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**The Interactive Effects of Nontoxic Levels of Microplastics -
Textile Microfibers and Tire Wear Particles, on the Acute Toxicity
of Bifenthrin in the Estuarine Grass Shrimp, *Palaemonetes pugio***

Xiaoxuan Fan

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The Interactive Effects of Nontoxic Levels of Microplastics - Textile
Microfibers and Tire Wear Particles, on the Acute Toxicity of Bifenthrin in
the Estuarine Grass Shrimp, *Palaemonetes pugio*

by

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DEDICATION

To dedicate my family whose unyielding love, support, and encouragement have enriched my spirit and inspired me to pursue and complete this research.

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I would like to acknowledge everyone who played a role in my academic accomplishment. First of all, it is a great pleasure to acknowledge my sincere appreciation to Dr. Geoffery I. Scott of the University of South Carolina for suggesting and supporting my research and thesis, and his kind supervision during my whole master's program. Then, I would like to express my thanks to my committee members: Dr. Dwayne E. Porter of the University of South Carolina and Dr. Paul Pennington of the National Oceanic and Atmospheric Administration Charleston Center, for their encouragement, creative and comprehensive advice until this work came to existence. In addition, I would like to convey my extreme gratitude to Lieutenant Colonel Hildehardo “JR” Viado for the kind help, generous advice and support during the study. Then, I want to express my appreciation to Dean G. Thomas Chandler and Ms. Maddie Meyer for their sharing of digital scale in their lab to support my research. I also want to thank Ms. Carson Maggie for her help to do water exchange and Mr. Samuel Putnam for his support to test chemical solution. Finally, this research would not be possible without the warm love and continued support from my family.

ABSTRACT

Contemporary pollution issues are primarily focused on Contaminants of Emerging Concern (CECs) which may include Brominated and Fluorinated flame retardants, Pharmaceuticals and Personal Care Products (PPCPs), Contemporary Use Pesticides (CUPs), nanomaterials and microplastics (Muraya et al., 2014). Tire wear particles and textile microfibers are examples of CECs which may gradually increase in the environment and may cause adverse effect on the health of some aquatic species (Marwood et al., 2011; Taylor et al., 2016). Microplastic particles are so small that they can be ingested by many marine species such as filter feeders and small particle feeders including mollusk and crustaceans, which will bioaccumulate within their body and some of the smallest particles may pass into individual cells where they are transparent and may cause harmful effects on a molecular level. Many studies (Gray and Weinstein, 2017; Qiao et al., 2019; Scherer et al., 2018) have observed that bioaccumulation of different sizes, types and shapes of microplastics and nanoplastics may occur. However, there is very little evidence about interaction between microplastics and coexisting contaminants. Therefore, the objective of this study was to evaluate toxicity of textile microfibers and tire wear particles and their influence on toxicity effect of bifenthrin, a contemporary use pyrethroid insecticide, toward estuarine adult grass shrimp, *Palaemonetes pugio*, a very widely distributed shallow water benthic macroinvertebrates which constitutes around 56% population of pelagic macrofaunal in tidal creeks of the southeastern coast of the United States.

Adult grass shrimps were initially exposed to microfibers, tire wear particles and bifenthrin individually and then later in mixtures of bifenthrin and microfibers, bifenthrin and tire wear particles (TWPs), and bifenthrin, microfibers (MFs) and tire wear particles. All bioassays were acute 96 hours, static renewal toxicity tests. Survival of grass shrimp was assessed after every 24 hours and each toxicity test was conducted for 96 hours under standard temperature and salinity conditions (20°C, 20 psu). Results of each exposed groups were made with the unexposed control group to assess acute toxic effects. Results were analyzed using SAS (Probit, Logit, Weibell) and Trimmed Spearman Karber Analysis (Hamilton et al, 1988). For the individual microplastics tested, there was no mortality after 96 hours of exposures for both MFs and TWPs at the highest doses tested, which were the Maximum Exposure Concentration (MEC) reported in scientific literature for each microplastic (Gray and Weinstein, 2017). Mixture tests with bifenthrin were conducted by selecting one significant concentration of each microplastic which was close to environmental concentration reported by Lead and Weinstein, (2019) for both TWPs and MFs. Results of bifenthrin had a 24 hours LC_{50} of 53.84 ng/L (95% CI = 45.15 – 66.79 ng/L, NOEC = 32 ng/L, LOEC = 56 ng/L), a 48 hours LC_{50} of 32.07 ng/L (95% CI = NC, NOEC = 18 ng/L, LOEC = 32 ng/L); a 72 hours LC_{50} of 25.31 ng/L (95% CI = 20.66 – 31.44 ng/L, NOEC = 10 ng/L, LOEC = 18 ng/L) and a 96 hours LC_{50} of 12.92 ng/L (95% CI = 3.01 – 24.03 ng/L, NOEC = <10 ng/L, LOEC = 10 ng/L). Results of mixture of bifenthrin and TWPs ad a 24 hours LC_{50} of 45.16 ng/L (95% CI = 30.60 –71.07 ng/L, NOEC = 18 ng/L, LOEC = 32 ng/L), a 48 hours LC_{50} of 21.00 ng/L (95% CI = 17.94 – 26.87 ng/L, NOEC = 18 ng/L, LOEC = 32 ng/L); a 72 hours LC_{50} of 19.51 ng/L (95% CI = 16.66 – 24.99 ng/L, NOEC = 10 ng/L, LOEC = 18 ng/L); and a and a 96 hours LC_{50} of

18.14 ng/L (95% CI = 15.39 – 22.69 ng/L, NOEC = 10 ng/L, LOEC = 18 ng/L). Results of mixture of bifenthrin and MFs had a 24 hours LC₅₀ of >100 ng/L (95% CI = 91.55 – 223.12 ng/L, NOEC = 32 ng/L, LOEC = 56 ng/L), a 48 hours LC₅₀ of 61.06 ng/L (95% CI = 47.31 – 83.40 ng/L, NOEC = 18 ng/L, LOEC = 32 ng/L); a 72 hours LC₅₀ of 40.53 ng/L (95% CI = 20.31 – 101.16 ng/L, NOEC = 32 ng/L, LOEC = 56 ng/L) and a and a 96 hours LC₅₀ of 19.69 ng/L (95% CI = 15.11 – 25.43 ng/L, NOEC = 10 ng/L, LOEC = 18 ng/L). Comparison of 96 hour results of the mixture tests of bifenthrin and MFs found 96-hour LC₅₀ of 19.69 ng/L (95% CI = 15.11 – 25.43 ng/L, NOEC = 10 ng/L, LOEC = 18 ng/L) while the mixture of bifenthrin and TWPs has a 96-hour LC₅₀ of 18.14 ng/L (95% CI = 15.39 – 22.69 ng/L, NOEC = 10 ng/L, LOEC = 18 ng/L), compared with bifenthrin test which has a 96-hour LC₅₀ of 12.92 ng/L (95% CI = 3.01 – 24.03 ng/L, NOEC <10 ng/L, LOEC = 18 ng/L). Overall these results showed that initially overall greater than additively toxic effect on bifenthrin caused by TWPs after 48 hours of exposure but less than additively toxic effects caused by MFs after 24-48 hours of exposure. As exposure times increased to 96 hours of exposure, both TWPs and MFs generally decreased the onset of toxicity of bifenthrin as evidenced by higher NOEC and LOEC values when compared to bifenthrin exposure *per se*; however, the 96-hour LC 50 values of binary mixtures of TWPs+ bifenthrin and MFs + bifenthrin were not significantly different than bifenthrin *per se*. Histological observations and analysis indicated that MFs caused clogging of the gill in grass shrimp which may reduce bifenthrin uptake at the gill which would reduce toxicity as was initially observed. Behavioral observations also indicated the bifenthrin + MF binary mixture enhanced hyperactive behavior, as grass shrimp actively bumped their head/rostrum against the sides of exposure containers. This effect was not observed in

either the bifenthrin nor the bifenthrin + TWP binary mixture exposures. This increased hyperactivity in the bifenthrin + MF binary mixture would likely alert predators and result in greater ecological death. These sublethal effects observed in the bifenthrin + MF binary mixture should be further evaluated with bifenthrin as well as other CECs in future studies.

TABLE OF CONTENTS

Dedication	iii
Acknowledgement	iv
Abstract	v
List of Table	viii
List of Figures	ix
List of Abbreviations	x
Chapter 1: Introduction	1
Chapter 2: Materials and Methods	21
Chapter 3: Results	29
Chapter 4: Discussion	33
Chapter 5: Conclusion.....	44
References	48

LIST OF TABLES

Table 2.1 Water quality parameters	23
Table 3.1 The results of acute toxicity tests for grass shrimp exposed to the mixture of bifenthrin with TWPs (97.5 mg/L = 50,000 particle/L= MEC) and Microfibers (100 g/= 88,500 particle/L = MEC).	31

LIST OF FIGURES

Figure 1.1 Chemical structures of the Pyrethroids Insecticides, Bifenthrin	17
Figure 2.1 Map of collection site for grass shrimp located at the CTL site on the western branch of Leadenwah Creek on Wadmalaw Island, SC22	24
Figure 3.1 Comparison of different LC50 values curves for adult grass shrimp exposures to bifenthrin, mixtures of bifenthrin and TWPs and mixtures of bifenthrin and microfibers	32
Figure 4.1 Dead adult grass shrimps with microfibers on their gill.....	39

LIST OF ABBREVIATIONS

CECs	Contaminants of Emerging Concern
CUPs	Contemporary Use Pesticides
DOM	Dissolved Organic Matter
HOCs.....	Hydrophobic Organic Chemicals
LOEC	Lowest Observable Effects Concentrations
MFs	Microfibers
MPs	Microplastics
MEC	Maximum Exposure Concentration
NOEC.....	No Observable Effects Concentration
PCPs	Personal Care Products
PE	Polyethylene
PP	Polypropylene
PVC.....	Polyvinyl Chloride
PS	Polystyrene
PET.....	Polyethylene Terephthalate
TWPs.....	Tire Wear Particles
WWTPs.....	Wastewater Treatment Plants

CHAPTER 1

INTRODUCTION

1. 1 Worldwide Plastic Consumption and Their Effects on Marine Ecosystems

The interaction between human and environment may result in a variety of environmental exposures to toxic chemicals which may adversely affect public health issue to people all over the world including increased risks of food safety, increased frequencies of asthma, chronic obstructive pulmonary disease, heart disease and stroke, and poor nutrition. Many environmental problems such as environmental degradation, global warming, and air/water pollution makes access to drinking water unsafe, while industrial pollution may cause severe air and water pollution all play roles in affecting these public health issues. Directly exposed to harmful pollutants, or indirectly influence from disturbed ecosystems pose risk to human health. It has been estimated that avoidable environmental problems can prevent 25% global disease burden among adult, and more than 30% disease burden among children annually (Pruss-Ustun et al., 2006). Therefore, efficient environmental protection can help decrease the development of death and disease. Due to close connected food web, great amounts of news, reports, research has shown existence of some kinds of pollutants in all parts of global ecosystems all over the world. For example, the ingestion of plastic particles has been proven among not only animals like seabirds, turtle, and whale, but also human organs due to contaminated food or trophic transfer (Campanale et al., 2020). With the detection and measurement of plastic materials in many

aquatic organisms, especially marine seafood species such as mollusks and crustaceans, the seafood consuming public is at risk not only of the microplastics but any other chemical, microbial and biological toxins which may absorb to these microplastic particles. Thus, new research should focus on the public health risk posed by microplastics within the aquatic environment.

