# Maternal Effect of Polyethylene Microplastic Fragments Containing Benzophenone-3 in Different Ages and Broods of *Daphnia Magna*

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#### Abstract



The maternal effect of microplastics (MPs) toxicity is likely influenced by age and brood of test species. This study investigated the maternal effect of polyethylene MP fragments ( $18.23 \pm 8.02 \mu m$ ) with benzophenone-3 (BP-3;  $2.89 \pm 0.20\%$ w/w) on chronic toxicity to *Daphnia magna* over two generations. Neonate (<24 h old) and adult (5 d old) daphnids in the F0 generation were exposed until 21 d old, then first and third brood neonates in the F1 generation were recovered in clean M4 medium for 21 d. Higher chronic toxicity and maternal effect of MP/BP-3 fragments were observed in the adult group compared with the neonate group, reducing growth and reproduction in both F0 and F1 generations. First brood neonates in the F1 generation showed a higher maternal effect of MP/BP-3 fragments than third brood ones, resulting in enhanced growth and reproduction relative to the control. This study provided insights into the ecological risk of MPs containing plastic additives in the natural environment.

Keywords Chronic toxicity · Microplastic · Multigeneration · Plastic additive · Water flea

## Introduction

Microplastic (MP) pollution is ubiquitous in the aquatic environment, from riverine and coastal waters to remote lakes and open seas (Ajith et al. 2020; Li et al. 2020; Dusaucy et al. 2021). MPs are a growing concern due to their potential harms: intestinal damage, oxidative stress, neurotoxicity, and behavioral change when ingested by organisms (De Sá et al. 2018; Botterell et al. 2019; Gola et al. 2021). Furthermore, MPs may introduce chemical additives into the environment, such as plasticizers, flame retardants, and ultraviolet (UV) stabilizers, which are known to be toxic to aquatic organisms (Cole et al. 2011; Koelmans 2015; Gunaalan et al. 2020). Previous studies have focused on plasticizers and flame retardants that are more commonly found in plastics, thus the adverse effect of UV stabilizers on aquatic organisms is relatively unknown (Gunaalan et al. 2020; Carve et al. 2021). Several studies have reported

Jinho Jung jjung@korea.ac.kr the toxicity of UV stabilizers, such as endocrine disruption, stress response, and neurotoxicity (Kim et al. 2014; Martín-Folgar et al. 2018; Tao et al. 2020). In particular, benzophenone-3 (BP-3), one of the most widely used UV stabilizers (Kim and Choi 2014; Bratkovics et al. 2015), is known to inhibit somatic growth, embryonic development, and reproduction of the water flea *Daphnia magna* (Im et al. 2022). Moreover, our previous studies have reported the combined effect of polyethylene (PE) MP fragments and BP-3 on acute toxicity (Na et al. 2021) and chronic toxicity (Song et al. 2021) in *D. magna*.

Most *D. magna* chronic toxicity studies with MPs have been conducted in the standard condition with neonates under 24 h old (OECD 211, 2012). However, in the real environment, populations consist of different age groups, which have different sensitivities to toxic chemicals (Muyssen and Janssen 2007; Wagner et al. 2017; Ellis et al. 2020). For instance, neonates have shown higher toxicity than adults when exposed to insecticides (Ginjupalli and Baldwin 2013), plastic additives (Shen et al. 2019), pharmaceuticals (Wagner et al. 2017), and microplastic (Eltemsah and Bøhn 2019). Therefore, chronic toxicity assessments with different age groups are required to better predict the ecological outcome of MPs.

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Parental exposure to MPs may induce maternal effects that influence offspring fitness, including development, somatic growth, and reproduction (Mousseau and Fox 1998; LaMontagne and McCauley, 2001). Several studies have demonstrated the maternal effect in *D*.magna under MPs exposure (Martins and Guilhermino 2018; Liu et al. 2022; Song et al. 2022). For instance, Liu et al. (2022)demonstrated that parental exposure to polystyrene MPs and roxithromycin decreased the reproduction of *D*. magna in the subsequent generation. In addition, Song et al. (2022) reported the transgenerational effect of PE MP fragments containing BP-3 across four generations. However, there is limited knowledge about the maternal effects of MPs in *D*. magna at different ages and broods.

The aim of this study was to investigate the maternal effect of PE MP fragments containing BP-3 additive (MP/ BP-3 fragments) on chronic toxicity to *D. magna*. We evaluated the difference between (1) neonate (<24 h) and adult (5 d) exposure groups and (2) the first and third brood groups over two subsequent generations. We hypothesized that (1) the neonate exposure will induce higher chronic toxicity and maternal effect than the adult exposure and (2) the maternal effect will be higher on the first brood than on the third brood offspring.

