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DOSE DEPENDENT EFFECTS OF TWO POLYMERIC MATERIALS AND SIMILARITIES WITH CERTAIN DRUGS

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Abstract
Polymeric materials became of crucial interest in the research area over the recent years due to worldwide production increase of these materials not always being followed by knowledge of their possible detrimental effects. Therefore, in the present study we analyzed the different effects of polymeric materials at various exposure times. Our results focused on some general effects in terms of anxiety and swimming performance over 120h of exposure and multiple repeated doses of both Polyethylene and Polypropylene microplastics. Polyethylene showed an anxiogenic potential, while Polypropylene exhibited an anxiolytic potential at the first two doses. In terms of swimming performance, an increase of specific parameters was observed throughout the repeated doses in the case of Polyethylene, whereas in the case of Polypropylene they decrease. These results were compared with those from the specialized existing literature on the effects of some of the most common hallucinogenic and psychoactive substances. Our results highlight that the mechanism of action of the aforementioned materials is different and show toxic effects from the first dose. Polyethylene has a similar outcome to the one in the case of some substances, especially hallucinogenic, such as LSD, mescaline, MDMA, phencyclidine, and nicotine. On the other hand, polypropylene has a similar effect to psychoactive drugs, cannabidiol, tetrahydrocannabinol, but also MDMA at certain doses. Bases on these observations, further studies should be conducted on the similarities of the detrimental effects.

Keywords: microplastics, zebrafish, drugs, toxicology

1. INTRODUCTION

Polymeric materials became of crucial interest in the research area over the recent years mainly due to the worldwide increase production of these materials that is not always followed by studies on their possible detrimental effects. Moreover, microplastic pollution is an emerging problem because the larger plastics gradually degrade into microplastics (<5 mm diameter), which are difficult to be further broken down (Xue et al., 2020). Microplastics were reported in several types of environments, such as waters (Dusaucy et al., 2021; Terzi et al., 2022), biota (Ugwu et al., 2021), agricultural soils (Kumar et al., 2020), air and even drinking water (Zhang et al., 2020).

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Microplastics were also found recently in human tissues, such as lungs (Amato-Lourenço et al., 2021; Jenner et al., 2022), placenta (Ragusa et al., 2021) and breastmilk (Ragusa et al., 2022). The most common plastics found in these are polyethylene (PE) and polypropylene (PP). These two types of polymers are mainly used in construction and in the packaging industry. Therefore, in the present study we analyzed the different effects of these polymeric materials at various exposure times, focused on some general effects in terms of anxiety and swimming performance over 120 h of exposure of both Polyethylene and Polypropylene microplastics. The results were compared with those from the specialized existing literature on the effects of some of the most common hallucinogenic and psychoactive substances.

2. MATERIALS AND METHODS

For our study, we used adults of zebrafish species (*Danio rerio*), purchased from an authorized local breeder. The zebrafish used in this experiment had an acclimatization period under experimental laboratory conditions for 10 days, in 10 L aquariums, equipped with oxygen pumps and water changed daily. After this period, zebrafish were randomly distributed into 3 experimental groups (n= 10) and were exposed to 2 mg L^{-1} concentration of polypropylene (PP) and polyethylene (PE) microplastics, administered by dietary exposure, daily for 120 h. The diet was in line with the husbandry requirements for this species, meaning that 8% of their food weight combined with the required amount of polymeric material was administered. The behavioral response was recorded using the EthoVision XT 14 video tracking software (Noldus Information Technology, The Netherlands) and the data were statistically analyzed by ANOVA analysis. To observe the effects of these polymeric materials we performed the Novel Tank Test. The results were then compared with those from the specialized existing literature regarding the effects of some of the most common hallucinogenic and psychoactive substances. Animals were treated and maintained in accordance with the EU Commission Recommendation (2007), Directive 2010/63/EU of European Parliament, and the Council of 22 September 2010 guidelines for accommodation, care and protection of animals used for experimental and other scientific purposes. The protocol we followed received approval from the Ethics Committee of the Faculty of Biology, “Alexandru Ioan Cuza” University of Iasi, with the registration number 343/09.02.2023.

3. RESULTS AND DISCUSSIONS

In the Novel Tank Test, the following parameters were monitored in particular: Distance moved, Velocity, Latency to reach the upper part of the tank for the first time, frequency of entries in the upper part, time spent in the upper part, circling behavior and freezing durations. In the case of polyethylene, the swimming performance parameters (distance moved, velocity) increased with the dose compared to the pretreatment, indicating a non-specific agitated behavior, with the exception of the 96 h exposure, where they decreased. Whereas the swimming parameters of the polypropylene group decreased in a dependent manner with the doses. For the other monitored parameters, presented in Table 1 and graphically representative in Figure 1, the latency to reach the upper part of the aquarium decreases compared to the pretreatment at all doses, in the case of both polymers as the doses increase, and the time spent in the upper part of the aquarium also increases.

