October 2023 Progress Report

"Assessing the threat of tire leachate and urban runoff on Matagorda Bay fish populations"

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Aquatic Toxicology. Tire wear particles (TWPs) represent complex mixtures of chemicals that are both introduced during the manufacturing process and generated through environmental degradation processes (e.g., photo-transformation of 6PPD to 6PPD-quinone by solar radiation). In the aquatic environment, these chemicals may leach from TWPs creating a potentially complete exposure pathway for biota. Prior laboratory studies have shown that exposure to TWP leachate during development can lead to deformities and mortality in freshwater fish and invertebrates (Chang et al., 2023; Cunningham et al., 2022); however, little is known about the toxicity of these complex chemical mixtures to ELS marine species, including the mechanisms of toxic action of particular ecological relevance in estuarine and marine ecosystems.

Photo-induced toxicity is a mechanistic phenomenon by which certain photodynamic compounds (i.e., those that are capable of absorbing certain wavelengths of solar radiation) become significantly more toxic in the presence of sunlight (Roberts et al., 2017). This increase in toxicity may happen through one of two mechanisms, (1) photo-sensitization, or (2) photo-modification. Photo-sensitization, which is the phototoxic mechanism investigated in the present study, occurs in transparent/translucent organisms (e.g., most early life stages of fish and shellfish) that have accumulated a body burden of photodynamic compounds from the surrounding environment (Nielsen, Lay, et al., 2018; Roberts et al., 2017). As sunlight penetrates into clear organism, it interacts with photodynamic compounds present in tissues of the animal, leading to oxidation of nearby biomolecules in a self-propagating reaction that results in death of the organism at much lower exposure concentrations than would otherwise cause toxic effects (Alloy et al., 2016; Alloy et al., 2017; Damare et al., 2018; Roberts et al., 2017; Sweet et al., 2018).

We investigated the potential for the complex mixture of chemicals present in TWP leachate to cause developmental toxicity in ELS red drum (*Sciaenops ocellatus*), including the potential for adverse effects to occur via mechanisms of photo-induced toxicity. Fertilized red drum embryos (n = 20 embryos/dish) were exposed to a range of nominal concentrations of TWP leachate (0, 5, 9, 14, and 18%), both under ambient laboratory lighting (visible light spectrum only) or simulated full spectrum solar radiation, in a fully factorial design. The intensity of simulated solar radiation for the test was designed to be representative of incident surface UV experienced by red drum embryos during the Deepwater Horizon oil spill (Lay et al., 2015; Nielsen, Lay, et al., 2018). All other environmental parameters were held constant between treatments, with successful hatch assessed at 24-h, and larval survival evaluated at 48-h and 72-h. Results indicate that TWP-leachate does not significantly affect hatch success of red drum via photo-induced toxicity. However, TWP-leachate adversely impacted the survival of larval red drum in a dose dependent manner aby the 48-h assessment, but only in the treatment co-exposed to simulated solar radiation. This indicates that interactions between UV radiation and photodynamic constituents present in TWP leachate may significantly increase the toxicity of TWPs to developing aquatic biota.