#### **Materials and Methods**

# Preparation and Physiochemical Characterization of MP/BP-3 Fragments

Virgin PE pellets and BP-3 were purchased from Sigma-Aldrich, USA. The PE pellets were washed with hexane and methanol (J.T. Baker, USA), then rinsed with distilled water to eliminate plastic additives (Lee et al. 2014). After drying in an oven (30 °C), the BP-3 was incorporated into the PE pellets(3% w/w) in a mixing extruder (LME, Dynisco, USA). The BP-3 content was chosen based on the study by Hahladakis et al. (2018), where they reported that UVstabilizer composition in plastic is generally less than 3%. Then, the MP/BP-3 fibers were cut into fragments (<1 mm) and ground in a freezer mill (Freezer/Mill 6875, SPEX® Sample Prep, USA). The products were dried in a desiccator and sieved using a stainless-steel mesh ( $\leq 20 \ \mu m$ ) to obtain MP/BP-3 fragments. The morphology and size of MP/BP-3 fragments were analyzed under a field emission scanning electron microscope (FE-SEM, Quanta 250 FEG, FEI, USA). The BP-3 content in MP/BP-3 fragments was analyzed by high-performance liquid chromatography (HPLC, Agilent, USA) after extraction in methanol (Song et al. 2021).

The leaching of the BP-3 additive was determined by stirring MP/BP-3 fragments (1.0 mg  $L^{-1}$ ) in a 2 L M4 medium for 48 h. The samples (2 mL) were collected periodically, filtered with 0.45  $\mu$ m PTFE syringe filters (Whatman, USA), and analyzed for BP-3 concentration using HPLC, as mentioned above.

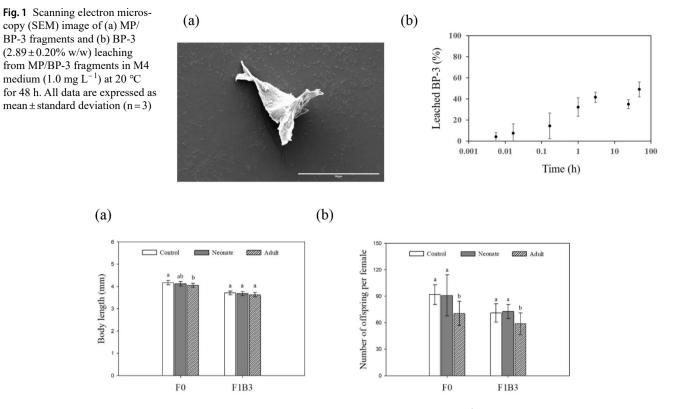
### Multigenerational Chronic Toxicity Testing of MP/ BP-3 Fragments

*Daphnia magna* clones, provided by the National Institute of Environmental Research (Republic of Korea), have been cultured since 2019 in accordance with the Organization for Economic Cooperation Development (OECD) Test Guideline 211 (OECD 2012). Daphnids were incubated in 2 L of M4 medium at 20±0.1 °C with a light/dark cycle of 16:8 h, and the medium renewal was every 3 days. The daphnids were fed with the freshwater algae, *Chlorella vulgaris*  $(5.0 \times 10^5$  cells mL<sup>-1</sup>; Aquanet, Republic of Korea) daily. Female neonates (<24 h old and ≥ 3rd brood) were used to maintain the laboratory culture.

Two generational chronic toxicity tests (n=15) were conducted in compliance with the OECD Daphnia magna reproduction test (OECD 2012). The third brood female neonates under 24 h old produced by same-aged mothers were collected from the laboratory culture for F0 generation. Each daphnid was grown in a 100-mL glass beaker containing 50 mL M4 medium under the same conditions as the culture ( $20 \pm 0.1$  °C and light/dark cycle 16:8 h). The daphnids were fed with  $5.0 \times 10^5$  cells mL<sup>-1</sup> of C. vulgaris every day, and the medium was refreshed every two days. Only the parental generation (F0) was exposed to 1.0 mg  $L^{-1}$  of MP/BP-3 fragments at neonate (<24 h old) and adult (5 d old) stages. To compare the somatic growth and reproduction of daphnids at the same age (21 d old), neonate and adult groups were exposed for 21 d and 16 d, respectively. For recovery in the F1 generation, the first (F1B1) and third (F1B3) brood neonates were grown in a clean M4 medium. Growth (adult and offspring body length) and reproduction (number of offspring per female, days to first brood) were determined for 21 d.

#### **Statistical Analysis**

Statistical analyses were performed with SAS version 9.4 (SAS Institute Inc., Cary, NC, USA). One-way analysis of variance (ANOVA) and post-hoc Tukey's honest significance test were used to test the significance of differences (p < 0.05) in neonate and adult groups. Student's t-tests were used to measure significant differences (p < 0.05) in the first and third brood groups.



