Matt Roberts
Graduate Research Proposal

A Comparative Analysis of the Toxicity of Tire Leachate in Marine Environments

Introduction:

The significance of this research is based on the emerging body of work on the impacts of microplastics accumulated in marine environments. While no internationally agreed definition of a microplastic exists, many researchers use a definition of particles in the size range 1 nm to less than 5 mm (GESAMP 2015). Particles from tires are increasingly identified as a type of microplastic in environmental samples (Barboza et al. 2018, Sommer et al. 2018). It is estimated that 1.7 billion tires are produced annually with light-truck and passenger vehicles making up the greatest proportion of the tire industry and that as much as 10% of the annual plastic inputs to oceans is tire wear particles (TWP) from automobiles (Kole et al. 2017).

TWP are a secondary microplastic, produced through abrasion of tire treads from contact with road surfaces (Grigoratos and Marini 2014, Kole et al. 2017, Kreider et al. 2010). Studies indicate that TWP are generated in a variety of sizes from approximately 10 nm to several 100 µm (Kreider et al. 2010). While most of these TWP are likely deposited near roadways, they can also be transported to the environment via air and water, most notably in stormwater run-off (Wik and Dave 2008). Kole et al. (2017) determined that urban areas with high amounts of city driving (stop and go versus highway driving) tend to be areas with the greatest accumulations of TWP. Given the diffuse nature of the generation of TWP, the range of physical sizes, and the potential to be transported, these particles ultimately are deposited in receiving waters where they can leach toxicants to marine and aquatic environments (Peter et al. 2018, Siegfreid et al. 2017, Wik and Dave 2008).

The general composition of modern tires is a complex mixture of rubber, metals, and various chemical additives. The primary components of tire tread are known; Edil (2008) lists common components as natural rubber, synthetic polymers such as styrene-butadiene copolymer, carbon black, extender oil (mixtures of aromatic hydrocarbons), metals (primarily zinc oxide), and sulfur compounds used in the vulcanization process. While those are the primary materials used in most tires, variations of metals, and other additives will exist from different manufacturers and different tire types; these differences can affect the rate of wear, the size of particles generated and potentially many other characteristics such as the leachate potential and components of leachate from tire particles (Grigoratos and Marini 2014, Khan et al. 2019). It has also been demonstrated that tires can sorb chemicals from roadways such as metals: aluminum, silicone, iron, sodium, calcium, potassium and magnesium (Krieder et al. 2010); and organic compounds such as toluene and xylene in laboratory experiments (Alamo-Nole et al. 2011). Consequently TWP can transport these toxicants to aquatic and marine systems (Kole et al. 2017). Additionally, chemicals from the environment may sorb to TWP once they enter the environment. Rochman et al. (2013) demonstrated that various types of common microplastics sorbed organic compounds while in marine environments and similar findings have resulted from the pellet watch program (Ogata et al. 2009), yet studies of this nature have not been conducted on TWP.

The toxicity from TWP can be caused by the chemicals from the tires as manufactured, the chemicals sorbed to the tires during use, and the chemicals that sorb to TWP once they are released into

the environment (Krieder et al. 2010, Alamo-Nole et al. 2011). Studies have demonstrated that tires and TWP can leach chemicals to soils and freshwater aquatic systems (Edil 2008, Wik and Dave 2009, Sheehan et al. 2006, Halle et al. 2019). The extent of leaching is less understood in marine environments, but data indicates that salinity likely acts to reduce the leaching of certain chemicals out of the tires when compared to aquatic systems (Hartwell et al. 2000). Toxicity may occur when exposures are from the aquatic environment as the chemicals leach from the tires, or when exposure occurs through ingestion where desorption of any chemicals associated with the tire particles can occur. Results from studies on other forms of microplastics indicate that exposure to simulated artificial conditions (artificial gut juice with pH of 4) could result in increased chemical desorption rates compared to exposure to seawater alone (Bakir et al. 2014, Stock et al. 2020), but this has not been investigated with TWP. Ultimately there are several gaps in our understanding of the role of TWP and their interactions with the environment related to toxicity in marine organisms.

Purpose of the Study:

The purpose of this study is to compare and evaluate leachate toxicity from tires in marine systems. Specifically, I will assess the toxicity from: a) new and used tires, b) under two leaching procedures, and c) before and after they have been deployed in a marine environment. As designed, I will be able to make comparisons that address how: a) age of the tire, b) exposure through the water or ingestion, and c) weathering of tires in a marine environment alter toxicity.

Research Design:

I propose to evaluate a common passenger vehicle all-season tire. The tires will be tested in four different ways: unweathered leached in saltwater, weathered leached in saltwater, unweathered leached in artificial gut juice, and weathered leached in artificial gut juice (Figure 1). The weathering of the tires will be carried about by a three-month field deployment in Bellingham Bay.

