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Quantitative investigation of the mechanisms of microplastics and nanoplastics toward zebrafish larvae locomotor activity

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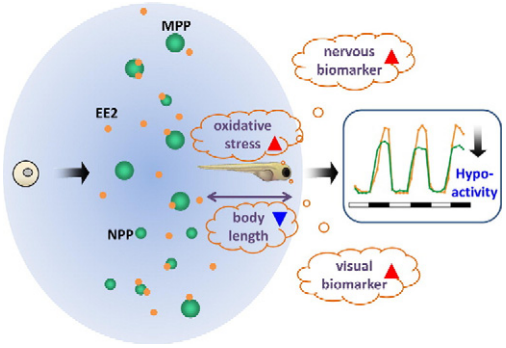
HIGHLIGHTS

- Nanoplastics, other than microplastics alone inhibited zebrafish larvae locomotion.
- Co-exposure with EE2, adsorption can alleviate locomotion hypoactivity.
- Co-exposure with a high EE2 concentration, enhanced hypoactivity can be found.
- Oxidative stress and body length reduction are main reasons for hypoactivity.

GRAPHICAL ABSTRACT



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ABSTRACT

This study investigated the direct and indirect toxic effects of microplastics and nanoplastics toward zebrafish (*Danio rerio*) larvae locomotor activity. Results showed that microplastics alone exhibited no significant effects except for the upregulated *zfrho* visual gene expression; whereas nanoplastics inhibited the larval locomotion by 22% during the last darkness period, and significantly reduced larvae body length by 6%, inhibited the acetylcholinesterase activity by 40%, and upregulated *gfap*, *α1-tubulin*, *zfrho* and *zfblue* gene expression significantly. When co-exposed with 2 μg/L 17 α-ethynylestradiol (EE2), microplastics led to alleviation on EE2's inhibition effect on locomotion, which was probably due to the decreased freely dissolved EE2 concentration. However, though nanoplastics showed stronger adsorption ability for EE2, the hypoactivity phenomenon still existed in the nanoplastics co-exposure group. Moreover, when co-exposed with a higher concentration of EE2 (20 μg/L), both plastics showed an enhanced effect on the hypoactivity. Principal component analysis was performed to reduce data dimensions and four principal components were reconstituted in terms of oxidative stress, body length, nervous and visual system related genes explaining 84% of total variance. Furthermore, oxidative damage and body length reduction were evaluated to be main reasons for the hypoactivity. Therefore, nanoplastics alone suppressed zebrafish larvae locomotor activity and both plastic particles can change the larvae swimming behavior when co-exposed with EE2. This study provides new insights into plastic particles' effects on zebrafish larvae, improving the understanding of their environmental risks to the aquatic environment.

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1. Introduction

Scientific studies have shown that plastics greatly contribute to the littering of the aquatic environment. Microplastics (commonly defined to be <5 mm) can be discharged into the environment not only directly (primary microplastics), but can also be generated via the fragmentation of larger plastic items (secondary microplastics) (Shim and Thomposon, 2015; Cole et al., 2011). Previously, these small particles have been proved to be numerically abundant in the marine environments (Cozar et al., 2014; Claessens et al., 2011; Law and Thompson, 2014; Andrady, 2011). However, investigations on the occurrence in fresh water system, still in an early stage, has commenced in lakes, rivers, shore sediments and estuaries(Faure et al., 2012; Morritt et al., 2014; Klein et al., 2015; Eerkes-Medrano et al., 2015). Microplastics can reach high densities in aquatic waters with maximum estimated densities in the thousands to 100 000 items per m^3 in surface waters (Eerkes-Medrano et al., 2015) and 1445 items per kg in sediments (Vianello et al., 2013). It has also been suggested that plastic particles with lengths in two or three dimensions between 1 and 100 nm ("nanoplastics") can be formed in the aquatic environment (Klaine et al., 2008; Koelmans et al., 2015). These findings emphasize the potential severity of plastic particles pollution in the aquatic environment today. Plastic particles in the aquatic environment will interact with environment and aquatic organisms. Nevertheless, due to the paucity of information about their effects on aquatic ecosystem, the environmental risk is essentially uncertain. Given the limited data, there is an urgent need to assess and quantify the effects of plastic particles on aquatic organisms.

Exposure to plastic particles can lead to a series of sub-lethal effects on fish and other aquatic organisms, such as decreased feeding, growth retardants, oxidative damage, and behavioral abnormality (Cedervall et al., 2012; Kashiwada, 2006; Browne et al., 2013; Bhattacharya et al., 2010). As for fish, it has been reported that the feeding speed was lowered in the carp *Carassius carassius*, when nanoplastics were dosed through food chain (Cedervall et al., 2012). In addition, nanoplastics are able to pass biological barriers, to penetrate and accumulate in tissues, to enhance the reactive oxygen species (ROS) production, and to cause subsequent effects on cellular level (Kashiwada, 2006; Browne et al., 2013; Bhattacharya et al., 2010). Polystyrene nanoplastics have been detected in adult fish medaka (*Oryzias latipes*) organs, in the blood with 10.5–16.5 ng/mg protein after 7 d exposure to 10 mg/L nanoplastics, and they are capable of penetrating the blood-brain barrier and eventually reached the brain (Kashiwada, 2006). Moreover, increased oxidative damage has been observed in the lugworm (*Arenicola marina*) and *Chlorella* and *Scenedesmus* algae after exposure to micro/nanoplastics, with increased ROS production level or decreased total antioxidant capacity, respectively (Browne et al., 2013; Bhattacharya et al., 2010). The above evidence demonstrates that effects on aquatic organisms generally occur rapidly and can serve as an early warning system to assess the potential toxicological effects in aquatic systems (Wernersson et al., 2015), which deserves more attention in the research field of toxicology of micro- and nanoplastics (Wagner et al., 2014).

