Matagorda Bay Mitigation Trust 2022-2023 Funding Cycle

RFP # 2022-2023-1

Title: Reproductive & Developmental Toxicity of "Forever Chemicals" to Matagorda Bay's prey fishes Reproductive & Developmental Toxicity of "Forever Chemicals" to Matagorda Bay's prey fishes

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Q4 July 2024 Progress Report

Q4 Update:

We have continued to build the PFAS profile of Matagorda Bay as part of Phase 1, as we have received analyses for both water and sediment samples. We have begun trials for sheepshead minnow larval exposures as part of Phase 3, and will begin collecting sheepshead minnows for PFAS body burden analyses and fecundity studies as part of Phases 2 and 3.

Phase 1:

During Q3, we collected additional samples from Matagorda Bay. We made the decision to take samples from an additional "refence site" east of the previous reference site to determine if we could find a "PFAS free" location in the Bay where we can collect fish expected to have lower or no PFAS exposure as part of Phase 3. Both sediment and water samples were collected following the protocols used at our previous four sampling locations. We also collected additional paired water and sediment samples downstream from the effluent of both the Point Comfort Wastewater Treatment Plant and the Formosa Plastics Corporation and downstream of the Chocolate Bay Wastewater Treatment Plant (Figure 1) for confirmatory analyses.

Sediment samples have been sent to an analytical chemistry lab – Eurofins Environmental Testing Northern California, LLC – for analysis using EPA Draft Method 1633. This is the same EPA Method that was used to analyze the sediment samples collected from our previous Matagorda Bay Reference Site and the site that is downstream of the Palacios Sewage Treatment Plant (as discussed in our Q3 Progress Report). This method will test for the 40 PFAS listed in Table 1. The paired water samples for these sediment samples are currently being extracted for analyses by the UT MSI Core Facilities.

Phase 2:

Our Phase 2 sampling was placed on hold due to Hurricane Beryl and will be completed during the upcoming quarter. During the next quarter, we will be collecting sheepshead minnows (*Cyprinodon variegatus*) from our sampling site downstream of the Palacios Sewage Treatment Plant to assess PFAS body burdens in their tissues. We will be using seining for collection of sheepshead minnows. Sheepshead minnows body burdens will be determined using EPA Method 1633 (Table 1) for Phase 2.

Phase 3:

During Q3, we began PFAS testing on sheepshead minnow embryos and larvae in the lab as part of Phase 3. We are using commercially purchased embryos for these studies to establish concentrations of PFAS that impact the development of these embryonic and larval fish. To date, we have collected data looking at the impacts of PFOS on the development of embryonic and larval sheepshead minnows. We chose to begin our lab testing with PFOS because as this was the most consistently found PFAS detected in Matagorda Bay sediment samples (as detailed in our Q3 report). The UT MSI Core Lab also found the highest concentrations of PFOS among our water samples as well, with an average concentration of ~15 parts per trillion (ppt). Therefore, we anchored our exposure concentrations to these environmentally relevant data for Matagorda Bay.

To do this, we spawned commercially-purchased broodstock sheepshead minnows at the Fisheries and Mariculture Laboratory (FAML) at UT MSI. Sheepshead minnows were spawned first thing in the morning and collected embryos within 2 hours of spawning. Embryos were assessed for viability and left to develop for 24 hours. At ~24 hours post fertilization (hpf), embryos were staged and only embryos within the 16-17 embryonic stages[2] were used for the study. As previously mentioned, the PFOS doses for this study were anchored to the average concentration of PFOS detected in samples collected in Matagorda Bay (~15 ppt). We performed a graded dose-response using the following doses: 0 ppt (control), 2 ppt, 6 ppt, 16 ppt, and 44 ppt (Figure 2). At the start of the experiment, embryos (n=10/dish; 50 total per dose across dishes) were placed in the experimental treatments (Figure 2). Survival was assessed every 24 hours and

embryos were checked for proper developmental milestones (e.g., eye pigment development). Following these checks, debris was removed from each dish and 50% water changes were made with water dosed with the relevant concentration. The experiments were performed in our lab's environmental chamber to ensure all dishes were maintained at the appropriate temperature (25°C) and were covered with transparent sheeting to prevent evaporation. Water quality parameters (temperature, pH, salinity, and dissolved oxygen) were measured daily prior to each dish's 50% water changes. Water samples were collected at the start of the experiment, from each dish during daily water changes, and at the end of the experiment for quantification of PFOS. These samples are currently being prepared for analyses at the UT MSI Core Laboratory.

After the final assessment when all the fish had hatched (7dfp), half the fish (n=5/dish) were euthanized in buffered MS222, placed in a 3% methyl cellulose solution, and imaged using a Nikon SMZ800N fitted with a camera and accompanying software. We are currently in the process of measuring a suite of morphological parameters to compare the impacts of PFOS on development (Figure 3). These results will be presented at the North American Society of Environmental Toxicology and Chemistry (SETAC) Annual Meeting this upcoming October in Fort Worth, Texas.

