Chemosphere 362 (2024) 142519

Contents lists available at ScienceDirect

Chemosphere

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

Aged polystyrene microplastics cause reproductive impairment via DNA-damage induced apoptosis in *Caenorhabditis elegans*

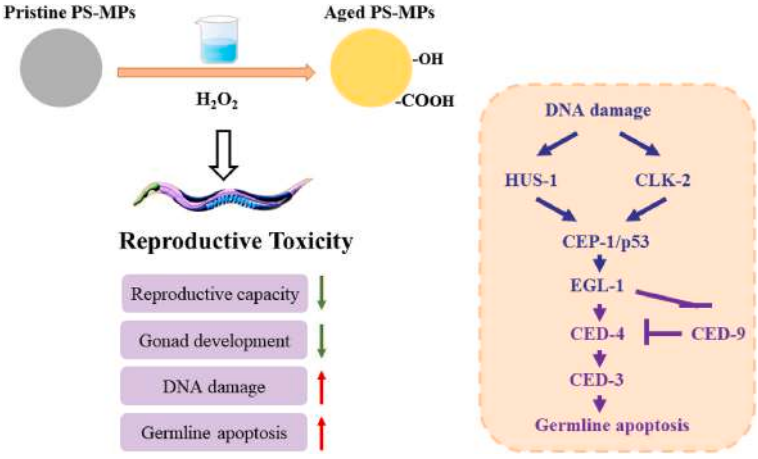
Tiantian Xu [a,b], Haibo Chen [a,c], Luohong Zhang [b], Dongli Xie [a,d], Shihui Tan [a,e], Hongzhi Guo [a,d], Mingdeng Xiang [a], Yunjiang Yu [a,*]

[a] State Environmental Protection Key Laboratory of Environmental Pollution Health Risk Assessment, South China Institute of Environmental Sciences, Ministry of Ecology and Environment, Guangzhou, 510655, China
[b] School of Environmental and Chemical Engineering, Xi'an Polytechnic University, Xi'an, 710048, China
[c] Institute for Environmental Pollution and Health, School of Environmental and Chemical Engineering, Shanghai University, Shanghai, 200444, China
[d] College of Environmental and Chemical Engineering, Chongqing Three Gorges University, Wanzhou, 404100, China
[e] School of Public Health, China Medical University, Shenyang, 110122, China

HIGHLIGHTS

- APS-MPs exposure caused more severe reproductive toxicity than pPS-MPs in nematodes.
- Exposure to aPS-MPs caused germline apoptosis and DNA damage in *C. elegans*.
- Alterations in related gene expression were observed.
- EGL-1-CEP-1-HUS-1-CED-3-CED-4-CED-9 pathway may be involved in regulating apoptosis.

GRAPHICAL ABSTRACT



ARTICLE INFO

Handling editor: X. Cao

ABSTRACT

Although polystyrene microplastics (PS-MPs) could induce toxic effects on environmental organisms, the toxicity of aged PS-MPs with H_2O_2 on soil organisms remains unclear. Our study utilized *Caenorhabditis elegans* as model organism to examine the reproductive toxicity of pristine PS-MPs (pPS-MPs) and aged PS-MPs (aPS-MPs) at environmentally relevant concentrations (0.1–100 μg/L). Acute exposure to aPS-MPs could induce greater reproductive impairment compared to pPS-MPs, as evidenced by changes in brood size and egg release. Assessment of gonad development using the number of mitotic cells, length of gonad arm, and relative area of gonad arm as parameters revealed a high reproductive toxicity caused by aPS-MPs exposure. Furthermore, aPS-MPs exposure promoted substantial germline apoptosis. Additionally, exposure to aPS-MPs (100 μg/L) markedly

* Corresponding author.
E-mail address: yuyunjiang@scies.org (Y. Yu).

https://doi.org/10.1016/j.chemosphere.2024.142519
Received 11 April 2024; Received in revised form 23 May 2024; Accepted 31 May 2024
Available online 1 June 2024

altered the expression of DNA damage-induced apoptosis-related genes (e.g., *egl-1*, *cep-1, clk-2*, *ced-3*, *-4*, and *-9*). Alterations in germline apoptosis caused by aPS-MPs were observed in mutants of *cep-1*, *hus-1*, *egl-1*, *ced-3*, *-4*, and *-9*. Consequently, the augmentation of reproductive toxicity resulting from aPS-MPs exposure was attributed to DNA damage-triggered cellular apoptosis. Additionally, the EGL-1-CEP-1-HUS-1-CED-3-CED-4-CED-9 signaling pathway was identified as a key regulator of germline apoptosis in nematodes. Our study provides insights into potential environmental risk of aPS-MPs with H_2O_2 on environmental organisms.

