The Toxicity of (Nano)Microplastics on *C. elegans* and Its Mechanisms

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Contents

Abstract Microplastics (MPs) and nanoplastics (NPs) are respectively defined as plastic debris with sizes of <5 mm and <100 nm. In recent years, (nano)microplastics (N/MPs) have been widely detected in air, water, soil, and other environmental matrices. Despite knowledge gap of the risks of N/MPs, more and more researchers pay attention to the adverse effects of this type of fine plastic items on biota. *Caenorhabditis elegans* (*C. elegans*) is an ideal model organism for toxicology study on N/MPs. In this chapter, we have reviewed research progress in the toxicity of N/MPs and its mechanism basing on this model. At the individual level, N/MPs can cause lethality on nematodes and the inhibition of growth and reproduction.

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Defu He and Yongming Luo (eds.), *Microplastics in Terrestrial Environments - Emerging Contaminants and Major Challenges*,
Hdb Env Chem (2020) 95: 259–278, DOI 10.1007/698_2020_452,

The alteration of locomotion behavior has been demonstrated in nematodes after N/MPs exposure. Moreover, the behavioral toxicity was revealed to be involved in the especial neurotoxicity, including damages of GABAergic and cholinergic neurons. In addition, intestine damages and oxidative stress were found in nematodes exposed to N/MPs. Several studies proved that the N/MPs-induced effects might be closely dependent on the size and dose of N/MPs. Recent studies showed that the toxicity of N/MPs was mediated by the insulin signaling pathway and p38 signaling; the intestinal signaling cascade of PMK-1-ATF-7-XBP-1 and PMK-1-SKN-1-XBP-1/GST-5 could regulate the responses to nanopolystyrene particles in nematodes. Although the toxicity of N/MPs has been largely investigated basing on *C. elegans*, the toxic mechanisms are still unclear. Moreover, current studies are most relying on a special type of pure polystyrene sphere, which might not be the representative of all N/MPs types. Therefore, more researches on environmental (nano)microplastics with different chemical compositions and shapes need to be done in the future.

Keywords *Caenorhabditis elegans*, Microplastics, Nanoplastics, Polystyrene, Toxicity

1 Introduction

1.1 Nanoplastics

Microplastics (MPs) are usually considered as plastic debris with sizes below 5 mm, which has reached a consensus among researchers. Similarly, nanoplastics (NPs) are referred to smaller debris with the size between 1 and 100 nm, which is consistent with the European Commission nanomaterials definition [1, 2]. Despite some scholars suggested to set the upper limit of the size of NPs as 1 μm [3–6], NPs were commonly regarded as in the size of smaller than 100 nm. The sources of NPs can be mainly divided into two categories. Primary NPs are mostly stemmed from industrial plastic products including ink of 3D printers, cosmetic products used for skin exfoliators, and synthetic fibers from clothes [4, 7, 8]. In addition, the breakdown of larger debris results in secondary NPs. The fragmentation of larger plastics may be attributed to both abiotic processes such as UV radiation, thermooxidation, and mechanical crushing and biotic driving processes including microbiological activity, animal digestion, etc. [9–12]. For example, *Antarctic krill* were proved to ingest MPs (31.5 μm) and break them into NPs in the size of less than 1 μm [13].

Due to small sizes, NPs in environments cannot be accurately quantified. It is lack of effective methods for extracting, counting, and identifying NPs [14–17]. There is also no uniform standard method for sampling and analyzing NPs [18]. Some researchers have predicted that the environmentally relevant concentration of NPs is ≤ 1 μg L^{-1} in freshwater environments [19], yet it needs further support of

experimental evidence. Considering the fragmentation process in their formation, NPs have variable presence of their morphology and types in environmental compartments including water, air, soil, and sediment. Up to date, there is limited knowledge about the fate and potential toxicity of NPs [14, 20].

1.2 Toxicology of Nanoplastics

Previous studies about the toxicity of NPs are mostly based on nanoscale-sized polystyrene (PS); yet commonly used types of plastic such as polypropylene (PP), polyethylene (PE), and polyvinyl chloride (PVC) have rarely been investigated. It is mostly due to available PS products from commercial corporations [21]. Additionally, PS can technically produce into nanobeads; however, it is difficult for other types of plastics. According to a recent study, LC50 of PS-NPs on *D. pulex* was 76.69 mg L^{-1} for 48 h exposure; PS-NPs would induce obvious inhibitions on animal growth and reproduction. In addition, a significant increase in the expression of HSP70 was demonstrated, which means the exposure of PS-NPs arouses the defense of antioxidant systems [22]. Another study showed that PS-NPs could cross cell membranes and cause tissue damages of zebra fish under conditions of laboratory exposure; however there is no considerable toxicity under natural conditions after exposure to environmentally relevant concentration of NPs [23].

Both MPs and NPs can be ingested by organisms and exert toxic effects. Some researchers have compared the potential effects between MPs and NPs and found size-dependent toxicity of N/MPs. For example, Sjollema et al. exposed three sizes, i.e., 50 nm, 500 nm, and 6 μm, of PS-M/NPs to *Dunaliella tertiolecta*. They found that smaller-sized NPs caused serious adverse effects including microalgal photosynthesis and the growth of *Dunaliella tertiolecta* [24]. Additionally, the toxicity of N/MPs may be related to size-dependent ingestion by different organisms. For example, 1–100 μm MPs can be ingested by the isopod *Idotea emarginata* [25]; while MPs with sizes of 11–700 μm MPs could be easily taken in by the marine amphipod, *Allorchestes compressa* [26]. Nevertheless, PS-NPs particles in sizes of about 1 μm can be easily taken in and accumulated in the digestive system of nematodes [27]. It is generally speculated that smaller-sized particles would be more toxic than larger-sized particles because of their larger specific surface area [28]. But there are still arguments of size-dependent toxicity of N/MPs among different research groups. For example, Lu et al. reported that 5 μm MPs induced higher activities of SOD and CAT than 70 nm NPs [29].