Plastic materials were introduced into commerce nearly 60 years and are a unique material used every day in human society. It is an inexpensive, lightweight, strong, durable and corrosion resistant material that can be modeled into various shapes for a variety of uses including storage containers, packaging, grocery store bags and surface coating on cookware. Due to its excellent performance, it is estimated that there will be > 33 billion metric tons of plastic production by 2050, which is about 4 times more than current production levels (Geyer et al., 2017). Polyethylene (PE), polypropylene (PP), polyvinyl chloride (PVC), polystyrene (PS) and polyethylene terephthalate (PET) are synthetic plastics most commonly used in our daily life, which account for about 90% of the overall global plastic production (Andrady and Neal, 2009).

As the result of its widespread use all over the world, plastic debris, especially microplastics (MPs), has permeated throughout the marine ecosystems of the globe including the deep ocean, as one kind of prolific persistent pollutant that accounts for > 90% of global marine debris (Derraik, 2002). Back in 1972, the alarm of plastic pollution in the area of North Atlantic Ocean was sounded by E. J. Carpenter and K. L. Smith. They stated that there will be increasingly greater amounts of plastic debris on the sea surface (Carpenter and Smith, 1972). The rapid growth of plastics production has surpassed almost other widely used anthropogenically produced materials now, which has resulted in severe

plastic pollution in the environment. Mismanaged plastic use in commerce and by society results in initial discharges of plastics as litter and marine debris which may decompose due to physical, chemical and biological processes into smaller size plastic particles termed “microplastics” (MPs). In aquatic ecosystems, light plastic debris tends to float on the surface and dense plastic debris tends to partition onto particles which can still be transported by currents, but may also settle down into benthic sediments (Engler, 2012). Moreover, with long exposure to the natural environment, the physicochemical properties of marine debris and litter can be changed by abiotic factors (e.g. sunlight) or biological factors (e.g. microbial films), which may alter their physical size and chemical structure to form MPs, often equipping them with the ability to cause unpredictable adverse effects. In the end, from pole to pole, from the ocean surface to the seabed, MPs are ubiquitous and persistent, which threaten both aquatic environments and the living marine resources which reside in these habitats. However, as plastic usage increases along with the increasing world population, technology and concepts for waste management are still needed to be explored by researchers to reduce the input of MPs into aquatic ecosystems.

In addition to the environmental formation of MPs from macrolastics, these smaller MPs may also originate from a number of original commercial sources including the primary microplastics that are direct discharge of manufactured microplastics found in cosmetics, detergents and plastic products. Thus, there are both primary MPs used in these commercial activities which may be discharged directly into the environment as well as, the secondary MPs that arise from physical, chemical or biological degradation of improperly managed plastic wastes (Carbery et al., 2018). Textile fibers are one kind of primary MPs released from synthetic textiles during laundering (De Falco et al., 2019).

Wastewater that contains personal care products, cleaners or laundry effluent will discharge microplastic into wastewater treatment plants (WWTPs), which are inefficient at removing these particles. This inability to effectively remove primary MPs from WWTP effluent results in their direct discharge into aquatic ecosystems and this route of exposure can play a pivotal role in the increasing microplastic pollution in marine environments that often results in deteriorating ecosystem health (Fendall and Sewell, 2009). For instance, in coastal environments, compared to a control (more pristine and less polluted) site, coastal WWTPs discharged >250% more MPs in the United Kingdom (Browne et al., 2011). Despite the significant discharge of primary MPs such as MFs from WWTPS, the input of secondary MPs is generally been found to be greater in terms of the total MP pollution reported in most marine environments (Barnes et al., 2009). In addition, it is demonstrated that rivers contribute the most proportion of MPs into the oceans, with approximately 80% of MP pollution originating from land (Rochman, 2018). Recently, under the circumstance of the COVID-19 pandemic in 2019-2021, there has been a significant increase in the use of plastics for both disposable gloves and medical and non-medical single-use face masks that are produced from plastic materials adding another environmental challenge due to the increased primary and secondary MP pollution that has resulted for this increased consumption of these bulk plastics into our environment as solid wastes (Aragaw, 2020). Obviously, with increased population growth, urbanization and globalization as the progress of human society continues, the environment, humans, wildlife and aquatic biota will face more and more challenges of plastic pollution from different sources, forms and levels resulting from human activities.

1.2 Contaminants of Microplastics in the Estuarine Environment

Coastal areas contribute the majority of microplastics into the ocean, which partially originated from estuaries (Cheung et al., 2016). Due to urbanization in coastal areas, high population density and urban development bring great amounts of plastic debris that influx into the ocean mostly through point and nonpoint source runoff into estuaries. Estuaries have been recognized as microplastics hotspots (Wright et al., 2013). Bessa et al. (2018) found that microplastics have been measured in 38% of total fish in the Mondego estuary, which indicates the significant exposure on to aquatic life in estuarine environments. However, based on current studies, even though distribution of MP pollution in the marine oceanic environments has been well reported, data in estuarine waters is still poorly recorded and explored (Yonkos et al., 2014). Therefore, determining the source and occurrence of microplastics in estuaries is meaningful for MP pollution assessment and management.

As an important urban estuary in the southeastern Atlantic coast of the United States, Charleston Harbor in South Carolina is the 9th busiest port in the US with more than 1.9 million Twenty Foot Equivalent Container Units (TEUs), serving 100 foreign ports, providing an economic impact of \$63.4 Billion and supporting the direct and indirect employment of 187,200 people (ShipA/Freight, 2021). Charleston Harbor faces increased urban runoff that contains pollutants including microbes, nutrients, legacy pollutants such as petroleum hydrocarbons and trace metals and Contaminants of Emerging Concern (CECs) such as polybrominated flame retardants (Fair et al, 2007) and perfluorinated compounds (Soloff et al. 2017) as well as microplastics (Gray et al 2018), as there is an estimated 7 tons of plastic littering the shoreline of the Charleston Harbor including plastic

bags, plastic bottles and other items which are broken down into microplastics over time. This pollution results from urbanization and industrial/commercial development in this coastal area. The discharge of these pollutants damages the function of estuaries as a natural transition and buffer zone between land and sea. Damages on ecosystems and water quality in some estuaries have been reported in response to continuous influx of contaminants (Freeman et al., 2019). Several studies have found the appearance of MPs in Charleston Harbor. Gray et al., 2018 reported an average concentration of 413.8 ± 76.7 MP particles/m² in the intertidal sediment and 6.6 ± 1.3 MP particles/m² in the sea surface microlayer, which were dominated by black microplastics fragments (73% of total microplastics) which were suspected to be tire wear particles (TWPs). Additional studies have also reported that blue fibers and TWPs contributed most of the particle types, which were 26.2% and 17.1% of total microplastics, respectively (Leads and Weinstein, 2019).

1.3 Tire Wear Particles (TWPs) and Textile Microfibers Pollution

With the ongoing awareness of adverse effects of microplastics on the aquatic ecosystem, tire wear particles (TWPs) (Wagner et al., 2018) and texture microfibers (MFs) (De Falco et al., 2019) increasingly get attention as main sources of primary microplastic pollutants. Both MFS and TWPs are commonly observed in marine ecosystems.

1.3.1 Tire Wear Particles (TWPs) Pollution

The shear and heat caused by the contact between tire and road cause abrasion of the tire, resulting in shredding and shedding of bits of tire wear particles (Kole et al., 2017). TWPs can be dispersed in air as suspended particles and deposited onto the roadside which

then be further moved into nearby aquatic ecosystems mainly due to rainfall and resulting nonpoint source runoff (Kovochich et al., 2020). Studies have reported that as much as 11% of the total receiving water pollutant budget originates from highway runoff, which demonstrates their role as the major source of pollutants to receiving waters (Ellis and Mitchell, 2006). It has been estimated that 10% of total microplastic wastes in the ocean worldwide was caused by tires (Kole et al., 2017), and TWPs accounted for 42% of all microplastic emissions from rivers to the sea (Siegfried et al., 2017). Noren and Naustvoll, (2010) visually identified black particles which come from tires when investigated MP distribution in the environment. A study in the U.S. detected great amounts of black fragments which are consistent with TWPs. Black fragment particles have been recognized as the most abundant microplastic pollution in Charleston Harbor, SC, accounting for 73% and 69.7% of total microplastics in both Charleston Harbor and Winyah Bay intertidal sediments respectively (Gray et al., 2018). Other researchers from other countries such as Japan, Germany and France also found high TWPs concentration in aquatic environments (Dubaish et al., 2013; Kumata et al., 2000; Unice et al., 2019). By calculated annual TWPs generation on the German road network, 75,200-98,400 t/a ‘non-airborne’ particles are emitted, within which 66-67% are transported to road banks and soils near roads and 12-20% are discharged into surface waters (Baensch et al., 2021).

Except for its direct pollution as a potential contaminant by being released into the environment, TWPs are able to release heavy metals into the environment (Goonetilleke et al., 2017). It has been reported that as a major contributor of metals (e.g. Zn), tire wear particles are able to transport a significant amount of metals into the environment when compared to atmospheric inputs (Councell et al., 2004). Wang et al., (2021) found a co-

relationship between metals release and different traffic activities and sources. They demonstrated that emission factors of some metals (e.g. Ti, Fe, Cu) are more than two times higher at the highway sites where they are full of heavy-duty vehicles. Besides, emission factors on weekday for some kinds of metal like Mn, Zn, Ca and Fe are also relatively higher than weekends due to different frequency of traffic activities (Wang et al., 2021). Release of TWPs is also closely related to air quality because particles generated from the abrasion of road surfaces contribute to PM of mineral origin (Tervahattu et al., 2006).

