed greater variation in toxicity and spanned four statistically distinct toxicity categories as determined through ratio testing and the rank order of toxicity of these tire groups changed after weathering. The rank order of toxicity for weathered tire groups (highest to lowest by statistically distinct toxicity categories) was 2014 and 2013; 2017 and 2015; 2018 (New); 2018 (Used) and 2016. There was a weak correlation for the unweathered tire groups (R^2 = 0.3) as shown in Figure 8 between age of tire and LC50. This correlation is being driven by the two oldest used tire groups (2013 and 2014) that had low LC50s and were included in the most toxic statistical toxicity category with the unweathered tire groups. This weak correlation overall indicates that the length of time the tires were

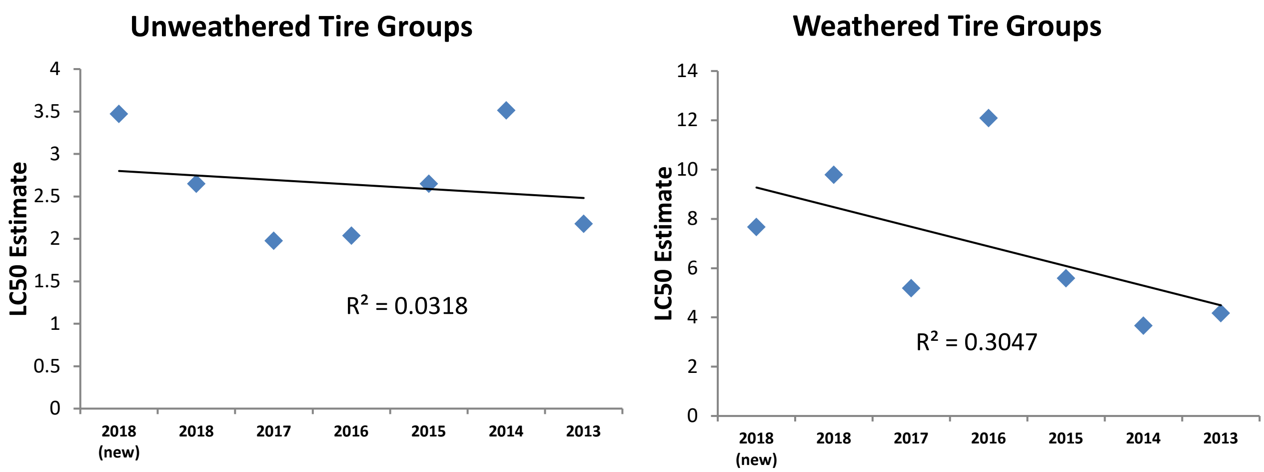

Figure 8. Correlation plots of the LC50 versus the year of tire manufacture for both the unweathered and weathered tire groups.

in use does not explain toxicity, however we are seeing evidence of differences based on year especially in relation to the 2014 tire group. The 2014 tire groups showed the smallest change in toxicity following weathering, and also was the only tire groups to show an increase in a metal following weathering. Further analysis would be required to fully understand whether the differences in the 2013 and 2014 tires are consistent.

Considering that the used tires were collected from different cars, the driving conditions of these tires is unknown and may have confounded differences between tire manufacture year. To assess this, one tire set from a single car contained tires from two different years (2018 and 2015). These tire groups were maintained as two separate tire groups for this comparison. Additionally, other work conducted in the Sofield lab using the same methods described in this thesis, compared tires manufactured in 2016 and 2017 from the same car (results not presented here). In both cases, there was no difference in toxicity based on year for unweathered tires and differences were only found after weathering. The 2018 and 2015 unweathered tire groups had the same toxicity prior to weathering (LC50 = 2.65 g/L). Following weathering the toxicity of the 2015 tire decreased by a factor of 2 (5.59 g/L) and the 2018 tire decreased by a factor of 3.6 (9.79 g/L). Weathering also impacted the toxicity of the other tire groups tested: the LC50 of the 2018 new tire changed from 3.47 g/L to 7.67g/L; the LC50 of the 2017 tire groups increased from 1.97 g/L to 5.19 g/L; the LC50 of the 2016 tire groups increased from 2.04 g/L to 12.09 g/L; the LC50 of the 2014 tire group increased from 3.51 g/L to 3.67 g/L; the LC50 for the 2013 tire group increased from 2.18 g/L to 4.17 g/L. These findings appear to indicate that both the year of the tire and driving condition do not relate to toxicity of unweathered tires but may play a role in weathered tires. However, all used tires in this study were obtained from Bellingham area tire distributors and the characteristics of this region could potentially explain some of these findings.

The city of Bellingham is in Whatcom County in the northwestern portion of Washington State. The County's Comprehensive Plan indicates that Whatcom County is a low-density area where the primary land uses consist of forestry and farmland (approximately 74% of the county), followed by residential, industrial, and commercial uses (Whatcom County Comprehensive Plan, 2021). Most of the population in Whatcom County resides in the western third of the county in urban areas (the cities of Bellingham[4], Blaine, Everson, Ferndale, Lynden, Nooksack, and Sumas) which comprise approximately 2.3% of the county land use (Whatcom County Comprehensive Plan, 2021). The low-density demographic of this region may explain the lack of differentiation between different ages of used tires. Whatcom county has relatively low traffic volumes, minimal industrial activity, and the urban centers are small (Bellingham, the largest city, has a population of 87,000 people (United State Census Bureau, 2018)), in turn minimizing the chemical load available for sorption to tires during use when compared to used tires obtained from higher density urban settings or areas with higher levels of industry.

4.1.3 Weathering in the marine environment on tire eluate toxicity

During weathering, chemicals associated with TWP are expected to change. Some organic and inorganic compounds will sorb from the environment, while others may leach from the TWP groups. In my study, all tire years were weathered in the marine environment (as described in section 2.1) for a

[4] All tires were collected from Bellingham area tire distributors.

period of 82 days. This weathering period did impact the toxicity of most tire groups as discussed in Section 4.1 and the weathered tire groups had a toxicity range of 3.67 g/L to 12.09 g/L compared to the unweathered tire groups with a toxicity range of 1.97 g/L to 3.51 g/L. While the toxicity within the unweathered tire groups were not statistically different, the weathered tire groups showed a much wider variability in toxicity resulting in four statistically different toxicity categories based on ratio testing of the LC50 values. Weathered tire groups 2013 and 2014 showed no statistical difference in toxicity from the unweathered tire groups (category 1) however the remaining weathered tire groups showed reduced toxicity to *A. bahia*.

Metal concentrations were analyzed for in all tire groups and both tire treatments (unweathered and weathered) at the 10 g/L concentration as discussed in section 2.8.3 and 3.3. The weathering period (82 days) was long enough to show a decrease in metals between unweathered and weathered tire treatments, with the exception of Zn in the weathered 2014 tire treatment, which was the only sample to show an increase following weathering. Statistically significant decreases in concentrations were only measured for Cu and Ni. All remaining analytes (Al, Mn, Co, and Zn) did decrease after weathering (with one exception, weathered 2014 Zn concentration); however, these reductions did not produce statistically significant differences in concentrations of these metals.

The impact of weathering on organic compounds in these TWP groups and the subsequent impact to measured toxicity for *A. bahia* has yet to be determined and analysis is currently in progress. From the literature, conventional microplastics such as PVC, PET, PE, PP, PS are found in the environment as ridged crystalline fragments (Hüffer et al., 2020; Wang et al., 2020). In the marine environment these microplastics adsorb organic compounds such as PCBs, PAHs, PFAS, and pharmaceutics and other non-polar compounds predominantly through hydrophobic interactions but also through electrostatic interactions, and pore filling mechanisms (Torres et al., 2020; Wang et al., 2020). Weathering can increase the sorption abilities of microplastics as they become fragmented and surfaces become more abraded as does the environment they are deposited and several researchers have measured higher sorption affinities for microplastics found in seawater (Alimi et al., 2018; Torres et al., 2020; Wang et al. 2020). Sorption of organic compounds to plastics has been shown to be 100 times greater than that of naturally occurring particulate organic matter making them a potentially significant vector of toxicants from aqueous systems to organisms (Bellasi et al., 2020, Nagash et al., 2020). Additionally, dissolved metals can be adsorbed to the surface of microplastics increasing their potential toxicity to marine organisms (Holmes et al., 2011; Nagash et al., 2020).

These characteristics are not shared equally with TWP which do not have the same physical qualities of conventional microplastics such as ridged crystalline structures. Additionally, TWP are a complex chemical mixture of rubbers, metals, and organic compounds and while they have the capacity to sorb non-polar substances, the predominant environmental interactions may be much different. In relation to TWP, the primary mode of mass transfer may be from TWP to the less concentrated contacting solution. From the literature, toxicity tests using weathered tires from a floating tire breakwater for an estimated period of 10 years showed no toxicity to *D. magna*, *O. mykiss*, or *P. promelas* (Day et al., 1993). The researchers found that tires in marine systems can potentially reach equilibrium with the surrounding contacting solutions (through the sorption and desorption of compounds), in a relatively short period. The compounds leached from the tires to test solutions stored in the lab for a least 32 days, was shown to remain toxic to *D. magna* when used in toxicity tests. The literature also suggests that tire particles can be a continuing source of Zn to contacting solutions where the release of Zn can occur for at least 30 days (Halsband et al., 2020).

4.1.4 Sediment Testing

Initially it was unclear whether sediments retained by weathered TWP would contribute to the toxicity of the TWP eluates. An acute toxicity test was performed using sediment only eluate solutions of 1 and 5 g/L to determine if these sediments contribute to toxicity to *A. bahia*. These test concentrations were based on the quantity of a sediment retrieved from the TWP eluate stock solutions of four weathered tire groups (2018 - new tire, 2014, 2016, and 2017). The average sediment concentration from these tire groups was 0.00083 g sediment/g weathered TWP. Based on these results, the highest concentration of sediment in a weathered tire eluate solution was 2.08E-2 g/L from the weathered used tire groups 2016 and 2018 tested at 25 g of TWP/L. The chosen sediment test concentrations (1 g/L and 5 g/L) for sediment toxicity evaluation were at least 2 orders of magnitude greater than measured sediment concentrations in weathered tire eluate solutions. Both sediment test treatments (1 g/L and 5 g/L) showed less than 10% mortality to *A. bahia* (the acceptable level for control mortality). These results indicate that the sediment accumulated by the weathered tire treatments did not contribute to the toxicity in this testing and that toxicity is being driven by compounds leached from the TWP.

4.2 Chemical analysis (metals)

In this study, metal concentrations were determined in the 10 g/L eluate stock solutions for all tire years and both tire treatments (unweathered and weather). At the LC50 concentration, all tire years and treatments were found to exceed the EPA Aquatic Life Criteria (EPA, 2016) for Cu, Ni, and Zn for both the CMC (acute) and CCC (chronic) concentrations (Figure 6). For all tire years, LC50s of both unweathered and weathered exceeded the EPA Aquatic Life Criteria (EPA, 2016) for both acute and chronic concentrations for Cu and Zn and, in some instances, Ni as shown in Table 8. When metal concentrations at the LC50s were correlated with the LC50, Cu and Ni showed higher concentrations associated with higher LC50 values. The tire groups that had the highest measured concentrations of both Cu and Ni were the weathered used 2018 tire group and weathered 2016 tire group. These two were the only groups that required a higher concentration series as discussed in Section 2.4.2. as these groups exhibited the lowest toxicity (highest estimated LC50s). This inverse relationship appears to indicate that the tires were the source of these metals, and that toxicity could not be attributed to those metals because greater metal concentrations resulted in less toxicity (great LC50s). In contrast, the Zn concentration at all LC50s was relatively constant. Two tire groups had notably lower Zn concentrations at the LC50: the TWP LC50 of 0.165 mg/L (unweathered 2014) and 0.135 mg/L (unweathered 2016). With those tire groups removed, the range of Zn concentrations at the LC50 was 0.557 - 0.954 mg/L and an average concentration of 0.704 mg/L. These Zn LC50s are similar to results measured by Ho et al. (1999) and Lussier et al. (1985). These researchers performed toxicity tests using single metal solutions to determine LC50 for *A.bahia*. These researchers measured LC50s for Zn to *A.bahia* of 0.35 mg/L and 0.6 mg/L under similar test conditions (Table 6). This suggests that while Zn may be responsible for some of the observed toxicity, further analysis is required to determine its contribution to the measured toxicity values. An investigation of the mixture toxicity should also be considered once the organic chemistry is completed.