Actually, environmental N/MPs usually contain not only additives but also other contaminants, such as organic chemicals and inorganic salts. For instance, Besseling et al. analyzed PCB concentrations on PS-NPs after joint exposure to *Arenicola*

marina and found bioaccumulations of PCBs companying with the increasing toxicity such as the loss of animal weight [30]. Another study showed that PE (10–106 μm) MPs were ingested by *Danio rerio*, accompanied with silver ions; adverse effects were increased with the increasing percentage of silver found in the intestines of fish [31]. A mass of studies has demonstrated that N/MPs can play the part of transport vectors for adhesion and accumulation of other coexisting contaminants [31–33]. Compared with MPs, NPs have a larger specific surface and a higher accessibility to cross cell membranes and result in higher risks to organisms according to more researchers [24–28].

1.3 Caenorhabditis elegans

Caenorhabditis elegans (*C. elegans*), a free-living nematode often found in soil environments, has been established as model organism for toxicology [34]. This type of nematode is mostly hermaphrodite and self-reproductive and includes a life cycle of about 3 days, which can be divided into eggs, larva (L1, L2, L3, and L4), and adult stages [35, 36]. Germ line in nematode hermaphrodite produces male and female gametes, i.e., sperm and oocytes. Under normal circumstances, a hermaphrodite nematode can produce about 300 offspring [34, 37]. Therefore, nematodes have advantage as model organisms, such as short experimental period, easy reproduction, and convenient observation and operation.

The nematode *C. elegans* has a simple and well-defined anatomy suitable for toxicology. Normal food, bacteria *OP50*, accompanied with N/MPs particles can be ingested by the nematode, through the pharynx and transferred into the intestine [34]. Despite simple structures, the nematodes were composed of multiple types of organs, such as muscles, nervous system, gland cells, and so on. Meanwhile, researchers have fully mapped the complete cell lineages in the nematode body. *C. elegans* contains a total of about 20,000 genes, 40% of which have homology with human genes [38]. Moreover, nematode is the first multicellular animal whose genome has fully revealed. Additionally, *C. elegans* hermaphrodite has 302 neurons: 282 neurons in the somatic nervous system and 20 neurons in the pharyngeal nervous system [39, 40]. These neurons have different neurotransmitter characteristics, including cholinergic, dopaminergic, GABAergic, etc., which are comparable to higher animals [40]. A number of toxicology indicators including reproductive or developmental toxicity, behavioral toxicity, and neurotoxicity and molecule changes can be assayed, especially basing on various types of transgenic strains. As sensitive to contaminants, the nematode *C. elegans* is an ideal model organism, especially for NPs toxicology [34].

2 Toxicities of (Nano)Microplastics in *C. elegans*

2.1 Developmental and Reproductive Toxicity

2.1.1 Developmental Toxicity

Several studies have investigated effects of NPs or MPs on the nematode *C. elegans* [27, 41–44]. Most of these researchers used different-sized particles or microspheres of PS. For example, Lei et al. exposed nematodes to PS particles with five diameter sizes of 0.1, 0.5, 1.0, 2.0, and 5.0 μm, in the same concentration of 1 mg L^{-1} and for 48 h [44]. Their results demonstrated the significant inhibition of survival rate after exposure (Fig. 1a). Of five sizes, 1.0 μm MPs showed strongest lethality, i.e., an average reduction of 32.27% of survival rate. In another study, nematodes were exposed to PA, PE, PP, PVC, or PS particles on the surface of solid medium, with a