Obviously, TWPs are increasingly an environmental threat because of their ability to adversely affect environmental quality. However, until now, most MP research studies have focused on thermoplastic materials like polyethylene or polystyrene while elastomers like rubber were less considered. Data on TWP accumulation and distribution are still rare due to the difficulties accurately collecting NPS runoff data (Wagner et al., 2018). TWPs are difficult to study due to change of morphology and composition following interactions with pavement materials, in addition to interacting with other components of the environment, such as sediments and other particulate matter. Because of the complexities, TWPs are much more difficult to be distinguished, tracked and measured (Wagner et al., 2018). Due to the increasing threat of TWPs to our environment, new technology and measurements that help to explore adverse effects on environmental and biological health of aquatic organisms are necessary to be developed.

1.3.2 Texture Fibers Pollution

In addition to tire wear products, textile microfibers, a microplastic sub-group made up of a number of polymers including nylon, polyester (PE), rayon, polyethylene

terephthalate (PET), polypropylene (PP) and acrylic, that are discharged from household laundry are also an important source of microplastics. Textile washing was ranked as seventh on scale of 10 in terms of MPs discharges (Verschoor et al., 2014). It was estimated that every washing discharges about 1,900 fibers (Browne et al., 2008). The median length of microfibers trapped by the filter with 60 μm pore size in washing machines is 510 μm , which is compatible with those coming from WWTPs and marine sediments (De Falco et al., 2019).

Microfibers occupy a high proportion of primary MPs contaminants, among which, according to a 2017 report of global apparel fiber consumption survey, synthetic fibers accounted for 65% of the total fiber demand. Large amounts of microfibers have been found in the aquatic ecosystems. The proportion of polyester and acrylic fibers from sediments is similar with those habitats that are downstream of WWTPs discharges of sewage effluent, which means that wastewater produced by the washing of clothes contribute to a great number of microfibers found in the marine and aquatic environments (Browne et al., 2011). The origination of microfibers from WWTPs containing effluents of waste water from clothes washing has been well established. Even though they can be removed from discharged laundry waste water efficiently, consecutive daily discharge of effluents with low concentrations of microfibers can still cause their accumulation in the aquatic environments (Lares et al., 2018). It was demonstrated that microfibers are the most abundant form of plastic debris in marine samples of the Gulf of Marine in the U.S. and the English Channel in the U.K (Lindeque et al., 2020). Some studies found bioaccumulation of microfibers in some marine species like the crab (Watt et al., 2015) and bivalve mollusks (Mankin et al., 2020), which resulted in adverse effects on their

health, including reduced food consumption and altered energy budgets. Large fibers with mean length more than 500 μm were detected within the marine zooplankton copepod and the euphausiid (e.g. krill) in the Northeast Pacific Ocean (Desforges et al., 2015). Other studies have reported that differences in the chemical nature of the textile microfibers can result in various degrees of the biodegradability of the microorganisms, which alter the diversity of microorganisms and the balance of microbial community (Zambrano et al., 2020). While there is significant information on environmental exposure levels in aquatic ecosystems and organisms about the widespread use of MFs, there is much less information about adverse effects including acute and chronic toxicity and bioconcentration/bioaccumulation in aquatic and marine species.

Driving and clothes washing, these two kinds of basic and essential human activities release a very large number of MP particles into the environment, which have made TWP and MFs become more and more abundant and widespread environmental pollutants. Presently over 3 billion people or nearly 50% of the world's population lives within 200 kilometers of the coast. In the US, > 40% of the population lives within an area that is only 17% of the land mass and produces more than 50% of the nation's Gross Domestic Product (NOAA, 2020). This increased population density and economic activity in the coastal zone increases ecotoxicological pressures on coastal ecosystems. The combined emissions of textile MFs and car TWPs result in almost 70% of global MPs released into the oceans. Currently most toxicity tests with MPs have been conducted with individual microplastic particles, there are few if any studies which have examined MP mixtures. For example, will the toxicity of TWPs be enhanced by co-exposure to MFs? Conversely, will the toxicity of MFs be enhanced by co-exposure to TWPs? Interactions

between MPs and subsequent toxicity is governed by bioaccumulation and individual differences in inherent toxicity of each different type of MP particle as well as differences in sensitivity among different aquatic species. The subsequent bioconcentration/bioaccumulation and trophic transfer of MPs, it is also meaningful to study the risk related to microplastics exposure and bioaccumulation in different aquatic species with different feeding types such as carnivores, herbivores and omnivores as well as particle and deposit feeders, particularly those species that may translocate and accumulate MPs into the different organs and tissues of organisms, which would help researchers better understand the ecotoxicology and trophic transfer of these pervasive environmental pollutants.

1.4 Bioaccumulation and Toxicity of Microplastics to Aquatic Species

With invasion of MPs into marine environments, >690 marine species from diverse trophic levels have been observed microplastic particles in their bodies (Parker et al., 2020). It is well-verified that aquatic species exposed to microplastics can ingest microplastics that were accidentally consumed as foods because of their small size that is similar to plankton species, and then the transformation of microplastics through trophic food chains may occur (Windsor et al., 2019). Bioaccumulation occurs when the uptake of contaminants exceeds their egestion (Maher et al, 2016). Because of continuous ingestion as food, fish (Lusher et al., 2013), bivalves (Browne et al., 2008) and crustaceans (Murray and Cowie, 2011) have been reported to bioconcentrate/bioaccumulate MPs throughout different tissues in their body. A recent study showed that mayfly and caddisfly, two riverine macroinvertebrates, contain 0.01-0.04 MPs mg⁻¹ dry weight and intestinal

accumulation of 250nm nanoplastics and whole-body distribution of 24 nm plastic particles in mollusk, *Pecten maximus*, after 6h exposure (Al-Sid-Cheikh et al., 2018). MPs are usually accumulated in the digestive system of aquatic species because it is the primary pathway of MPs ingestion for most aquatic organisms by accidental ingestion. Based on properties of MPs (e.g. shape, size, component), the accumulation of MPs in different organs of marine species are extremely variable (Akhbarizadeh et al., 2019). Moreover, other kinds of non-ingestive accumulation such as adherence or absorption to outer tissues can also contribute to the bioaccumulation of MPs in non-digestive tissues such as the mantle in oysters or other bivalves (Abbasi et al., 2018).

Plastic products provide convenience to consumers, but they may also pose ecotoxicological threats to aquatic life. It has been widely shown that adverse effects may occur including reduced organ system functions (Lei et al., 2018), gene damage (Arias-Andres et al., 2018), endocrine disruption (Rochman et al., 2014), mortality (Jemec et al., 2016), and bioaccumulation of exotic pollutants (Brennecke et al., 2016) may occur. The harmful impacts of MPs on survival, food intake, reproductive capacity and gene expression among different marine species has been widely studied (Ma et al., 2019). When it comes to the possible mechanisms, MPs can cause physical impairments that affect feeding and growth by blocking the digestive system, which results in reduced sensation for feeding, less food consumption and reduced fitness (Gray and Weinstein, 2017). As it was reported by Besseling et al. (2013) after 28 days exposure of polystyrene spheres, lugworms suffered weight loss due to inactive feeding and mussels experienced augmented pseudofeces egestion and decreased filtering rate. The copepod *Centropages typicus* showed descending feeding after exposure of polystyrene beads (Cole et al., 2011). This

physical obstruction, blocking digestive system and disrupting feeding, is the most common adverse effect of MPs (Setälä et al., 2014). Moreover, by passing through cell membranes, they may translocate into other tissues such as the circulatory (Browne et al., 2008) and digestive systems (Lu et al., 2016). Lu et al., 2016 found accumulation of 5µm microplastics in zebrafish gills, liver, and gut after 7 days exposure, which altered their normal function and metabolism. On the other hand, as a vector of numerous pollutants, MPs can also cause health damages by releasing toxins from harmful algae and microbes as well as toxicants from legacy pollutants and CECs into the body of aquatic organisms (Ma et al., 2019). Contaminants including hydrophobic organic chemicals (HOCs), heavy metals, microbes, antibiotics, microbes and harmful algae can be adsorbed to microplastic particles which may concentrate these pollutants and then transmit them to aquatic consumers that ingest the particles (Brennecke et al. 2016). Until now, based on their simultaneous exposure, the majority of studies focused on the relationship (e.g. additive or less than additively toxic) between microplastics and adhered contaminants (Wang et al., 2019). However, very little research has been conducted on the effects of microplastic mixtures which may co-occur in the environment. Toxic concentrations that cause adverse effects have also been explored. For example, Polypropylene (PP) MP fibers had a 10-d LC50 of 71.43 fibers per mL in *Hyalalella azteca* which was > two times more toxic than Polyethylene (PE) MPs, with 10-d LC50 of 4.64×10^4 particles per mL (Au et al., 2015). Similarly. The algae feeding rate in the copepod (*Centropages typicus*) was decreased at >4000 mL/L Polystyrene (PS) microspheres (Cole et al., 2013).

Uptake of MPs occurs due to accidental ingestion by aquatic organism creatures has been widely reported for marine species. It is astonishing to know that MPs appear to

be present in aquatic food chains throughout the globe including the deep ocean (Fossi et al., 2014). It is well-known that trophic transfer of microplastics can result in increasingly higher concentration of exposure to consumers in higher trophic levels including humans that may consume seafood species. The existence of MPs in seafood, drinking water, in the air they breath and an even edible salt has been demonstrated (Belzagui et al., 2019), which means humans are exposed to MPs continuously. Li et al. (2015) demonstrated the transmission of microplastics through marine food chains from mussels (e.g. mollusk) to crabs (crustaceans) higher in the food web. Trophic transfer of microplastics can result in increasingly higher concentration of exposure to consumers at higher trophic levels. However, due to effective methods to estimate and distinguish between the contributions of both primary and secondary microplastic pollutants in the environment as well as the different types of MPs, estimating related risks to human health is still unclear. Estimates suggest that each person consumes the equivalent amount of plastic in a credit card annually (Weinstein, 2021). As the highest-level consumer, definitely, humans are vulnerable to MPs exposure and bioaccumulation (Carbery et al., 2018).

