Science of the Total Environment 643 (2018) 324–334

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Maternal transfer of nanoplastics to offspring in zebrafish (*Danio rerio*): A case study with nanopolystyrene

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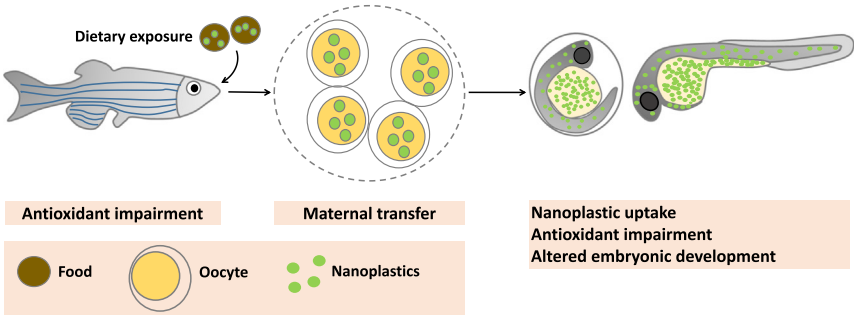
HIGHLIGHTS

- Is parental transfer a relevant route of exposure to nanoplastics in fish?
- Adult zebrafish were dietary exposed to nanopolystyrene and F1 embryos analyzed.
- Nanoplastic uptake was detected in maternally and co-parentally exposed F1 embryos.
- Plastic exposure affected the antioxidant system in both the F0 and F1 organisms.
- Despite the low toxicity, nanoplastics can affect multiple generations of fish.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 25 April 2018
Received in revised form 14 June 2018
Accepted 14 June 2018
Available online xxxx

Editor: D. Barcelo

Keywords:
Plastics
Zebrafish
Cross-generational
Diet exposure
Maternal transfer

ABSTRACT

Plastics are ubiquitous anthropogenic contaminants that are a growing concern in aquatic environments. The ecological implications of macroplastics pollution are well documented, but less is known about nanoplastics. The current study investigates the potential adverse effects of nanoplastics, which likely contribute to the ecological burden of plastic pollution. To this end, we examined whether a dietary exposure of adult zebrafish (*Danio rerio*) to polystyrene nanoparticles (PS NPs) could lead to the transfer of nanoplastics to the offspring, and whether nanoplastics exposure affects zebrafish physiology. Specifically, adult female and male zebrafish (F0 generation) were exposed to PS NPs via diet for one week and bred to produce the F1 generation. Four F1 groups were generated: control (unexposed females and males), maternal (exposed females), paternal (exposed males), and co-parental (exposed males and females). Co-parental PS NP exposure did not significantly affect reproductive success. Assessment of tissues from F0 fish revealed that exposure to PS NPs significantly reduced glutathione reductase activity in brain, muscle, and testes, but did not affect mitochondrial function parameters in heart or gonads. Assessment of F1 embryos and larvae revealed that PS NPs were present in the yolk sac, gastrointestinal tract, liver, and pancreas of the maternally and co-parentally exposed F1 embryos/larvae. Bradycardia was also observed in embryos from maternal and co-parental exposure groups. In addition, the activity of glutathione reductase and the levels of thiols were reduced in F1 embryos/larvae from maternal and/or co-parental exposure groups. Mitochondrial function and locomotor activity were not affected in F1 larvae. This study demonstrates that (i) PS NPs are transferred from mothers to offspring, and (ii) exposure to PS NPs modifies the antioxidant system in adult tissues and F1 larvae. We conclude that PS NPs could bioaccumulate and be passed on to the offspring, but this does not lead to major physiological disturbances.

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https://doi.org/10.1016/j.scitotenv.2018.06.186
0048-9697/

1. Introduction

Plastic pollution is a rapidly developing research field. The abundance of plastic in the oceans is estimated at 5.25 trillion particles and the number of particles continues to increase (Eriksen et al., 2014); however, the potential adverse ecological implications of micro and nanoplastics (plastic particles with at least 1 dimension <100 nm) are largely unknown (Andrady, 2011). Similarly, the ecological implications of micro and nanoplastics in freshwater ecosystems are also poorly understood (Anderson et al., 2016).

Some information is available for microplastics. As reviewed by Anderson et al. (2016), microplastics are ubiquitous in freshwater and marine environments and are regarded as contaminants of emerging concern. Microplastics are present in several personal care products for product stabilization, viscosity regulation, and skin conditioning and some of these particles reach the aquatic environment via sewage effluents. They can also form through degradation of larger pieces, and their fate and behavior depend on their composition – low-density plastics are typically buoyant, whereas high-density plastics are more likely to sink and accumulate in sediment (Anderson et al., 2016). Concentration of microplastics in aquatic environments is variable at both temporal and spatial scales, but usually ranges from as low as few particles to dozens of thousands particles per cubic meter of surface water (Li et al., 2016). The ingestion of microplastics by invertebrates and fish has been shown in different species and areas of the globe (Wesch et al., 2016) at concentrations as low as <1 to hundreds of particles per organism (Vandermeersch et al., 2015). For instance, 27% of red mullet (*Mullus surmuletus*) caught near Balearic Islands and 15% of European pilchards (*Sardina pilchardus*) and anchovies (*Engraulis encrasicolus*) near Spanish Mediterranean coast had measureable quantities of microplastics in the gastrointestinal tract (up to 1 particle/individual) (Alomar et al., 2017; Compa et al., 2018). Their toxicity is thought to be linked to stress of ingestion (blockage of digestive tract), leakage of additives within plastics, and presence of adsorbed organic pollutants on the surface of microplastics (Anderson et al., 2016). Due to the potential environmental risks of microplastics several European countries and a few of the states within US banned microplastics in consumer products (Anderson et al., 2016).