1. Introduction

Microplastics (MPs), generally refer to plastics with sizes <5 mm, are the most plentiful plastic debris in the environment and have aroused broad attention in recent years (Chae and An, 2018). Polystyrene microplastics (PS-MPs), are known as representative plastics owing to its widespread applications in various fields (Lambert and Wagner, 2016). PS-MPs ubiquitously exist in freshwater, soil, ocean, atmosphere, and even organisms (Song et al., 2020), PS-MPs were also detected in human skin, hands, saliva, hair, stool and placenta (Abbasi and Turner, 2021; Ragusa et al., 2021). As we can see that PS-MPs have posed a direct threat to the environment and organisms. Reproductive toxicity exhibits adverse effects on organisms, including organs, tissue, endocrine system, and pregnancy. PS-MPs treatment could cause reproductive dysfunction. Many reports have indicated that PS-MPs treatment induce reproductive toxicity in different species, including zebrafish (*Danio rerio*), freshwater prawns, mice, and *Oryzias melastigma* (Wang et al., 2019a; Zhang et al., 2021; Liu et al., 2022; Sun et al., 2022). However, original PS-MPs are selected as contaminants for toxicology experiments in studies, which are absence of environmental relevance (Kik et al., 2020; Pelegrini et al., 2023; Siddiqui et al., 2023). Thus, the reproductive toxicity induced by MPs in environmentally relevant condition needs to be further evaluated.

MPs are vulnerable to undergo extensive aging processes in the environment, including ultraviolet (UV) irradiation, thermal degradation, chemical oxidation, and biodegradation (Luo et al., 2022). Laboratory-accelerated technologies are considered to efficiently simulate complex natural aging processes in the environment, which have advantages of artificial regulation of aging conditions and the time costs can be saved. Advanced oxidation processes (AOPs) are extensively used for the degradation of organic pollutants by generating a mass of reactive oxygen species (ROS) to disrupt their internal composition, which have efficient influence on detecting the alternations in characterization of aged MPs and illustrating the biosynthesis pathways of secondary MPs (Esplugas et al., 2007; Tsitonaki et al., 2010). AOPs enable to simulate and accelerate aging processes of MPs rapidly as an alternative because of the strong oxidizing ROS, which provide insights for understanding natural aging behavior in the environment (Liu et al., 2021). Hydrogen peroxide (H_2O_2) is an efficient approach to accelerate MPs chemically aging owing to containing large amounts of free radicals (Jia et al., 2018). The active oxidizing species generated in H_2O_2 are mainly hydroxyl radicals (•OH). Previous studies have indicated that H_2O_2 aging can effectively alter the physicochemical characteristics of PS-MPs, including surface morphology, particle size, oxygen-containing groups, crystal structure, and environmental behavior (Hüffer et al., 2018; Lang et al., 2020). Additionally, many studies have reported that aged PS-MPs exhibit multiple adverse effects in organisms (Wang et al., 2020c, 2021b; Huang et al., 2021) In microalgae *Skeletonema costatum*, aPS-MPs in seawater and their leachate caused greater significant inhibitory effect on the growth of microalgae than virgin PS-MPs, which was hypothetical to be induced by the synergistic effect of aged PS itself and leaching solution (Ni et al., 2023). In human, exposure to UV-photodegraded polystyrene nanoplastics (PS-NPs) elevated cytotoxicity than pristine PS-NPs on human lung epithelial A549 cells (Shi et al., 2021). Recently, in male ICR mice, acute exposure to aged PS-MPs enhanced potential metabolic disorders than pristine PS-MPs, resulting in more serious immune damage and reproductive toxicity (Cui et al., 2023). Nonetheless, the toxic effects of aged MPs with H_2O_2 on organism remain largely unknown, especially for soil organism.

