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The Influence of Polyethylene Nanoplastics on The Toxicity of Methoxychlor on D. magna

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The influence of polyethylene nanoplastics on the toxicity of methoxychlor on *D. magna*

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Introduction

Microplastics (particles <5mm) have become an emerging contaminant of concern in aquatic environments due to its multiple pathways of entry via wastewater treatment plant (WWTP) effluent, household discharges, and industrial outflows (Fendall & Sewell, 2009; Anderson et al., 2016; Mason et al., 2016). Recent studies have focused on 1) the presence of microplastics in aquatic environments and 2) their impacts on the physiology of exposed organisms. Microplastics are able to be ingested by organisms, leading to a multitude of physiological threats including gastrointestinal obstruction and toxicity from the leaching of organic contaminants (Wang et al., 2016).

Methoxychlor is an organochlorine pesticide that has posed a threat to biological systems as an estrogenic and antiandrogenic endocrine disrupting chemical (Akgul et al., 2011), resulting in the alteration of reproduction and behavior in exposed mammals (Haschek, 2013; Monneret, 2017). Although its use is currently banned in the United States, methoxychlor remains relevant as a model legacy contaminant with implications as a transgenerational toxicant (Aoyama et al., 2012). Due to plastic's ability to sorb and transport organic pollutants (Wang et al., 2016), it is possible that it may interact with methoxychlor and result in a synergistic impact on exposed biota.

Objectives

In this study, the effects of polyethylene nanoplastics (d = 10-20 µm) on the toxicity of methoxychlor on the freshwater invertebrate *Daphnia magna* will be assessed. Mortality after 48hrs and mobility after 24hrs of exposure will be used as indicators of toxicity.

Hypotheses

Mortality of *D. magna* was expected to be significantly greater when exposed to a solution of methoxychlor and polyethylene nanobeads in comparison to when exposed individually to methoxychlor or nanobeads. Mobility was expected to decrease more significantly when exposed to nanobeads and methoxychlor in mixture than when exposed to singular toxicants.

Materials and Methods

- Adult *D. magna* were exposed to 0, 12.5, 25, 50, and 100 mg/L of polyethylene nanobeads (Cospheric, d=10-20 µm) or methoxychlor at 0 (EtOH vehicle), 1, 2.5, 5, and 10 ug/L) for 48 hours.
- Mortality was assessed at 24 and 48 hours (n=9 for 0, 12.5, 25, 100 mg/L, n=8 for 50 mg/L for nanobeads) (n= 11 for 0 µg/L, 14 for 1 µg/L, 17 for 2.5 µg/L, 15 for 5 µg/L, 16 for 10 µg/L for methoxychlor).
- Mobility assays were performed (n=9 for 0, 12.5, 25, 100 mg/L, n=8 for 50 mg/L for nanobeads, n = 11 for 0 µg/L, 14 for 1.0 µg/L, 16 for 2.5 µg/L, 15 for 5.0 µg/L, 15 for 10 µg/L for methoxychlor) in a lightbox chamber to ensure a controlled light source with 3 min of acclimation followed by 3.5 min of recording. Videos were analyzed using tracking software ToxTrac (v2.61). Speed, mobile speed, acceleration, distance traveled, and frozen events were assessed over a 3-minute period.

Results

Concentration	Mortality	Sample Size
0 mg/L	0	9
12.5 mg/L	1	9
25 mg/L	1	9
50 mg/L	4	8
100 mg/L	1	9

Table 1 – Mortality results after 48 hours of exposure to varying concentrations of polyethylene nanobeads.

Concentration	Mortality	Sample Size
EtOH Control	0	11
1.0 µg/L	0	14
2.5 µg/L	1	17
5.0 µg/L	2	15
10.0 µg/L	4	16

Table 2 – Mortality results after 48 hours of exposure to varying concentrations of methoxychlor in ethanol vehicle.