Table 6. Comparison of metal concentrations at their respective LC50 concentrations from tire eluate in this study and aqueous metals solutions from Ho et al. (1998), and Lussier et al. (1985), to *A. bahai*.

Analyte	This study (LC50)	Ho et al. 1999	Lussier et al. 1985
Al	0.024 - 0.172 mg/L		
Mn	0.104 - 2.89 µg/L		
Co	0.099 - 0.817 µg/L		
Ni	0.049 - 0.221 mg/L	0.72 mg/L	0.387-0.635 mg/L
Cu	0.021 - 0.135 mg/L	0.36 mg/L	0.146-0.25 mg/L
Zn	0.135 - 0.954 mg/L	0.58 mg/L	0.35-0.6 mg/L

The values from this study are the range measured at the LC50 test concentrations. The values from both the Ho et al. (1998) and Lussier et al. (1985) studies are the median lethal concentrations for the given analyte.

Few studies have looked at metal concentrations from TWP eluate using a saltwater contacting solution. An early study by Hartwell et al. (1998) created eluate solutions using cut up tires (1 cm^3 tire chips from what was assumed to be whole tires)[5] at 23°C, to create 50 g/L eluate solutions. These researchers analyzed metal concentrations in their eluate and found lower concentrations for all

[5] The assumption of whole tires is based on two reasons: first there is a statement that all tires contain some sort of steel belting and that observations of steel cords were recorded (although not presented). Second their metal results are very high for Fe indicating that the steel belts are likely part of the tire chips.

analytes when compared to the measurements in this study (Table 6). A second study conducted by Halsband et al. (2020) measured metals from several treatments of tire crumb rubber granulate. The authors measured comparable concentrations of Cu and Zn, and lower concentrations of Co, Mn, and Ni in their crumb rubber samples, when compared to these results (Table 7).

Both the Halsband et al. (2020) and Hartwell et al. (1998) studies had long leachate periods for their tire and crumb rubber eluate solutions. Intuitively this should produce greater metal concentrations in their respective eluate solutions. The differences in metal concentrations between this study and the Hartwell et al (1998) study may be attributed to differences in tire formulations and the size of tire particles used to create eluate. Hartwell et al (1998) used tire chips (1 cm^3) to create test eluate using EPA TCLP method 1311, using synthetic seawater at 25 ppt, a rotary extractor at 30 rpm, at 23°C, over a 7-day period. In this study the eluate procedure consisted of using TWP between 0.64-1000 µm, filtered natural seawater contacting solution of 25 ppt, at 21°C, for 48-hours. These three studies appear to indicate that metal concentrations in tire derived eluate solutions are being driven primarily by tire particle size.

Table 7. Metal concentrations from this study (weathered and unweathered, Hartwell et al. (1998), and Halsband et al. (2020).

Analyte	Unweathered	Weathered	Hartwell et al. 1998	Halsband et al. 2020
Al	0.077 - 0.225 mg/L	0.071 - 0.225 mg/L	0.00007 mg/L	
Mn	0.657 - 5.197 µg/L	0.250 - 2.95 µg/L	0.29 µg/L	2.6 - 8.4 µg/L
Co	0.414 - 3.204 µg/L	0.444 - 1.575 µg/L	0.004 µg/L	2.5 - 7.5 µg/L
Ni	0.181 - 0.304 mg/L	0.093 - 0.191 mg/L	0.000006 mg/L	< 0.002 mg/L
Cu	0.093 - 0.121 mg/L	0.082 - 0.103 mg/L	0.000011 mg/L	0.023 – 0.058 mg/L
Zn	0.473 - 3.729 mg/L	0.455 - 2.004 mg/L	0.000364 mg/L	0.67 - 2 mg/L

Eluate concentrations in all three studies are compared at 10 g tire/L. Eluate in the Hartwell et al. (1998) study was created at 50 g/L and is presented here corrected to 10 g/L for comparison. Values for the Halsband et al (2020) study are mean concentrations of each analyte.

Halsband et al. (2020) utilized tire crumb rubber granulate from end-of-life tires; one treatment collected from a sports turf field (of unknown age), one treatment that had not been used on a sports field, and one treatment that was purchased from a crumb rubber distributor. Tire crumb rubber is a waste byproduct of automotive tires and the use of crumb rubber in applications such as turf fields is considered a waste diversion measure for used tires (Halsband et al. 2020). These researchers used crumb rubber classified as "Medium Infill" in the size range of 1.0–2.8 mm, a 14-day leachate process using filtered natural seawater with a salinity of 34 - 35 psu, pH 8 - 8.2, temperature of 20°C, on an orbital shaker at 250 rpm. These parameters are close to the eluate procedures used in this study, with the primary differences being the leaching period (14-days compared to 48-hours), the salinity of the contacting solution (34 - 35 psu compared to 25 ppt in this study), and the size of the particles. Eluate creation methods of the three different studies are presented in Table 8.

Table 8. Eluate procedures from this study, Hartwell et al. 1998, and Halsband et al. 2020.

Study	Temperature	Salinity	Leachate period	Particle size	Source of Particles
This study	21°C	25 ppt	48-hours	64-1000 µm	Artificially created particles from new and used tires of same brand and model
Hartwell et al. 1990	23°C	25 ppt	7-days	1 cm^3 chips	Various cut up tires from scrap yard (both car and truck)
Halsband et al. 2021	20°C	34 - 35 psu	14-days	1 - 2.8 mm	Tire crumb rubber from a turf sports field

5.0 Conclusion

The dispersal and accumulation of microplastics and tire particles in marine systems is irrefutable. The exponential growth of production and consumption of both microplastics and tires indicate that emissions of these compounds to marine systems are likely to increase in the future. Marine organisms are being subject to continuous environmental exposures to compounds from tires and these compounds are capable of leaching from these particles into contacting solutions. Quantities of TWP and microplastics entering marine systems are not well characterized and secondary uses of end-of-life tires likely contribute to both the particle and chemical load from these uses.

This study demonstrated that eluate from tire wear particles exhibit a toxic effect to *A. bahia.* Particles from both new and used tires exhibited similar toxicity to *A. bahia* prior to weathering in the marine environment and *A. bahia* demonstrated a clear dose-dependent response to TWP eluates. Inorganic elements of these eluate solutions, possibly driven by Zn, have the potential to produce mortality to *A. bahia,* however organic elements likely play a role in toxicity and further analysis is required to identity and quantify these impacts. Additionally, sub-lethal effects are also probable under these exposure conditions and further analysis is required to identify these mechanisms of toxicity. Weathering did impact the toxicity of TWP eluates, however inorganic elements were not shown to be statistically affected during the 82-day weathering period.

While the eluates in this study were created using concentrations of TWP that exceed currently established environmental conditions, this study furthers our understanding of the toxicity of these environmental inputs. *A. bahia* may not be the most sensitive marine organism, mortality is the least sensitive effect endpoint, and the leaching of organic and inorganic compounds to contacting solutions is not the only route of exposure to marine organisms. However, these results and results of other studies focusing on tire related impacts to freshwater organisms should raise attention to the potential consequences of this environmental exposure and should warrant further analysis to identify and quantify how these substances are altering both freshwater and marine environments.

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Appendix A

Rainfall Data - Bellingham WA (September 2020 - December 2020)

Station Name: BELLINGHAM 3 SSW, WA US
Station ID: USC00450587
Station Location: Elevation: 15 ft. Latitude: 48.7177° N, Longitude: -122.5113° W
Source: NOAA National Centers for Environmental Information (NCEI), Climate Data Online (CDO)

			Temperature (°F)			Precipitation (in)				
			MAX	MIN	Average	Total				
September	1	2020	74	56	65	0				
September	2	2020	74	57	65.5	0				
September	3	2020	73	51	62	0				
September	4	2020	73	50	61.5	0				
September	5	2020	72	58	65	0				
September	6	2020	74	51	62.5	0				
September	7	2020	82	52	67	0				
September	8	2020	81	48	64.5	0				
September	9	2020	75	47	61	0				
September	10	2020	78	50	64	0				
September	11	2020	77	53	65	0				
September	12	2020	65	51	58	0				
September	13	2020	60	49	54.5	0				
September	14	2020	63	49	56	0				
September	15	2020	74	54	64	0.06				
September	16	2020	66	56	61	0.02				
September	17	2020	66	56	61	0				
September	18	2020	66	54	60	0				
September	19	2020	64	57	60.5	0				
September	20	2020	68	54	61	0				
September	21	2020	67	47	57	0				
September	22	2020	67	53	60	0	TWP deployed 9/23/20			
September	23	2020	66	56	61	0.32				
September	24	2020	64	53	58.5	1.06				
September	25	2020	63	54	58.5	0.41				
September	26	2020	62	53	57.5	0.51				
September	27	2020	63	46	54.5	0.02				
September	28	2020	65	44	54.5	0				
September	29	2020	66	46	56	0	September Weathering Period			
September	30	2020	70	48	59	0	Average temperature (°F)	57.4	Total precipitation (in)	2.32
October	1	2020	69	48	58.5	0				
October	2	2020	67	50	58.5	0				
October	3	2020	64	49	56.5	0				
October	4	2020	59	53	56	0				
October	5	2020	62	48	55	0.02				
October	6	2020	60	47	53.5	0				
October	7	2020	59	48	53.5	0.02				
October	8	2020	61	53	57	0				
October	9	2020	63	54	58.5	0				
October	10	2020	63	51	57	0.69				
October	11	2020	55	48	51.5	0.21				
October	12	2020	58	44	51	0.31				
October	13	2020	61	45	53	0.46				
October	14	2020	58	46	52	0				
October	15	2020	56	41	48.5	0				
October	16	2020	59	49	54	0.18				
October	17	2020	61	49	55	0				
October	18	2020	55	46	50.5	0.58				
October	19	2020	55	51	53	0				