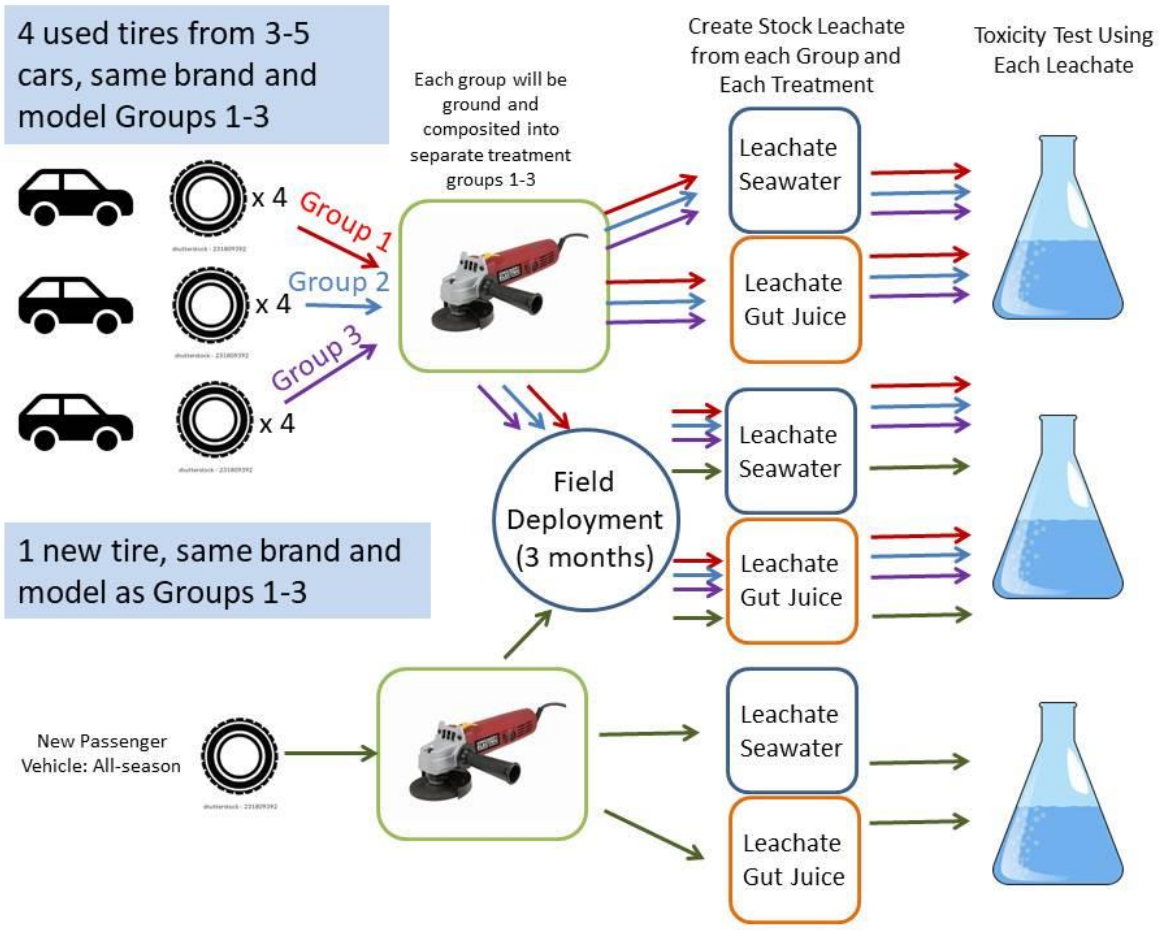

Figure 1:

Proposed experimental design with used tires from three cars. Tires will be ground using an angle grinder, tire particles will be separated in to different groups for generation leachate stock solution or weathering and then generation of leachate stock solution. All leachate formulations will then be used to conduct acute toxicity tests (n=16 with used tires from 3 cars).

Methods:

Used tires will be collected from three to five cars; all tires will be the same brand and model. The tires from each car will be ground and mixed in equal proportions to create three to five used tire composites. A new tire of the same brand and model will be purchased. For this experiment, the thread of tires will be ground into particles for use in the leachate tests. This will be carried out using an angle grinder with a special grinding blade. Tire particles will be collected using a shop vacuum connected to the guard on the angle-grinder. This method is used by the McIntyre laboratory from WSU Puyallup (Washington State University; personal communication) and would represent TWP for my work. Following the grinding of tires, tire particles will be sieved to determine the relative size distribution of the particles generated. The tire particles from the used tire composites and the new tire will then be divided in half; one that will be tested as is (unweathered; will be stored in the lab) and one that will be weathered.