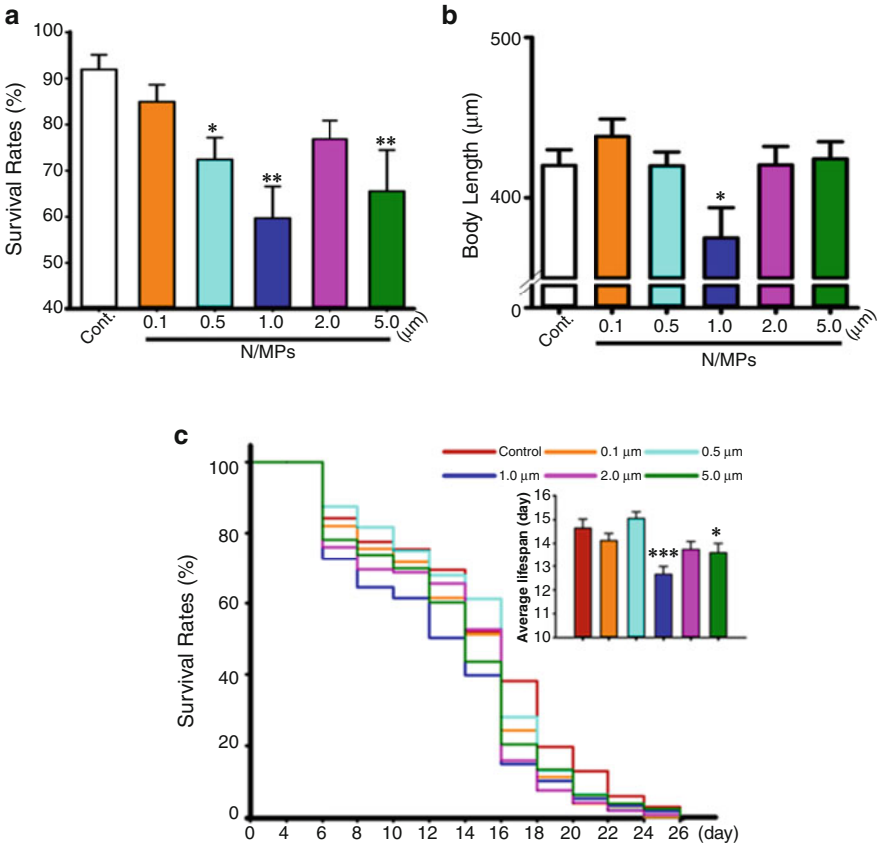

Fig. 1 Effects of different-sized PS (nano)microplastics (N/MPs) in *C. elegans* after 3-day exposure with concentration of 1 mg L^{-1} (**a**, survival rate; **b**, body length; **c**, life span) [44]

series concentration of 0.5, 1.0, 5.0, and 10.0 mg L^{-1} [27]. Despite no obvious dose-effect relationship, 1.0 μm PS particles also caused the biggest reduction of survival rate of nematodes among several sizes of N/MPs. These results indicate that N/MPs can exert size-dependent lethality; 1.0 μm PS particles seem to be the most toxic to *C. elegans*. These results imply that 1 μm is an appropriate size of N/MPs to be taken in and accumulated in the digestive tract of nematodes.

Similarly, 1.0 μm PS particle exposure could induce remarkable decreases in body length of nematodes; however, there were not significant changes in other groups of 0.1, 0.5, 2.0, or 5.0 μm MPs (Fig. 1b) [44]. Lei et al. also compared the effects of different polymer types of MPs including PA, PE, PP, PVC, and PS with the same sizes [27]. They found similar toxicity of MPs on nematodes, which included slight lethality and the inhibition on the body length of nematodes. Additionally, the inhibition of nematode life span was demonstrated after exposure to PS particles in five size groups. Among them, 1.0 and 5.0 μm PS particle exposure resulted in a noteworthy decrease of average life span (Fig. 1c). In 1.0 μm PS-exposed group, nematodes presented the shortest average life span. Collectively, these studies disclosed that the toxicity of N/MPs particles was mainly dependent on the size of MPs instead of their polymer types [27].

2.1.2 Reproductive Toxicity

N/MPs exposure can result in the inhibition of reproduction of nematodes. A study investigated multiple types of N/MPs including PA, PE, PP, PVC, and PS particles on reproductive activity of nematodes [27]. Results showed five common types of MPs that induced the decrease of embryo numbers and brood size (Fig. 2). Of the exposed groups, the PP group had the lowest embryo numbers. Both the embryo numbers and brood size decreased remarkably in PE, PVC, or PS group. The biggest

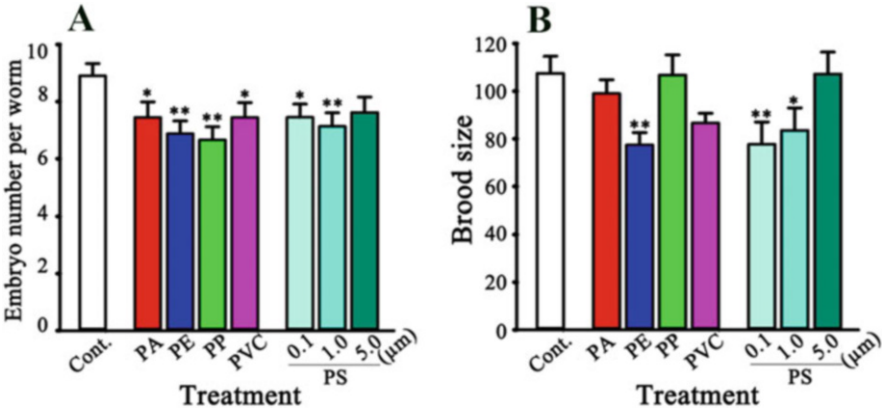

Fig. 2 Effects of PS N/MPs in *C. elegans* after exposure to 5.0 mg m^{-2} different type or size particles for 2 days (**a**, embryo numbers; **b**, brood size) [27]

inhibition rates, 25.22% of embryo number and 28.02% of brood size, were found in PP and PE exposure groups. Reproductive toxicity seems to be associated with both plastic polymer and the sizes of N/MPs.

2.2 Behavioral and Neural Toxicity

2.2.1 Effects of Micro-sized PS Particles on Locomotion Behaviors

According to a recent study, exposure to 1 mg L^{-1} PS N/MPs could cause obvious changes in locomotion behaviors of the nematodes in a size-dependent manner [44]. For instance, small-sized particles (0.1 and 0.5 μm) induced the increase in the average number of head thrashes and body bends, but microscale particles of 1.0, 2.0, and 5.0 μm PS resulted in decreases of the nematodes' locomotion behavior. Furthermore, crawling movements of the nematodes were demonstrated to significantly change after exposed to PS MPs of different sizes. According to the analysis results of crawling tracks, 0.1 and 2.0 μm PS MPs induced significant increases in mean crawling speed. Moreover, angles of body bending also changed in exposed groups. Body bending angles reflect the coordination and balancing ability. A zero bending angle is an indication of no directional bias, while positive and negative body bending angles mean forward and backward bias. The results showed that 0.1 μm PS particles induced a significant decrease in body bending frequency. These results indicated that N/MPs particles could cause locomotion behavior deficits in the nematodes.