Much less is known about nanoplastics, which are predicted to be present in the aquatic environment (Cozar et al., 2014), and are thought to form primarily via photo- and physical degradation of larger plastic particles (Andrady, 2011). The presence of nanoplastics is not yet quantified in the aquatic environment and biota due to limitations in analytical methods (Koelmans et al., 2015). Recent studies suggest that plastic nanoparticles accumulate in various aquatic invertebrates, which could lead to their accumulation within the food web (Bergami et al., 2016; Della Torre et al., 2014; Mattsson et al., 2015; von Moos et al., 2012). Moreover, several studies document that plastic nanoparticles not only accumulate in various fish species, but also lead to physiological alterations when using polystyrene nanoparticles (PS NPs) as a model. For example, PS NPs have been shown to accumulate in the brain of adult Crucian carp (*Carassius carassius*; dietary exposure of ~130 mg of particles per feeding), leading to morphological changes in the brain and behavioral changes (lower activity and longer feeding time) (Mattsson et al., 2017). Accumulation of PS NPs (1–50 mg/L) in zebrafish (*Danio rerio*) embryos/larvae has also been reported (Chen et al., 2017; van Pomeren et al., 2017), which was associated with behavioral alterations, oxidative stress, and a reduction in acetylcholinesterase activity (Chen et al., 2017).

Our previous study independently corroborated the ability of PS NPs to penetrate the zebrafish chorion and accumulate in the yolk sac and other regions upon a waterborne exposure (Pitt et al., 2018). We also showed several effects on embryos/larvae (e.g. bradycardia and locomotor hypoactivity). Notably, accumulation of PS NPs in the embryo yolk sac suggests that egg yolk is a potential target for PS NPs accumulation in adult female fish. Such accumulation of PS NPs in maternal gametes could transfer to the offspring, potentially altering physiology and development. While the cross-generational transfer of PS NPs has been already documented in invertebrate models (e.g. Brun et al., 2017; Cui et al., 2017; Lee et al., 2013; Zhao et al., 2017), it has not yet been examined in a vertebrate model. The current study used a dietary exposure to PS NPs as an ecologically-relevant route of exposure to assess the potential transfer of nanoplastics to the offspring. Taking into account that nanoplastics were shown to induce developmental issues, changes on locomotor activity and oxidative stress in zebrafish (Chen et al., 2017; van Pomeren et al., 2017; Pitt et al., 2018), the adverse effects of PS NPs were also evaluated in both the F0 and F1 generation using a set of biomarkers to investigate if these effects persist after a parental exposure.

2. Materials and methods

2.1. Materials

Fluorescent and non-fluorescent PS NPs (cat. #FSDG001 and #PS02002, respectively) were purchased from Bangs Laboratories, Inc. (Fishers, IN, USA). The fluorescent stock solution contained 1% (internally labeled with Dragon Green; ex./em. 480/520) PS NPs with a nominal mean diameter of 42 nm. The non-fluorescent stock solution contained 10% PS NPs also with a nominal mean diameter of 42 nm. Additionally, the stock solutions contained 0.1% sodium dodecyl sulfate (SDS; surfactant to prevent particle aggregation) and 0.05–0.09% sodium azide (bacteriostatic preservative). All other chemicals were purchased from Sigma-Aldrich (St. Louis, MO, USA) unless otherwise specified.

2.2. PS NPs characterization and preparation

Non-fluorescent PS NPs were characterized using a dynamic light scattering (DLS) (Zetasizer Nano, Malvern Instruments Ltd., Malvern, UK). The hydrodynamic diameter and zeta potential of 5 mg/L PS NPs were assessed in 0.065‰ artificial seawater (ASW; Instant Ocean, Blacksburg, VA, USA). The particle size characterization of fluorescent PS NPs was assessed in our previous study in 0.065‰ ASW (Pitt et al., 2018).

Prior to addition of PS NPs to zebrafish food (see Section 2.3), the particles were centrifuged using a Vivaspin® 2 mL Ultrafiltration Device (300,000 molecular weight cut-off, cat. #AA022) (Bangs Laboratories, Inc., Fishers, IN, USA) at 4000*g* for 10 min intervals to remove the sodium azide and SDS present within the solution. The PS NPs solution was then washed three times in deionized (DI) water and filtered in the same manner. The PS NPs were then brought up to a final volume to reach 5% of the total solution by mass in DI water.

2.3. Diet preparation

Two diets were prepared: a control diet and PS NPs diet with fluorescent or non-fluorescent particles, depending on the experiment. To prepare the control diet, crushed Zeigler's Adult Zebrafish Complete Diet (Aquatic Habitats, Inc., Gardners, PA, USA), decapsulated brine shrimp egg, and gelatin (Carolina Biological Supply Company, Burlington, NC, USA) were mixed in DI water, such that the final concentrations of the aforementioned components were 90, 45, and 120 mg/mL, respectively. (Bisesi et al., 2015; Blickley et al., 2014). For the treated diet, PS NPs were added such that the final concentration of the particles was approximately 10% of the food by mass (the gelatin content was not considered part of the diet). This concentration was chosen based on a previous study with medaka and low-density polyethylene microplastics (Rochman et al., 2013). This mixture was heated to 60 °C and then vortexed. Enough food was prepared for a week of feeding based upon the average fish mass of each tank. Food was transferred to vials in aliquots of the daily amount of food required for an average 1% of