October	20	2020	58	42	50	0				
October	21	2020	54	43	48.5	0.02				
October	22	2020	52	34	43	0				
October	23	2020	49	37	43	0.6				
October	24	2020	47	36	41.5	0.08				
October	25	2020	45	27	36	0				
October	26	2020	46	27	36.5	0.07				
October	27	2020	52	40	46	0				
October	28	2020	56	50	53	0				
October	29	2020	59	50	54.5	0.02				
October	30	2020	56	48	52	0.17	October Weathering Period			
October	31	2020	51	33	42	0	Average temperature (°F)	50.9	total precipitation (in)	3.43
November	1	2020	55	32	43.5	0				
November	2	2020	52	34	43	0				
November	3	2020	53	39	46	0.7				
November	4	2020	61	50	55.5	0.3				
November	5	2020	61	50	55.5	0.09				
November	6	2020	61	40	50.5	0				
November	7	2020	48	33	40.5	0.05				
November	8	2020	47	29	38	0				
November	9	2020	44	26	35	0				
November	10	2020	42	34	38	0.24				
November	11	2020	41	29	35	0				
November	12	2020	46	34	40	0.02				
November	13	2020	46	41	43.5	1.2				
November	14	2020	45	41	43	0.01				
November	15	2020	50	39	44.5	0.28				
November	16	2020	45	40	42.5	0.63				
November	17	2020	59	39	49	0.08				
November	18	2020	52	43	47.5	0.47				
November	19	2020	48	41	44.5	0.35				
November	20	2020	49	44	46.5	0				
November	21	2020	48	34	41	0.08				
November	22	2020	44	33	38.5	0.01				
November	23	2020	44	39	41.5	0.07				
November	24	2020	49	42	45.5	0.12				
November	25	2020	44	38	41	0.36				
November	26	2020	46	40	43	0.19				
November	27	2020	52	43	47.5	0.01				
November	28	2020	51	39	45	0.24				
November	29	2020	42	30	36	0	November Weathering Period			
November	30	2020	52	36	44	0.29	Average temperature (°F)	43.5	Total precipitation (in)	5.79
December	1	2020	44	30	37	0				
December	2	2020	44	27	35.5	0.01				
December	3	2020	42	29	35.5	0.01				
December	4	2020	46	30	38	0				
December	5	2020	51	27	39	0				
December	6	2020	54	35	44.5	0				
December	7	2020	51	43	47	0.01				
December	8	2020	57	46	51.5	0.72				
December	9	2020	46	42	44	0.32				
December	10	2020	44	33	38.5	0				
December	11	2020	43	36	39.5	0.13				
December	12	2020	39	30	34.5	0.02				
December	13	2020	42	30	36	0.17	December Weathering Period			
December	14	2020	47	37	42	0.18	Average temperature (°F)	40.2	Total precipitation (in)	1.57
December	15	2020	47	39	43	0.26	TWP retreived 12/14/20			
December	16	2020	49	42	45.5	0.27				
December	17	2020	49	42	45.5	0.87				
December	18	2020	48	41	44.5	0.19				

Appendix B

Bellingham, WA Tidal Data (September 2020 - December 2020)

Tide Station: Station: 9449211 Bellingham, WA

Source: NOAA Tides and Currents - https://tidesandcurrents.noaa.gov/noaatidepredictions.html?id=9449211&legacy=1

Date	Time	Tide Prediction (Ft MLLW)	Tide Stage High/Low		
9/21/2020	2:27 AM	-0.15	L		
9/21/2020	9:14 AM	7.61	H		
9/21/2020	2:39 PM	3.88	L		
9/21/2020	8:22 PM	8.31	H		
9/22/2020	3:20 AM	-0.52	L		
9/22/2020	10:34 AM	7.54	H		
9/22/2020	3:39 PM	4.94	L		
9/22/2020	8:59 PM	8.01	H	TWP deployed 9/23/20	
9/23/2020	4:18 AM	-0.59	L		
9/23/2020	12:08 PM	7.63	H		
9/23/2020	4:56 PM	5.71	L		
9/23/2020	9:43 PM	7.58	H		
9/24/2020	5:20 AM	-0.42	L		
9/24/2020	1:38 PM	7.92	H		
9/24/2020	6:51 PM	6	L		
9/24/2020	10:38 PM	7.09	H		
9/25/2020	6:28 AM	-0.14	L		
9/25/2020	2:47 PM	8.24	H		
9/25/2020	8:49 PM	5.71	L		
9/25/2020	11:51 PM	6.65	H		
9/26/2020	7:37 AM	0.14	L		
9/26/2020	3:39 PM	8.44	H		
9/26/2020	9:52 PM	5.2	L		
9/27/2020	1:19 AM	6.43	H		
9/27/2020	8:41 AM	0.39	L		
9/27/2020	4:20 PM	8.48	H		
9/27/2020	10:31 PM	4.63	L		
9/28/2020	2:41 AM	6.47	H		
9/28/2020	9:34 AM	0.66	L		
9/28/2020	4:54 PM	8.39	H		
9/28/2020	10:58 PM	4.06	L		
9/29/2020	3:47 AM	6.65	H		
9/29/2020	10:19 AM	0.99	L		
9/29/2020	5:21 PM	8.22	H		
9/29/2020	11:20 PM	3.48	L		
9/30/2020	4:41 AM	6.84	H		
9/30/2020	10:58 AM	1.41	L		
9/30/2020	5:42 PM	8.04	H		
9/30/2020	11:43 PM	2.86	L	September tide average:	4.94

10/1/2020	5:29 AM	7	H
10/1/2020	11:33 AM	1.92	L
10/1/2020	5:57 PM	7.9	H
10/2/2020	12:08 AM	2.24	L
10/2/2020	6:14 AM	7.14	H
10/2/2020	12:08 PM	2.54	L
10/2/2020	6:12 PM	7.81	H
10/3/2020	12:36 AM	1.64	L
10/3/2020	6:58 AM	7.27	H
10/3/2020	12:43 PM	3.22	L
10/3/2020	6:30 PM	7.75	H
10/4/2020	1:06 AM	1.1	L
10/4/2020	7:44 AM	7.38	H
10/4/2020	1:21 PM	3.94	L
10/4/2020	6:52 PM	7.65	H
10/5/2020	1:40 AM	0.68	L
10/5/2020	8:34 AM	7.47	H
10/5/2020	2:02 PM	4.66	L
10/5/2020	7:16 PM	7.48	H
10/6/2020	2:16 AM	0.4	L
10/6/2020	9:28 AM	7.53	H
10/6/2020	2:48 PM	5.32	L
10/6/2020	7:41 PM	7.26	H
10/7/2020	2:55 AM	0.25	L
10/7/2020	10:30 AM	7.55	H
10/7/2020	3:46 PM	5.87	L
10/7/2020	8:05 PM	7	H
10/8/2020	3:41 AM	0.24	L
10/8/2020	11:46 AM	7.59	H
10/8/2020	5:09 PM	6.24	L
10/8/2020	8:22 PM	6.75	H
10/9/2020	4:33 AM	0.3	L
10/9/2020	1:13 PM	7.73	H
10/10/2020	5:34 AM	0.36	L
10/10/2020	2:17 PM	7.92	H
10/10/2020	9:46 PM	5.92	L
10/10/2020	10:18 PM	6.25	H
10/11/2020	6:40 AM	0.38	L
10/11/2020	2:59 PM	8.09	H
10/11/2020	9:19 PM	5.53	L
10/12/2020	12:04 AM	6.15	H
10/12/2020	7:43 AM	0.37	L
10/12/2020	3:30 PM	8.21	H
10/12/2020	9:25 PM	4.86	L
10/13/2020	1:35 AM	6.31	H
10/13/2020	8:41 AM	0.42	L
10/13/2020	3:57 PM	8.31	H
10/13/2020	9:51 PM	3.91	L
10/14/2020	2:54 AM	6.7	H

10/14/2020	9:33 AM	0.66	L
10/14/2020	4:22 PM	8.43	H
10/14/2020	10:25 PM	2.75	L
10/15/2020	4:05 AM	7.19	H
10/15/2020	10:21 AM	1.15	L
10/15/2020	4:48 PM	8.56	H
10/15/2020	11:02 PM	1.49	L
10/16/2020	5:10 AM	7.68	H
10/16/2020	11:07 AM	1.89	L
10/16/2020	5:16 PM	8.68	H
10/16/2020	11:42 PM	0.27	L
10/17/2020	6:13 AM	8.11	H
10/17/2020	11:53 AM	2.82	L
10/17/2020	5:45 PM	8.74	H
10/18/2020	12:25 AM	-0.75	L
10/18/2020	7:16 AM	8.43	H
10/18/2020	12:41 PM	3.83	L
10/18/2020	6:16 PM	8.7	H
10/19/2020	1:10 AM	-1.43	L
10/19/2020	8:19 AM	8.62	H
10/19/2020	1:33 PM	4.79	L
10/19/2020	6:50 PM	8.52	H
10/20/2020	1:57 AM	-1.71	L
10/20/2020	9:26 AM	8.71	H
10/20/2020	2:31 PM	5.59	L
10/20/2020	7:26 PM	8.16	H
10/21/2020	2:48 AM	-1.58	L
10/21/2020	10:37 AM	8.72	H
10/21/2020	3:45 PM	6.12	L
10/21/2020	8:07 PM	7.64	H
10/22/2020	3:42 AM	-1.11	L
10/22/2020	11:53 AM	8.71	H
10/22/2020	5:43 PM	6.22	L
10/22/2020	8:58 PM	7	H
10/23/2020	4:42 AM	-0.44	L
10/23/2020	1:04 PM	8.73	H
10/23/2020	7:59 PM	5.74	L
10/23/2020	10:06 PM	6.34	H
10/24/2020	5:47 AM	0.29	L
10/24/2020	2:03 PM	8.74	H
10/24/2020	9:03 PM	5.03	L
10/24/2020	11:38 PM	5.81	H
10/25/2020	6:57 AM	0.96	L
10/25/2020	2:51 PM	8.69	H
10/25/2020	9:44 PM	4.29	L
10/26/2020	1:26 AM	5.67	H
10/26/2020	8:02 AM	1.55	L
10/26/2020	3:28 PM	8.57	H
10/26/2020	10:15 PM	3.56	L

10/27/2020	3:00 AM	5.91	H		
10/27/2020	8:58 AM	2.09	L		
10/27/2020	3:57 PM	8.41	H		
10/27/2020	10:38 PM	2.86	L		
10/28/2020	4:08 AM	6.33	H		
10/28/2020	9:45 AM	2.65	L		
10/28/2020	4:18 PM	8.23	H		
10/28/2020	10:56 PM	2.16	L		
10/29/2020	5:03 AM	6.77	H		
10/29/2020	10:26 AM	3.25	L		
10/29/2020	4:33 PM	8.09	H		
10/29/2020	11:15 PM	1.46	L		
10/30/2020	5:50 AM	7.19	H		
10/30/2020	11:05 AM	3.87	L		
10/30/2020	4:47 PM	8.02	H		
10/30/2020	11:38 PM	0.8	L		
10/31/2020	6:34 AM	7.58	H		
10/31/2020	11:42 AM	4.49	L		
10/31/2020	5:03 PM	7.97	H	October tide average:	5.04
11/1/2020	12:04 AM	0.21	L		
11/1/2020	6:16 AM	7.92	H		
11/1/2020	11:21 AM	5.08	L		
11/1/2020	4:24 PM	7.89	H		
11/1/2020	11:33 PM	-0.26	L		
11/2/2020	6:58 AM	8.2	H		
11/2/2020	12:02 PM	5.61	L		
11/2/2020	4:46 PM	7.75	H		
11/3/2020	12:05 AM	-0.57	L		
11/3/2020	7:42 AM	8.4	H		
11/3/2020	12:48 PM	6.06	L		
11/3/2020	5:08 PM	7.54	H		
11/4/2020	12:40 AM	-0.72	L		
11/4/2020	8:29 AM	8.51	H		
11/4/2020	1:41 PM	6.41	L		
11/4/2020	5:24 PM	7.3	H		
11/5/2020	1:20 AM	-0.7	L		
11/5/2020	9:22 AM	8.54	H		
11/5/2020	2:50 PM	6.62	L		
11/5/2020	4:39 PM	7.07	H		
11/6/2020	2:04 AM	-0.55	L		
11/6/2020	10:19 AM	8.54	H		
11/7/2020	2:55 AM	-0.27	L		
11/7/2020	11:17 AM	8.54	H		
11/8/2020	3:52 AM	0.1	L		
11/8/2020	12:07 PM	8.56	H		
11/9/2020	4:55 AM	0.54	L		
11/9/2020	12:48 PM	8.6	H		
11/9/2020	7:49 PM	4.79	L		
11/9/2020	10:58 PM	5.57	H		