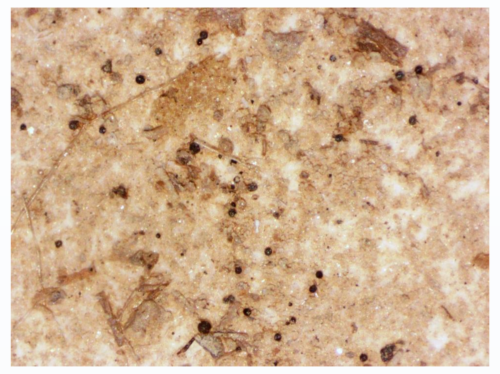
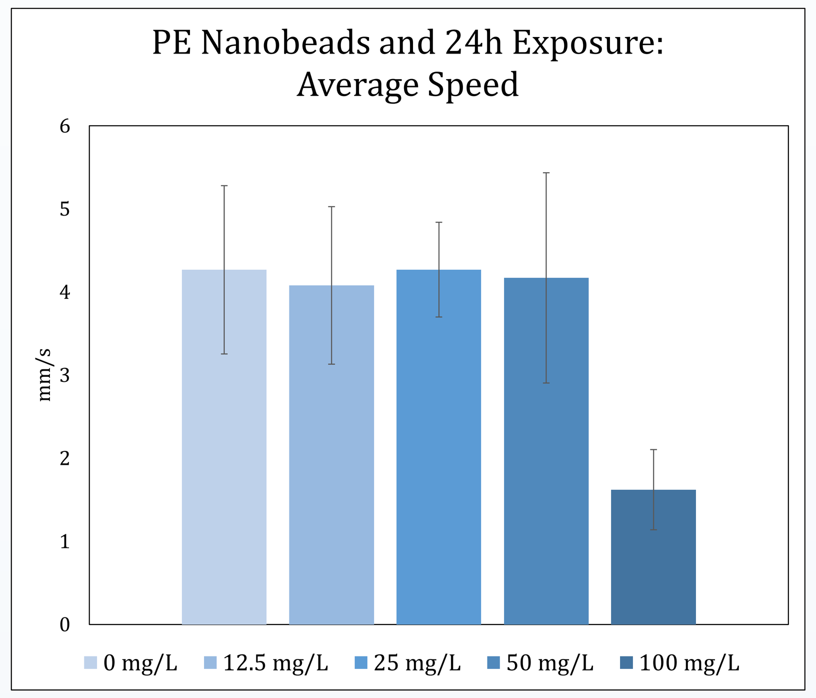
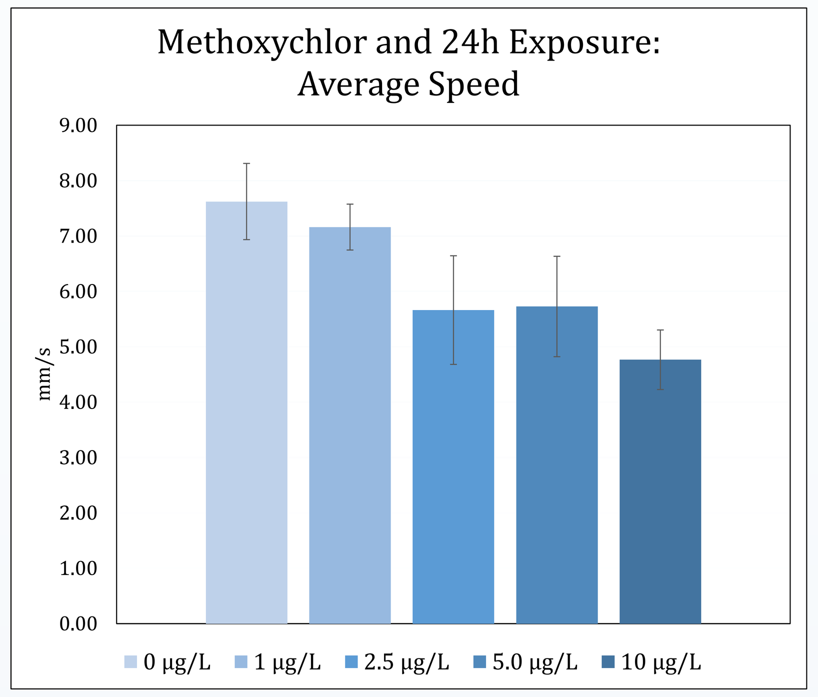


Image 1 – Microplastic beads observed in sediment samples collected from Rappahannock River, Virginia, USA. Diameter of particles range from 9 - 66 µm.



