4-1-2019

Toxicological effects of tire wear particles on mummichogs and fathead minnows

Stephanie B. LaPlaca
Clemson University

Peter van den Hurk
Clemson University

Toxicological effects of tire wear particles on mummichogs and fathead minnows

Stephanie B. LaPlaca, Peter van den Hurk
Clemson University, Clemson, SC

Background

Recent studies on the distribution of microplastics in the Charleston Harbor, SC, revealed that a large part of the microplastic particles that are found in the intertidal sediments are tire wear particles. These particles originate from the wear of tire treads on roadways, and wash into the estuary during rain events. The synthetic rubber in car tires consist of a large variety of chemicals, which can be different between brands, but usually contains styrene-butadiene rubber, carbon black and zinc. To investigate the potential toxicity of tire wear particles, both fathead minnow and Atlantic killifish were exposed to different concentrations of tire crumb particles (38-355 µm) in a 7 day exposure. Dissection of the fish revealed that particles were ingested and accumulated in the intestinal tract.
To investigate if polynuclear aromatic hydrocarbons were leaching from the particles, bile fluorescence was measured, together with potential induction of cytochrome P450-1A through the EROD assay. In addition, glutathione S-transferase was measured as a general stress parameter.

Research Questions

1. Do fish ingest tire wear particles (TWP)?
2. What toxic effects can be measured from exposure to TWP?
   - 2a. Do fish absorb PAHs leaching from TWP?
   - 2b. Is a biological response as induction of biotransformation enzymes (CYP1A and GST) observed?

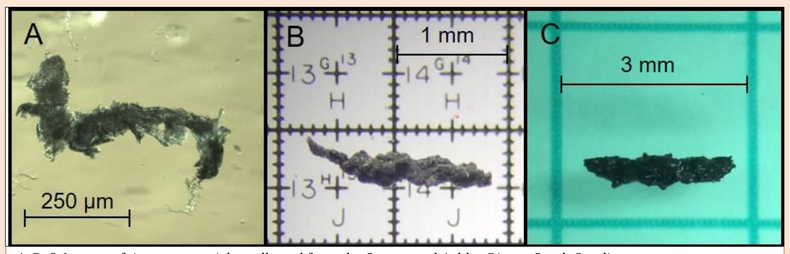
Methods

Exposure set up:
- Fathead minnows (*P. promelas*) from Clemson University culture
- Mummichogs (*F. heteroclitus*) collected from North Inlet - Winyah Bay NERR, SC

- Micronized tire particles, commercially obtained (size 38-355 µm)
Experiment A – Mummichogs, 0.0, 0.3, 1.9, 6.0 g/l
Experiment B – Mummichogs, 0.0, 0.1, 0.33, 1.0 g/l
Experiment C – Fathead minnows, 0.0, 0.3, 1.9, 6.0 g/l

- 5 fish per concentration, individually exposed in 4L jars
- 7 day static-renewal, water & particles renewed every other day

Biomarkers:
Bile Fluorescence – fluorescence units were measured at wavelength pairs typical for 2-ring, 4-ring, and 5-ring PAHs
Cytochrome P450-1A (Ethoxyresorufin O-deethylase [EROD] assay)
Glutathione S-transferase (GST assay using 1-chloro-2,4-dinitrobenzene)

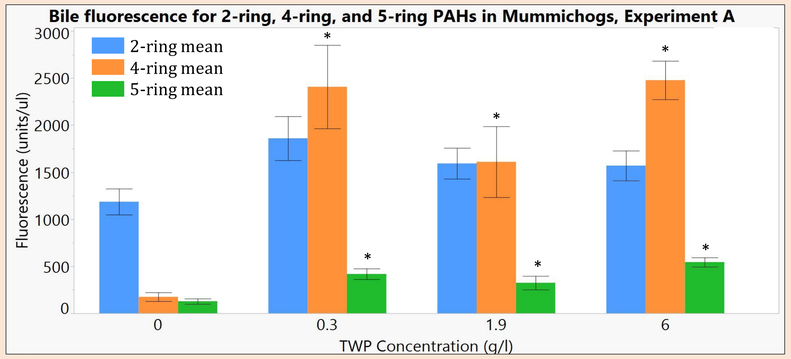


A, B, C: Images of tire wear particles collected from the Cooper and Ashley Rivers, South Carolina.
From: Weinstein et al. (2018). Microplastic contamination in Coastal South Carolina: Sources, trophic transfer, and abundance in biota. SC Sea Grant Report.

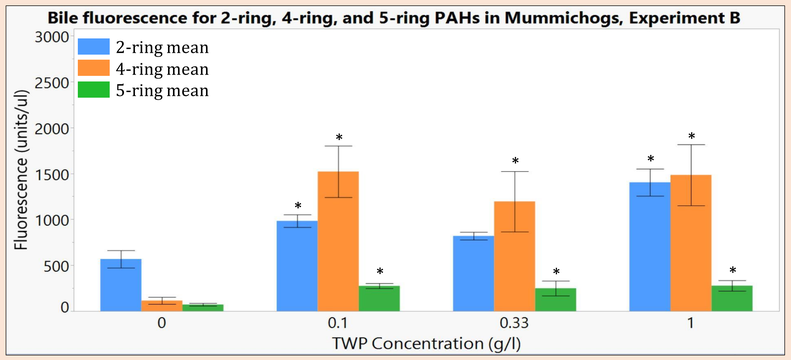
Fathead minnow (P. promelas) from Experiment C with tire wear particles in intestinal tract.