References:

[1]https://www.sgsaxys.com/2021/09/14/epa-announces-availability-of-epa-1633-draft-pfas-method-developed-by-sgs-axys-sgs-axys-continues-to-expand-range-of-pfas-testing-methods/

[2]Lencer, E. S., & McCune, A. R. (2018). An embryonic staging series up to hatching for Cyprinodon variegatus: An emerging fish model for developmental, evolutionary, and ecological research. *Journal of Morphology*, *279*(11), 1559-1578.

Figures:

Figure 1. Expected point sources for introduction of PFAS into the Matagorda Bay system and the proposed sampling sites for characterization of PFAS in the Bay.

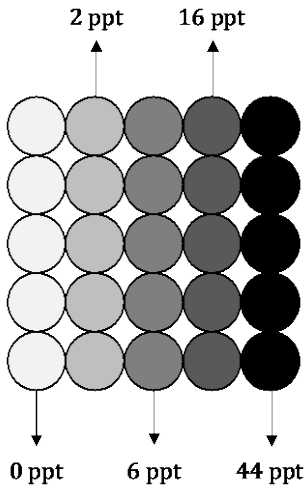

Figure 2. Depiction of the graded dose-response experiment performed in embryonic and larval sheepshead minnows. There was a total of five environmentally-relevant doses in the study, ranging from 0 parts per trillion (ppt) to 44 ppt. Each circle represents one replicate (i.e., dish), for a total of 5 replicates per dose. Briefly, fish (n=10/dish) were added at ~24 hours post-

fertilization (dpf) and monitored through hatch (~ 6-7 dpf). At the end of the experiment (7 dpf), half the fish in each dish (n=5/dish) were imaged using a microscope for morphological analysis.

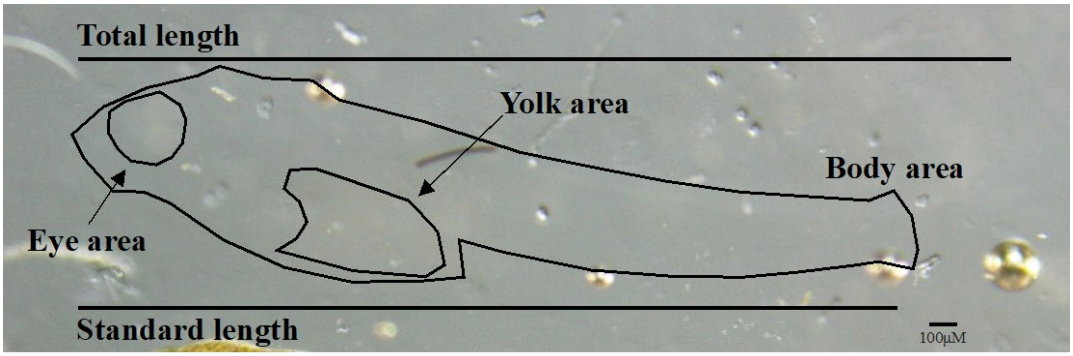

Figure 3. Morphological measures taken of each sheepshead minnow larva.

Tables:

FAMILY	ANALYTE	TYPICAL REPORTING LIMITS
PFCA	PFBA, PFPeA, PFHxA, PFHpA, **PFOA**, PFNA, PFDA, PFUnA, PFDoA, PFTrDA, PFTetrDA	0.4-1.6 ng/L water 0.04-0.16 ng/g solid 0.1-0.4 ng/g tissue 0.1-0.4 ng/mL serum 10-40 ppb AFFF
PFSA	PFBS, PFPeS, PFHxS, PFHpS, **PFOS**, PFNS, PFDS, PFDoS	0.4 ng/L water 0.04 ng/g solid 0.1 ng/g tissue 0.1 ng/mL serum 10 ppb AFFF
FTS and FTCA	4:2, 6:2 and 8:2 FTS, 3:3, 5:3 and 7:3 FTCA	3.2- 10 ng/L water 0.32 – 1 ng/g solid 0.8 – 2.5 ng/g tissue 40-250 ppb AFFF
Sulfonamides	EtFOSAA, MeFOSAA, PFOSA, EtFOSA, MeFOSA, EtFOSE and MeFOSE	0.4-4 ng/L water 0.04-0.4 ng/g solid 0.1-1 ng/g tissue 10-100 ppb AFFF
Ether carboxylates and sulfonates	HFPO-DA (GEN-X), ADONA, F-53B, NFDHA, PFMBA, PFMPA, PFEESA	0.4-1.6 ng/L water 0.04 – 0.16 ng/g solid 0.1-0.4 ng/g tissue 10-40 ppb AFFF

Table 1. PFAS to be analyzed using EPA Method 1633 for sediment (i.e., solid) samples taken from each sampling site in Matagorda Bay. Table provided by SGS AXYS Analytical Services, LTD[1].