**Fig. 2** Chronic toxicity and maternal effect of MP/BP-3 fragments in the neonate and adult *Daphnia magna* in terms of (a) body length and (b) number of offspring per female. F0 and F1 indicate parental and first generation, respectively, and B3 indicates the third brood. Only the F0 generation (neonate <24 h and adult 5 d) was exposed to MP/

#### **Results and Discussion**

#### **Physiochemical Properties of MP/BP-3 Fragments**

The shape of synthesized MP/BP-3 fragments was irregular thin pointy flakes (Fig. 1). The size of the fragments was  $18.23 \pm 8.02 \ \mu m$  (n=60), which is an edible size for *D. magna* (Rehse et al. 2016; Frydkjær et al. 2017; Canniff and Hoang 2018). The amount of BP-3 in MP/BP-3 fragments was measured as  $2.89 \pm 0.20\%$  w/w. In the M4 medium, BP-3 was gradually released from MP/BP-3 fragments(Fig. 2), thus, the leaching was up to 49.1% for 48 h. The leaching of BP-3 can further aggravate the toxicity of MP fragments in *D. magna* (Na et al. 2021).

# Different Chronic Toxicity and Maternal Effects in Neonate and Adult Exposure Groups

MP/BP-3 fragments significantly (p < 0.05) reduced the growth (body length) and reproduction (number of off-spring per female) in the adult group of F0 generation relative to the control (Fig. 2), but not in the neonate group. Our previous study reported that pristine PE MP fragments

BP-3 fragments (1.0 mg L<sup>-1</sup>) until 21 d old, and F1 generation (third brood) was recovered in M4 medium (control) for 21 d. All data are expressed as mean±standard deviation (n=15). Lowercase letters indicate significant differences (p < 0.05) among the three treatment groups

(44.39±11.16 µm) were not acutely toxic to neonates (<24 h old) but toxic to juveniles (4 d old) (Song et al. 2021). Additionally, Liu et al. (2018) demonstrated that the oldest daphnids (21 d old) were the most sensitive to polystyrene nanoplastics compared to younger individuals (1, 4, 7, and 14 d old). Considering that the filtering and feeding rate of *D. magna* increase with body size (McMahon 1965), faster and greater uptake of adult daphnids may aggravate the chronic toxicity of MP/BP-3 fragments. However, the bioaccumulation kinetics of MP/BP-3 fragments in *D. magna* should be investigated to evaluate the toxicity difference between neonates and adult daphnids. On the other hand, *D. magna*, in its early life stage, may have more possibility of acclimation to MP/BP-3 stress (Klerks and Weis 1987; Dietrich et al. 2010).

Several studies have reported the negative effects of MPs on *D. magna* reproduction and growth (Martins and Guilhermino 2018; An et al. 2021; Trotter et al. 2021). For instance, An et al. (2021) demonstrated that polyethylene MP fragments ( $17.23 \pm 3.43 \mu m$ ) reduced the total number of offspring and adult body length in *D. magna*. Ingestion of MP particles can hinder the feeding activities of organisms and decrease the energy budget for reproduction and growth

(Cole et al. 2013; Murphy and Quinn 2018). Moreover, the irregular shape of MP fragments can result in interstitial damage and a longer retention time in the gut (An et al. 2021; Silva et al. 2021). Recent studies emphasize the potential harm of plastic additives in MPs (Schrank et al. 2019; Zimmermann et al. 2020; Koelmans et al. 2022). BP-3 is known to act as an endocrine-disrupting chemical (Kim and Choi 2014; Wang et al. 2016), possibly by mimicking the ecdysone hormone (Ozáez et al. 2014). Ecdysone is a type of steroid hormone that regulates molting and development in insects and invertebrates, including D. magna (Pan et al. 2021). In particular, Lambert et al. (2021) observed that BP-3 significantly altered the expression of genes related to endocrine activity in D. magna, leading to a significant decrease in the number of offspring and body length. Song et al. (2021) also reported that BP-3 significantly decreased the embryonic development of D. magna.

Parental exposure to MP/BP-3 fragments did not influence the life-history traits of the F1 generation in the neonate group (F1B3). However, the number of offspring significantly (p < 0.05) decreased in the adult group of the F1 generation relative to the F1 control (Fig. 2b), indicating a maternal effect of MP/BP-3 fragments. Parental exposure in the adult group may influence the fitness of progeny (Beyer and Hambright 2017; Shaw et al. 2017), possibly leading to reduced reproduction in the F1 generation. In addition, the adverse effect of MPs may be exacerbated since the BP-3 additive is known to hinder development in D. magna embryos (Song et al. 2021). Moreover, the embryos (F1 generation) in the brood chamber can be directly exposed to MP/BP-3 fragments during parental exposure. Several studies have reported that MPs were found in the brood chamber (Guilhermino et al. 2021) and in developing embryos (Rosenkranz et al. 2009) of daphnids.