Caenorhabditis elegans (*C. elegans*), a soil-dwelling nematode with a free-living lifestyle, serves as a superb model organism owing to its brief life cycle, ease of cultivation, large-scale offspring, hermaphroditic fertilization, highly homologous with human genes and favorable reproductive capacity, which has been used for toxicological assessment of environmental pollutants(Yu et al., 2022a). To date, *C. elegans* as a powerful tool is widely applied for investigating reproductive toxicity evaluation and its underlying molecular mechanisms using sublethal endpoints of fertility, DNA damage activation, germline apoptosis, gonad development and gene expression (Zhao et al., 2016; Qu et al., 2019a; Sun et al., 2021; Youssef et al., 2021). Some studies suggested that PS-MPs exposure resulted in reproductive impairments in *C. elegans* (Mueller et al., 2020; Schultz et al., 2021; Huang et al., 2022b). In addition, other toxic effects such as deficient brood size and egg ejection rate were detected in nematodes after exposure to PS-MPs. Nevertheless, these studies are mainly focused on reproductive toxicity of pristine PS-MPs exposure in nematodes, the reproductive abnormalities and underlying molecular mechanisms induced by aged PS-MPs with H_2O_2 require further study.

In the present study, exposure concentration of 0.1–100 μg/L were chosen based on the environmentally relevant concentration of MPs ranged from ng/L to μg/L (Lenz et al., 2016; Al-Sid-Cheikh et al., 2018). Pristine PS-MPs and aged PS-MPs were used to assess reproductive toxicity of MPs in nematodes. Firstly, we measured brood size, egg ejection, and gonad development to assess reproductive toxicity of pristine PS-MPs and aged PS-MPs. Next, the endpoints of germline apoptosis, and the expression of DNA damage-induced apoptosis associated genes were investigated for exploring the potential underlying mechanisms. Furthermore, the roles of DNA damage signaling pathways in inducing germline apoptosis were examined by related nematodes. Our findings provide new insights for understanding the reproductive toxicity and environmental health risks of aged MPs with H_2O_2.

2. Materials and methods

2.1. Characterization of pPS-MPs and aPS-MPs

The commercial PS-MPs were purchased from Janus New Materials (Nanjing, China). The procedure for PS-MPs aging with H_2O_2 was adopted from a previous study (Lang et al., 2020). Pristine PS-MPs (pPS-MPs) in water were added with H_2O_2 (30 %) in quartz glass petri dishes for 7 days continuously in shaken flasks (200 rpm) under dark condition. Subsequently, the aged PS-MPs (aPS-MPs) were then rinsed using ultra-pure water for 3 times and oven-dried at 60 °C. Eventually, PS-MPs before and after aging solid samples were prepared for research. Similar experiments were repeated 2 times, and the resulting samples were mixed. Characterization of characterization was analyzed using Fourier-transform infrared spectroscopy (FTIR), and scanning electron microscopy (SEM) were examined to characterize the physical and chemical properties of PS-MPs. Additional details of PS-MPs characterization are presented in Text S1.

2.2. C. elegans maintenance and exposure

All nematodes were supplied by the *Caenorhabditis* Genetics Center

and cultivated in the standard nematode growth medium (NGM) in the sterile environment at 20 °C. Strains we examined in this study contained wild-type (N2), *cep-1* (*w40*), *egl-1* (*n497*), *hus-1* (*op241*), *ced-3* (*n717*), *ced-4* (*n1162*), and *ced-9* (*n1950*). The gravid hermaphrodites were cleaved using bleaching solution (1 N NaOH/5% NaOCl, 5:2), collected by centrifugation 3600 rpm for 2 min and washed with K-medium (32 Mm KCL, 51 mM NaCl) for 3 times to obtain eggs. The eggs were incubated on new NGM plates containing *Escherichia coli* OP50 as food for 48 h at 20 °C to acquire synchronized L4 larvae (Williams, 1990). Moreover, PS-MPs were diluted in K -medium for working concentrations of 0.1, 1, 10, 100 μg/L. The method of acute exposure (24-h) and endpoint tests were determined according to previous study (Chen et al., 2022). L4 larvae underwent a 24-h exposure to working concentrations ranging from 0.1 to 100 μg/L of both pPS-MPs and aPS-MPs. The control and treated nematodes were exposed to PS-MPs liquid solutions in 6-well plates (10 mL/300 nematodes) for 24 h per concentration. Besides, PS-MPs were stably suspended in liquid solutions during the exposure period, and the remaining solution of PS-MPs were autoclaved and disposed of in the waste liquid. Subsequently, toxic evaluations were performed on the exposed nematodes and this process was replicated across three separate, independent experiments.