1.5 Bifenthrin (BF) and Its Toxicity Effect to Aquatic Species

The pesticide bifenthrin ((2-methyl-1, 1-biphenyl-3-yl)-methyl-3-(2-chloro-3,3,3-trifluoro-1-propenyl)-2,2-dimethylcyclopropanecarboxylate) (Figure 1) has been listed as Toxicity Class II moderately toxicant by the United States Environmental Protection Agency (WHO, 2009). As a third-generation type I synthetic pyrethroid, it has been widely used in farming, veterinary, household and landscape maintenance. Its usage has increased significantly the past few years due to the phase out of the “dirty dozen” organophosphate

insecticides due to public health concerns for pregnant women and children. It is estimated that the monthly amount of bifenthrin that was used for insect control in California has peaked at more than 2800 kg/month during July to October in 2006 (Weston et al., 2013). Based on the greater and greater amount of usage of bifenthrin, almost 60% of the agricultural and urban streams samples reported the detection, which makes bifenthrin as the most frequently detected insecticide in the United States (Kuivila, 2012). Another recent study estimated that nearly 80% environmental sample sites showed bifenthrin pollution (Jeppe et al., 2017). Therefore, with the gradually increased use of bifenthrin because of its greater insecticidal efficiency, enhanced hydrostability and photostability, it has attracted public concerns about its environmental damage and toxicological effects, particularly to aquatic invertebrates.

As a primary contributor to the toxicity in aquatic environments, bifenthrin has been recognized as one kind of toxicant that can pose high acute lethal toxicity to aquatic species at levels commonly measured in the environment. Pennington et al., (2014) demonstrated that after 24- and 96-hours exposure, the calculated median lethal concentration of shrimp to bifenthrin were 0.061 and 0.051 $\mu\text{g/L}$ respectively. Furthermore, compared to adult grass shrimp, younger grass shrimp were more sensitive to bifenthrin with the 96-h median lethal concentration of 0.013 $\mu\text{g/L}$ (Harper et al., 2008). Other aquatic animals like rainbow trout and sheepshead minnow showed 96-h median lethal concentration of 0.15 (Werner and Moran 2008) and 17.5 $\mu\text{g/L}$, respectively (Velisek et al. 2009a). In addition, the R-enantiomer was determined as the only active isomer that contributes to the acute toxicity of bifenthrin (Liu et al., 2005b). Liu et al., (2005b) also indicated that 1R-cis-bifenthrin isomer was the primary cause toxicity of bifenthrin to *C.*

dubia, with a 96-h LC₅₀ for the R-enantiomer was 0.0796 ± 0.018 µg/L. Results clearly indicated that the 1R- cis-bifenthrin was more active and toxic than S-enantiomer to *C. dubio* (Liu et al., 2005c).

Except for acute toxic effects, bifenthrin can also result in long-term and sublethal toxic effects, including but not limited to development toxicity, oxidative adverse effects, neurobehavioral toxicity, harmful immunological effects and endocrine disruption. In stream sediments, bifenthrin has a very long half-life, ranging from 251 ~ 1,950 days, and it is stable and persistent in aquatic environments, which poses chronic health threat to aquatic species (Gan et al., 2005). It is well-known that for many environmental compounds and some aquatic species adverse health effects are often more severe in early life stages due to their more sensitive and susceptible characteristics (e.g. larger surface area to volume ratio) when compared to later life stages. DeMicco et al., (2010) demonstrated reduced and altered growth and development in zebrafish embryos following exposure to bifenthrin. Bifenthrin has been found to cause developmental toxicity and effects in many non-target aquatic species by accelerating the hatching process, resulting in apparent morphological damages in embryos and early life history stages (DeMicco et al., 2010).

Many chemical contaminants induce oxidative free radicals which may cause oxidative damage and related stress responses, which are useful and sensitive biomarkers for assessing sublethal toxicological effects. There is evidence that bifenthrin can induce high relative molecular levels of stress response (Jin et al., 2010a). In addition, bifenthrin has been shown to cause immunological effects in aquatic species (Jin et al., 2010a).

In conclusion, with the increased of bifenthrin to aquatic animals and the increased use and subsequent exposure in aquatic ecosystems, more investigations about its lethal and sublethal toxicity among various species and under a broader range of environmental conditions is needed. Moreover, the interaction of bifenthrin with other chemicals present in the environment as environmental mixtures is needed to enhance our toxicological knowledge and to allow more realistic environmental risk assessment.

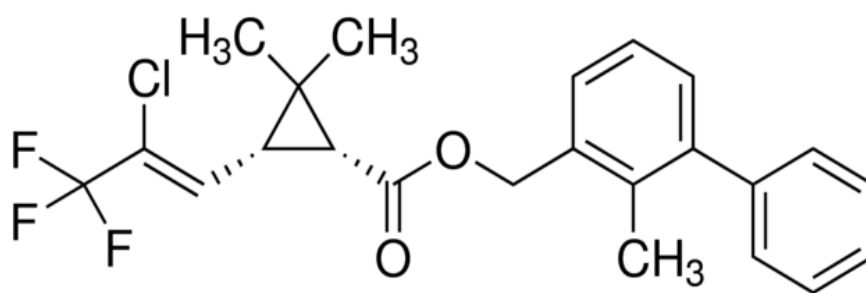


Figure 1.1 Chemical structures of the Pyrethroids Insecticides, Bifenthrin (After Sigma Chemicals, 2019).

1.6 Statement of Purpose and Objectives

1.6.1 Statement of Purpose

While initial toxicity tests have been conducted with both bifenthrin and individual MP particles, there are few if any studies which have examined interaction of MP particles and bifenthrin as chemical mixtures. Bifenthrin, TWP and MFs are all reported to co-occur within aquatic ecosystems. This poses a question - “Will the toxicity of bifenthrin be enhanced or decreased by co-exposure to TWP and MFs. Interactions between MP particles and chemicals such as bifenthrin and subsequent toxicity is governed by

interactions between the chemical to affect uptake/depuration kinetics and individual differences in inherent toxicity between each different type of MP particle and bifenthrin. The subsequent trophic transfer and accumulation are also meaningful to study as the risk related to MP exposure as there may be significantly alterations in the uptake/depuration rate kinetic and toxic interactions between each chemical, possibly affecting and altering the disposition and accumulation in different organs and tissues within an organism, which would help researchers better understand the ecotoxicology and trophic transfer of these pervasive environmental pollutants.

In this study we will evaluate two kinds of MP particles - TWPs from tire abrasion and MFs from textiles washing and one widely used insecticide, bifenthrin. Driving and clothes washing, these two kinds of basic and essential human activities release a very large number of MP particles into the environment, which resultantly discharge high levels of TWPs and MFs into the environment, thus becoming more abundant environmental pollutants. Bifenthrin is widely used in agriculture, horticulture, and for residential pest control and is very toxic to aquatic life and persistent in aquatic environments than previous generations of pyrethroids (Mokry and Hoagland, 1990). To do this both the toxicity of these individual MP particles and bifenthrin will be studied along with binary mixture studies of MPs and bifenthrin. This approach will determine the role MP particles may play when co-occurring with highly toxic chemicals such as bifenthrin.

1.6.2 Objectives

The objectives of this research will be determining the individual 96-h toxicity of bifenthrin, TWPs and MFs to adult grass shrimp, *Palaemonetes pugio*, under normal

condition of 20 °C and 20 psu salinity. In addition, the interaction between bifenthrin and individual TWPs and individual MFs as binary mixtures will be assessed to determine the potential for joint toxicity interactions (Additive Toxicity, Synergism or Antagonism). These findings will provide evidence for a better understanding of the risks posed by the co-occurrence of these compounds in aquatic ecosystems.

1.7 Hypothesis

The following hypothesis will be tested:

H1₀: Tire wear microplastic particles are not toxic to the estuarine grass shrimp, *P. pugio*.

H1_a: Tire wear microplastic particles are toxic to the estuarine grass shrimp, *P. pugio*.

H2₀: Microfiber microplastic particles are not toxic to the estuarine grass shrimp, *P. pugio*.

H2_a: Microfiber microplastic particles are toxic to the estuarine grass shrimp, *P. pugio*.

H3₀: The combination of exposure to tire wear particles and bifenthrin is additively toxic to the estuarine grass shrimp, *P. pugio*.

H3_a: The combination of exposure to tire wear particles and bifenthrin is either additively, greater than additively toxic or less than additively toxic to the estuarine grass shrimp, *P. pugio*.

H4₀: The combination of exposure to microfiber microplastic particles and bifenthrin is additively toxic to the estuarine grass shrimp, *P. pugio*.

H4_a: The combination of exposure to microfiber microplastic particles and bifenthrin is neither additively, greater than additively toxic nor less than additively toxic to the estuarine grass shrimp, *P. pugio*.

CHAPTER 2

MATERIALS AND METHODS

2.1 Test Species

As a very widely distributed, shallow water benthic macroinvertebrates, the grass shrimp, *Palaemonetes pugio*, constitutes around 56% population of pelagic macrofaunal in tidal creeks of the southeastern coast of the United States (Scott et al., 1994). As a habitat for various kinds of grasses, salt marsh ecosystems in estuaries are a challenging and dynamic environment which shows dramatic fluctuations of dissolved oxygen, salinity, temperature and pH as well as other environmental conditions, during each tidal cycle as well as diurnally and seasonally (Cochran and Burnett, 1996). Fortunately, the special ability of low-oxygen adaptation of grass shrimp in tidal marsh embayment provides an ideal environment with limited predation and competition for them to grow into a large population (Welsh, 1975). Cochran and Burnett, 1996 also demonstrated that grass shrimp show excellent adaptation to the environment of periodic hypoxia and hypercapnia. In addition, grass shrimp has euryhaline and eurythermal characteristics, which also allow them to maintain high population abundances in their habitat which would be stressful many other species, particularly predatory (Buikema et al., 1980).

Margalef (1968) indicated that grass shrimp act as an ecological pivot point for interacting with dominant trophic components to channel large quantities of energy in the coastal marine ecosystem. Among the food webs of Atlantic and Gulf coast estuaries, grass

shrimp play an important role in energy and nutrients cycling among numerous estuarine trophic levels by not only being a prey of many recreationally and commercially aquatic species but also acting as detritivores and bottom consumers (Anderson, 1985). Grass shrimp produce large amounts of waste produces used by other species including large amounts of fecal pellets, dissolved organic matter (DOM) and biomass production, which constitute 60% of total ingestion, large quantities of ammonia and phosphate excretion and unique way of detritus decomposition. Thus, they are important for speeding up detritus breakdown, preventing blockages and efficiently transferring energy all over the food web in the embayment (Welsh, 1975).