Results – Bile Fluorescence

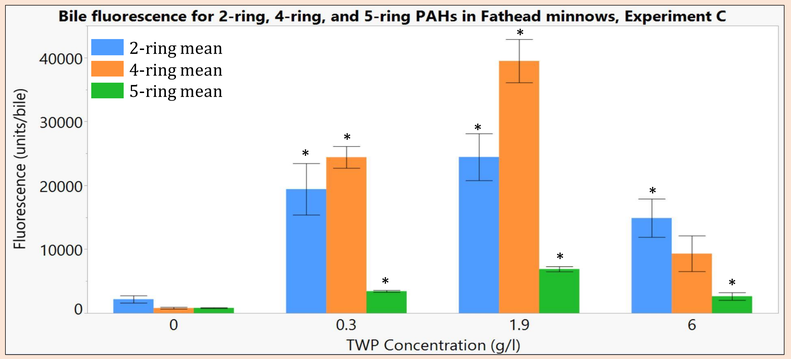
Experiment A – Mummichogs (0.3, 1.9, 6.0 g/l)



Experiment B – Mummichogs (0.1, 0.33, 1.0 g/l)



Experiment C – Fathead minnows (0.3, 1.9, 6.0 g/l)

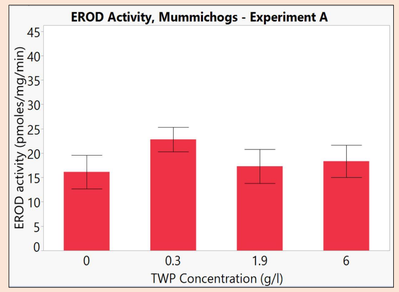


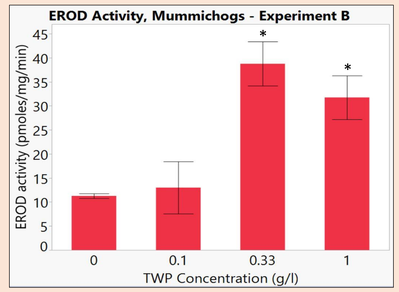
Average fluorescence units per µl of bile. Asterisks indicate significant differences from the control treatment (0 g/l). Significant increase in bile fluorescence, indicating exposure to PAHs, when exposed to TWP.

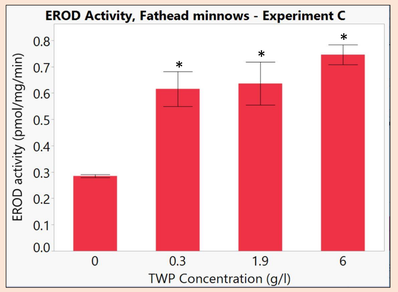
References: Gray, AD, Wertz, H, Leads, RR, Weinstein, JE. (2018). Microplastic in two South Carolina estuaries: Occurrence, distribution, and composition. *Marine Pollution Bulletin, 128,* 223-233. Gray, AD and Weinstein, JE. (2017). Size and shape dependent effects of microplastic particles on adult daggerblade grass shrimp, *Palaemonentes pugio. Environmental Toxicology and Chemistry, 36*(11), 3074-3080. Kreider, ML, Panko, JM, McAtee, BL, Sweet, LI, Finley, BL. (2010). Physical and chemical characterization of tire-related particles: Comparison of particles generated using different methodologies. *Science of the Total Environment, 408*(3), 652-659. Van den Hurk, P. (2006). Bile fluorescence, heme oxygenase induction, and increased biliverdin excretion by mixtures of environmental toxicants. *Aquatic Toxicology,* 77:202-209.
Acknowledgements: John Weinstein, Rachel Leads, Sarah Kell (The Citadel, College of Charleston); John Smink (Clemson University); SC Sea Grant Consortium, award N468 (R/ER-46).

For more information: Stephanie B. LaPlaca, slaplac@clemson.edu

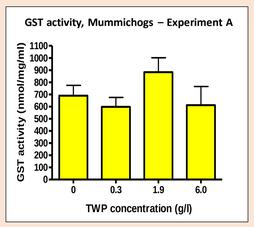
Results – EROD Assay

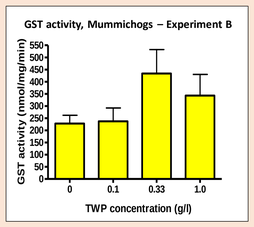


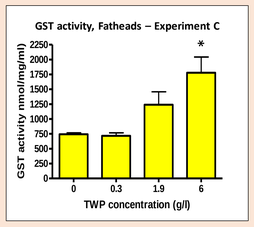




Results – GST Assay







Conclusions

1. Fish are more sensitive to tire wear particles than grass shrimp.
   - Shrimp: no mortality at 100 g/l (Gray & Weinstein, 2017), fathead minnows 40% mortality at 6 g/l.
2. Major route of uptake & exposure to tire particles is ingestion.
3. PAHs from tire particles are absorbed in the fish, processed in the liver, then excreted into the bile.
4. Exposure to tire particles leads to upregulation of CYP1A and GST enzyme activity in both mummichogs and fathead minnows.
5. EROD activity may be suppressed at concentrations > 1 g/l in mummichogs.
6. Biomarker responses show effects at environmentally relevant concentrations of tire wear particles (1 – 2 g/l).

Future Work

- Composition of tire particles – which compounds are leaching out?
- Variability in pH or salinity may impact leachability.
- Trophic transfer of tire particles and their absorbed contaminants?
- Repeat exposures with larger fish and larger sample size.