11/10/2020	5:59 AM	1.06	L
11/10/2020	1:21 PM	8.66	H
11/10/2020	8:00 PM	3.72	L
11/11/2020	12:42 AM	5.75	H
11/11/2020	7:00 AM	1.68	L
11/11/2020	1:51 PM	8.75	H
11/11/2020	8:29 PM	2.41	L
11/12/2020	2:13 AM	6.33	H
11/12/2020	7:56 AM	2.42	L
11/12/2020	2:19 PM	8.88	H
11/12/2020	9:03 PM	0.99	L
11/13/2020	3:29 AM	7.12	H
11/13/2020	8:49 AM	3.26	L
11/13/2020	2:48 PM	9.01	H
11/13/2020	9:41 PM	-0.37	L
11/14/2020	4:34 AM	7.93	H
11/14/2020	9:40 AM	4.15	L
11/14/2020	3:18 PM	9.1	H
11/14/2020	10:21 PM	-1.5	L
11/15/2020	5:34 AM	8.63	H
11/15/2020	10:31 AM	5.01	L
11/15/2020	3:49 PM	9.09	H
11/15/2020	11:03 PM	-2.27	L
11/16/2020	6:32 AM	9.14	H
11/16/2020	11:24 AM	5.74	L
11/16/2020	4:23 PM	8.93	H
11/16/2020	11:47 PM	-2.6	L
11/17/2020	7:29 AM	9.43	H
11/17/2020	12:22 PM	6.27	L
11/17/2020	5:00 PM	8.59	H
11/18/2020	12:33 AM	-2.48	L
11/18/2020	8:26 AM	9.52	H
11/18/2020	1:28 PM	6.56	L
11/18/2020	5:40 PM	8.07	H
11/19/2020	1:21 AM	-1.99	L
11/19/2020	9:24 AM	9.47	H
11/19/2020	2:57 PM	6.55	L
11/19/2020	6:27 PM	7.39	H
11/20/2020	2:12 AM	-1.21	L
11/20/2020	10:22 AM	9.33	H
11/20/2020	5:27 PM	6.09	L
11/20/2020	7:25 PM	6.62	H
11/21/2020	3:07 AM	-0.28	L
11/21/2020	11:18 AM	9.17	H
11/21/2020	6:47 PM	5.31	L
11/21/2020	8:42 PM	5.84	H
11/22/2020	4:05 AM	0.72	L
11/22/2020	12:08 PM	9	H
11/22/2020	7:37 PM	4.45	L

11/22/2020	10:20 PM	5.25	H		
11/23/2020	5:07 AM	1.7	L		
11/23/2020	12:49 PM	8.82	H		
11/23/2020	8:14 PM	3.59	L		
11/24/2020	12:27 AM	5.14	H		
11/24/2020	6:10 AM	2.62	L		
11/24/2020	1:22 PM	8.64	H		
11/24/2020	8:43 PM	2.75	L		
11/25/2020	2:15 AM	5.61	H		
11/25/2020	7:09 AM	3.46	L		
11/25/2020	1:45 PM	8.47	H		
11/25/2020	9:05 PM	1.94	L		
11/26/2020	3:26 AM	6.31	H		
11/26/2020	8:02 AM	4.24	L		
11/26/2020	2:03 PM	8.34	H		
11/26/2020	9:24 PM	1.17	L		
11/27/2020	4:20 AM	7.02	H		
11/27/2020	8:51 AM	4.94	L		
11/27/2020	2:20 PM	8.28	H		
11/27/2020	9:44 PM	0.46	L		
11/28/2020	5:07 AM	7.66	H		
11/28/2020	9:37 AM	5.54	L		
11/28/2020	2:39 PM	8.24	H		
11/28/2020	10:08 PM	-0.18	L		
11/29/2020	5:47 AM	8.19	H		
11/29/2020	10:21 AM	6.04	L		
11/29/2020	3:01 PM	8.2	H		
11/29/2020	10:35 PM	-0.71	L		
11/30/2020	6:25 AM	8.61	H		
11/30/2020	11:04 AM	6.43	L		
11/30/2020	3:25 PM	8.11	H		
11/30/2020	11:05 PM	-1.11	L	November tide average:	5.12
12/1/2020	7:02 AM	8.9	H		
12/1/2020	11:49 AM	6.71	L		
12/1/2020	3:49 PM	7.96	H		
12/1/2020	11:39 PM	-1.35	L		
12/2/2020	7:40 AM	9.08	H		
12/2/2020	12:36 PM	6.88	L		
12/2/2020	4:09 PM	7.77	H		
12/3/2020	12:16 AM	-1.43	L		
12/3/2020	8:20 AM	9.16	H		
12/3/2020	1:30 PM	6.93	L		
12/3/2020	4:09 PM	7.54	H		
12/4/2020	12:56 AM	-1.34	L		
12/4/2020	9:02 AM	9.18	H		
12/4/2020	2:36 PM	6.83	L		
12/4/2020	3:51 PM	7.26	H		
12/5/2020	1:40 AM	-1.07	L		
12/5/2020	9:45 AM	9.15	H		

12/6/2020	2:28 AM	-0.59	L		
12/6/2020	10:26 AM	9.11	H		
12/7/2020	3:19 AM	0.1	L		
12/7/2020	11:06 AM	9.08	H		
12/7/2020	6:33 PM	4.95	L		
12/7/2020	9:12 PM	5.48	H		
12/8/2020	4:15 AM	1	L		
12/8/2020	11:42 AM	9.07	H		
12/8/2020	6:52 PM	3.8	L		
12/8/2020	11:05 PM	5.2	H		
12/9/2020	5:15 AM	2.06	L		
12/9/2020	12:16 PM	9.1	H		
12/9/2020	7:25 PM	2.44	L		
12/10/2020	1:04 AM	5.52	H		
12/10/2020	6:18 AM	3.18	L		
12/10/2020	12:48 PM	9.17	H		
12/10/2020	8:02 PM	0.99	L		
12/11/2020	2:44 AM	6.42	H		
12/11/2020	7:21 AM	4.25	L		
12/11/2020	1:21 PM	9.26	H		
12/11/2020	8:41 PM	-0.39	L		
12/12/2020	3:57 AM	7.49	H		
12/12/2020	8:22 AM	5.2	L		
12/12/2020	1:54 PM	9.33	H		
12/12/2020	9:21 PM	-1.56	L		
12/13/2020	4:56 AM	8.46	H		
12/13/2020	9:20 AM	5.95	L		
12/13/2020	2:29 PM	9.33	H		
12/13/2020	10:03 PM	-2.38	L		
12/14/2020	5:48 AM	9.18	H		
12/14/2020	10:17 AM	6.47	L		
12/14/2020	3:06 PM	9.21	H		
12/14/2020	10:45 PM	-2.8	L	December tide average:	5.22
12/15/2020	6:37 AM	9.62	H		
12/15/2020	11:14 AM	6.75	L		
12/15/2020	3:46 PM	8.94	H		
12/15/2020	11:29 PM	-2.81	L		
12/16/2020	7:24 AM	9.81	H		
12/16/2020	12:14 PM	6.81	L		
12/16/2020	4:30 PM	8.51	H		

Appendix C

Water Quality Measurements for Guemes Channel
WWU Shannon Point Marine Center

Date	Time	Location	Replicate	Chlorophyll	Phaeopigments	Temperature	Salinity	Dissolved O2	pH
29-Sep-20	10:20	pump	1	0.593	0.817	11.2	29.4	6.76	7.8
29-Sep-20		pump	2	0.665	0.833	11.2	29.3	7	7.81
29-Sep-20		pump	3	0.669	0.888	11.2	29.3	6.86	7.82
5-Oct-20	10:00	pump	1	0.635	1.808	11	29.7	7.4	7.79
5-Oct-20		pump	2	0.659	1.394	11	29.7	7.57	7.8
5-Oct-20		pump	3	0.592	1.526	11	29.7	7.47	7.81
12-Oct-20	10:40	pump	1	0.936	2.011	11.2	29.2	7.94	7.81
12-Oct-20		pump	2	0.832	2.075	11.1	29.2	8.14	7.81
12-Oct-20		pump	3	0.791	2.256	11.1	29.2	8.08	7.82
19-Oct-20	10:30	pump	1	0.476	1.877	10.6	29.8	8.16	7.79
19-Oct-20		pump	2	0.607	1.573	10.6	29.9	10.16	7.8
19-Oct-20		pump	3	0.487	1.806	10.5	29.8	10.11	7.81
26-Oct-20	10:30	pump	1	0.49	0.819	9.9	29.1	8.98	7.85
26-Oct-20		pump	2	0.472	0.951	9.9	29.2	9.2	7.87
26-Oct-20		pump	3	0.477	0.904	9.85	29.2	8.86	7.88
2-Nov-20	10:45	pump	1	0.347	0.774	9.8	29.9	8.19	7.78
2-Nov-20		pump	2	0.344	0.812	9.8	30	8.24	7.79
2-Nov-20		pump	3	0.367	0.774	9.8	29.9	7.98	7.8
9-Nov-20	10:45	pump	1	0.507	0.591	9.3	29.4	5.84	7.84
9-Nov-20		pump	2	0.507	0.579	9.3	29.5	7.54	7.84
9-Nov-20		pump	3	0.46	0.604	9.3	29.6	6.04	7.85
16-Nov-20	10:30	pump	1	0.71	1.972	9.2	29.4	9.18	7.84
16-Nov-20		pump	2	0.655	1.977	9.2	29.4	9.11	7.85
16-Nov-20		pump	3	0.642	1.936	9.1	29.5	8.83	7.86
23-Nov-20	9:50	pump	1	0.331	0.438	9.1	29.6	7.14	7.82
23-Nov-20		pump	2	0.306	0.449	9	29.6	7.17	7.82
23-Nov-20		pump	3	0.299	0.449	9.05	29.7	7.16	7.82
21-Dec-20	10:30	pump	1	0.325	0.575	8.9	29.4	7.57	7.8
21-Dec-20		pump	2	0.317	0.581	8.7	29.6	8.33	7.84
21-Dec-20		pump	3	0.365	0.658	8.7	29.6	8.18	7.85