2.2.2 Effects of (Nano)Microplastics on GABAergic, Cholinergic, and Dopaminergic Neurons

Multiple types of neurons, such as GABAergic neurons, cholinergic neurons, and dopaminergic neurons, are in charge of the control of locomotion behavior in the nematode. Recently, two studies revealed the neuronal damages associated with nanopolystyrene particles in *Caenorhabditis elegans* [44, 45]. It indicates that exposure to nano-/micro-sized PS particles could be involved with neurotoxicity, which may be the mechanisms of behavioral toxicity.

In *C. elegans*, γ-aminobutyric acid (GABA) is an important inhibitory neurotransmitter, which plays an important role in motor functions. The effects of exposure to PS particles on GABAergic neurons were assayed by using the transgenic strain EG1285 (*unc-47p::gfp*), in which GABAergic neurons are visualized by the translational expression of *unc-47*. After exposure to 1.0 μm PS particles at concentration of 1 mg/L, the fluorescence intensity was significantly decreased (Fig. 3a–d), indicating the downregulated expression of *unc-47*. However, exposure to PS particles of other sizes had no impacts on the expression of *unc-47*. Besides, Qu et al. found that 100 nm PS-NPs could also induce neurodegeneration of D-type

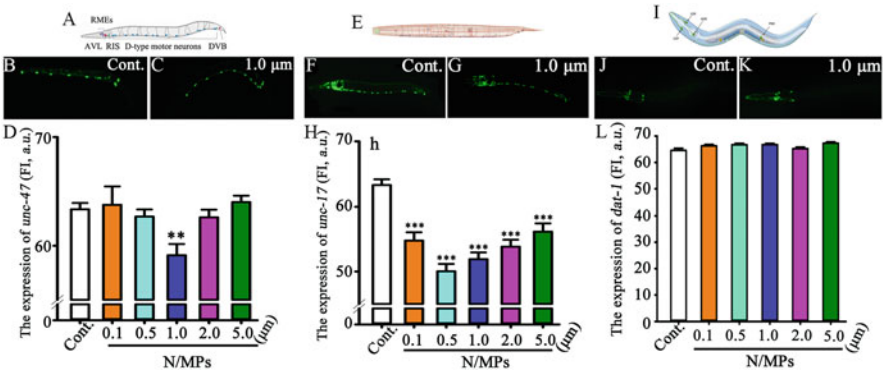

Fig. 3 Effects of PS N/MPs on *unc-47::gfp*, *unc-17::gfp*, and *dat-1::gfp* expression in EG1285, LX929, and BZ555 nematodes. The diagrammatic figure of GABAergic neurons (**a**), cholinergic neurons (**e**), and dopaminergic neurons (**i**). Fluorescent images of EG1285 (**b**, **c**), LX929 (**f**, **g**), and BZ555 (**j**, **k**), in the control nematodes and the nematodes exposed to 1 μm PS particles; *unc-47::gfp* (**d**), *unc-17::gfp* (**h**), and *dat-1::gfp* (**l**) expression pattern in control nematodes and nematodes exposed to 0.1, 0.5, 1.0, 2.0, 5.0 μm PS particles for 2 days [44]

GABAergic motor neurons in the nematodes [45]. In *C. elegans*, GABAergic neurons are comprised of RMEs, D-type neurons, RIS, AVL, and DVB. D-type neurons have responsibility for the control of ventral body and dorsal body muscles. REM neurons have control of the head. AVL and DVB control enteric movement. RIS are internuncial neurons. During the locomotion of the worm, D-type neurons suppress the contraction of ventral and dorsal body muscles. When the nematode bends its body, it will contract the muscles on the side of the body and relax the muscles on the other side of the body at the same time, enabling the nematode to keep moving in a wavy way [46–48]. The results showed that the exposure of PS particles can suppress the function of D-type GABAergic neurons. So, the special neurotoxicity may be involved with behavioral damages in crawling movement.

Cholinergic neurons can influence the posterior rhythm during the worm's forward locomotion [49]. Acetylcholine (ACh) is an important neurotransmitter in organisms, which mainly specially distributed widely in the nerve endings at neuromuscular junctions. ACh is synthesized by choline acetyltransferase (ChAT, encoded by *cha-1*) and encapsulated in synaptic vesicles by the vesicular Ach transporter (VAChT, encoded by *unc-17*) [47, 50]. In the transgenic strain LX929, cholinergic neurons can be visualized by the translation expression of green fluorescence protein (GFP) driven by the promoter of cholinergic transporter *unc-17* gene (Fig. 3e, f). After exposure to PS microparticles at the concentration of 1 mg L^{-1}, the fluorescence intensity was significantly decreased in the exposed groups [44]. It indicates the downregulated expression of *unc-17* induced by MPs. Broken and atrophied ciliated dendrites can be observed after exposure to PS particles, especially in the groups exposed to 0.5 and 1.0 μm PS particles (Fig. 3f–h). It reveals that PS particles can cause the downregulation of *unc-17* and may prevent ACh from transferring into synaptic vesicles and make the ciliated dendrites