The weathering process will be a field deployment of tire particles, in Bellingham Bay, for three months. This duration is comparable to previous work done with microplastics by Allie Johnson (WWU MS student) and has been shown by Rochman et al. (2013) to be a sufficient deployment time for steady-state to occur for some chemicals sorbing to microplastics. The weathering procedure will consist of placing tire particles into fine mesh nylon bags. Bags will be sewn shut and fitted with strong tethers of various colors to indicate which bag contains which particular tire particle type. This method has been adapted from methods developed by students in the Sofield laboratory (work in progress; unpublished). We are proposing a deployment site in Bellingham Bay, located at established United States Geological Survey (USGS) sites. The bags containing the tire particles will be tied to the top of cable that is anchored to the sediment of the seafloor at the site following methods developed by students in the Sofield laboratory (work in progress; unpublished). Grab samples of water from the deployment locations will be collected and analyzed for metals concentrations.

Leachate Testing:

I am proposing two separate leachate tests for tire particles in this experiment. One will be performed using filtered natural seawater (0.2 µM) at 21^{o}C for 48 hours (following methods currently used in the Sofield lab) and one will be performed using an artificial gut juice solution composed of 15.5 mM sodium taurocholate in seawater at 21^{o}C for 48 hours (Teuten et al. 2007, Bakir et al. 2014); this solution has been used to replicate leaching in benthic marine worms (Teuten et al. 2007). Stock leachate will be generated for each weathered and unweathered sample for the creation of 16-24[1] separate stock solutions (Figure 1). Leachate solutions will be analyzed for metals with ICP-MS[2] and used in acute toxicology tests utilizing an existing, in-house culture of mysid shrimp (*Americamysis bahia*). The test endpoint will be mortality and will follow the methods described in USEPA (2016). Dose-response curves will be generated to demonstrate the toxicity of the tire formulations using leachate from both used and new and weathered and unweathered tire particles. LC50s will be compared between samples and to expected environmental concentrations of tire particles in the environment.

Anticipated Results:

The test involving new, unweathered tires will provide a baseline for the toxicity of TWP to compare across the remaining tests. I anticipate that used tires will exhibit higher toxicity due to the sorption of substances from roadways during use. The difference between the new and used tires will provide insight into what substances are being sorbed and the relative hazards of those substances to marine organisms. It is less clear how weathering will affect toxicity. We may see an increase in toxicity from the sorption of organic compounds from the marine environment to TWP, conversely, we may see the weathering process promotes leaching of the tire chemicals and desorption of chemicals sorbed during use, resulting in less toxic TWP when compared to new or used tires.

[1] 16 if we have used tires from three cars, up to 24 if we have used tires from five cars.
[2] Another student will analyze these leachate samples and the site water samples with non-target analysis to identify organic chemicals

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Schedule for Completion:

Steps	Timeline
Fieldwork	
Grind tires	6/1/2020-7/1/2020
Preparation for deployment	7/1/2020
Deploy tires in Bellingham Bay	3-month time frame
Laboratory Work	
Leachate testing	Summer 2020 - Fall 2020
Reporting	
Data analysis and report preparation	Fall 2020-Spring 2021
Thesis defense	Spring 2021

Budget:

Some of the supplies required for this project are already available. These are included in Table 1. The detailed breakdown of the required materials needed to complete the project is in Table 2 and is the basis for this Grant Proposal.

Table 1. A partial list of required supplies, already available.

Previously Obtained Materials

Material Description	Supplier	Cost per unit	Number of units	Total
Angle Grinder Blades	Miller Tire	$100	3	$300
Nylon Bags	Duda Energy LLC	$14	17	$238

Total Expense: **$538**

Table 2. Supplies required to complete the project as proposed.

Material Description	Supplier	Cost per unit	Number of units	Total
Angle Grinder and Dust Shroud (Milwaukee Model # 6142-31S)	Home Depot	$180	1	$180.00
Angle Grinder Blades (Model #46MCM120)	Millers Tire	$130	4	$520.00
Shop Vacuum (Ridgid: 9-gallon Model# HD09001)	Home Depot	$70	1	$70.00
Shop Vacuum Bags (12 pack Model # VF3503B)	Home Depot	$75	1	$75.00
One new and 12-20 Used All-season tires	Discount Tire	$300	13-21	$300.00
Test Animal Food	Amazon	$50	2	$100.00
Tire Disposal (21 tires)	RDS	$7/tire	21	$147.00

Total:	$1,392.00
Tax: (Bellingham tax rate of 8.7%)	$121.10
Total Required:	**$1,513.10**

The figure for tire disposal would require that the tires be transported to a disposal facility.

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