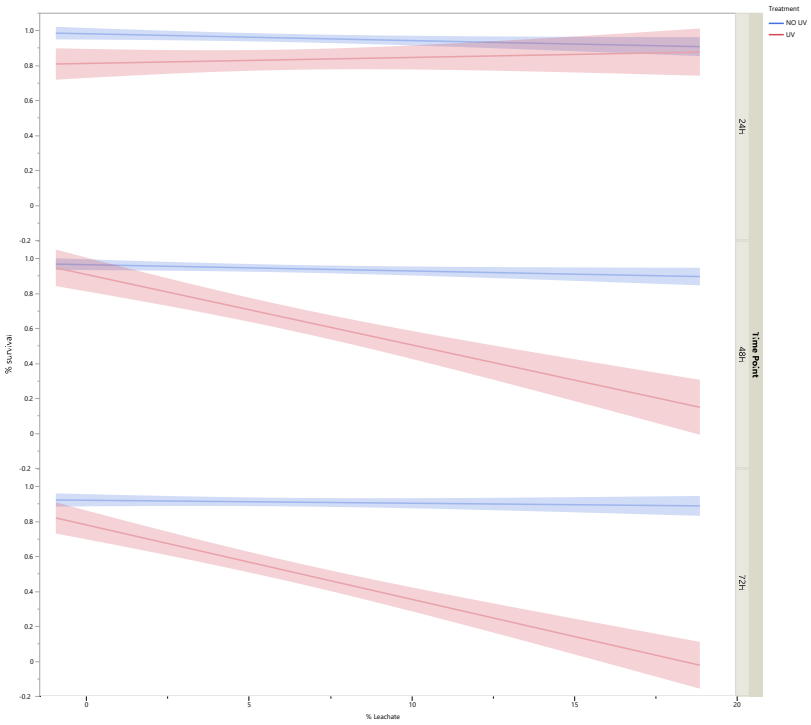

Figure 1. Hatch success (24-hr) and survival of embryo-larval red drum exposed to TWP leachate, both with and without co-exposure to UV radiation.

It should be noted that we used commercially-obtained (i.e., un-weathered) TWPs to generate the leachate. The weathering process generally increases the photo-induced toxicity of crude oil to ELS marine species; thus, the use of unweathered TWPs in the present study may be underestimating the toxicity of TWP to red drum larvae. We are currently planning follow-up studies to examine the potential sub-lethal effects of TWP exposure, as well as the potential effects of natural weathering processes on the toxicity of TWPs, including photo-induced mechanisms. We are finalizing a manuscript describing the results of our 6PPD-quinone studies and will begin drafting a second manuscript describing the findings of our photo-induced toxicity studies after follow-up studies have been completed.

Analytical Environmental Chemistry. This aspect of the project involves both field and laboratory components. Main activities for the quarter are briefly reviewed below in their respective sections below.

***Field work*.**

During the past quarter, we conducted several field trips to collect samples. (1) During the heavy rain on August 22nd, we collected water samples from Mission River and Aransas River, as well as from the stormwater discharge site at Cole Park in the city of Corpus Christi. The goal of this sampling trip was to capture peaks of 6PPD and 6PPD-Q in the runoff of the rainwater. The unfiltered samples were spiked with deuterium labeled 6PPD-Q (D5-6PPD-Q) and were subsequently extracted using the Oasis HLC Prime solid phase extraction cartridges, following our established protocol in the lab. Cartridges were preserved at -20 ℃ before elution and further LC-MS quantification. (2) We also conducted a three-day field trip (September 6 - 8) to San Antonio Bay, Matagorda Bay, and two major rivers that run into the bay (Guadalupe River and Lavaca River) to collect water samples for 6PPD-Q analysis (Figure 2). The unfiltered samples were extracted via SPE following the previously described protocol once returned to lab at UTMSI. Cartridges were preserved at -20 ℃ before elution and further LC-MS quantification.

(3) A one-day field trip (September 19th) to NERR was conducted to collect water samples for 6PPD-Q analysis. The unfiltered samples were extracted via SPE following the previously described protocol once returned to lab at UTMSI. Cartridges were preserved at -20 ℃ before elution and further LC-MS quantification.