2.3. Reproductive capacity measurement

Reproductive capability was tested by evaluating the endpoints of brood size (BS) and egg ejection (EE) rate as previously described (Chen et al., 2022). BS was ascertained by count the number of offspring at all stages beyond the egg. The rate of EE was assessed by quantifying the egg count on the plate per hour subsequent to transferring the individual ovipositing nematode onto a fresh NGM plate (Zhao et al., 2016). Thirty nematodes were examined per concentration.

2.4. Gonad development measurement

We investigated the gonad development by utilizing the number of mitotic cells, the length of the gonad arm, and relative area of the gonad arm as key parameters for evaluation (Qu et al., 2019a; Hua et al., 2023). The 40,6-diamidino-2-phenylindole (DAPI) is an essential fluorescent dye which penetrate intact cell membranes and binds strongly to DNA. DAPI can be widely used to stain germline cells under fluorescence microscopy (Lant and Derry, 2014). The experimental method was performed as previous study reported (Qu et al., 2019a). After washing with K medium, the nematodes were immobilized with 4% paraformaldehyde for 30 min. Then, the nematodes were strained with 0.2 μg/mL DAPI for 30 min. Finally, the nematodes were observed using a fluorescence microscope (Olympus BX51, Japan). Each treatment group was subjected to analysis involving thirty nematodes.

2.5. Germline apoptosis measurement

Acridine orange (AO) straining is an effective approach for assaying apoptotic germline cells. After acute exposure, forty nematodes were stained with AO solutions (25 μg/mL), maintained OP50 and incubated for 60 min at 20 °C. The nematodes were rinsed 3 times with K medium to remove excrete, followed by immobilization with levamisole for 30 min. The data were assessed by quantifying fluorescent images. Thirty nematodes were evaluated for each treatment.

2.6. RT-qPCR assays

High purified RNA was obtained from *C. elegans* using RNAsimple Total RNA TRIzol reagent (Invitrogen, USA). Evo M-MLV kit (Accurate Biotechnology, China) was applied for the synthesis of cDNA through reverse transcription. RT-qPCR analysis was executed with a Applied Biosystems StepOnePlus Real-Time PCR system (Thermo Fisher Scientific, USA) using SYBR Green Supermix (TOYOBO, Japan). The conditions were as follows: 30 s at 95 °C, 40 cycles of 5 s at 95 °C and 30 s at 60 °C. The relative gene expression was normalized against the reference *tba-1*. All assays were repeated three times. The primer pairs are listed in Supplementary Table 1.

2.7. Data analysis

The results are presented as mean ± S.E.M using SPSS software v21. Data were all continuous and passed the normality test and homogeneity test of variance. Statistical differences between groups were analyzed with ANOVA with Turkey's test as a post-hot test. Two-way ANOVA was further performed for the comparison between groups for certain statistical analysis. $*p < 0.05$ or $**p < 0.01$ was deemed statistically significant.