Appendix D

Definitive Testing Data

Unweathered Tire Treatments - Final Test Data

Treatment ID	Treatment Year	Dose (g/L)	Number Affected	Total Mysids	Percent Mortality	Initial Water Quality Parameters						Final Water Quality Parameters					
						pH	Salinity (ppt)	DO (mg/L)	DO (% sat)	Temperature °C	Date	pH	Salinity (ppt)	DO (mg/L)	DO (% sat)	Temperature °C	Date
JS-New	2018 (new tire)	15	10	10	100							7.9	25	6.52	77.3		3/26/2021
JS-New	2018 (new tire)	15	10	10	100							7.9	25	6.26	74		3/26/2021
JS-New	2018 (new tire)	15	10	10	100							7.9	25	6.05	71.7		3/26/2021
JS-New	2018 (new tire)	7.5	10	10	100							7.9	25	6.28	74.3		3/27/2021
JS-New	2018 (new tire)	7.5	10	10	100							7.9	25	6.17	73		3/28/2021
JS-New	2018 (new tire)	7.5	10	10	100							7.9	25	6.16	72.7		3/27/2021
JS-New	2018 (new tire)	3.75	10	10	100							8	25	6.67	78		3/29/2021
JS-New	2018 (new tire)	3.75	8	10	80	7.8	25	6.96	82.6	25°C	3/25/2021	8	26	6.62	78.1	25°C	3/29/2021
JS-New	2018 (new tire)	3.75	8	10	80							8	26	6.45	75		3/29/2021
JS-New	2018 (new tire)	1.875	0	10	0							7.9	26	5.81	68		3/29/2021
JS-New	2018 (new tire)	1.875	0	10	0							7.8	27	5.73	67.4		3/29/2021
JS-New	2018 (new tire)	1.875	0	10	0							7.8	27	6.07	71.6		3/29/2021
JS-New	2018 (new tire)	0.9	0	10	0							7.7	26	5.31	60.3		3/29/2021
JS-New	2018 (new tire)	0.9	0	10	0							7.7	27	5.16	60.6		3/29/2021
JS-New	2018 (new tire)	0.9	0	10	0							7.7	27	4.92	58.2		3/29/2021
Control	2018 (new tire)	0	1	10	10							7.9	25.5	6.22	73.8		3/29/2021
Control	2018 (new tire)	0	0	10	0	8	25	7.71	89.7	25°C	3/25/2021	8	25.5	6.7	79.6	25°C	3/29/2021
Control	2018 (new tire)	0	0	10	0							8	26	6.24	73.6		3/29/2021
JS-1	2014	15	10	10	100							7.8	25	5.82	69		3/27/2021
JS-1	2014	15	10	10	100							7.8	25	5.87	69.6		3/27/2021
JS-1	2014	15	10	10	100							7.8	25	5.84	69.9		3/27/2021
JS-1	2014	7.5	10	10	100							7.9	26	6.34	74.9		3/28/2021
JS-1	2014	7.5	10	10	100							7.9	25	6.32	74.7		3/28/2021
JS-1	2014	7.5	10	10	100							7.8	25	5.95	70.2		3/27/2021
JS-1	2014	3.75	8	10	80							7.9	26	6.05	71		3/29/2021
JS-1	2014	3.75	8	10	80	7.8	25	6.96	83	25°C	3/25/2021	7.9	26	6.2	72.9	25°C	3/29/2021
JS-1	2014	3.75	9	10	90							7.9	27	6.29	74.4		3/29/2021
JS-1	2014	1.875	0	10	0							7.8	26	5.27	61.5		3/29/2021
JS-1	2014	1.875	0	10	0							7.8	26	5.34	62.8		3/29/2021
JS-1	2014	1.875	0	10	0							7.6	26	4.84	54.2		3/29/2021
JS-1	2014	0.9	0	10	0							7.6	27	4.28	50.3		3/29/2021
JS-1	2014	0.9	0	10	0							7.6	27	4.51	53.6		3/29/2021
JS-1	2014	0.9	0	10	0							7.5	27	3.52	41.6		3/29/2021
Control	2014	0	1	10	10							7.9	25.5	6.22	73.8		3/29/2021
Control	2014	0	0	10	0	8	25	7.71	89.7	25°C	3/25/2021	8	25.5	6.7	79.6	25°C	3/29/2021
Control	2014	0	0	10	0							8	26	6.24	73.6		3/29/2021

IG-1	2013	15	10	10	100							7.7	26	5.37	63.1		4/22/2021
IG-1	2013	15	10	10	100							7.7	26	5.51	65.1		4/22/2021
IG-1	2013	15	10	10	100							7.7	26	5.22	61.4		4/22/2021
IG-1	2013	7.5	10	10	100							8	26	5.41	63.6		4/23/2021
IG-1	2013	7.5	10	10	100							8	25	5.62	65.6		4/23/2021
IG-1	2013	7.5	10	10	100							7.8	25	5.75	67.5		4/22/2021
IG-1	2013	3.75	10	10	100							8	26	5.86	69		4/24/2021
IG-1	2013	3.75	9	10	90	7.6	26	6.83	79.5	25°C	4/20/2021	8	26	5.83	68.5	25°C	4/24/2021
IG-1	2013	3.75	9	10	90							8	26	5.92	69.3		4/24/2021
IG-1	2013	1.875	4	10	40							7.8	26	4.82	56.4		4/24/2021
IG-1	2013	1.875	3	10	30							7.8	26	5.17	60.7		4/24/2021
IG-1	2013	1.875	3	10	30							7.8	26	5.58	65.8		4/24/2021
IG-1	2013	0.9	0	10	0							7.7	26	5.61	65.4		4/24/2021
IG-1	2013	0.9	0	10	0							7.7	25	4.91	57.5		4/24/2021
IG-1	2013	0.9	0	10	0							7.7	25	5.32	62.4		4/24/2021
Control	2013	0	0	10	0							7.4	25	3.41	38.4		4/24/2021
Control	2013	0	0	10	0	8.6	25	6.79	80	25°C	4/20/2021	7.4	26	3.5	41.6	25°C	4/24/2021
Control	2013	0	0	10	0							7.4	26	3.47	39.5		4/24/2021
IG-3-15	2015	15	10	10	100							7.8	26	6.31	74.8		5/6/2021
IG-3-15	2015	15	10	10	100							7.8	27	6.12	72.2		5/6/2021
IG-3-15	2015	15	10	10	100							7.8	27	6.37	75.2		5/6/2021
IG-3-15	2015	7.5	10	10	100							8	25	6.76	79.7		5/8/2021
IG-3-15	2015	7.5	10	10	100							8	26	6.83	80.3		5/8/2021
IG-3-15	2015	7.5	10	10	100							8	26	7.15	84.3		5/8/2021
IG-3-15	2015	3.75	10	10	100							8.1	27	7.22	85.2		5/8/2021
IG-3-15	2015	3.75	8	10	80	7.4	26	8.2	93.1	25°C	5/4/2021	8.1	26	7.03	82.3	25°C	5/8/2021
IG-3-15	2015	3.75	9	10	90							8.1	26	7.05	83.1		5/8/2021
IG-3-15	2015	1.875	0	10	0							8	26	6.92	81.6		5/8/2021
IG-3-15	2015	1.875	2	10	20							8	26	6.53	77.2		5/8/2021
IG-3-15	2015	1.875	1	10	10							7.9	26	6.29	74.4		5/8/2021
IG-3-15	2015	0.9	0	10	0							7.6	26	4.35	51.4		5/8/2021
IG-3-15	2015	0.9	0	10	0							7.6	26	4.4	52		5/8/2021
IG-3-15	2015	0.9	0	10	0							7.6	27	5.45	64.4		5/8/2021
Control	2015	0	0	10	0							8.1	27	6.96	82.1		5/8/2021
Control	2015	0	0	10	0	8.2	25	7.97	93.3	25°C	5/4/2021	8.1	27	6.75	80	25°C	5/8/2021
Control	2015	0	0	10	0							8.1	27	6.89	81.4		5/8/2021

IG-3-18	2018	15	10	10	100							7.7	25	6.5	77.5		5/21/2021
IG-3-18	2018	15	10	10	100							7.7	25	6.61	79.3		5/21/2021
IG-3-18	2018	15	10	10	100							7.7	25	6.13	72.9		5/21/2021
IG-3-18	2018	7.5	10	10	100							8	25	7.14	84.7		5/23/2021
IG-3-18	2018	7.5	10	10	100							8	26	6.97	82.4		5/23/2021
IG-3-18	2018	7.5	10	10	100							8	26	6.85	81.2		5/23/2021
IG-3-18	2018	3.75	10	10	100							7.9	25	7.19	84.2		5/23/2021
IG-3-18	2018	3.75	9	10	90	7.6	25	7.94	90.7	25°C	5/20/2021	8	26	7.12	83.8	25°C	5/24/2021
IG-3-18	2018	3.75	9	10	90							8	26	7.03	82.8		5/24/2021
IG-3-18	2018	1.875	0	10	0							7.8	26	5.81	66.5		5/24/2021
IG-3-18	2018	1.875	0	10	0							7.8	27	5.3	62.5		5/24/2021
IG-3-18	2018	1.875	0	10	0							7.8	27	6.27	72.9		5/24/2021
IG-3-18	2018	0.9	1	10	10							7.4	25	3.27	37.4		5/24/2021
IG-3-18	2018	0.9	0	10	0							7.4	26	3.02	35.6		5/24/2021
IG-3-18	2018	0.9	1	10	10							7.3	26	3.55	41.4		5/24/2021
Control	2018	0	1	10	10							8	25	6.22	72.7		5/24/2021
Control	2018	0	0	10	0	8.1	26	8.8	98.4	25°C	5/20/2021	7.9	25	6.3	73.4	25°C	5/24/2021
Control	2018	0	0	10	0							7.9	25	6.25	73.2		5/24/2021
JS-3	2016	15	10	10	100							7.5	26	6.31	73.6		5/11/2021
JS-3	2016	15	10	10	100							7.7	26	6.91	82.1		5/12/2021
JS-3	2016	15	10	10	100							7.6	26	6.28	74.2		5/11/2021
JS-3	2016	7.5	10	10	100							8	25	7.78	92.3		5/14/2021
JS-3	2016	7.5	10	10	100							8	26	7.28	86.4		5/14/2021
JS-3	2016	7.5	10	10	100							8	25	7.15	85		5/14/2021
JS-3	2016	3.75	7	10	70							7.9	26	6.51	77.6		5/15/2021
JS-3	2016	3.75	10	10	100	7.4	26	8.24	93.8	25°C	5/11/2021	8	26	6.5	81.4	25°C	5/15/2021
JS-3	2016	3.75	9	10	90							8	26	6.51	77.5		5/15/2021
JS-3	2016	1.875	5	10	50							8	26	6.77	80.1		5/15/2021
JS-3	2016	1.875	6	10	60							7.9	26	6.09	72.5		5/15/2021
JS-3	2016	1.875	4	10	40							7.9	26	6.18	73.3		5/15/2021
JS-3	2016	0.9	0	10	0							7.7	27	4.35	51.4		5/15/2021
JS-3	2016	0.9	0	10	0							7.6	27	3.97	49.3		5/15/2021
JS-3	2016	0.9	0	10	0							7.6	26	4.12	49		5/15/2021
Control	2016	0	1	10	10							8	25	6.67	79.9		5/15/2021
Control	2016	0	1	10	10	8.1	25	8.34	97.5	25°C	5/11/2021	8	26	6.77	80.8	25°C	5/15/2021
Control	2016	0	1	10	10							8	27	6.38	76		5/15/2021

JS-4	2017	15	10	10	100							7.7	26	5.83	69		4/15/2021
JS-4	2017	15	10	10	100							7.7	26	5.51	65.2		4/15/2021
JS-4	2017	15	10	10	100							7.8	25	6.03	70.5		4/15/2021
JS-4	2017	7.5	10	10	100							7.8	27	5.6	66.1		4/15/2021
JS-4	2017	7.5	10	10	100							8	25	5.9	69.6		4/15/2021
JS-4	2017	7.5	10	10	100							8	26	6.05	71.7		4/16/2021
JS-4	2017	3.75	10	10	100							8	26	6.28	74.8		4/17/2021
JS-4	2017	3.75	10	10	100	7.4	25	7.16	80.7	25°C	4/11/2021	7.8	25	5.7	69.5	25°C	4/17/2021
JS-4	2017	3.75	10	10	100							7.9	25	6.01	73.8		4/17/2021
JS-4	2017	1.875	3	10	30							7.9	26	5.64	64.8		4/17/2021
JS-4	2017	1.875	4	10	40							7.9	25	5.92	72.2		4/17/2021
JS-4	2017	1.875	0	10	0							7.9	25	5.34	65.4		4/17/2021
JS-4	2017	0.9	0	10	0							8	25	5.75	69.5		4/17/2021
JS-4	2017	0.9	0	10	0							7.9	26	5.76	69.9		4/17/2021
JS-4	2017	0.9	0	10	0							7.9	26	5.36	65.5		4/17/2021
Control	2017	0	1	10	10							7.9	25	5.78	69.4		4/17/2021
Control	2017	0	0	10	0	8.8	25	7.68	87.6	25°C	4/11/2021	8	25	5.99	71.6	25°C	4/17/2021
Control	2017	0	0	10	0							8	25	6.22	74.2		4/17/2021