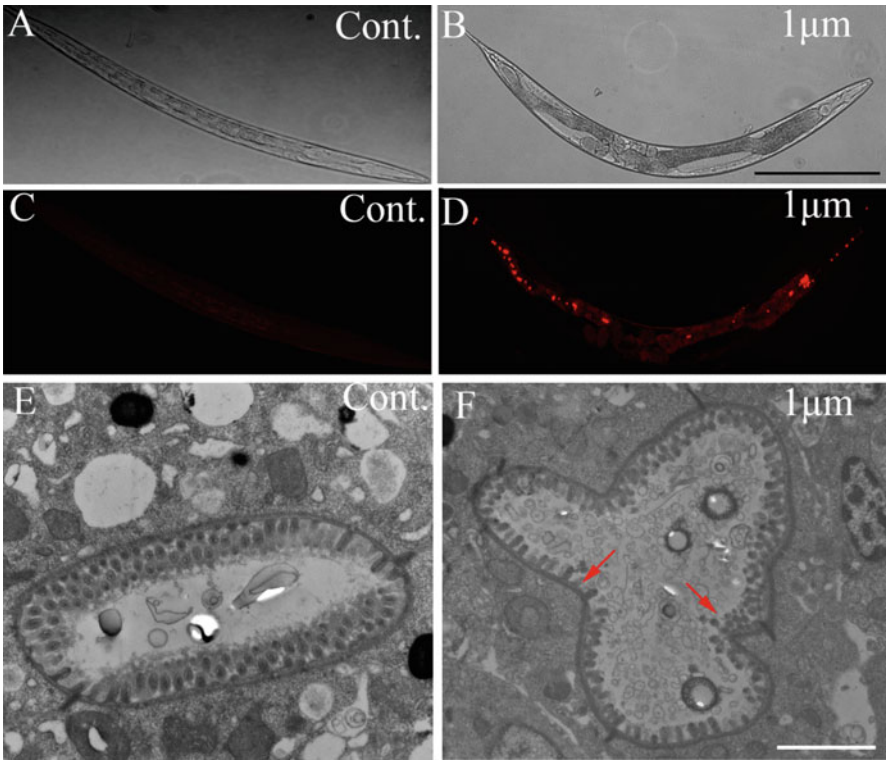

Fig. 4 Accumulation of 1 μm PS particles and intestinal damages induced by MPs exposure. (**a**–**d**) Light field (**a**, **b**) or fluorescence images (**c**, **d**) of the control and the MPs-exposed nematodes; (**e**–**f**) photomicrographs of the intestine of the control and the MPs-exposed nematodes. The red arrows indicate the intestinal damages. The black bar, 200 μm; the white bar, 0.6 μm

broken and atrophied, causing excitatory activities in nematodes. These results support PS-induced behavior toxicity that exposure to 0.1 and 0.5 μm PS particles can induce increases in head thrashes and body.

Dopamine (DA), encoded by *dat-1*, is another important neurotransmitter regulating locomotion behavior in *C. elegans*. The neurons containing DA as the neurotransmitter are called dopaminergic neurons [36, 51]. In a recent study in our laboratory [44], the transgenic strain BZ555 (*dat-1::gfp*) was used to assay the effects of PS particles on the dopaminergic neurons. In BZ555, dopaminergic neurons are labeled by green fluorescent protein, including four cephalic (CEP) neurons, two anterior deirid (ADE) neurons, and two posterior deirid (PDE) neurons (Fig. 4i). However, after 48 h exposure to 0.1, 0.5, 1.0, 2.0, and 5.0 μm PS particles, there was no obvious change in expression of *daf-1::gfp* (Fig. 3j–l). It indicates that the N/MPs exert no or slight toxicity on dopaminergic neurons in nematodes.

2.3 Intestine Damages

2.3.1 Distribution of PS Nanoplastics in *C. elegans*

After nematodes were exposed to fluorescently labeled PS particles with sizes of 0.1, 1.0, and 5.0 μm, PS particles can be observed distributing in the digestive system, from lumen of pharynx to gut lumen and rectum. Among three sizes of N/MPs, 1.0 μm particles have the strongest fluorescence intensity in the body of nematodes. It indicates 1.0 μm PS particles can more easily accumulate in the intestine of *C. elegans* (Fig. 4a–d). So, this result supports the strongest toxicity of 1.0 μm PS particles, including developmental, reproductive, and neural toxicity. Additionally, we found noticeable damages in the nematode intestine, such as fracture of villi and the rupture of epithelial cells, especially in 1.0 μm MPs-exposed group (Fig. 4e–f). We speculate that the accumulated MPs may interact with intestinal epithelial cells through physical or chemical impacts, which further exert intestine damages.

2.3.2 Changes in Intestinal Calcium Levels of *C. elegans*

According to Lei et al., the potential effects on intestinal calcium levels were observed after exposure to PS particles (0.1, 1.0, 5.0 μm) [44]. In the KWN190 strain of *C. elegans*, the calcium indicator protein D3cpv was expressed throughout the cytoplasm of intestinal cells [27]. Results showed that 1.0 μm PS particles caused a significant decrease in intestinal calcium levels but no remarkable change in 0.1 and 5.0 μm PS groups (Fig. 5). It is consistent with size-dependent toxicity of N/MPs on intestinal damages and implies that in the activity of intestinal calcium, it is involved in the toxic mechanism.