Given the rapid accumulating rate of plastic particles in the aquatic environment, they could function as contaminant vectors in principle. The adherence of hydrophobic contaminants has been reported (Wright et al., 2013). Koelmans et al. reviewed that they can either contribute to the transportation or to the bioaccumulation of co-exposure chemicals in organisms (Koelmans et al., 2016). For example, microplastics can provide a feasible pathway to transfer attached pollutants into gut tissues of lugworm *Arenicola marina* (Browne et al., 2013), even at a low dose of 0.074% (dry weight ratio of plastic to sediment), the polychlorinated biphenyl's bioaccumulation increased by 1.1–3.6 fold (Besseling et al., 2013). According to our knowledge, the limited vector studies in aquatic organisms are depend on ingestion (Wright et al., 2013; Koelmans et al., 2016). Furthermore, so far little attention has been given to the factors that can influence organisms' fitness. To understand the plastic particles' effects more comprehensively, it is certainly necessary to investigate these parameters.

Zebrafish (*Danio rerio*) is a small freshwater teleost, with broad homologies to other vertebrate species in terms of genome, brain patterning, and the structure and function of neural and physiological systems (Lieschke and Currie, 2007; Strahle et al., 2012), rapidly emerging as an important model animal, including behavioral neuroscience (Gerlai, 2011), because altered neurological function is generally behaviorally apparent (Tierney, 2011). Behavioral response is related to neuronal and physiological integrity of the developing zebrafish, which is highly sensitive to pollutants, and has become a validated tool to evaluate the comprehensive sublethal toxic effects on zebrafish larvae (Rihel et al., 2010; MacPhail et al., 2009; Nusser et al., 2016). As plastic particles mainly lead to sublethal effects on aquatic organisms, monitoring the locomotor activity of zebrafish can be used as a sensitive endpoint (Kulig et al., 1996).

The aim of the present study was to quantify the potential effects of plastic particles alone and their effects on co-exposed chemicals. Endocrine disrupting chemicals (EDCs) have a variety of adverse health effects in organisms, and the primary toxic effects of EDCs were reported to be related to infertility and reduction in sperm count in adults (Choi et al., 2004). But other important toxic effects such as neurotoxicity and carcinogenicity have also been demonstrated (Choi et al., 2004). Here, 17α-ethynylestradiol (EE2) was chosen as a positive control for neurotoxicity, because EE2 can alter the zebrafish brain structure and involved in many aspects of the neuroendocrine system development influencing zebrafish behavior (Chen et al., 2016a; Maranho et al., 2014; Reyhanian et al., 2011). The focus was on the locomotion behavior of zebrafish larvae and several other parameters that could elucidate underlying mechanisms of action were assessed. The mRNA expression levels of genes related to zebrafish neurobehavioral system (*gfap* and *α1-tubulin*) were examined, which are only expressed in the central nervous system and considered as important neurodevelopment biomarkers (Fan et al., 2010). In addition, as the larval locomotion behavior was observed under light/dark stimulation environment, we also monitored two photoreceptor opsin biomarkers (*zfrho* and *zfblue*). Moreover, the increase of ROS could render zebrafish brain development and subsequently affect the swimming behavior (Salminen and Paul, 2014). Therefore, we evaluated the oxidative stress responses with sensitive biomarkers – catalase (CAT) activity, glutathione peroxidase (GPx) activity and the reduced form of glutathione (GSH). Besides, the body length, which directly influences the swimming ability, was measured. Finally, acetylcholinesterase (AChE) activity was measured, because it is an important neurotoxic biomarker and is in relative to fish growth (Pereira et al., 2012; Worek et al., 2002). The specific objectives were to determine the effect of plastics alone on zebrafish larvae locomotion behavior; to investigate the effect of plastics when co-exposed with EE2 on zebrafish larvae behavior; and to explore the underlying mechanisms of locomotor activity alteration with a series of biomarkers. To the best of our knowledge, this is the first study to systematically examine the mechanisms of plastic particles toxicity toward zebrafish larvae locomotor activity, and we believe that the findings will be helpful in ecological risk evaluations of micro- and nanoplastics.

2. Materials and methods

2.1. Chemicals and characterization of plastic particles

17α-Ethinylestradiol (EE2) was used as positive control of zebrafish larval locomotor activity, purchased from Dr. Ehrenstorfer Co., Germany. Polystyrene microplastics (45 μm) and polystyrene nanoplastics (50 nm, fluorescent or unfluorecent labeled) were purchased from Polysciences Co. (Warrington, PA, USA). All other chemicals used throughout experiments were of analytical grade.