3. Results and discussion

3.1. Alternations in characterizations of PS-MPs

As revealed by dynamic light scattering, the size of pPS-MPs and aPS-MPs were 1.05 ± 0.02 μm and 0.98 ± 0.01 μm, respectively. No significant alterations in the sizes of pPS-MPs and aPS-MPs were observed. As presented in Fig. 1A and B, the pPS-MPs were uniform spherical with smooth surface, while obvious fragmentation, wrinkles, and cracks were detected on the aPS-MPs surface, which is consistent with previously reported study. As depicted in Fig. 1C, more pronounced and additional peaks emerged at 1657 cm^{-1} for aPS-MPs were attributed to C═O bonds, respectively. The carbonyl index (CI) was defined to reflect MPs aging degree based on changes resulting from oxidation. CI was determined by calculating the ratio of the absorbance of both carbonyl and methylene peaks (Liu et al., 2019c). The CI values exhibited an increase from 0.23 in pPS-MPs to 0.90 in aPS-MPs, indicating surface oxidation of PS-MPs due to H_2O_2 treatment. These results were agree with previous literature (Lang et al., 2020). During the aging process, excessive free radicals were generated and led to rapid oxidation of the PS-MPs surface, then C═O bonds are formed. Therefore, H_2O_2 process enhances the aging degree and affects the physicochemical characteristics of PS-MPs.

3.2. Comparison of toxicity between pPS-MPs and aPS-MPs on reproductive capacity

Reproductive capacity at different reproductive stages was evaluated as reflected by the endpoints of BS and EE rate in nematodes. As shown in Fig. 2A, pPS-MPs (100 μg/L) have significant influence on the BS and EE rate than that in control nematodes. However, relatively fewer of BS and EE rate were observed in nematodes exposed to aPS-MPs (10 μg/L) than exposed to pPS-MPs (Fig. 2B). In addition, alternations of reproductive capacity were not significant in nematodes after exposed to leachate from aPS-MPs (Fig. S1).

The findings illustrated that exposure to PS-MPs remarkably decreased the reproductive capacity of nematodes. Prior reports suggested that exposure to PS-MPs significantly exerted an adverse effect on the reproductive capacity as reflected by the endpoints of BS and embryo number in *C. elegans* (Lei et al., 2018; Mueller et al., 2020; Yu et al., 2020). Besides, significant reduction of reproductive capacity by the endpoints of the BS and number of fertilized eggs were observed after exposed to surface-charged PS-NPs in nematodes (Hanna et al., 2018; Qu et al., 2019a; Sun et al., 2021). Recently, Yu and co-workers reported that prolonged maternal exposure to PS-NPs declined transgenerational reproduction with endpoint of BS in nematodes, which was responsible for germline toxicity and epigenetic regulation (Yu et al., 2021a). Other studies also indicated that PS-MPs exposure reduced reproductive capacity in different organisms such as *Daphnia magna*, freshwater prawns, and mice (Jaikumar et al., 2019; Jin et al., 2021; Sun et al., 2022). Furthermore, many studies demonstrated that acute exposure to aPS-MPs remarkably decreased reproductive capacity compared with

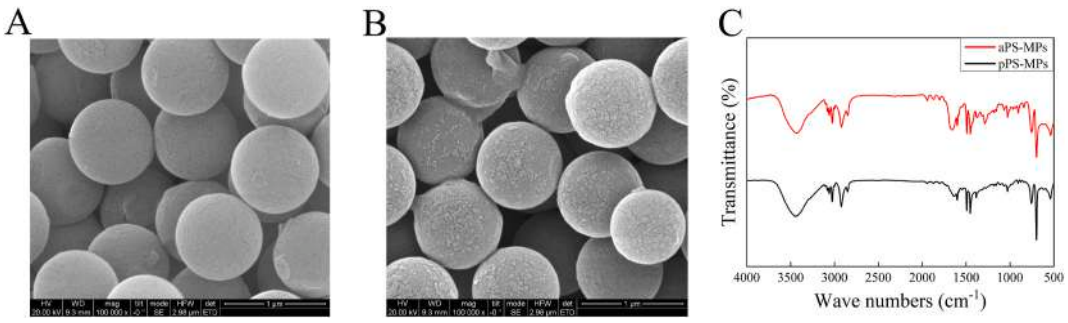


Fig. 1. Alternations in characterizations of aPS-MPs. (A) SEM image of pPS-MPs. (B) SEM image of aPS-MPs. (C) FTIR spectrum.