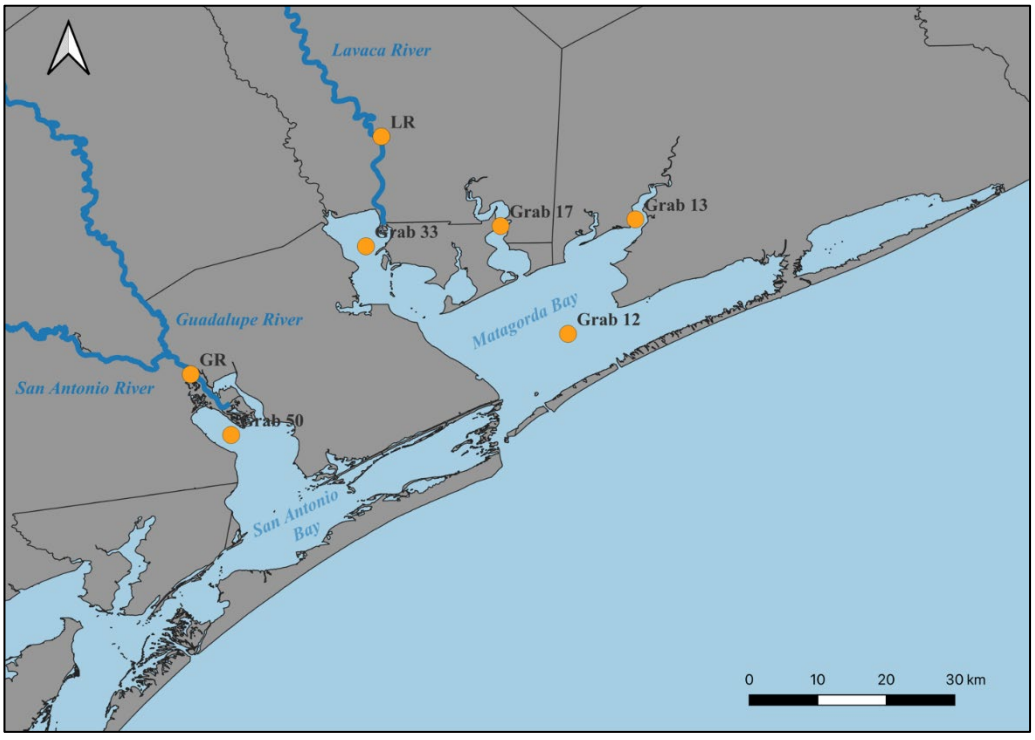

Figure 2. Sampling map for the September field trip.

Lab work.

Characterizing tire particle leachates. Tire particle leachates were created by dissolving the cryo-milled tire particles (acquired from U.S. Tire Manufacturers Association) in artificial seawater for 10 days. The concentration of DOC is on the level of 120 ppm, determined by a TOC analyzer. To study the molecular composition of the leached DOM, water samples were collected, filtered through a 0.2 µm filter, and were extracted via solid phase extraction with PPL cartridges (Agilent), following our published work in DOM research. The extracted samples were then analyzed by the high-resolution liquid chromatography mass spectrometer (Agilent, 6560 Ion Mobility Quadrupole Time-of-Flight Mass Spectrometer). A total of 3309 unique molecular features were detected, with a total of 1,368 features can be assigned with a CHONS formula. The average H/C ratio and O/C ratio are 1.32 and 0.23, respectively (Figure 3). Quite different from natural dissolved organic matter collected from rivers/coastal regions/open oceans, over 60% of the assigned formulas in the tire particle leachate have a relatively low H/C ratio (< 1.5) and low O/C ratio, reflecting the hydrophobic and recalcitrant nature of the leachate.