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Table 1 - The results of the monitored parameters during the exposure in the novel tank test (- no data/no effect, ↑- increase, ↓- decrease)

		Distance moved	*Velocity*	*Latency to top*	*Fervency to enter in top*	*Time spent in top*	*Circling behavior*	*Freezing durations/Anxiogenic effect*
PE	24 h	↑	↑	↓	↑	↑	↑	↑
	48 h	↑	↑	↓	↑	↑	↓	↑
	72 h	↑	↑	↓	↑	↑	↓	↑
	96 h	↓	↓	↓	↑	↑	↓	↑
	120 h	↑	↑	↓	-	↑	↑	↑
PP	24 h	↓	↓	↓	↓	↑	↓	↓
	48 h	↓	↓	↓	↑	↑	↓	↓
	72 h	↓	↓	↓	↓	↑	↓	↓
	96 h	↓	↓	↓	↑	↑	↓	↓
	120 h	↓	↓	↓	↑	↑	↓	↑

The frequency of entering the upper part of the aquarium increases with the dose in the case of polyethylene, except for the last dose, in which case the increase was very subtle compared to the pretreatment. In the case of polypropylene, the frequency increases only after 48 hours of exposure and decreases after 24 and 72 hours of exposure.

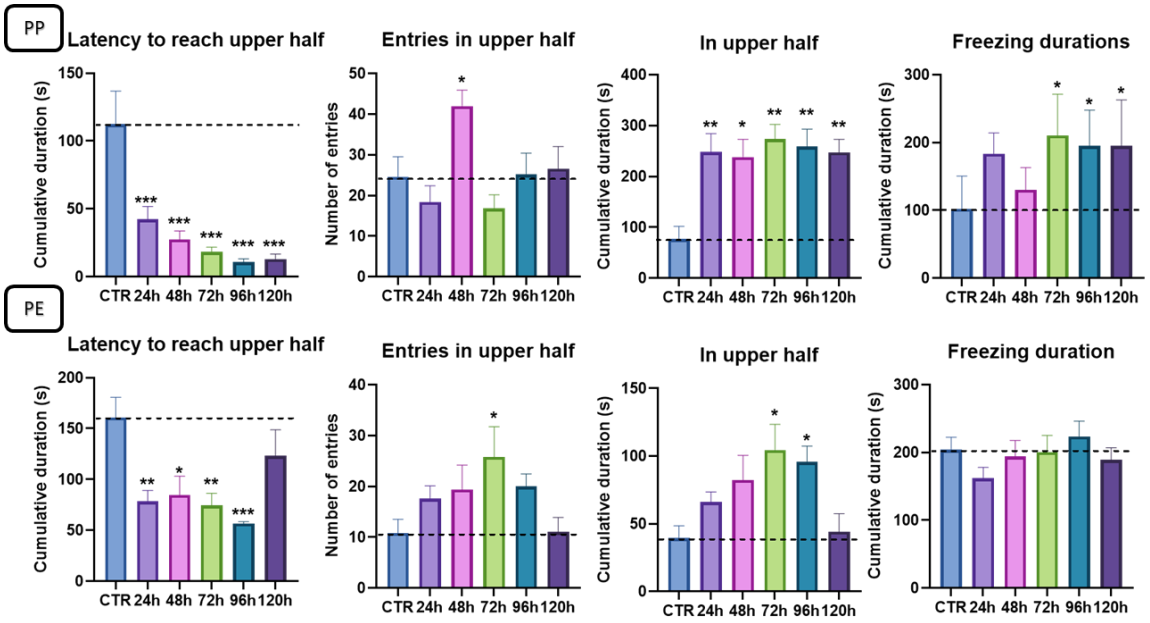

Figure 1. The results represented graphically and analyzed with Anova analysis, at a confidence level of 95%. Values are represented as mean per group±SEM. (* - p≤0.05, ** - p≤0.01, *** - p≤0.001)

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The circling behavior registered low values in the case of polypropylene and polyethylene at 48, 72 and 96 hours of exposure. The increase of this parameter was observed in the case of exposure to polyethylene for 24 and 120 hours. The freezing durations values significantly increased compared to the pretreatment, especially in the case of exposure to polyethylene. For an easier visualization of the behavior during the tests, the representative heatmaps and tracks for each individual situation are shown in Figure 2.