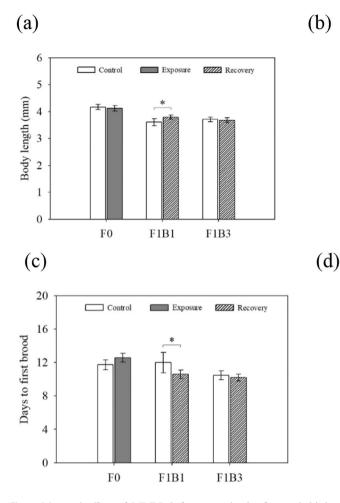
#### Different Maternal Effects in the First and Third Brood of Neonate Exposure Group

As discussed in the previous section, parental exposure of MP/BP-3 fragments to neonates did not influence the lifehistory traits of the third brood (F1B3). However, this exposure significantly (p < 0.05) increased the body length and offspring number of the first brood of *D. magna* (F1B1) relative to the control (Fig. 3). Several studies have reported increased maternal effect on the first brood of *D. magna*. For instance, Ellis et al. (2020) demonstrated that the first brood of offspring was the most sensitive to parental exposure to silver and titanium oxide nanomaterials. Additionally, Liu et al. (2012) reported that the third brood offspring showed enhanced recovery after parental exposure to the pesticide buprofezin compared to the first brood.

The first brood of *D. magna* is generally less stable than subsequent broods (Kim et al. 2014). The number of offspring of the F1B1 control ( $46.15 \pm 16.09$ ) was significantly (p < 0.05) lower than in the F1B3 control  $(71.2 \pm 10.49)$ . which is far lower than the number ( $\geq 60$ ) recommended in the OECD guideline (OECD 2012). However, parental exposure to MP/BP-3 fragments increased the number of offspring per female  $(71.2 \pm 13.74)$  to the control level in the F1B1 recovery group. Under this stress condition, D. magna may use more energy for growth and reproduction than for defense (Costantini 2014; Im et al. 2019). Indeed, the F1B1 recovery group reached primiparity significantly faster  $(10.6 \pm 0.51 \text{ d})$  than the control group  $(12 \pm 1.22 \text{ d})$ , leading to higher somatic growth and reproduction. However, the size of the F1B1 offspring  $(818.27 \pm 19.00 \,\mu\text{m})$  was significantly smaller than that of the control group ( $828.95 \pm 17.96 \mu m$ ). Given that offspring fitness increases with size (Smith and Fretwell 1974; Moran and Emlet 2001), the above findings suggest that the first brood of D. magna responded to MP/ BP-3 stress by accelerating somatic growth and reproduction, which resulted in less fit offspring.

### Conclusion

Contrary to the initial hypothesis, MP/BP-3 fragments induced greater chronic toxicity and maternal effect in adults than in neonates, inhibiting the growth and reproduction in both F0 and F1 generations of *D. magna*. On the other hand, the maternal effect of MP/BP-3 fragments was higher in the first brood neonates than in the third brood neonates, enhancing growth and reproduction in the F1 generation. This study demonstrates different MP toxicity and maternal effects in *D. magna* at different ages and broods, providing insight into the ecological risk of MPs. However, further studies are required over multiple generations to identify the transgenerational effect of MPs containing plastic additives.

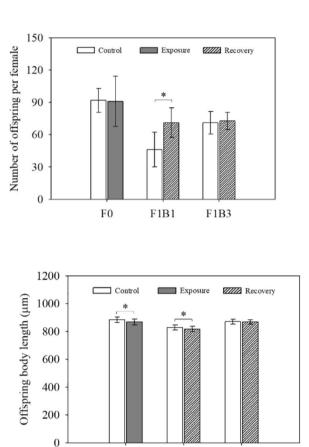


**Fig. 3** Maternal effect of MP/BP-3 fragments in the first and third brood of *Daphnia magna* in terms of (a) body length, (b) number of offspring per female, (c) days to first brood, and (d) offspring body length. F0 and F1 indicate parental and first generation, respectively. B1 and B3 indicate the first and third brood, respectively. Only the

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F0 generation (neonate <24 h) was exposed to MP/BP-3 fragments (1.0 mg L<sup>-1</sup>) until 21 d old, and the F1 generation was recovered in M4 medium (control) for 21 d. All data are expressed as mean  $\pm$  standard deviation (n=15). Asterisks indicate significant differences (p<0.05) between the control and treatment groups

F1B1

F1B3

F0

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