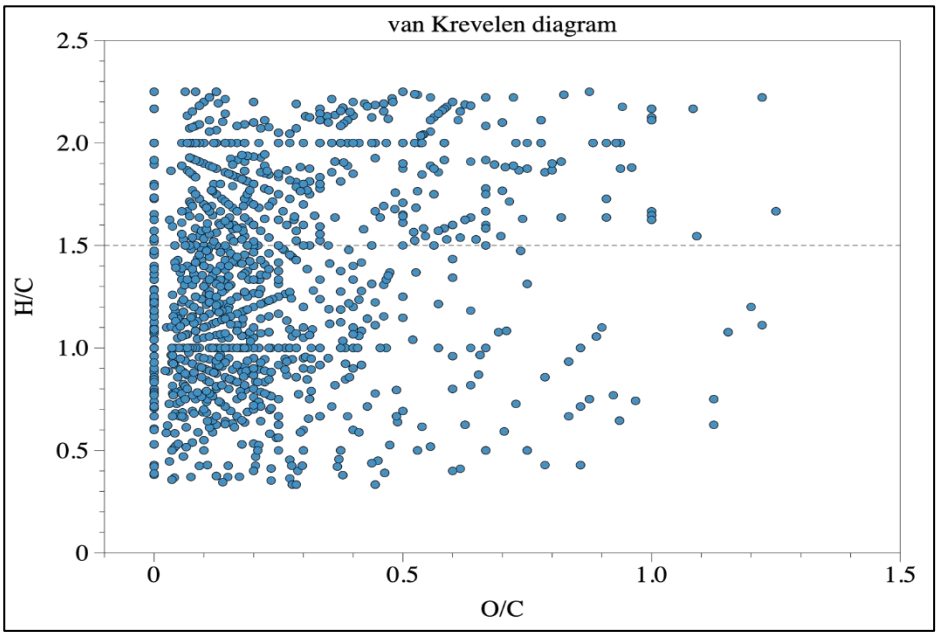

Figure 3. van Krevelen diagram of detected formulas in the tire particle leachate. Each dot represents a unique formula. A total of 1,368 formulas were calculated and assigned.

Detecting the levels of PAHs in tire particle leachates. The extracted samples were also run on GC/MS for a preliminary screen of polycyclic aromatic hydrocarbons (PAHs). However, none of the 16 EPA-priority PAHs were detected. This is probably because the used sorbent is specially designed to extract dissolved organic matter (PPL), not PAHs. To acquire a more comprehensive picture of the presence of these compounds, which play important roles in photoreactions (including photo-induced toxicity), we have acquired new standards containing more alkylated PAHs (e.g., C1 – C4 anthracene; C1 – C4 chrysene; etc.) from Chiron and NIST. We are currently developing new analytical methods to measure these PAHs and their corresponding derivatives. Samples collected from incubation experiments have already been extracted with a special SPE cartridge (ISOLUTE TPH) designed for a max recovery of PAHs from water samples and will be analyzed shortly.

Refining the protocols of 6PPD-Q measurements. Our previous measurements of 6PPD-Q (specifically in incubation samples) showed some discrepancies between the measured concentration and the expected/spiked concentrations. Specifically, in some cases, the measured concentrations of 6PPD-Q were only 50% (or even less) of the expected values. After excluding possible errors during the preparation of the stock solution, we speculated that salinity may play a role, due to potential interactions with the carrier solvent (e.g., the “salting-out” effect). We conducted additional experiments to determine whether salinity-driven effects may explain the analytical discrepencies; however, the results did not suggest that this was the case.

On the other hand, we did find an effect of the solvent used to dissolve 6PPD-quinone into exposure solutions. Dimethylsulfoxide (DMSO) is a common solvent for preparation of 6PPD-quinone stock solutions in biological experiments, while acetonitrile (ACN) is generally recommended for chemical analysis. During the salinity test experiment, we found there is a systematic difference between these two difference solvents. DMSO can increase the

response factor of 6PPD-quinone (i.e., increase the peak area on the LC-MS), which can lead to errors. We also found different preparation approaches (i.e., whether the stock solution has been frozen and thawed; whether the stock solution has been sonicated; whether the stock solution was prepared in glass or plastic container) can affect the measured concentrations of 6PPD-quinone (Figure 4). Based on these results, it is recommended that future measurements of 6PPD-quinone via LC-MS be carried out using the same solvents utilized in the experiment. Moreover, there is a need to establish a consistent procedure for the preparation of 6PPD-quinone stocks in DMSO to ensure comparability between studies conducted in different labs. We are currently working on a manuscript describing the analytical methods we have developed for the detection of 6PPD-quinone in environmental media.