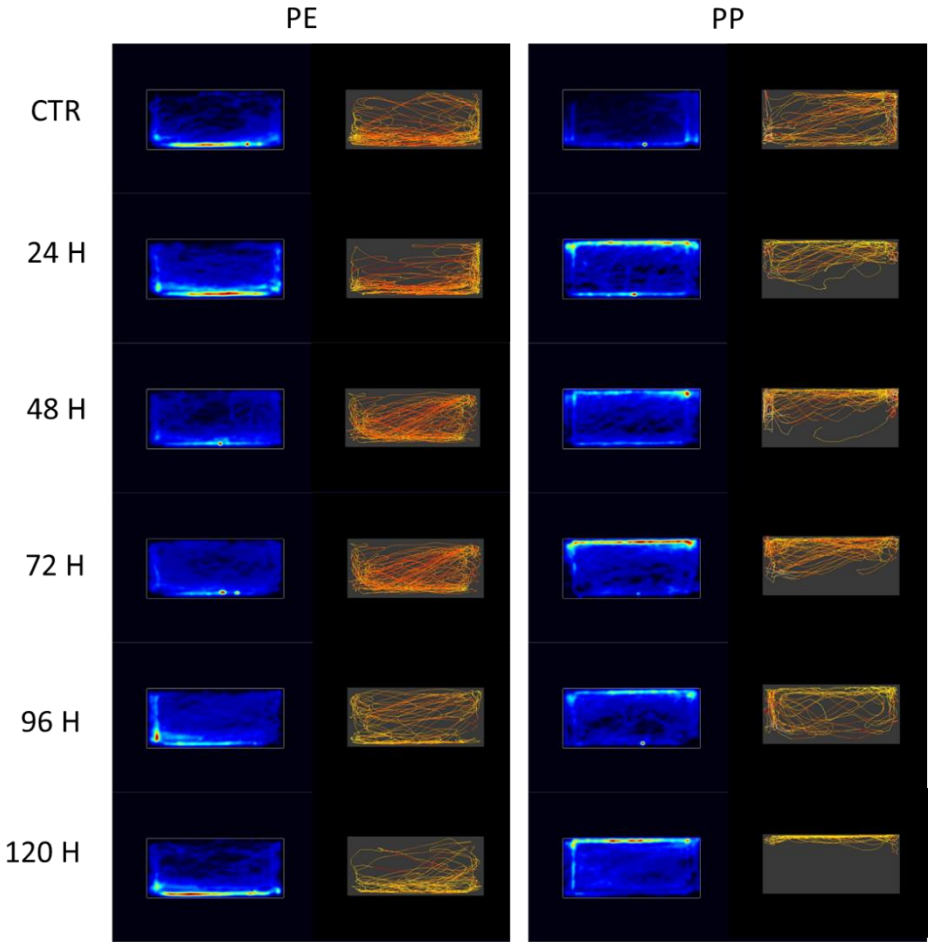

Figure 2. Representative tracks and heatmaps illustrating the average behavior of each group following treatment.

Regarding the specialized literature results related to the testing of certain substances used as drugs, the activity of the monitored parameters is presented in Table 2.

Based on these results, the effects of polyethylene were similar to those of hallucinogenic drugs, in particular Mescaline, lysergic acid diethylamide (LSD) and 3,4-methylenedioxymethamphetamine (MDMA), whose effects in humans include euphoria, hallucinations, depersonalization, and psychosis. Moreover, the effects of this polymer were also similar to Nicotine and also Phencyclidine (PCP) which is another strong hallucinogenic drug, originally developed as an anesthetic, with a similar action to ketamine, in the sense that it acts as a glutamatergic antagonist for the N-methyl- D-aspartate receptor (NMDAR). In humans, PCP induces analgesia, ataxia, euphoria, anxiety, hallucinations, and psychosis (Kyzar et al., 2012).