Due to these significant ecological properties, grass shrimp have become a “Corner Stone Species” that can be used for laboratory, mesocosm and field research. Its transparent carapace allows researchers to observe bioaccumulation of various contaminants such as MPs in the gut and gills directly and easily. In addition, it has been reported that grass shrimp can be an ideal bioindicator of human health and estuarine status, which makes them be involved in various biomonitoring studies and toxicity tests (Key et al., 2006). The spatial and temporal trends of population abundances, overall size-frequency distributions and sex ratios such as the percentage of male and gravid females are closely related to adjoining land use environmental conditions near estuarine systems (Leight et al., 2005). Besides, it has been shown that grass shrimp are highly sensitive to a variety of legacy pollutants such as pesticides, polycyclic aromatic hydrocarbons (PAHs), and trace metals as well as CECs such as triclosan and contemporary use pesticides (Al-Yassein, 2019; Leight et al., 2005). Therefore, based on these characteristics and utility in past studies, grass shrimp can be a good candidate for this research to detect the toxicity effects of tire

wire particles and textile microfibers and their influence on the acute toxicity effect of the Bifenthrin.

2.2 Collection and Acclimation

Adult *Palaemonetes pugio* were collected from Leadenwah Creek (32.6367°N, 80.2017°W) (Figure 2.1), a long-term study site used by the National Oceanic and Atmospheric Administration (NOAA), by using a dip net and transported back to laboratory at the University of South Carolina. Then, the grass shrimps were cultured, in glass aquaria with artificial seawater at room temperature (20°C) under 14 hours:10 hours light/dark cycle, at 20 practical salinity units (psus) salinity for at least 14 days prior to toxicity tests and other experiments. Aquarium water was changed at the middle of the acclimation period. Daily, shrimp will be fed Tetramin fish flakes (0.5 to 1.5 scoops per tank) once per day based on the amount of the grass shrimp in each tank (200-300/tank). Daily, water quality parameters in holding tanks were measured (e.g. water temperature (°C), salinity (practical salinity units = psu), pH, and dissolved oxygen (mg/L) in the entire acclimation period. However, they were not fed 24h prior to being introduced into the assays. Supplemental aerations were provided to each aquaria to ensure adequate oxygen levels (> 6 mg/L) during acclimation.

Table 2.1 Water quality parameters

Water Quality Parameter	Value Measured
Temperature	20 °C
Salinity	20 psu
PH	7.7- 8.1
Dissolved oxygen	≤ 6.80 mg/L

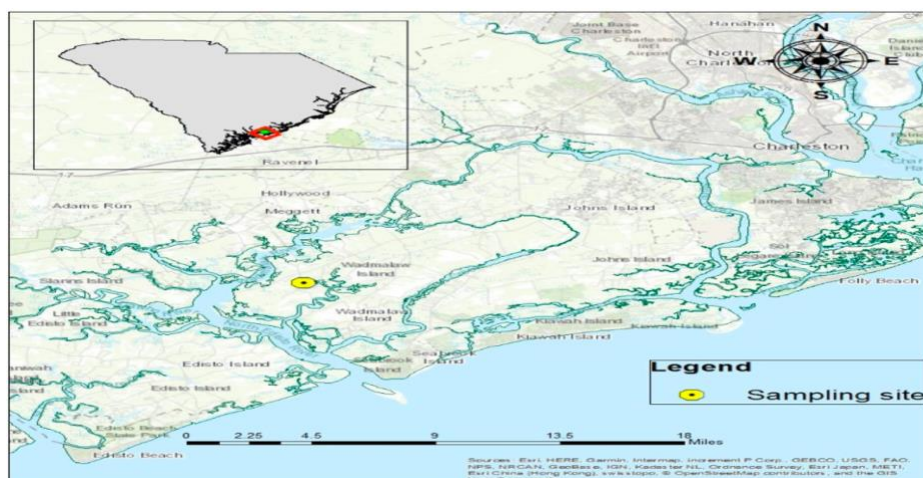


Figure 2.1 Map of collection site for grass shrimp located at the CTL site on the western branch of Leadenwah Creek on Wadmalaw Island, SC.

2.3 Toxicity Tests for TWP and Textile MFs

2.3.1 Preparation of Stock Concentrations of TWP and Textile MFs

TWPs with 38-68 μm diameter, which include the size of the most abundant TWPs ranging from 25 to 50 μm in the environment, were obtained from JR Viado. The small MFs were manually produced from a commercially manufactured 100% acrylic black yarn, which is one kind of yarn used in a wide range of clothing products. The acrylic yarn has a density of 1.18 g/cm^3 . Black color was selected to assure a better identification and tracking during toxicity tests. The yarn was manually cut into short fibers by using methods from other studies to produce microfibers (Bour et al., 2020; Gray and Weinstein, 2017). Briefly, the yarn was cut into possible short lengths with sharp scissors. Using sieves to measure a part of fibers that were produced at the initial cutting can help to roughly test whether this method of cutting can produce fibers with length at least below 500 μm , which are compatible with those of microplastics found in wastewater (>300 μm) discharged from

WWTPs and within the range of the most abundant fraction of microfibers reported during laundry washing reported by De Falco et al. (2019). By measuring the length of fibers created partially with sieves, most of the fibers were trapped between sieves with 300 μm and 500 μm pore sizes. Therefore, these fibers can be recognized as microplastics based on their estimated length at around 300-500 μm (an average of 400 μm) (Mattsson et al., 2015). In order to prepare the concentrations needed for each toxicity test, TWPs and MFs were weighted in advance by using digital microscale to get satisfied weight needed. Weighted particles were wrapped in solvent cleaned, dried aluminum small foil cup to avoid other pollutants. Gentle agitation and shaking from a magnetic field machine ensured suspension homogeneity before each toxicity test. Toxicity tests with TWPs were conducted with nominal concentrations ranging from 97.5 to 1,365 mg/L with the highest dose based upon the Maximum Exposure Concentrations reported in published studies (Gray and Weinstein, 2017). Similarly, toxicity tests with textile MFs were conducted with nominal concentrations ranging from 1 to 1,000 mg/L, with the highest dose based upon the Maximum Exposure Concentrations reported in published studies (Gray and Weinstein, 2017).

2.3.2 Preparation of Stock Concentrations of Bifenthrin

Bifenthrin purchased from Chem ServiceTM (purity 96.7%) was weighed and mixed with pesticide grade acetone and used to produce a concentrated bifenthrin stock solution of 44.482 mg/L. Daily working stocks with exposure concentrations of 100, 56, 32, 18 and 10 ng/L were created daily by mixing stock solution with Instant Ocean Seawater. The final concentration of acetone carrier was kept at 0.1% in each bifenthrin exposure treatment.

2.3.3 96 Hours Static Renewal Toxicity Tests

Adult grass shrimp were exposed in beakers containing 1L instant ocean seawater at 20 psu salinity and at 20°C temperature. A total of 5 animals per beaker will be used for each test, and three replicates for each exposure concentrations and controls (n=15 shrimps/treatment). MP particle tests (e.g. TWP - tire wear particles and MF - textile microfibers) were will be prepared by adding appropriate amounts (mg) of microplastics that had been weighted in advanced into beakers with 1L artificial salt water. Initially, unreplicated 96 hours range finding bioassays were conducted, using log spaced doses (MF group: control, 1, 10, 100, and 1,000 mg/L; TWPs group: control, 97.5, 975 and 1,365 mg/L), to identify the final exposure concentration to use for the definitive toxicity tests. Shrimp were not be fed. All toxicity tests were conducted at standard conditions (20°C, 20 psu) and 14 hours:10 hours light/dark cycle. Water changes were made every 24hours to and supplement aeration was not provided. Mortality and survival were the test end points used in all range finding bioassays.

The results of the range finding bioassays with the TWPs and textile MFs indicated that there was no grass shrimp mortality at the Maximum Exposure Concentration reported for each MP particle (100 mg/L (= 88,500 particle/L) for microfibers and 97.5 mg/L (= 50,000 particle/L) for TWPs) based upon our toxicology research and environmental monitoring research conducted by (Lead and Weinstein (2019). Thus, no further toxicity tests with individual MPs were conducted. Rather the MEC for each MP particle type - TWPs and textile MFs were used in mixture toxicity tests conducted with bifenthrin. The average weight of each TWPs and textile microfibers particles was calculated by weighting 100 particles repeatedly (five times) and then calculating the mean weight. Furthermore,

the concentration (mg/L) of TWPs and textile MFs were transferred from particle/L by multiplying by the mass of 100 particles.

Static renewal toxicity tests with bifenthrin individually and in mixture assessments with MPs were conducted using methods described by Al-Yassein (2019). Five different concentrations of bifenthrin were tested (10, 18, 32, 56, 100 ng/L) individually and in mixtures with TWPs (97.5 mg/L = 50,000 particle/L = MEC) and textile MFs (100 mg/L = 88,500 particle/L = MEC). All grass shrimp were not be fed after 24 hours prior to exposure. All toxicity tests were conducted at standard conditions (20°C, 20 psu salinity) and 14 hours:10 hours light/dark cycle. Water changes were made every 24 hours and supplemental aeration was not provided. Survival, mortality and alterations in behavior were test endpoints for assessment. Survival and mortality data were further analyzed statistically (Probit, Logit, ANOVA, Dunnetts and Chi Square) to calculate the 24-96h No Observable Effect Concentration (NOEC = highest concentration with no mortality), Lowest Observable Effect Concentration (LOEC = initial concentration with mortality > than the controls), and the Median Lethal Concentration (LC₅₀ = concentration where 50% mortality occurs).

2.3.4 Behavioral Observations

In addition to mortality and survival, alterations in swimming behavior of grass shrimp was used as a sublethal measure of effects from bifenthrin individually and in binary mixtures of bifenthrin-TWPs and bifenthrin-textile MFs. Grass shrimp swimming behavior in each beaker was observed for five minutes at time = 0 (prior to exposure) and after each water change at 24, 48, 72 and 96 hours of exposure in each toxicity test to count

the number of displays of altered behavior (increased swimming burst laterally and vertically versus no apparent movement in controls). Both the number of these altered behaviors and the number of individuals affected were noted for each individual replicate at each dose. In addition, the estimated relative physiological intensity (extreme, medium, slight) based on energy expenditure was also estimated. Symbols that were used for recording these altered behavioral results were decided in advance and kept consistent through the whole period of observation for both controls and all exposure treatments (bifenthrin and binary mixtures of bifenthrin-TWPs and bifenthrin-textile MFs). The behavior observation was conducted after every time of water exchange which were recognized as initial dose. The records of 0-, 24-, 48- and 72-hours exposures will be arranged into a mixing sheet with levels (extreme, medium, slight) based on energy consumption of different behaviors for further analysis.