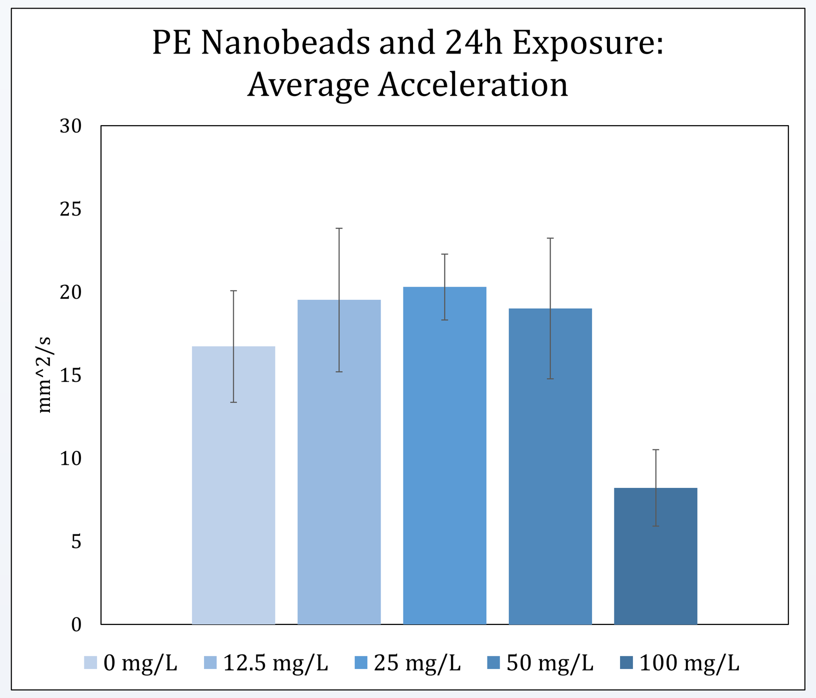
Test details	Adjusted P Value
0 vs. 12.5	0.9959
0 vs. 25	0.994
0 vs. 50	0.9976
0 vs. 100	0.0597
12.5 vs. 25	>0.9999
12.5 vs. 50	>0.9999
12.5 vs. 100	0.0309
25 vs. 50	>0.9999
25 vs. 100	0.0232
50 vs. 100	0.0431

Figure 1 – Average speed of Daphnia magna after 24 hours of static exposure to varying levels of polyethylene nanobeads. Error bars represent S.E.M. Sample size for treatment levels are: 9 for 0 mg/L, 9 for 12.5 mg/L, 9 for 25 mg/L, 8 for 50 mg/L, 9 for 100 mg/L.



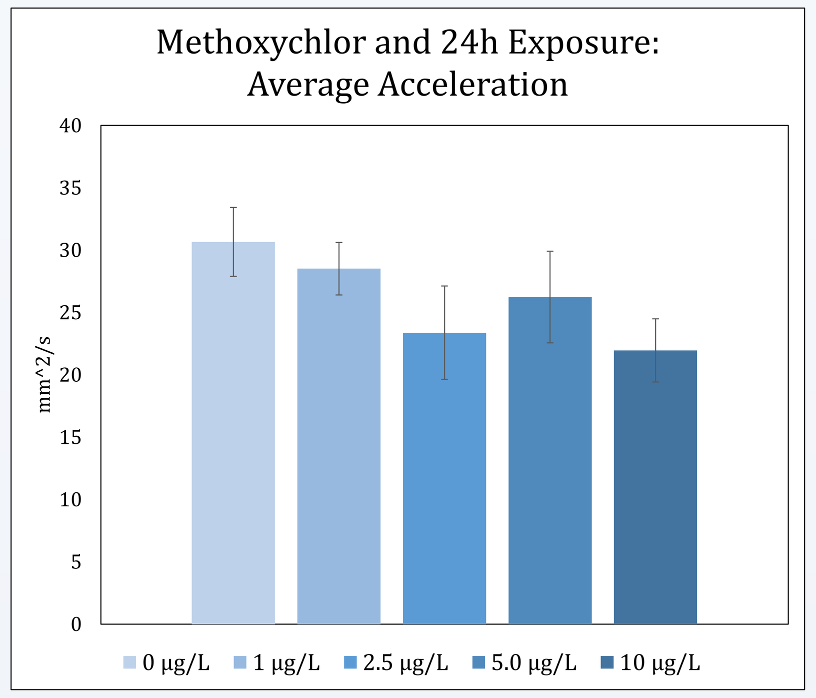
Test details	Adjusted P Value
0 vs. 1	>0.9999
0 vs. 2.5	0.1993
0 vs. 5	0.7273
0 vs. 10	0.5296
1 vs. 2.5	0.1908
1 vs. 5	0.7527
1 vs. 10	0.5438
2.5 vs. 5	0.8513
2.5 vs. 10	0.9646
5 vs. 10	0.9969

Figure 4 – Average speed of Daphnia magna after 24 hours of static exposure to varying levels of methoxychlor (in ethanol vehicle). Error bars represent S.E.M. Sample size for treatment levels are: 11 for 0 µg/L, 14 for 1 µg/L, 16 µg/L, 15 for 5.0 µg/L, and 15 for 10 µg/L.