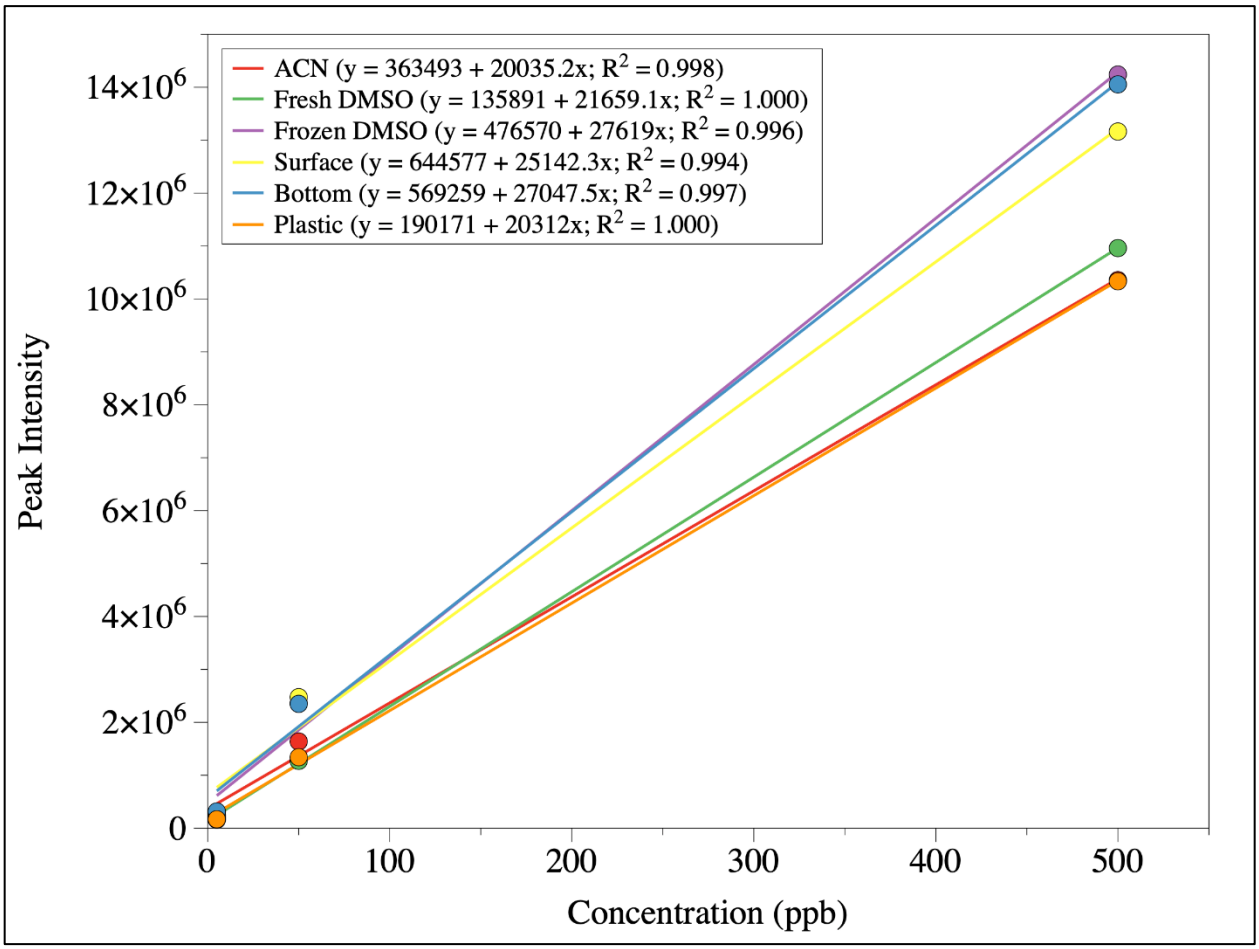

Figure 4. Effects of solvents and preparation approaches on the measurement of 6PPD-quinone.

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