2.3.5 Statistical Analysis

All statistical analysis was performed with SAS 9.4. The 24-96 hours LC₅₀ values and their 95% confidence limits (95% CI) were determined by using the Probit Analysis and the Logit transformation method was selected as it was the model that best fits these data. The No Observable Effects Concentration (NOEC) and Lowest Observable Effects Concentrations (LOEC) were calculated and determined using ANOVA and Dunnett's test after 96-hours exposure. Chi Square Analysis was used to detect time points where LC₅₀ values were significantly ($p < 0.05$) between bifenthrin individually and in the binary mixture toxicity tests of bifenthrin-TWPs and bifenthrin-textile MFs. The level of significance for all statistical analysis was set at $p \leq 0.05$.

CHAPTER 3

RESULTS

After 24 hours exposure, LC_{50} for bifenthrin and the binary mixtures of bifenthrin + TWPs and bifenthrin + textile MFs were 53.84 ng/L (95% Confidence Interval (CI) = 45.15 – 66.79 ng/L), 45.16 ng/L (95% CI = 30.60 – 71.07 ng/L) and > 100ng/L, (No 95% CI calculatable) respectively. These results indicated that during the initial 24 hours exposure period, the textile MFs were less than additively toxic, reducing the initial toxicity of bifenthrin, while TWPs were additively toxic, slightly increased the toxicity of bifenthrin. After 24 hours exposure, as shown by Figure 2, the protective abilities of the textile MFs decreased sharply, then became milder in the following subsequent exposure periods (48-96 hours). After 96 hours of exposures, the less than additively toxic effect of textile MFs continued to help moderate the toxic effects of bifenthrin but less so than was observed during the initial 24 hours of exposure (Figure 2).

As for the bifenthrin + TWPs binary mixture, initially the LC_{50} values were initially lower than bifenthrin during the 24 - 48 hours exposure periods, suggesting that TWPs increased the toxicity of bifenthrin; however, after 72 to 96 hours of exposure, the TWPs their interactive additive toxicity effects were diminished (Figure 2). After 96 hours exposure, the bifenthrin + TWPs binary mixture (LC_{50} = 18.14 ng/L, 95% CI = 15.39 – 22.69 ng/L) was slightly less toxic higher than bifenthrin exposure group (LC_{50} = 12.92 ng/L, 95% CI = 3.01 – 24.03 ng/L), indicating that the TWPs also appeared to reduced bifenthrin toxicity as well (Table 3.1).

The NOEC of bifenthrin and the binary mixtures of textile MFs and bifenthrin + TWPs after 96 hours of exposure were both 10 ng/L, which were all significantly ($p < 0.05$) higher (less toxic) than individual bifenthrin exposure of < 10 ng/L (Figure 3.1, Table 3.1). In addition, the LOECs were 18 ng/L for both binary mixtures of bifenthrin + TWPS and bifenthrin + textile MFs versus < 10 ng/L for bifenthrin, indicating that the onset of bifenthrin toxicity was reduced with exposure to TWPs and textile MFs. Temporal analysis of NOECs and LOECs for the entire 24-96 hours exposure period indicated that they were generally significantly higher ($P < 0.05$) than bifenthrin, suggesting that the binary mixtures of bifenthrin+ textile MFs and bifenthrin + TWPs mixture generally reduced the toxicity of bifenthrin, reducing both the onset of toxicity (LOEC) and increasing the tolerance of bifenthrin (NOEC) (Figure 3.1, Table 3.1).

Temporal analysis of NOECs and LOECs for the entire 24-72 hours exposure periods indicated that they were generally significantly higher ($P < 0.05$) than bifenthrin for B + TWPs at 24h of exposure and significantly higher ($p < 0.05$) than bifenthrin for B + textile MFs at 72h of exposure suggesting that the binary mixtures of bifenthrin+ textile MFs and bifenthrin + TWPs mixture generally reduced the toxicity of bifenthrin sporadically, reducing both the onset of toxicity (LOEC) and increasing the tolerance of bifenthrin (NOEC) (Table 3.1). The NOEC of bifenthrin and the binary mixtures of textile MFs and bifenthrin + TWPs after 96 hours of exposure were both 10 ng/L, which were all significantly ($p < 0.05$) higher (less toxic) than individual bifenthrin exposure of < 10 ng/L (Table 3.1). In addition, the LOECs were 18 ng/L for both binary mixtures of bifenthrin + TWPS and bifenthrin + textile MFs versus < 10 ng/L for bifenthrin, indicating that the onset of bifenthrin toxicity was reduced with exposure to TWPs and textile MFs.

Table 3.1 The results of acute toxicity tests for grass shrimp exposed to the mixture of bifenthrin with TWPs (97.5 mg/L = 50,000 particle/L= MEC) and Microfibers (100 g/= 88,500 particle/L = MEC). Temporal differences in LC50 values within each treatment are noted by different numbers (1-4). Comparisons between treatments at each time points where LC50, LOEC and NOEC values were significantly (p<0.05) different between treatments, are noted by different letters (A-C).

Duratio n	Treatment	LC ₅₀ (ng/L)	95% CI (ng/L)	NOEC (ng/L)	LOEC (ng/L)
24h	Bifenthrin ¹	53.84 ^A	45.15 – 66.79	32 ^A	56 ^A
	Bifenthrin + TWPs ¹	45.16 ^A	30.60 – 71.07	18 ^B	32 ^B
	Bifenthrin + Textile MFs ¹	119.5 ^B	91.55 – 223.12	32 ^A	56 ^A
48h	Bifenthrin ²	32.06 ^A	NC	18 ^A	32 ^A
	Bifenthrin + TWPs ²	21.00 ^B	17.94 – 26.87	18 ^A	32 ^A
	Bifenthrin + Textile MFs ²	61.06 ^C	47.31 – 83.40	18 ^A	32 ^A
72h	Bifenthrin ³	25.31 ^A	20.66 – 31.44	10 ^A	18 ^A
	Bifenthrin + TWPs ³	19.51 ^A	16.66 – 24.99	10 ^A	18 ^A
	Bifenthrin + Textile MFs ³	40.53 ^A	20.31 – 101.16	18 ^B	32 ^B
96h	Bifenthrin ⁴	12.92 ^A	3.01 – 24.03	<10 ^A	10 ^A
	Bifenthrin + TWPs ⁴	18.14 ^A	15.39 – 22.69	10 ^B	18 ^B
	Bifenthrin + Textile MFs ⁴	19.69 ^A	15.11 – 25.43	10 ^B	18 ^B

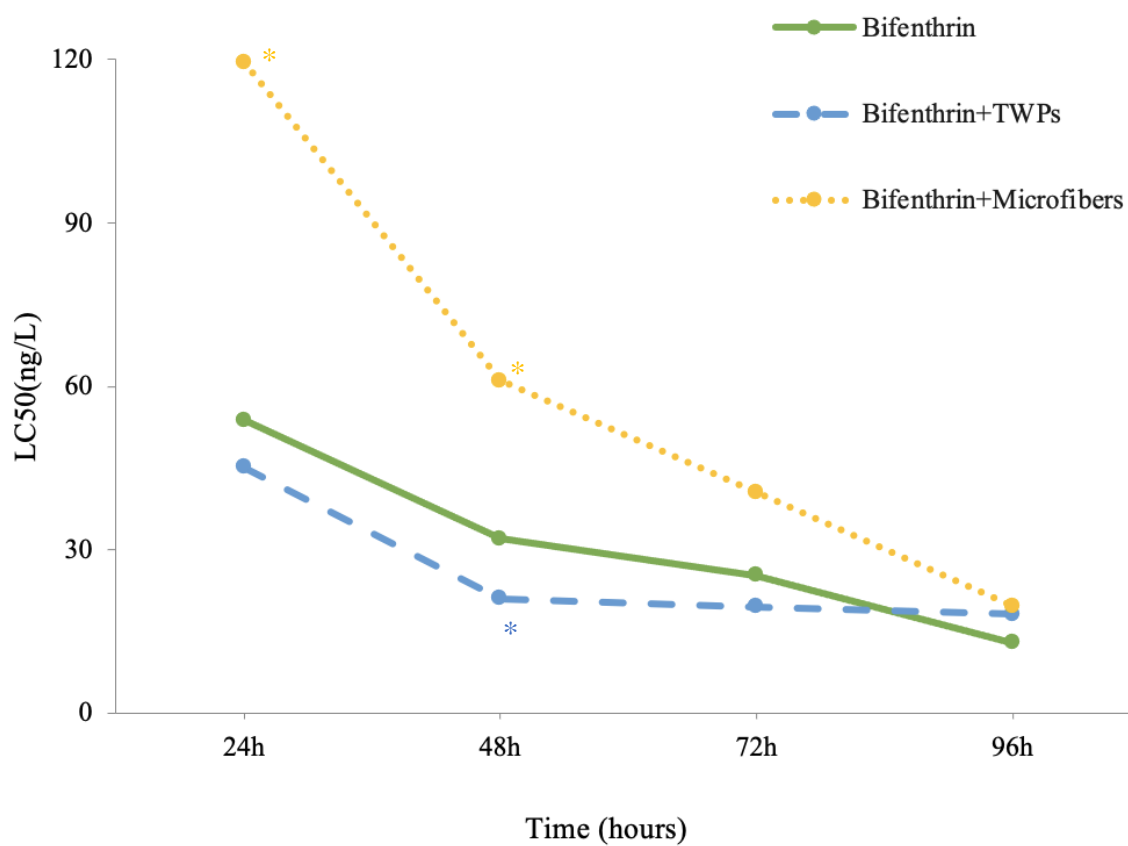


Figure 3.1 Comparison of different LC50 values curves for adult grass shrimp exposures to bifenthrin, mixtures of bifenthrin and TWPs and mixtures of bifenthrin and microfibers. Asterisks (*) indicated where binary mixture of bifenthrin + TWPs and bifenthrin + textile MFs were significantly different than individual bifenthrin exposure.

CHAPTER 4

DISCUSSION

4.1 96h Acute Toxicity of Individual Tire Wear Particles and Textile Microfibers

4.1.1 96h Acute Toxicity of Tire Wear Particles

In this study, there was no mortality after 96 hours exposure of the tire wear particles at the MEC of 100 mg/L reported in SC coastal environments (Lead and Weinstein (2019)). Similarly, there was another study that showed non-lethal effects of TWPs exposure after 96h of exposure and results further suggested that the LC₅₀ value of TWPs may > 50,000 particles/L (Leads and Rachel, 2018). However, another study reported a 96h LC₅₀ of 65% leachate and LOEC for grass shrimp of 50% respectively, with the salinity of 5 ‰ and tire chip concentrations of 50 g/L, which was 18% truck, 50% car-blackwall, and 32% car-whitewall TWPs (Hartwell et al., 1998). In addition to tests concerning impact on survival, recent research indicates that certain kinds of microplastics exposure may infect the immune function of *Palaemonetes pugio*. For example, Leads and Rachel (2018) suggested that the immune system of grass shrimp would be stimulated after exposed to polyethylene spheres based on the result of 100% survival (a 12% increase in survival) compared to controls following bacterial challenges, suggesting that exposure to microplastics may interact with the immune system. However, they didn't find any effects on immune function following exposure to TWPs. In our study, we did not observe any

sublethal effects on behavior (Section 4.3 of the Discussion). These findings suggest that MPs at MEC concentrations in the environment are likely to be more chronically toxic than acutely toxic. Thus, it is important that the fate and chronic sublethal effects of TWP on grass shrimp, *Palaemonetes pugio*, be further examined at a cellular and molecular level.