Definitive Testing Data

Weathered tire treatments

						Initial water quality						Final water quality					
Treatment ID	Treatment Year	Dose (g/L)	Number Affected	Total Mysids	Percent Mortality	pH	Salinity (ppt)	DO mg/L	DO %	Temperature (°C)	Date	pH	Salinity (ppt)	DO mg/L	DO %	Temperature (°C)	Date
W-JS-New	2018 (new tire)	15	10	10	100							7.8	25	5.97	69.3		4/8/2021
W-JS-New	2018 (new tire)	15	10	10	100							7.8	26	5.88	68.3		4/8/2021
W-JS-New	2018 (new tire)	15	10	10	100							7.8	25	5.6	65.6		4/8/2021
W-JS-New	2018 (new tire)	7.5	5	10	50							7.8	25	5.92	67.9		4/10/2021
W-JS-New	2018 (new tire)	7.5	4	10	40							7.8	25	5.88	69.7		4/10/2021
W-JS-New	2018 (new tire)	7.5	3	10	30							7.9	25	5.93	70		4/10/2021
W-JS-New	2018 (new tire)	3.75	0	10	0							7.9	25	6.01	70.9		4/10/2021
W-JS-New	2018 (new tire)	3.75	0	10	0	7	25	6.62	74.4	25°C	4/6/2021	7.9	26	6.1	72.3	25°C	4/10/2021
W-JS-New	2018 (new tire)	3.75	0	10	0							8	26	5.91	69.9		4/10/2021
W-JS-New	2018 (new tire)	1.875	0	10	0							7.8	25	6.06	71.6		4/10/2021
W-JS-New	2018 (new tire)	1.875	0	10	0							7.9	26	6.17	73.3		4/10/2021
W-JS-New	2018 (new tire)	1.875	0	10	0							8	26	6.1	72.2		4/10/2021
W-JS-New	2018 (new tire)	0.9	0	10	0							7.8	26	6.17	72.9		4/10/2021
W-JS-New	2018 (new tire)	0.9	0	10	0							7.9	25	6.26	74.2		4/10/2021
W-JS-New	2018 (new tire)	0.9	0	10	0							7.9	25	6.22	74		4/10/2021
Control	2018 (new tire)	0	1	10	10							8.1	25.5	6.48	76.5		4/10/2021
Control	2018 (new tire)	0	0	10	0	8.7	25	7.56	87.5	25°C	4/6/2021	8.1	25	6.15	73	25°C	4/10/2021
Control	2018 (new tire)	0	0	10	0							8.1	25	6.03	71.2		4/10/2021

W-JS-1	2014	15	10	10	100							7.5	25	5.67	65.2		4/8/2021
W-JS-1	2014	15	10	10	100							7.6	25	5.73	66.8		4/8/2021
W-JS-1	2014	15	10	10	100							7.6	25	5.54	65.5		4/8/2021
W-JS-1	2014	7.5	10	10	100							8	25	6.35	75		4/10/2021
W-JS-1	2014	7.5	10	10	100							7.9	25	6.23	73.2		4/9/2021
W-JS-1	2014	7.5	10	10	100							7.9	25	6.18	73		4/9/2021
W-JS-1	2014	3.75	5	10	50							8	26	5.88	69		4/10/2021
W-JS-1	2014	3.75	6	10	60	7	25	6.41	71.7	25°C	4/6/2021	7.9	25	5.6	66.1	25°C	4/10/2021
W-JS-1	2014	3.75	7	10	70							7.9	25	5.7	67.5		4/10/2021
W-JS-1	2014	1.875	0	10	0							8	25	6.21	73.4		4/10/2021
W-JS-1	2014	1.875	0	10	0							7.9	25	5.99	70.5		4/10/2021
W-JS-1	2014	1.875	0	10	0							7.9	25	5.82	68.9		4/10/2021
W-JS-1	2014	0.9	0	10	0							8	26	6.14	72.5		4/10/2021
W-JS-1	2014	0.9	0	10	0							7.9	26	6	70.7		4/10/2021
W-JS-1	2014	0.9	0	10	0							7.9	25	5.95	70.3		4/10/2021
Control	2014	0	1	10	10							8.1	25.5	6.48	76.5		4/10/2021
Control	2014	0	0	10	0	8.7	25	7.56	87.5	25°C	4/6/2021	8.1	25	6.15	73	25°C	4/10/2021
Control	2014	0	0	10	0							8.1	25	6.03	71.2		4/10/2021
W-IG-3-18	2018	25	10	10	100							7.9	26	6.4	75.6		5/23/2021
W-IG-3-18	2018	25	10	10	100							7.9	27	6.11	72.3		5/23/2021
W-IG-3-18	2018	25	10	10	100							7.9	27	6.37	75.4		5/23/2021
W-IG-3-18	2018	15	8	10	80							7.8	26	6	70.4		5/24/2021
W-IG-3-18	2018	15	9	10	90							7.9	26	6.27	73.5		5/24/2021
W-IG-3-18	2018	15	9	10	90							7.8	27	5.7	67		5/24/2021
W-IG-3-18	2018	9	4	10	40							7.6	26	2.77	33.2		5/24/2021
W-IG-3-18	2018	9	6	10	60	7.6	26	7.18	82.1	25°C	5/20/2021	7.5	26	2.68	32.1	25°C	5/24/2021
W-IG-3-18	2018	9	3	10	30							7.5	26	2.32	28.6		5/24/2021
W-IG-3-18	2018	5.4	1	10	10							7.4	26	2.2	25.2		5/24/2021
W-IG-3-18	2018	5.4	0	10	0							7.4	26	2.01	22.8		5/24/2021
W-IG-3-18	2018	5.4	0	10	0							7.4	27	1.76	20.3		5/24/2021
W-IG-3-18	2018	3.24	0	10	0							7.4	26	3.98	43		5/24/2021
W-IG-3-18	2018	3.24	0	10	0							7.3	26	2.05	23.5		5/24/2021
W-IG-3-18	2018	3.24	0	10	0							7.3	26	2	23.3		5/24/2021
Control	2018	0	1	10	10							8	25	6.22	72.7		5/24/2021
Control	2018	0	0	10	0	8.1	26	8.8	98.4	25°C	5/20/2021	7.9	25	6.3	73.4	25°C	5/24/2021
Control	2018	0	0	10	0							7.9	25	6.25	73.2		5/24/2021

W-JS-4	2017	15	10	10	100							7.9	26	5.8	68		4/23/2021
W-JS-4	2017	15	10	10	100							7.9	26	5.95	70.2		4/23/2021
W-JS-4	2017	15	10	10	100							8	26	5.56	65.6		4/23/2021
W-JS-4	2017	7.5	8	10	80							7.8	26	5.04	59.2		4/24/2021
W-JS-4	2017	7.5	8	10	80							7.8	27	5.07	58.5		4/24/2021
W-JS-4	2017	7.5	8	10	80							7.8	26	5.13	59.2		4/24/2021
W-JS-4	2017	3.75	2	10	20							7.7	26	4.44	51.9		4/24/2021
W-JS-4	2017	3.75	2	10	20	7.8	26	6.78	78.7	25°C	4/20/2021	7.5	26	4.25	48.6	25°C	4/24/2021
W-JS-4	2017	3.75	2	10	20							7.4	26	4.08	42.6		4/24/2021
W-JS-4	2017	1.875	0	10	0							7.5	25	3.56	42.1		4/24/2021
W-JS-4	2017	1.875	0	10	0							7.3	25	3.62	43.1		4/24/2021
W-JS-4	2017	1.875	0	10	0							7.3	26	3.49	40.8		4/24/2021
W-JS-4	2017	0.9	1	10	0							7.4	25	3.31	39.2		4/24/2021
W-JS-4	2017	0.9	0	10	0							7.5	26	3.39	40		4/24/2021
W-JS-4	2017	0.9	0	10	0							7.4	26	3.32	39.5		4/24/2021
Control	2017	0	0	10	0							7.4	25	3.41	38.4		4/24/2021
Control	2017	0	0	10	0	8.6	25	6.79	80	25°C	4/20/2021	7.4	26	3.5	41.6	25°C	4/24/2021
Control	2017	0	0	10	0							7.4	26	3.47	39.5		4/24/2021
W-JS-3	2016	25	10	10	100							7.8	27	6.21	74		5/13/2021
W-JS-3	2016	25	10	10	100							7.8	27	6.3	75.1		5/13/2021
W-JS-3	2016	25	10	10	100							7.8	27	5.5	65.6		5/13/2021
W-JS-3	2016	15	7	10	70							8	27	7.03	83.9		5/15/2021
W-JS-3	2016	15	5	10	50							7.9	27	6.54	78.1		5/15/2021
W-JS-3	2016	15	6	10	60							8	26	6.75	80.8		5/15/2021
W-JS-3	2016	9	2	10	20							8	27	6.62	78.7		5/15/2021
W-JS-3	2016	9	2	10	20	7.3	26	6.83	78.8	25°C	5/11/2021	8	26	6.7	79.9	25°C	5/15/2021
W-JS-3	2016	9	3	10	30							8.1	26	6.77	80.6		5/15/2021
W-JS-3	2016	5.4	0	10	0							8.1	26	6.7	79.8		5/15/2021
W-JS-3	2016	5.4	1	10	10							8	27	6.39	75.5		5/15/2021
W-JS-3	2016	5.4	2	10	20							8	27	6.48	77		5/15/2021
W-JS-3	2016	3.24	0	10	0							7.9	26	6.82	79.3		5/15/2021
W-JS-3	2016	3.24	0	10	0							7.9	26	5.9	70.2		5/15/2021
W-JS-3	2016	3.24	0	10	0							8	27	6	71.5		5/15/2021
Control	2016	0	1	10	10							8	25	6.67	79.9		5/15/2021
Control	2016	0	1	10	10	8.1	25	8.34	97.5	25°C	5/11/2021	8	26	6.77	80.8	25°C	5/15/2021
Control	2016	0	1	10	10							8	27	6.38	76		5/15/2021

W-IG-3-15	2015	15	10	10	100							7.8	27	5.71	67.4		5/6/2021
W-IG-3-15	2015	15	10	10	100							7.8	28	5.85	69.2		5/6/2021
W-IG-3-15	2015	15	10	10	100							7.9	28	6.4	75.7		5/7/2021
W-IG-3-15	2015	7.5	8	10	80							8	27	6.72	79.1		5/8/2021
W-IG-3-15	2015	7.5	9	10	90							8	27	6.51	76.6		5/8/2021
W-IG-3-15	2015	7.5	9	10	90							8	28	6.88	80.9		5/8/2021
W-IG-3-15	2015	3.75	0	10	0							8	26	6.66	77.6		5/8/2021
W-IG-3-15	2015	3.75	1	10	10	7.5	26	7.36	83.7	25°C	5/4/2021	8	26	6.43	75.7	25°C	5/8/2021
W-IG-3-15	2015	3.75	0	10	0							8	26	6.48	76.2		5/8/2021
W-IG-3-15	2015	1.875	1	10	10							7.9	27	6.51	76.8		5/8/2021
W-IG-3-15	2015	1.875	0	10	0							7.9	27	6.39	75.3		5/8/2021
W-IG-3-15	2015	1.875	0	10	0							7.8	27	5.54	65.4		5/8/2021
W-IG-3-15	2015	0.9	0	10	0							7.6	26	3.7	43.8		5/8/2021
W-IG-3-15	2015	0.9	0	10	0							7.5	26	3.27	37.6		5/8/2021
W-IG-3-15	2015	0.9	0	10	0							7.5	26	4.96	58.6		5/8/2021
Control	2015	0	0	10	0							8.1	27	6.95	82.1		5/8/2021
Control	2015	0	0	10	0	8.2	25	7.97	93.3	25°C	5/4/2021	8.1	27	6.75	80	25°C	5/8/2021
Control	2015	0	0	10	0							8.1	27	6.89	81.4		5/8/2021
W-IG-1	2013	15	10	10	100							7.8	26	5.77	68.1		4/23/2021
W-IG-1	2013	15	10	10	100							7.8	26	5.63	67.1		4/22/2021
W-IG-1	2013	15	10	10	100							7.8	26	5.48	64.8		4/23/2021
W-IG-1	2013	7.5	10	10	100							7.9	26	5.92	68.5		4/24/2021
W-IG-1	2013	7.5	10	10	100							7.9	26	5.66	66.7		4/23/2021
W-IG-1	2013	7.5	9	10	90							8	26	5.8	67		4/24/2021
W-IG-1	2013	3.75	5	10	50							7.9	26	5.4	62.3		4/24/2021
W-IG-1	2013	3.75	3	10	30	7.7	26	6.41	74.4	25°C	4/20/2021	7.9	26	5.26	61	25°C	4/24/2021
W-IG-1	2013	3.75	2	10	20							7.9	25	5.19	59.9		4/24/2021
W-IG-1	2013	1.875	0	10	0							7.8	26	5.1	59		4/24/2021
W-IG-1	2013	1.875	0	10	0							7.8	25	5.08	58.6		4/24/2021
W-IG-1	2013	1.875	1	10	10							7.9	26	5.46	63.2		4/24/2021
W-IG-1	2013	0.9	0	10	0							7.9	26	5.41	62.2		4/24/2021
W-IG-1	2013	0.9	0	10	0							7.9	26	5.66	65		4/24/2021
W-IG-1	2013	0.9	0	10	0							7.9	26	5.4	62		4/24/2021
Control	2013	0	0	10	0							7.4	25	3.41	38.4		4/24/2021
Control	2013	0	0	10	0	8.6	25	6.79	80	25°C	4/20/2021	7.4	26	3.5	41.6	25°C	4/24/2021
Control	2013	0	0	10	0							7.4	26	3.47	39.5		4/24/2021