2.4 Oxidative Stress

Oxidative stress reflects an imbalance between the production of free radicals and the ability to readily detoxify their harmful effects through neutralization by antioxidants. Oxidative damages of organisms are generally identified by assay of reactive oxygen species (ROS). The increase of ROS can cause damages in proteins, lipids, or DNA and then induce aging, diseases, or cell death [52]. Glutathione S-transferase (GST-4) is a major cellular detoxification enzyme and participates in oxidative response, which can sensitively reflect the level of oxidative stress [53]. Lei et al. used the transgenic strain CL2166 (*gst-4::gfp*) to assay the level of oxidative stress by fluorescence detection. After exposure to 0.1, 1.0, and 5.0 μm PS particles, the expression of *gst-4* was significantly increased in a size-dependent manner [27]. The expression level of *gst-4* in the 1.0 μm PS group is higher than those in the other two groups (Fig. 6). Lei et al. also investigated the oxidative stress caused by other

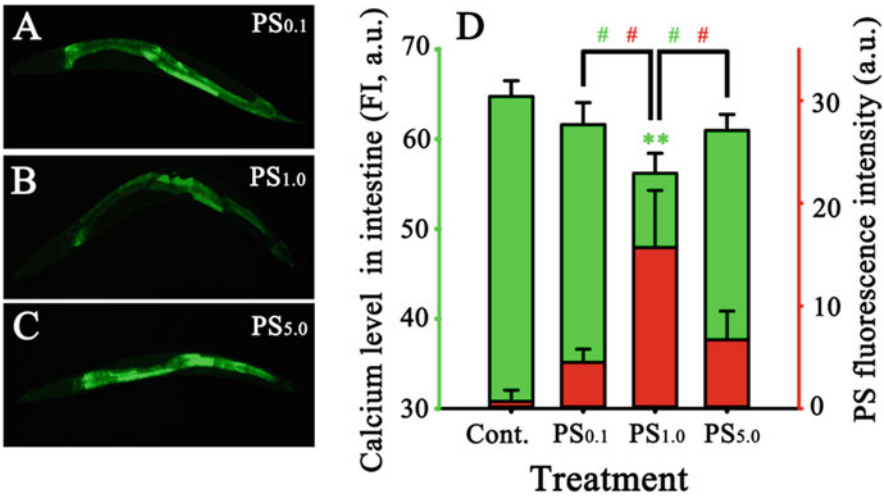

Fig. 5 Changes in calcium levels in *C. elegans*. (**a–c**) Calcium levels in the intestine after exposure to PS particles with different sizes. (**d**) Quantified values of calcium levels and PS particles accumulation in the intestine after PS exposure. Bar = 200 μm [27]

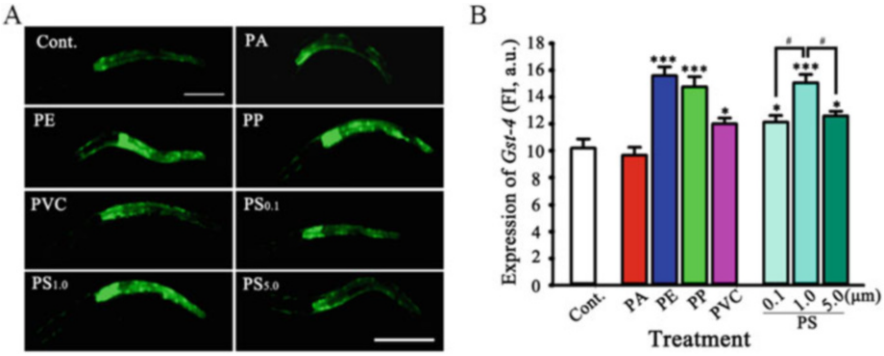

Fig. 6 Effects of MPs particles on the expression of *gst-4::gfp* in CL2166 nematodes of the control nematodes and the nematodes exposed to PA, PE, PP, PVC, and PS (0.1, 1.0, 5.0 μm) particles at concentration of 5 mg m^{-2}. (**a**, fluorescent images; **b**, expression of *gst-4::gfp*). The bar = 200 μm [27]

microplastics including PA, PE, PP, and PVC (in the size of 70 μm). The results showed that all of these MPs could cause significant increase in the expression of *gst-4*. It suggests that oxidative stress is a key characteristic of the toxicity of MPs on *C. elegans*.

Furthermore, Lei's study showed that two natural antioxidants, curcumin and oligomeric proanthocyanidins, could decrease the elevated expression of *gst-4* induced by PS particles. Curcumin is extracted from turmeric, a traditional herbal medicine, and used as traditional medicine for curing ulceration and skin infection in

India and other countries. It was reported that curcumin could induce resistances to inflammation, oxidation, or even cancer [54]. Oligomeric proanthocyanidins are extracted from pine or other plants and have been widely used as a strong natural antioxidant [55]. The results indicate that natural antioxidants are capable of alleviating oxidative stress induced by MPs [44].

3 Mechanisms of (Nano)Microplastics' Toxicities in *C. elegans*

3.1 The Insulin Signaling Pathway

Up to now, a few studies have investigated the potential mechanisms of toxic action between N/MPs and nematodes. According to a recent study, a signal cascade of DAF-2-AGE-1-AKT-1-DAF-16-SOD-3/MTL-1/GPD-2 in the insulin signaling pathway can respond to nanopolystyrene particle exposure in *C. elegans* [56]. Insulin signaling pathway is involved in numerous life activities, such as aging, reproduction, lipid metabolism, stress response, and so on. The insulin signaling pathway contains the upstream protein DAF-2, an insulin-IGF receptor ortholog, the downstream protein DAF-16, and multiple molecules [57, 58]. Some scholars demonstrated that the depression of DAF-2 pathway can induce a resistance to heat or oxidative stress, in order to protect animals from oxidative damage [59]. Shao et al. found polystyrene NPs could induce an obvious ROS production and the decrease in locomotion behavior in wild-type nematodes [56]. These toxic actions are closely related to the decreased expressions of *daf-2*, *age-1*, and *akt-1* and the increased expression of *daf-16*. Furthermore, the expression of *daf-16* was translocated from the cytoplasm to nuclei. Mutation of *daf-2*, *age-1*, or *akt-1* could significantly suppress ROS production and behavioral deficits, after the mutant nematodes being exposed to NPs, but the mutation of *daf-16* resulted in a significant increase in ROS production. These results indicate that mutation of *daf-2*, *age-1*, or *akt-1* can induce a resistance to the toxicity of nanopolystyrene particles but mutation of *daf-16* enhances a toxic susceptibility. Moreover, the resistance induced by mutation of *daf-2*, *age-1*, or *akt-1* can be suppressed by RNAi knockdown of *daf-16*. Additionally, they found that the intestine-specific activities of DAF-2, AGE-1, AKT-1, and DAF-16 could regulate the toxicity of nanopolystyrene particles in the nematodes. These results indicate that the signaling cascade of DAF-2-AGE-1-AKT-1-DAF-16 in the insulin signaling pathway is involved in a protective response to the toxicity of nanopolystyrene particles.