Except for grass shrimp, other species have been tested in acute toxicity tests of TWPs. Goudey and Barton (1992) indicated a 96h LC₅₀ of 10-33% of 50 g/L after exposing rainbow trout (*Oncorhynchus mykiss*) to tire pieces in tap water with the concentration of 200 g/L.

Since we didn't determine a LC₅₀, LOEC and NOEC value for TWPs at the MEC reported for SC coastal environments due to a lack of toxicity, long-term future studies should be conducted to determine the acute and chronic toxicity of TWPs in grass shrimp. This will allow a determination of a Margin of Safety for TWPs by comparing the MEC with the LC₅₀, NOEC and LOEC values. Moreover, we only included one type of TWPs and additional types of TWPs should be tested as was done by Goudey and Barton (1992) to gain a more comprehensive understanding of their toxicity to aquatic species.

4.1.2 96h Acute Toxicity of Textile Microfibers

After 96h exposure, we didn't find any mortality in the exposure groups of textile MFs at the MEC reported for SC coastal environments. A study that tested a closely related species, *Palaemonetes varians*, showed the ability of clearing their digestive tract via regurgitation, after ingestion of anthropogenic pollutants like microfibers (Saborowski et al., 2019). Due to this efficient protection mechanism, low mortality rate of *P. varians* was observed in their study (Saborowski et al., 2019). Gray and Weinstein (2017) found 33-55%

mortality of grass shrimp after 3h exposure of weathered PP fibers (30–105 μm in size) at a concentration of 50,000 particles/L. The textile fibers, found in the environment, were partially chemically and physically degraded. However, our fibers were only physically degraded. Some properties like density, surface structure, chemical components and other factors may contribute to the distribution and sinking of microplastics in the aquatic environment and further influence their interaction with biota (Wang et al., 2016). As for acrylic fibers made in our study, they were not degraded, and their density is heavier than seawater that was used in this study. As a result of many of them sank to the bottom of the beakers within several minutes, which produced a less homogeneous exposure. Future studies should use small stir bars separated from the grass shrimp and netting to keep the MFs in solution.

Currently, risk assessments related to plastic microfibers are limited (Jemec et al., 2016). Some studies with other species showed that starved daphnids had increased mortality after 48h exposure of MP fibers and majority of ingested fibers in the gut were about 300 μm (Jemec et al., 2016). It was further demonstrated that Asian clams had greater uptake of the middle-sized fibers (100-1000 μm), which was consistent with previous studies (Li et al., 2019). Based on our research, long-term chronic toxicity tests assessing the sublethal effects of textile microfibers are needed. Future studies should address the different sizes, shapes, material types and weathering condition of textile microfibers to gain a more comprehensive understanding of their toxicology, biological and environmental adverse impacts to aquatic ecosystems.

4.2 The Influence of Individual Tire Wear Particles and Textile Microfibers on the Acute Toxic of Bifenthrin

4.2.1 The Influence of Individual Textile Microfibers on the Acute Toxic of Bifenthrin

Many studies have shown that microplastics are able to sorb to other environmental chemicals in the environment, both legacy pollutants and CECs (Bakir et al., 2014; Ma et al., 2016; Van et al., 2021). However, demonstrations about the influence of these contaminants sorbed on to microplastics and resulting toxicity, sublethal biological effects and bioaccumulation are still rare. Microplastic play a role in transferring toxic to aquatic species by loading and carrying these contaminants, which may cause greater toxic effects and more bioaccumulation compared to exposure to these conventional contaminants *per se* (Paul-Point et al., 2016). Sleight et al., 2017 assessed the ability of microplastics to absorb other chemical contaminants and found that in some cases combined exposure can decrease adverse biological effects compared to exposure of individual contaminants. Plastic polymer type, size, age and degree of weathering of plastic, chemical properties of contaminants and other environmental factors may influence the sorption of environmental pollutants to microplastics (Menéndez et al., 2020). Based on lack of investigation on the interaction between microplastics and coexisting contaminants, it is necessary to explore related interaction.

We assessed the effects of MPs – TWPs and textile MFs on the co-occurrence of bifenthrin, a pyrethroid insecticide that is Super Toxic (96h LC₅₀ < 20 ng/L to aquatic species) which has been used widely and detected in various environmental samples. We

used MPs concentrations of TWPs and textile MFs microfibers at the MEC detected along the SC coast (Wagner et al., 2018), in binary mixture studies with bifenthrin and compared results with bifenthrin exposure *per se*. This approach allowed us to gain an understanding of effects of these two kinds of microplastic pollutants originating from human daily activities on this Super Toxic insecticide.

Our results showed that textile MFs have a strong ability to alter and mitigate toxic effects of bifenthrin toxicity to grass shrimp, especially during the first 48h exposure. Even though its protective ability started to wane after 48h exposure, these textile MFs still showed an ability to decrease toxicity of bifenthrin following 72-96 hours of exposure.

The microfibers we used in this research are made from acrylic, a polymer (polyacrylonitrile). The polymer must contain at least 85% acrylonitrile monomer in order to be called “acrylic” in the US. Polyacrylonitrile was used as an adhesive glue to produce nanotubes for detection of bifenthrin in tea brew, which showed its ability of adherence and absorption (Ren et al., 2018). Therefore, this ability can play a critical role in mitigating toxic of bifenthrin to aquatic species. Ziajahrom et al., (2019) found that polyethylene (PE) microplastics can absorb huge amount of bifenthrin, which significantly reduced the toxicity of bifenthrin when it was mixed with PE microplastics. PE is a polymer that is used in rope and other materials and are the most common plastic material worldwide. Some properties of this polymer would help decrease the toxicity of bifenthrin as our acrylic fibers did in this study.

Our results showed a gradually decreased protective effect after 24-hours exposure, and was then decreased after from 48-96 hours. There were dead grass shrimps which had great amounts of microfibers clogging at their gills when we did our water exchange at 48-

hours exposure (Figure 4.2.1). After that, the dead grass shrimps in subsequent exposure period generally had the same clogging of the gills as was observed earlier. Surviving grass shrimp were not observed to have gills clogged to the extent observed in dead shrimp. Attached microfibers would contribute to more opportunities for grass shrimp to come in contact with bifenthrin that was absorbed by microfibers. Therefore, it would be one of the reasons that the protective effect of microfibers showed a trend of decreased effectiveness after 24-hours exposure. On the other hand, blocked microfibers would cause difficulty for grass shrimp to respire across the gills which may have also contributed to the observed mortality. However, an interesting point is that there was no mortality in our previous test of individual textile MFs even though we found some shrimps had fibers attached to their gills. Higher respiration rates and altered behavior (which was observed) associated with MFs may affect the toxicity of bifenthrin when microfibers are attached to the gill. Therefore, more blocked fibers on the gills may alter the uptake of bifenthrin reducing the pattern of grass shrimp mortality as our results indicated an overall protective effect, particularly during the first 24-48h of exposure. Studies by Scott et al. (1993) have shown that most pesticide exposures from NPS runoff in SC last < 6 hours at the time of ebb tide and due to the extensive dilution effect of incoming flood tides are reduced by 90%. Thus, MFs co-exposure with pesticides following NPS runoff events would be predicted to be somewhat effective at reducing the toxicity of other similarly acting pesticides if MFs levels are at the concentrations used in this study. Further research in this area with other pesticides and other pollutants would be needed to fully understand these protective effects observed with bifenthrin in this study.

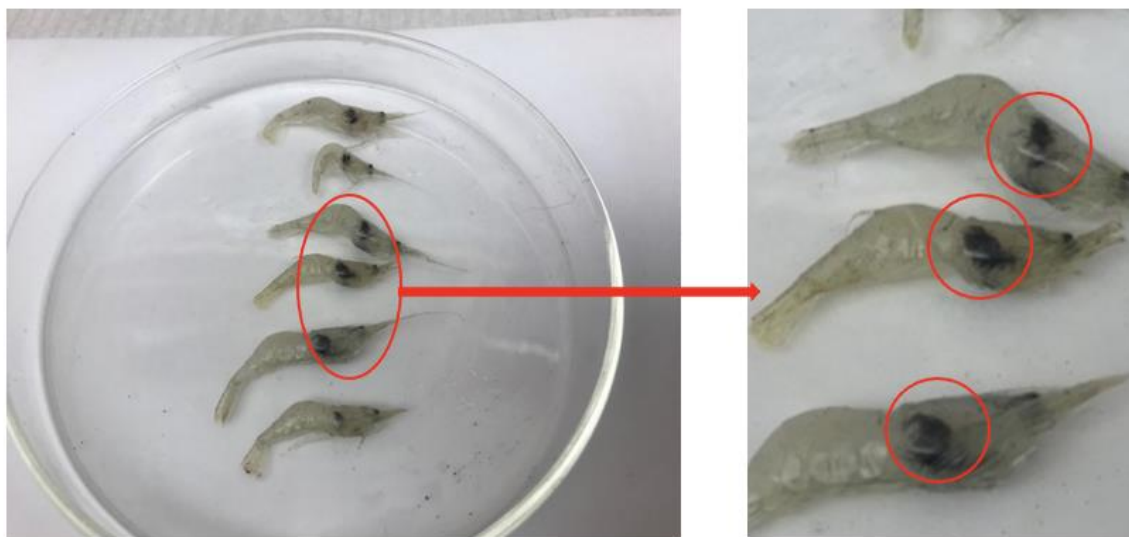


Figure 4.1 Dead adult grass shrimps with microfibers on their gill

4.2.2 The Influence of Individual Tire Wear Particles on the Acute Toxic of Bifenthrin

Tire wear particles are a mixture of tire tread material and road pavement material. Compare with traditional MPs, TWPs are generally more complex having multiple forms (e.g. auto tire, truck tire, tire tread, and tire sidewalls TWPs) with more complex chemistry. Rubber used in tires contains many different ingredients resulting in TWPs with various physical and chemical characteristics and properties that may affect their toxicity. In addition, tire treads and brake dust may contain various of trace metals that may be emitted into the environment. Tires also contain various organic compounds (e.g. rubber hydrocarbons, carbon black) and several inorganic materials are also included in tires and associated TWPs (Thorpe and Roy, 2008). Thus, TWPs can be a source of trace metals and many other chemical pollutants. Councell et al., (2004) demonstrated that tire wear

particles can be recognized as Zinc in the environment. Other metal like Mn, Fe, Co et al were also reported the presence in tire wear particles (Adachi and Tainosho, 2004). In addition, emissions of organic compounds from tire wear have also been detected (Rogge et al., 1993).