Appendix E

Definitive Testing Ratio Test Results
drc Package in R

Ratio Test Comparison	Estimate	Standard Error	T-value	P-value	Significance Code
JS-New/JS-1	0.988317	0.491877541	-0.023752	0.981050352	
JS-New/JS-3	1.702731	0.705905479	0.995503	0.319491604	
JS-New/JS-4	1.757572	0.823550338	0.919886	0.357632536	
JS-New/IG-1	1.595237	0.658504749	0.903923	0.366036327	
JS-New/IG-3-15	1.310143	0.541041652	0.573234	0.566486304	
JS-New/IG-3-18	1.310543	0.541555431	0.573427	0.566355473	
JS-New/W-JS-New	0.452852	0.191551032	-2.856409	0.004284634	**
JS-New/W-JS-1	0.945737	0.425519893	-0.127523	0.898526735	
JS-New/W-JS-3	0.287345	0.118732262	-6.002205	1.94656E-09	***
JS-New/W-JS-4	0.669828	0.278106955	-1.187214	0.235143186	
JS-New/W-IG-1	0.831757	0.34328903	-0.490091	0.624069494	
JS-New/W-IG-3-15	0.621014	0.256526355	-1.477377	0.139574631	
JS-New/W-IG-3-18	0.354759	0.145964784	-4.420527	9.84605E-06	***
JS-1/JS-3	1.72286	0.509794707	1.417942	0.156207588	
JS-1/JS-4	1.778349	0.654003051	1.19013	0.233995226	
JS-1/IG-1	1.614095	0.473581396	1.296705	0.19473287	
JS-1/IG-3-15	1.325631	0.389260977	0.836536	0.402853248	
JS-1/IG-3-18	1.326035	0.389875981	0.836253	0.403012751	
JS-1/W-JS-New	0.458205	0.140932022	-3.844369	0.000120863	***
JS-1/W-JS-1	0.956916	0.328890245	-0.130997	0.89577762	
JS-1/W-JS-3	0.290741	0.085472584	-8.298083	< 2.2e-16	***
JS-1/W-JS-4	0.677746	0.201132932	-1.602196	0.109112352	
JS-1/W-IG-1	0.84159	0.246846464	-0.641737	0.521044045	
JS-1/W-IG-3-15	0.628355	0.184611305	-2.013121	0.04410186	*
JS-1/W-IG-3-18	0.358952	0.104637028	-6.126393	8.98936E-10	***
JS-3/JS-4	1.032208	0.251612128	0.128005	0.898144889	
JS-3/IG-1	0.93687	0.094891408	-0.665288	0.505866078	
JS-3/IG-3-15	0.769436	0.078462625	-2.938514	0.003297897	**
JS-3/IG-3-18	0.769671	0.079312385	-2.904075	0.003683402	**
JS-3/W-JS-New	0.265956	0.036439877	-20.14397	< 2.2e-16	***
JS-3/W-JS-1	0.555423	0.114235534	-3.891755	9.95218E-05	***
JS-3/W-JS-3	0.168755	0.017372909	-47.84719	0	***
JS-3/W-JS-4	0.393384	0.043529078	-13.93588	1.91735E-44	***
JS-3/W-IG-1	0.488484	0.049343475	-10.36643	1.76216E-25	***
JS-3/W-IG-3-15	0.364716	0.037358199	-17.0052	3.75741E-65	***
JS-3/W-IG-3-18	0.208347	0.019928688	-39.7243	0	***
JS-4/IG-1	0.907637	0.218490273	-0.422733	0.672490224	
JS-4/IG-3-15	0.745428	0.179659191	-1.416972	0.156491163	
JS-4/IG-3-18	0.745655	0.180053903	-1.412604	0.157772143	
JS-4/W-JS-New	0.257658	0.06642494	-11.17566	2.68261E-29	***
JS-4/W-JS-1	0.538093	0.161423187	-2.861469	0.004216832	**

JS-4/W-JS-3	0.16349	0.039470989	-21.19305	5.535E-100	***
JS-4/W-JS-4	0.38111	0.093300619	-6.633294	3.28277E-11	***
JS-4/W-IG-1	0.473242	0.113866751	-4.62609	3.72633E-06	***
JS-4/W-IG-3-15	0.353336	0.085227667	-7.587487	3.26288E-14	***
JS-4/W-IG-3-18	0.201846	0.048121785	-16.58613	4.39008E-62	***
IG-1/IG-3-15	0.821284	0.077599035	-2.303066	0.021275108	*
IG-1/IG-3-18	0.821535	0.078573238	-2.271327	0.023127203	*
IG-1/W-JS-New	0.283877	0.03734006	-19.1784	2.80412E-82	***
IG-1/W-JS-1	0.59285	0.119793916	-3.398753	0.000676937	***
IG-1/W-JS-3	0.180127	0.01720832	-47.64401	0	***
IG-1/W-JS-4	0.419892	0.043581352	-13.31092	9.99996E-41	***
IG-1/W-IG-1	0.5214	0.048723468	-9.822778	4.49167E-23	***
IG-1/W-IG-3-15	0.389292	0.036974099	-16.51717	1.3802E-61	***
IG-1/W-IG-3-18	0.222386	0.019486273	-39.90572	0	***
IG-3-15/IG-3-18	1.000305	0.096400297	0.003161	0.997478208	
IG-3-15/W-JS-New	0.345651	0.045648972	-14.33437	6.67251E-47	***
IG-3-15/W-JS-1	0.721857	0.146111476	-1.903634	0.056957908	.
IG-3-15/W-JS-3	0.219323	0.021112982	-36.97616	1.3838E-299	***
IG-3-15/W-JS-4	0.511263	0.053408472	-9.150932	2.82223E-20	***
IG-3-15/W-IG-1	0.63486	0.059799476	-6.10608	1.02108E-09	***
IG-3-15/W-IG-3-15	0.474004	0.045367752	-11.59404	2.20897E-31	***
IG-3-15/W-IG-3-18	0.270779	0.023941861	-30.45801	4.6918E-204	***
IG-3-18/W-JS-New	0.345545	0.045921957	-14.25145	2.19527E-46	***
IG-3-18/W-JS-1	0.721637	0.146458591	-1.900623	0.057351381	.
IG-3-18/W-JS-3	0.219256	0.021355608	-36.55919	6.3697E-293	***
IG-3-18/W-JS-4	0.511107	0.053927681	-9.065715	6.18947E-20	***
IG-3-18/W-IG-1	0.634666	0.060517851	-6.036794	1.57206E-09	***
IG-3-18/W-IG-3-15	0.47386	0.045895271	-11.46392	1.00117E-30	***
IG-3-18/W-IG-3-18	0.270696	0.024268981	-30.05087	1.0635E-198	***
W-JS-New/W-JS-1	2.088401	0.463917696	2.346108	0.018970611	*
W-JS-New/W-JS-3	0.634522	0.084277063	-4.336623	1.44689E-05	***
W-JS-New/W-JS-4	1.479131	0.205418027	2.332469	0.019676034	*
W-JS-New/W-IG-1	1.836709	0.241208349	3.468821	0.000522748	***
W-JS-New/W-IG-3-15	1.37134	0.181592398	2.044907	0.040863999	*
W-JS-New/W-IG-3-18	0.783388	0.099684713	-2.172973	0.02978238	*
W-JS-1/W-JS-3	0.303832	0.061648203	-11.2926	7.13762E-30	***
W-JS-1/W-JS-4	0.70826	0.146552235	-1.990689	0.046515127	*
W-JS-1/W-IG-1	0.879481	0.177591939	-0.67863	0.4973723	
W-JS-1/W-IG-3-15	0.656646	0.133063306	-2.580383	0.009869086	**
W-JS-1/W-IG-3-18	0.375114	0.07476015	-8.358548	3.17475E-17	***
W-JS-3/W-JS-4	2.331095	0.245730359	5.416891	6.06443E-08	***
W-JS-3/W-IG-1	2.894633	0.275702361	6.872021	6.32983E-12	***
W-JS-3/W-IG-3-15	2.161216	0.209093264	5.55358	2.79877E-08	***
W-JS-3/W-IG-3-18	1.234611	0.110546251	2.122285	0.033813795	*
W-JS-4/W-IG-1	1.241748	0.128553574	1.880525	0.060036524	.
W-JS-4/W-IG-3-15	0.927125	0.097264415	-0.749245	0.453709337	
W-JS-4/W-IG-3-18	0.529627	0.052063103	-9.03467	8.22488E-20	***

W-IG-1/W-IG-3-15	0.746629	0.070696388	-3.583933	0.000338459	***
W-IG-1/W-IG-3-18	0.426517	0.037238749	-15.40016	8.16302E-54	***
W-IG-3-15/W-IG-3-18	0.571257	0.050810439	-8.438082	1.61294E-17	***

Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Appendix F

Reference Toxicant Test Data

Reference Toxicant Test Data Percent Mortality (10 organisms per replicate)					Initial Water Quality Parameters					Final Water Quality Parameters				
Treatment Type	Exposure Time (hours)													
	24	48	72	96	pH	Salinity (ppt)	DO (mg/L)	DO (% sat)	Date	pH	Salinity (ppt)	DO (mg/L)	DO (% sat)	Date
Control	0	10	10	10						7.9	25.5	6.22	73.8	3/29/2021
Control	0	0	0	0						8	25.5	6.7	79.6	3/29/2021
Control	0	0	0	0						8	26	6.24	73.6	3/29/2021
CdCl2 12.5 ug/L	0	0	0	0						7.9	25.5	6.43	76	3/29/2021
CdCl2 12.5 ug/L	0	0	0	0						7.9	26.5	6.32	74.5	3/29/2021
CdCl2 25 ug/L	0	0	0	0						7.9	26	6.29	74.4	3/29/2021
CdCl2 25 ug/L	0	0	0	0	8	25	7.71	89.7	3/25/2021	7.9	26	6.27	74.1	3/29/2021
CdCl2 50 ug/L	0	30	60	70						7.9	25.5	6.3	74.3	3/29/2021
CdCl2 50 ug/L	0	20	30	40						7.9	25.5	6.11	72	3/29/2021
CdCl2 100 ug/L	0	50	90	100						7.9	25.5	6.43	76	3/29/2021
CdCl2 100 ug/L	0	60	90	100						8	25.5	6.47	76.6	3/29/2021
CdCl2 200 ug/L	10	90	100	100						7.9	25.5	5.52	64.1	3/28/2021
CdCl2 200 ug/L	30	90	100	100						7.9	25.5	5.21	60.6	3/28/2021
Control	0	0	0	10						8.1	25.5	6.48	76.5	4/10/2021
Control	0	0	0	0						8.1	25	6.15	73	4/10/2021
Control	0	0	0	0						8.1	25	6.03	71.2	4/10/2021
CdCl2 12.5 ug/L	10	10	10	10						8.1	25	6.44	76.5	4/10/2021
CdCl2 12.5 ug/L	0	0	0	0						8.1	25	6.44	76.4	4/10/2021
CdCl2 25 ug/L	0	0	0	10						8.2	25	6.49	76.4	4/10/2021
CdCl2 25 ug/L	0	0	0	10	8.7	25	7.56	87.5	4/6/2021	8.1	25	6.53	77.2	4/10/2021
CdCl2 50 ug/L	0	0	0	20						8.1	25	6.48	76.7	4/10/2021
CdCl2 50 ug/L	0	0	0	30						8.1	25	6.44	76.1	4/10/2021
CdCl2 100 ug/L	0	0	80	100						8.2	25	6.43	76.2	4/10/2021
CdCl2 100 ug/L	0	0	80	90						8.2	25	6.47	76.4	4/10/2021
CdCl2 200 ug/L	0	70	100	100						8.1	25	6.19	73	4/9/2021
CdCl2 200 ug/L	0	100	100	100						8.2	25	6.13	72.3	4/8/2021
Control	0	10	10	10						7.9	25	5.78	69.4	4/17/2021
Control	0	0	0	0						8	25	5.99	71.6	4/17/2021
Control	0	0	0	0						8	25	6.22	74.2	4/17/2021
CdCl2 12.5 ug/L	0	0	0	0						7.9	25	5.74	69	4/17/2021
CdCl2 12.5 ug/L	0	0	0	0						7.9	26	5.68	68.3	4/17/2021
CdCl2 25 ug/L	0	0	0	0						7.9	26	5.74	69	4/17/2021
CdCl2 25 ug/L	0	0	0	10	8.8	25	7.68	87.6	4/13/2021	7.9	26	5.83	70	4/17/2021
CdCl2 50 ug/L	0	0	0	60						7.9	25	5.82	70.2	4/17/2021
CdCl2 50 ug/L	0	0	0	40						7.9	26	5.72	68.8	4/17/2021
CdCl2 100 ug/L	0	50	100	100						7.9	25	6.07	78.2	4/16/2021
CdCl2 100 ug/L	0	70	90	100						8	25	6.01	71.2	4/17/2021
CdCl2 200 ug/L	0	100	100	100						8.2	26	6.52	78.7	4/15/2021
CdCl2 200 ug/L	0	100	100	100						8.2	26	6.57	77.2	4/15/2021
Control	0	0	0	0						7.4	25	3.41	38.4	4/24/2021
Control	0	0	0	0						7.4	26	3.5	41.6	4/24/2021
Control	0	0	0	0						7.4	26	3.47	39.5	4/24/2021
CdCl2 12.5 ug/L	30	30	30	30						7.4	25	3.03	35.7	4/24/2021
CdCl2 12.5 ug/L	0	0	0	0						7.4	25	2.94	37.6	4/24/2021
CdCl2 25 ug/L	0	0	0	0						7.3	25	3.07	36.4	4/24/2021
CdCl2 25 ug/L	0	0	0	10	8.6	25	6.79	80	4/20/2021	7.4	25	3.36	39.2	4/24/2021
CdCl2 50 ug/L	10	10	20	70						7.4	25	3.04	34.5	4/24/2021
CdCl2 50 ug/L	0	10	10	80						7.4	25	2.94	33.7	4/24/2021
CdCl2 100 ug/L	10	30	30	100						7.4	28	2.81	32.8	4/24/2021
CdCl2 100 ug/L	0	30	60	100						7.4	25	2.75	36.2	4/24/2021
CdCl2 200 ug/L	10	90	100	100						7.9	25	6.11	66.3	4/23/2021
CdCl2 200 ug/L	40	80	100	100						7.8	25	5.87	64.2	4/23/2021
Control	0	0	0	0						8.1	27	6.95	82.1	5/8/2021
Control	0	0	0	0						8.1	27	6.75	80	5/8/2021
Control	0	0	0	0						8.1	27	6.89	81.4	5/8/2021
CdCl2 12.5 ug/L	0	0	0	0						8	26	6.52	77.4	5/8/2021
CdCl2 12.5 ug/L	0	0	0	0						8.1	26	6.48	76.5	5/8/2021

CdCl2 25 ug/L	0	10	10	10						8.2	26	7.35	86.7	5/8/2021
CdCl2 25 ug/L	0	0	0	0	8.2	25	7.97	93.3	5/4/2021	8.1	26	6.92	82.2	5/8/2021
CdCl2 50 ug/L	0	0	40	50						8.1	26	6.88	81.5	5/8/2021
CdCl2 50 ug/L	0	0	0	30						8.1	26	6.79	80.5	5/8/2021
CdCl2 100 ug/L	10	10	90	100						8.1	26	6.67	77.6	5/8/2021
CdCl2 100 ug/L	0	0	60	100						8.1	27	7.22	85.8	5/8/2021
CdCl2 200 ug/L	20	90	100	100						8.2	26	7.2	83.1	5/7/2021
CdCl2 200 ug/L	40	70	100	100						8.1	26	7.18	83.8	5/7/2021
Control	0	0	10	10						8	25	6.67	76	5/14/2021
Control	0	0	0	10						8	26	6.77	80.8	5/14/2021
Control	10	10	10	10						8	27	6.38	79.9	5/14/2021
CdCl2 12.5 ug/L	0	0	0	0						8	26	6.33	75.6	5/14/2021
CdCl2 12.5 ug/L	0	0	0	10						8	26	6.42	76.3	5/14/2021
CdCl2 25 ug/L	0	0	0	0						8	26	6.38	73.9	5/14/2021
CdCl2 25 ug/L	0	0	0	0	8.1	25	8.34	97.5	5/11/2021	8.1	26	6.56	77.7	5/14/2021
CdCl2 50 ug/L	0	0	30	30						8.1	26	6.68	79.6	5/14/2021
CdCl2 50 ug/L	0	0	20	30						8	25	6.51	77.1	5/14/2021
CdCl2 100 ug/L	0	10	50	100						8	26	6.36	73.6	5/14/2021
CdCl2 100 ug/L	0	10	60	100						8	26	6.55	77.7	5/14/2021
CdCl2 200 ug/L	10	70	100	100						8	26	6.34	75.5	5/13/2021
CdCl2 200 ug/L	40	70	100	100						7.9	26	6.2	74.4	5/13/2021
Control	0	0	0	10						8	25	6.22	72.7	5/24/2021
Control	0	0	0	0						7.9	25	6.3	73.4	5/24/2021
Control	0	0	0	0						7.9	25	6.25	73.2	5/24/2021
CdCl2 12.5 ug/L	0	0	0	0						8	25	6.57	76.2	5/24/2021
CdCl2 12.5 ug/L	0	0	0	0						8	25	6.53	77.1	5/24/2021
CdCl2 25 ug/L	0	0	0	0						8	25	6.58	77.3	5/24/2021
CdCl2 25 ug/L	0	0	0	0	8.1	26	8.8	98.4	5/20/2021	8	25	6.35	74.7	5/24/2021
CdCl2 50 ug/L	0	0	0	20						8	25	6.51	76.2	5/24/2021
CdCl2 50 ug/L	0	0	0	10						8	25	6.46	75.9	5/24/2021
CdCl2 100 ug/L	0	30	90	100						8	25	7	82.2	5/24/2021
CdCl2 100 ug/L	0	10	100	100						8	25	5.66	67	5/23/2021
CdCl2 200 ug/L	10	100	100	100						8.1	25	7.47	88.9	5/22/2021
CdCl2 200 ug/L	10	100	100	100						8.1	25	7.66	91	5/22/2021

Appendix G

Trace Metal Analysis Raw Data
Data presented in mg/L

Tire Group	Treatment	Al	Cr	Mn	Co	Ni	Cu	Zn	Cd	Sb	Pb
2018-New	unweathered	0.22494	ND	0.00170	0.00057	0.18103	0.09258	2.74721	ND	ND	ND
	weathered	0.22471	ND	0.00210	0.00044	0.09301	0.08241	0.72654	ND	ND	ND
2018	unweathered	0.09230	ND	0.00217	0.00072	0.28016	0.11549	2.51705	ND	0.00088	ND
	weathered	0.12547	ND	0.00295	0.00058	0.18680	0.10299	0.65391	ND	ND	ND
2017	unweathered	0.13522	ND	0.00228	0.00320	0.24843	0.10774	3.72962	ND	0.00242	ND
	weathered	0.07104	ND	0.00184	0.00158	0.16428	0.10001	1.15009	ND	ND	ND
2016	unweathered	0.11639	ND	0.00290	0.00049	0.29940	0.11830	0.66065	ND	ND	0.00254
	weathered	0.11999	0.00136	0.00150	0.00046	0.15745	0.09586	0.45499	ND	ND	ND
2015	unweathered	0.11049	ND	0.00230	0.00078	0.30404	0.12078	2.56762	ND	ND	ND
	weathered	0.08730	ND	0.00148	0.00056	0.19122	0.09913	1.13864	ND	ND	ND
2014	unweathered	0.07693	ND	0.00066	0.00041	0.20661	0.09739	0.47255	ND	ND	ND
	weathered	0.14620	ND	0.00230	0.00066	0.17832	0.09617	2.00356	ND	ND	ND
2013	unweathered	0.16728	0.00134	0.00520	0.00172	0.29385	0.11934	3.72962	ND	ND	ND
	weathered	0.10076	ND	0.00025	0.00107	0.16550	0.09695	1.90790	ND	ND	ND
Detection Limits		0.00160	0.00014	0.00003	0.00000	0.00060	0.00011	0.00078	0.00002	0.00001	0.00005
EPA Aqualtic Life Criteria	CMC		1.10000			0.07400	0.00480	0.09000	0.03300		0.21000
EPA Aqualtic Life Criteria	CCC		0.05000			0.00820	0.00310	0.08100	0.00790		0.00810

*Metals analysis carried out using an Agilent 7500ce ICP-MS

Appendix H

Sediment Test Data

	Sediment Toxicity - (Percent Mortality) Exposure Time (hours)				Initial Water Quality Parameters					Final Water Quality Parameters				
Concentration	**24**	**48**	**72**	**96**	**pH**	**Salinity (ppt)**	**DO (mg/L)**	**DO (% sat)**	**Date**	**pH**	**Salinity (ppt)**	**DO (mg/L)**	**DO (% sat)**	**Date**
1 (g/L) A	0	0	0	0						7.9	27	8.34	92.4	4/17/2021
1 (g/L) B	0	0	0	0						7.9	27	73.3	83	4/17/2021
1 (g/L) C	10	10	10	10	7.1	25	7.1	79.1	4/13/2021	7.9	26	8.17	90.9	4/17/2021
1 (g/L) D	0	0	0	0						7.9	26	8.14	91	4/17/2021
1 (g/L) E	0	0	0	0						7.9	27	7.87	88.8	4/17/2021
5 (g/L) A	10	10	10	10						7.8	25	6.98	80.1	4/17/2021
5 (g/L) B	0	10	20	20						7.8	27	6.88	78.5	4/17/2021
5 (g/L) C	0	0	0	0	6.8	25	8.09	80.9	4/14/2021	7.8	25	8.44	93.6	4/17/2021
5 (g/L) D	10	10	10	10						7.8	25	8.48	90.4	4/17/2021
5 (g/L) E	10	10	10	10						7.8	25	6.92	79	4/17/2021