In another study, researchers detected the expression of *sod-3*, *mtl-1*, and *gpd-2* gene in the intestine of nematodes and found the target gene of *daf-16* specially responded to nanopolystyrene exposure. As a superoxide dismutase, SOD-3 is involved in superoxide radical's removal in order to protect against oxidative stress [60]. MTL-1 is a metallothionein and responsible for metal detoxification and stress

adaption [61], while GPD-2 is a glyceraldehyde-3-phosphate dehydrogenase in organisms [62]. After exposure to nanopolystyrene particles, the mutation of *daf-16* could cause significantly decreases in expression of *sod-3*, *mtl-2*, and *gpd-2*; intestine-specific RNAi knockdown of these three genes could result in increase of ROS production. Furthermore, resistance to toxicity of nanopolystyrene in the transgenic strain over pressing *daf-16* could be suppressed by RNAi knockdown of these three genes. Therefore, SOD-3, MTL-1, and GPD-2 are the downstream targets of DAF-16 and play an important role in the protective response to the toxicity of NPs through the insulin signaling pathway.

3.2 *The Protective Response Mediated by the Intestinal p38 Signaling*

Using the model organism nematodes, Qu et al. investigated that a protective response to nanopolystyrene particles. They found the special protective response mediated by p38 mitogen-activated protein kinase (MAPK) signaling pathway, which could activate the endoplasmic reticulum unfolded protein response (UR EPR) [41]. In *C. elegans*, PMK-1 p38 MAPK signaling pathway is responsible for the regulation of oxidative stress response [63]. Stress can induce misfolding and aggregation of proteins, which will disrupt the protein homeostasis and make adverse effects on cellular viability. Eukaryotic cells have evolved specific signaling pathways known as unfolded protein responses to protect themselves from proteotoxicity, including heat shock response, endoplasmic reticulum unfolded protein response, and mitochondrial unfolded protein response [64]. So, p38 mitogen-activated protein kinase signaling pathway is an important mechanism that protects nematodes' cells from the toxic action of NPs.

Prolonged exposure to 100 nm nanopolystyrene particles ($\geq$1 μg L^{-1}) resulted in severe induction of ROS production and decreases in locomotion behavior [41]. In the p38 MAPK signaling pathway, NSY-1-SEK-1-PMK-1 is a classic signaling cascade. Under conditions of NPs exposure, PMK-1 needs to be phosphorylated in order to activate the p38 MAPK signaling. According to a recent study, the expression and phosphorylation level of *pmk-1* was significantly increased in nematodes after prolonged exposure to 100 nm nanopolystyrene particles at the predicted environmentally relevant concentration (1 μg L^{-1}). In addition, elevated toxicity susceptibility to nanopolystyrene was proved in *pmk-1(km25)* mutant nematodes. Though PMK-1 can be expressed in neurons and intestine cells, only mutation of intestine-specific PMK-1 can suppress the susceptibility of NPs-induced toxicity. It indicates that intestinal PMK-1 is the regulator of the response to nanopolystyrene particles in *C. elegans*. Exposure to 100 nm polystyrene particles (1 μg L^{-1}) can also induce the increased expression of *atf-7* and *skn-1*; both genes are considered as the downstream targets of PMK-1. In *pmk-1(km25)* mutant nematodes, the NPs-induced expression of *atf-7* and *skn-1* can be significantly decreased; intestine-specific RNAi

knockdown of *atf-7* or *skn-6* can increase ROS production. When PMK-1 in the intestine of nematodes was overexpressed, the nematodes can obtain a resistance to the toxicity to NPs. RNAi knockdown of *atf-7* or *skn-1* can also suppress the resistance to NPs. These results indicate that *atf-7* and *skn-1* are downstream of *pmk-1* in the response to nanopolystyrene particles. Collectively, current studies suggest that the intestinal signaling cascade of PMK-1-ATF-7-XBP-1 and PMK-1-SKN-1-XBP-1/GST-5 can regulate the responses to nanopolystyrene particles in *C. elegans*. It may be a pivotal mechanism involved in biota's response to N/MPs; however it needs more research in higher animals.

3.3 Other Mechanisms

In recent years, several studies on the behavioral and neural toxicities of N/MPs to nematode *C. elegans* have been done. All these studies show that exposure to PS N/MPs can induce changes in locomotion behaviors and neuronal damages [44], but only a part of the mechanism of the neurotoxicity of N/MPs has been revealed. Qu et al. found that there is an association between the neurotoxicity of PS-NPs and changes in autophagy induction in nematodes [45]. Autophagy is a pathway for intracellular macromolecules degradation, which can be activated by toxicants and have the capacity to protect organism against neurotoxicity [65, 66]. Since *lgg-1* is a key regulator of autophagy [67], Qu et al. used LGG-1::GFP as the marker to investigate the effects of NPs on autophagy. The results showed that exposure to PS-NPs (1,000 μg L^{-1}) could induce a decrease in autophagy induction and could result in behavioral deficits and damages in D-type GABAergic motor neurons at the same time. Moreover, RNAi knockdown of *lgg-1* could induce a susceptibility to the neurotoxicity of PS-NPs on the development and function of D-type GABAergic motor neurons. These results imply that the damages on D-type neurons induced by exposure to PS-NPs are related with the decrease in autophagy induction.