Studies about the toxicity effects of mixtures of tire wear particles with other chemical pollutants are rare. Our study found that TWPs can initially increase the toxicity of bifenthrin to grass shrimp during the first 24-48h of exposure (Figure 3.1). Thus, greater than additive toxic effects were observed during the 48 hours exposure. By 96 hours of exposure, the binary mixture of bifenthrin + TWPs was less toxic than was observed in the individual bifenthrin exposure (Figure 3.1). The mechanisms for these observed toxic effects have not been fully identified. Initially during the first 48 hours of exposure, it appeared that bifenthrin may possibly attach to the TWPs increasing the initial toxicity but after 48 hours TWPs appeared to reduce the toxicity of bifenthrin, possibly due to the gills becoming poisoned and/or clogged with TWPs thus reducing exposure.

Future TWPs studies should be conducted as different driving conditions can generate tire wear particles with different sizes and shapes (Kim and Lee, 2008). Therefore, it would be meaningful to explore further about the toxic effects of bifenthrin changed by co-exposure of different types, sizes and shapes of TWPs. Finally, because this study was conducted under consistent laboratory environmental conditions (20°C, 20 psu salinity), future studies under more extreme climate change conditions should be conducted.

4.3 The Change of Grass Shrimp Behavior under Toxicity Tests

Aquatic animals such as grass shrimp have evolved numerous behaviors which enhance survival in their surrounding estuarine environment. For example, grass shrimp at high tide migrate into the *Spartina* cord grass to escape predation while at low tide they hunker down into extremely shallow waters with depleted oxygen levels which prevents predation. Any chemical which disrupts this normal behavior will increase predation and cause declines in grass shrimp populations.

Exposure of the clam, *R. decussatus*, to Zn was assessed using behavioral parameters to measure time-dose-response relationship effects (Soliman et al., 2015). Normal behaviors (e.g. mate, prey capture, predator avoidance) are also ecologically important for reproduction and population continuity of a species. Disturbance of behaviors caused by contaminants are responsible for disruption of balance at the population level for most organism. Thus, animal behavior can be recognized as an effective sublethal parameter to assess the effects of toxic chemicals such as bifenthrin.

Kunz et al., (2006) demonstrated 12 kinds of different grass shrimp normal behavioral activities, including swimming behaviors such as swimming in the lower half of water column, being motionless and walking on the benthic pebble substrate were most frequently observed. According to their research, a sudden jab was used to illicit a change in behavior in response to a predator. This jab was a kind of offensive behavior that altered the behavior of grass shrimp, simulating what may occur when a grass shrimp is confronted by a predator, such as the estuarine fish, the mummichog (*Fundulus heteroclitus*) (Kunz et al., 2006). Our research found an interesting phenomenon when grass shrimp are exposed to binary mixtures of bifenthrin + textile MFs. In the bifenthrin + the textile MFs exposures,

jabbing behavior was observed in the absence of any predator but was only observed after 48 hours exposure. After 48 hours exposure, one out of 15 shrimps (6.67 % response) kept hitting the beaker for almost 5 mins in exposure of 10 ng/L concentration. In the next highest dose of 18 ng/L exposure, two shrimps out of 15 shrimp (13.33 % response) also displayed this jabbing behavior, similar to the shrimp in 10 ng/L group. At higher doses this effect was not observed. Furthermore, after 72 hours exposure a larger number of shrimps displayed this jabbing behavior as two shrimps out of 15 shrimps (13.33 % response) in 10 ng/L and four shrimps out of 15 shrimp (26.67 % response) in 18 ng/L displayed this offensive behavior. Another surprising point is that one shrimp in 32 ng/L exposure started to display this offensive behavior. According to Figure 3.1, the protective ability of microfibers to reduce bifenthrin toxicity decreased significantly after 24 hours exposure. After 48 hours exposure, the LC_{50} for the bifenthrin + textile MFs was increasingly close to the LC_{50} for bifenthrin and appeared to cause more and more stimulated effects on grass shrimps, which may explain the appearance of jabbing behavior that showed up and gradually got more aggressive after 48 hours exposure. None of this kind of behavior was observed in the bifenthrin + TWPs or the bifenthrin exposure alone.

It is rational to realize that this behavioral disturbance in grass shrimp caused by contaminants in this study can significantly impact their avoidance from predator and their quality of life. Due to altering effects of behavior on these aquatic organisms at the bottom level of food chain, it is predictable that population of these organisms will be impacted due to increased predation. What about effects on their predator species and other organism such as humans that are at the top of the food web. Pyrethroids are known to cause hyperactivity in children and this jabbing behavior may be considered similar to someone

banging their head on the wall when hyper excited, as the shrimp were banging themselves against the sides of their container. This is indeed abnormal behavior in shrimp which appeared to only be induced by bifenthrin + textile MFs exposure. Future research should more fully address this alteration in behavior.

CHAPTER 5

CONCLUSIONS

Studies about the impacts of MPs such as TWP and textile MFs microfibers on the uptake/depuration rates kinetics (e.g. bioavailability) and acute/chronic toxicity of both legacy pollutants and CECS are rare. In this study, the effects of TWP and textile MFs on the acute toxicity of the pyrethroid insecticide, bifenthrin, was assessed using the estuarine grass shrimp, *P. pugio*, as an exposure model for crustaceans. Individual acute 96h toxicity tests were conducted with bifenthrin, TWP and textile MFs, followed by binary mixture studies of bifenthrin + TWP and bifenthrin + textile MFs. Test end point in all toxicity tests were survival, mortality and alterations in behavior. Statistically analysis using Probit Analysis (with Logit, Weibull and Exponential Transformations) were used to calculate LC50 values at different time points (24-96h), NOECs and LOECs for bifenthrin and each binary mixture tested and additional statistical analysis (ANOVA, Dunnetts, Chi Square) was used to test differences among these exposure conditions. Results indicated:

1. TWP were not toxic to grass shrimp in acute exposures up to the MEC measured in SC coastal environments of 97.5, 975, 1365 mg/L.
2. Similarly, textile MFs were not toxic to grass shrimp in acute exposures up to the MEC measured in SC coastal environments of 10, 100, 1,000 mg/L.
3. As a result of these finding binary mixture studies with bifenthrin were conducted at the MEC for TWP and textile MFs in coastal SC.

4. The 96h LC for bifenthrin was 12.92 ng/L (95% CI = 3.01 – 24.03 ng/L).

5. Initially at 48 h of exposure the bifenthrin + TWPs binary mixture was significantly more toxic than the individual bifenthrin exposure ($p < 0.05$). This effect did not persist and by the end of the 96h of exposure time points the binary mixture was less toxic than the individual bifenthrin exposure ($p < 0.05$) as both the LOEC and NOEC values were significantly higher than in the bifenthrin exposure *per se*. This indicated that MFs shifted the onset of toxicity of bifenthrin. The 96h LC50 for bifenthrin + TWPs was 18.14 ng/L (95% CI = 15.39 – 22.69 ng/L), which was equivalently toxic as the individual bifenthrin exposure. This indicated that although TWPs reduced the onset of toxicity by altering the LOEC and NOEC at 96h of exposure, it did not alter the median LC 50 value.

6. Initially at 24-48 h of exposure the bifenthrin + Textile MFs binary mixture was very, very significantly less toxic ($p < 0.05$) than the individual bifenthrin exposure. This effect persisted as after 96 of exposure both the LOEC and NOEC values were significantly higher than in the bifenthrin exposure *per se*. The 96h LC50 for bifenthrin + Textile MFs was 19.69 ng/L (95% CI = 15.11 – 25.43 ng/L), which was equivalently toxic as bifenthrin *per se*. This indicated that although MFs reduced the onset of toxicity by altering the LOEC and NOEC after 96h of exposure, it did not alter the median LC 50 value.

7. The 96h LOECs for bifenthrin and the binary mixture of bifenthrin + TWPS and bifenthrin + textile MFS were 10, 18 and 18 ng/L, respectively. Similarly, the 96h NOECs for bifenthrin and the binary mixture of bifenthrin + TWPS and bifenthrin + textile MFS were ≤ 10 , 10 and 10 ng/L, respectively. Generally, the NOECs and LOECs for the binary mixtures of bifenthrin + TWPS and bifenthrin + textile MFs were significantly ($p < 0.05$) higher than the LOECs and NOECs for the individual bifenthrin toxicity tests, indicating

that MPs exposure to TWPs and textile MFS significantly reduced the onset of toxicity and increased the tolerance of bifenthrin in grass shrimp.

In conclusion, our results suggested compared to bifenthrin exposure group co-exposure to MPs altered the toxicity of bifenthrin, generally reducing the overall toxicity of this generally super toxic insecticide after 96 hours of exposure. It should be noticed that our study was conducted under stable and standard lab environment conditions (20°C and 20 psu) and the microfibers we used were not weathered. The butterfly effect has proved that a small environmental change in initial conditions can often set off a long-term chain reaction throughout an ecosystem. Our study shows how micro level plastic particles particle can influence the health of grass shrimp. Even though grass shrimp is a small aquatic species at a lower trophic level, because of close connection among living organisms in food webs in the entire global ecosystem including humans there are unknown public health effects as it is possible for humans to be exposed to both microplastics and bifenthrin. Our findings clearly indicate that nontoxic microplastics when they interact with other chemicals like bifenthrin may alter their toxicity posing unknown risk to public health. The existence of known Adverse Outcome Pathways for exposure rings a clear bell for more concern for human health effects of organisms at the top of food webs including man. Therefore, more studies related to this topic are needed. Future studies should be conducted to assess sublethal effects in grass shrimp such as ingestion kinetics of both bifenthrin as well as other CECs along with MPs including assessment of important cellular/molecular initiating events which may affect both aquatic and human species under both conventional exposure and climate change conditions of increased temperature and salinity. With these future studies a more complete and comprehensive understanding of harmful effects that

can be transferred to humans and affect public health will be gained. This One Health Approach will enable regulatory actions needed to protect both ecosystem and human health.

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