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Table 2 - The results of the monitored parameters during the different drugs exposure (*30 mins exposure with LSD solution added to the novel tank water, # in case of withdraw, - no data/no effect, ↑- increase, ↓- decrease)

	Distance moved	Velocity	Latency to top	Fervency to enter in top	Time spent in top	Circling behavior	Anxiety index/ Thigmotaxis/ Anxiogenic effect	Ref.
LSD	↑*	↑*	↓	↑	↑	-	-	(Grossman et al., 2010)
Mescaline	↑	↑	↓	↑	↑	↑	-	(E. J. Kyzar et al., 2012)
MDMA	-	-	↓	↑	↑	-	-	(Stewart et al., 2011)
Reserpine	↓	↓	↑	↓	↓	-	↑	(E. Kyzar et al., 2013)
Amphetamine	↑	↑	-	↓	↓	-	-	(Benvenutti et al., 2021; E. Kyzar et al., 2013)
Cocaine	↑	↑	-	-	↓	-	↑#	(López-Patiño et al., 2008; Pisera-Fuster et al., 2019)
Nicotine	↑	↑	-	-	↑	-	-	(Pisera-Fuster et al., 2019)
Ibogaine	↑	↑	↓	↓	↑	-	-	(Cachat et al., 2013)
PCP	↑	↑	↓	↑	↑	↑	-	(E. J. Kyzar et al., 2012)
Ketamine	-	-	↓	↓	↑	↑	-	(Riehl et al., 2011)
MK-801	↓	↑	-	-	↓	↑	-	(Benvenutti et al., 2021; Swain et al., 2004)
CBD	↓	↓	-	-	↑	-	↓	(Carty et al., 2019; Jensen et al., 2018; Nazario et al., 2015)
THC	↓	↓	-	-	-	-	↓	(Carty et al., 2019; Dahlén et al., 2021)

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In the case of polypropylene, the effects were similar to those of psychoactive drugs, such as 3,4-methylenedioxymethamphetamine (MDMA) for certain doses, but also with Cannabidiol (CBD) and Tetrahydrocannabinol (THC), the main cannabinoids in the Cannabis plant. THC is considered the psychoactive component, while CBD has no psychotropic activity, but has been reported to reduce epileptic seizures and modify anxiety-like behavior (Jensen et al., 2018; Licitra et al., 2022). The graphic projection of the similarity of the effects of the two polymers and certain drugs is presented in Figure 3.

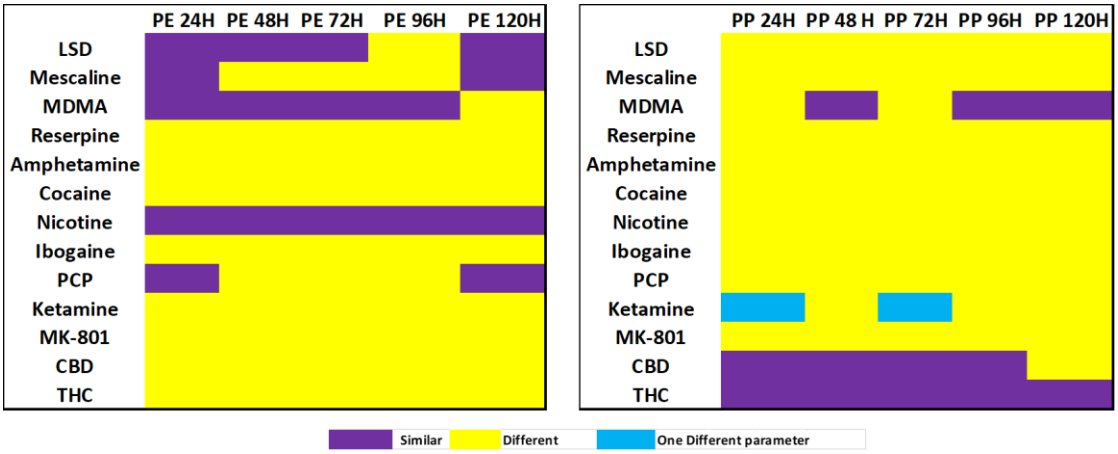

Figure 3. Representative similarity of the effects of the two polymers and certain drugs presented in Table 2.

4. CONCLUSIONS

Polyethylene showed an anxiogenic potential, while Polypropylene showed an anxiolytic effect at the first two doses. In terms of swimming performance, an increase of specific parameters was observed throughout the repeated doses in the case of Polyethylene and on the opposite pole, in the case of Polypropylene they decrease. These results were compared with those from the specialized existing literature on the effects of some of the most common hallucinogenic and psychoactive substances. Our results highlighted that the mechanism of action of the aforementioned materials is different and show toxic effects from the first dose. Polyethylene had a similar effect to the one identified in some of these substances, especially hallucinogenic, such as LSD, mescaline, MDMA, phencyclidine, and nicotine. Polypropylene had a similar effect to psychoactive drugs, cannabidiol, tetrahydrocannabinol but also MDMA at certain doses. Bases on these observation, further studies should be conducted on the similarities of the detrimental effects.

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