The molecular response to nanoplastics still remains largely unknown in organisms. Qu et al. employed *C. elegans* exposed to PS-NP (100 nm, 1 μg L^{-1}) to investigate the long noncoding RNAs (lncRNAs). They found that 37 lncRNAs were dysregulated, among which 22 lncRNAs were downregulated and 15 lncRNAs were upregulated [68]. Focused on the known lncRNAs (downregulated *linc-7*, *linc-50*, and *linc-169*; upregulated *linc-2*, *linc-9*, *linc-18*, *linc-32*, and *linc-61*), they examined their dynamic expression in PS-NP. Both the decreasing expression of *linc-7*, *linc-50*, and *linc-169* and increasing expression of *linc-2*, *linc-9*, *linc-18*, *linc-32*, and *linc-61* were dose-dependent in nematodes exposed to PS-NP (1–100 μg L^{-1}). Moreover, with intestinal reactive oxygen species (ROS) production and locomotion behavior sited as the endpoints, they conducted the effects of RNA interference (RNAi) knockdown of *linc-2*, *linc-7*, *linc-9*, *linc-18*, *linc-32*, *linc-50*, *linc-61*, and *linc-169* in nematodes. In results, the RNAi knockdown of *linc-2*, *linc-7*, *linc-9*, *linc-18*, *linc-32*, *linc-50*, *linc-61*, and *linc-169* in nematodes without PS-NP exposure did not induce the obvious intestinal ROS production and locomotion behavior;

however, compared with nanopolystyrene-exposed wild-type nematodes, nanopolystyrene-exposed *linc-2*, *linc-9*, or *linc-61* (all RNAi) nematodes were observed with the more severe ROS production and decreasing locomotion behavior; nanopolystyrene-exposed *linc-18* or *linc-50* (all RNAi) nematodes were observed with the inhibition of ROS production and increase of locomotion behavior. Among these five studied lncRNAs, *linc-2*, *linc-9*, *linc-50*, and *linc-61* alterations mediated a protective response to PS-NP, and the alteration of *linc-18* possibly mediated the toxicity of PS-NP, which is suggested by a further study associated with their biological processes and signaling pathways.

Qu et al. also observed the response of microRNAs (miRNAs) to PS-NP (100 nm, 1 μg L^{-1}) [69]. After exposure, seven miRNAs were dysregulated by PS-NP (*mir-39*, *mir-76*, *mir-794*, and *mir-1830* downregulated; *mir-35*, *mir-38*, and *mir-354* upregulated). According to the phenotypic analysis of both transgenic strains and mutant nematodes, *mir-35*, *mir-38*, *mir-76*, *mir-354*, and *mir-794* were found to be involved in the response to PS-NP. The expression of all these seven miRNAs above was dose-dependent in nematodes exposed to PS-NP (1–100 μg L^{-1}). The previous study on the function of insulin signaling pathway has shown its response in PS-NP, and meanwhile the KEGG analysis suggested that *mir-794* could medicate in the insulin signaling pathway, which also reveals a possible molecular response pathway candidated by *mir-794* and insulin signaling. Additionally, *mir-35*, *mir-38*, and *mir-354* may influence the Wnt signaling pathway, a related pathway of controlling toxicity induction of several environmental toxicants such as graphene oxide [70]. In particular, overexpression of *mir-354* could decrease the expression of cwn-1 which encodes a Wnt ligand. These results confirmed that *mir-354* could be an intervent to the function of Wnt signaling pathway in response to PS-NP in nematodes.

4 Summary

In this chapter, we have reviewed research progress in the toxicology of N/MPs using the model nematode *C. elegans*. Several studies have revealed that both NPs and MPs can cause multiple toxicity including lethality, reproductive and developmental toxicity, alteration of locomotion behavior, neurotoxicity, intestine damages, and ROS production in nematodes. These effects on nematodes were obviously both dose-dependent and size-dependent with (nano)microplastics. PS-MPs in the concentration ranging from 1 μg L^{-1} to 100 μg L^{-1} could induce multiple adverse effects [42, 56]. According to these studies, similar-sized MPs in different polymer types (PA, PP, PE, and PVC) showed nearly same toxicity in *C. elegans* [27, 44]. It indicates that the toxicity of MPs is closely dependent on their size, rather than their composition.

Up to date, the majority of researchers used pure PS spheres to carry out toxicology studies of NPs. However, the real environment includes various types of MPs or NPs in different chemical compositions or different shapes. Furthermore, environmental (nano)microplastics contain a variety of additives and other absorbed

pollutants such as hydrophobic organic chemicals [71]. Therefore, future toxicology researches need to focus on real environment-character N/MPs in the environmental relevant concentration level. Additionally, more studies need to reveal the toxic action mechanisms of MPs, especially about NPs' toxicity and its cellular or molecular pathway.

Acknowledgments The authors gratefully acknowledge the financial support by the National Science and Technology Major Project for Water Pollution Control and Treatment (No.2018ZX07208008) and the National Key Research and Development of China (No. 2016YFC1402204 and No. 2018YFC1901004).

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