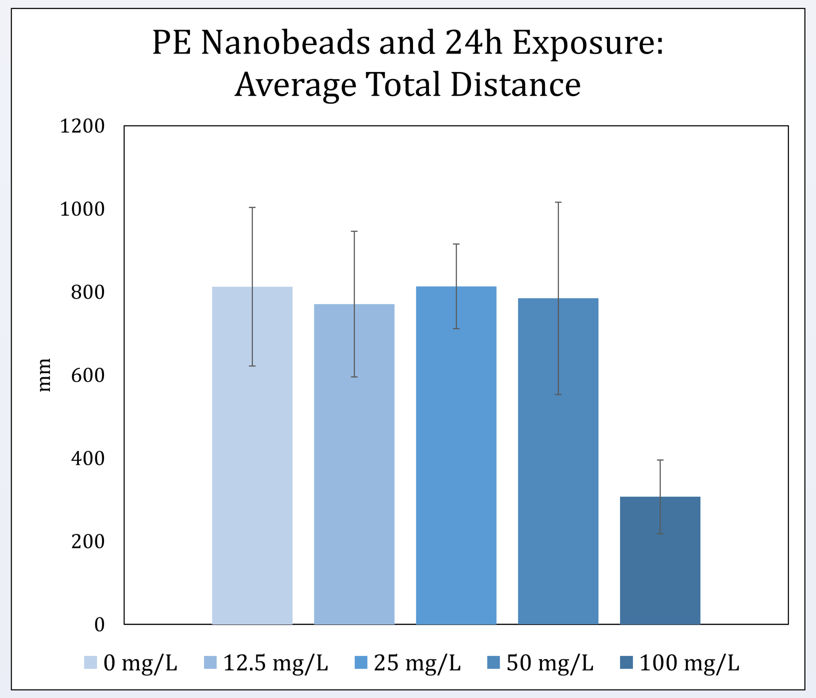
Test details	Adjusted P Value
0 vs. 12.5	0.821
0 vs. 25	0.8026
0 vs. 50	0.842
0 vs. 100	0.2128
12.5 vs. 25	>0.9999
12.5 vs. 50	>0.9999
12.5 vs. 100	0.0254
25 vs. 50	>0.9999
25 vs. 100	0.0201
50 vs. 100	0.0335

Figure 2 – Average acceleration of Daphnia magna after 24 hours of static exposure to varying levels of polyethylene nanobeads. Error bars represent S.E.M. Sample size for treatment levels are: 9 for 0 mg/L, 9 for 12.5 mg/L, 9 for 25 mg/L, 8 for 50 mg/L, 9 for 100 mg/L.



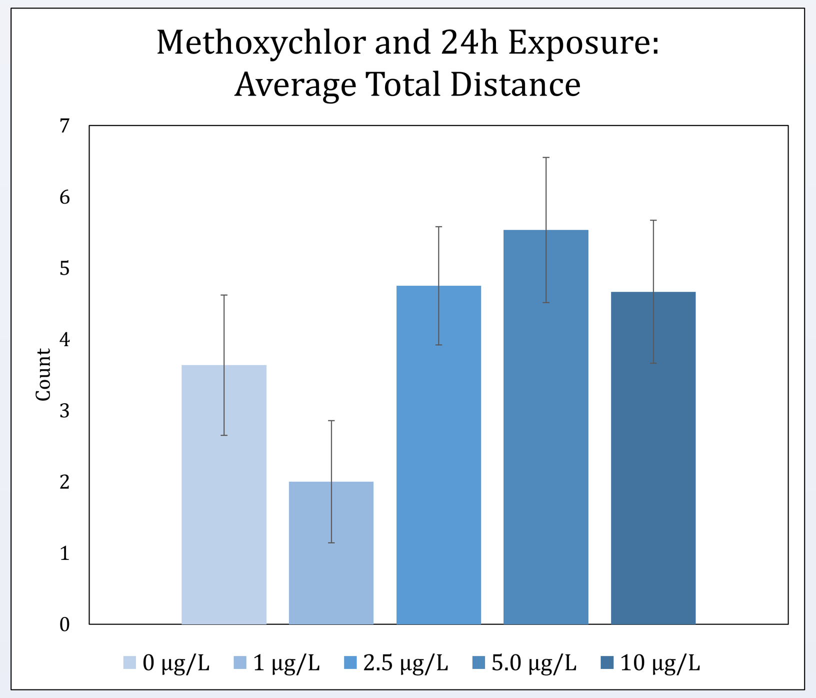
Test details	Adjusted P Value
0 vs. 1	0.9996
0 vs. 2.5	0.2516
0 vs. 5	0.86
0 vs. 10	0.6059
1 vs. 2.5	0.2875
1 vs. 5	0.9181
1 vs. 10	0.6812
2.5 vs. 5	0.7793
2.5 vs. 10	0.9656
5 vs. 10	0.9881

Figure 5 – Average acceleration of Daphnia magna after 24 hours of static exposure to varying levels of methoxychlor (in ethanol vehicle). Error bars represent S.E.M. Sample size for treatment levels are: 11 for 0 µg/L, 14 for 1 µg/L, 16 µg/L, 15 for 5.0 µg/L, and 15 for 10 µg/L.



Test details	Adjusted P Value
0 vs. 12.5	0.9959
0 vs. 25	0.994
0 vs. 50	0.9976
0 vs. 100	0.0597
12.5 vs. 25	>0.9999
12.5 vs. 50	>0.9999
12.5 vs. 100	0.0309
25 vs. 50	>0.9999
25 vs. 100	0.0232
50 vs. 100	0.0431

Figure 1 – Average distance traveled of Daphnia magna after 24 hours of static exposure to varying levels of polyethylene nanobeads. Error bars represent S.E.M. Sample size for treatment levels are: 9 for 0 mg/L, 9 for 12.5 mg/L, 9 for 25 mg/L, 8 for 50 mg/L, 9 for 100 mg/L.



Test details	Adjusted P Value
0 vs. 1	0.9998
0 vs. 2.5	0.1832
0 vs. 5	0.7553
0 vs. 10	0.5181
1 vs. 2.5	0.1937
1 vs. 5	0.8099
1 vs. 10	0.5656
2.5 vs. 5	0.8019
2.5 vs. 10	0.959
5 vs. 10	0.9936

Figure 6 – Average distance traveled by Daphnia magna after 24 hours of static exposure to varying levels of methoxychlor (in ethanol vehicle). Error bars represent S.E.M. Sample size for treatment levels are: 11 for 0 µg/L, 14 for 1 µg/L, 16 µg/L, 15 for 5.0 µg/L, and 15 for 10 µg/L.

Image 2 – Daphnia magna exhibiting ingestion of polyethylene nanobeads. Particles are found accumulated in the upper portion of the GI tract.

Image 3 – Daphnia magna exhibiting ingestion of nanoplastic beads. Accumulation is found throughout the entirety of the GI tract

Image 3 – Daphnia magna exhibiting ingestion of nanoplastic beads, found scattered throughout GI tract.

Results (cont.)

D. magna mortality after 48hrs of exposure was found in all treatment levels, except the controls for both polyethylene nanobeads and methoxychlor exposure. No clear correlation between nanoplastic ingestion and exposure concentration was found. Speed, acceleration, and distance traveled were shown to vary between treatment levels, with visual implications of a linear trend as treatment concentration increased. Mobile speed and frozen events showed no distinct trends throughout treatment levels.

Conclusions

Mortality results from 48h assays indicate that selected treatment levels of both nanoplastics and methoxychlor were not entirely lethal, and thus were appropriate for this study. Using mobility as an indicator of toxicity can better explicate the extent to which a substance may cause harm. Further replicates are needed to increase sample size for a more accurate assessment of each contaminants impact on freshwater invertebrates.

Future Studies

The next phase of this study will examine the potential synergistic effects of methoxychlor and nanoplastic exposure through the combination of treatments used in this study. Gas chromatography-mass spectrometry will be used to analyze exposure solutions to assess actual versus nominal concentrations. Additionally, levels of methoxychlor before and after contact with polyethylene nanobeads will be assessed to examine potential sorption interaction between toxicants. It is expected that the concentration of methoxychlor will be lower in solutions which contained polyethylene nanoplastics in mixture, compared to solutions of methoxychlor only.

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