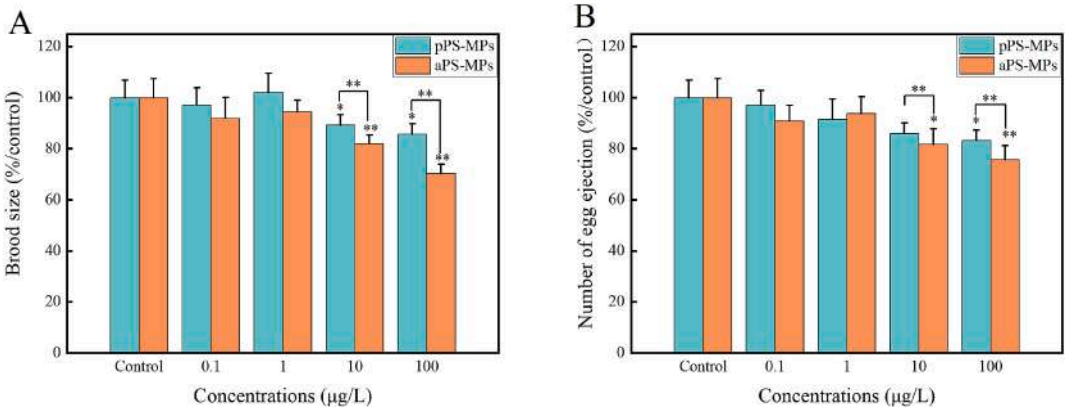


Fig. 2. Effects of pPS-MPs and aPS-MPs with H_2O_2 on the reproductive capacity of nematodes. (A) Brood size. (B) Egg ejection rate. *$p < 0.05$, **$p < 0.01$. Bars indicate means ± S.E.M. N = 30. **$p < 0.01$. *vs* control.

pPS-MPs in nematodes. Recently, acute exposure to aPS-MPs under UV treatment notably declined reproductive capacity than pPS-MPs in nematodes (Chen et al., 2022). The results illustrated that compared to virgin PS-MPs, wastewater-incubated PS-MPs significantly declined the growth and reproduction with reductive numbers of offsprings per surviving adult of *Daphnia magna* (Schur et al., 2021). Wang and colleagues reported that aPS-MPs were more inhibitive on the growth of *C. reinhardtii*, resulting in the declination of chlorophyll-a level in the cells (Wang et al., 2020a). Similarly, aPS-MPs exerted negative effect on growth and liver-related conditions in *Epinephelus moara* (Wang et al., 2020b). Therefore, acute exposure to aPS-MPs markedly reduced reproductive capacity than pPS-MPs.

3.3. Comparison of toxicity of between pPS-MPs and aPS-MPs on gonad development

We further assessed the gonad development using dye of DAPI in aPS-MPs exposed nematodes (Fig. 3A). As shown in Fig. 3B, pPS-MPs (10–100 μg/L) exposure significantly declined mitotic cells per gonad, the length of the gonad arm, and the relative area of gonad arm. Exposure to aPS-MPs (0.1–1 μg/L) showed no obvious changes compared to control; whereas, a noticeable decrease in the number of mitotic cells, was observed in the aPS-MPs exposure (10–100 μg/L). Additionally, exposure to 0.1 μg/L aPS-MPs exhibited no obvious changes compared to control; however, acute exposure to 1–100 μg/L aPS-MPs remarkably decreased the length of the gonad arm, and the relative area of gonad arm compared to the control group (Fig. 3C and D).

The findings demonstrated that exposure to PS-MPs markedly affected the gonad development. Qu and co-workers reported that that exposure to pristine PS-NPs (100 μg/L) could dramatically reduce both the length of gonad arm and relative area of gonad arm (Qu et al., 2019a). Similarly, exposure to pristine PS-NPs (100 μg/L) in parents (P0) caused damage on gonad development in the F1–F2 generations. Moreover, aPS-MPs has larger potential to enhance gonad development than pPS-MPs. For example, exposure to amino-modified PS-NPs (100–1000 μg/L) caused severe reduction in the length of gonad arm or relative area of gonad arm than pristine PS-NPs (Qu et al., 2019a). Analogously, exposure to amino-modified PS-NPs (10 μg/L) remarkably decreased both the length of gonad arm and relative area of gonad arm in the F1 generation. Furthermore, amino modified PS-NPs exposure had adverse effect on gonad development in the F1–F3 generations than pristine PS-NPs (Sun et al., 2021). Study reported that exposure to 1–100 μg/L aged polylactic acid microplastics (PLA-MPs) caused more obvious impairments on gonad development than pristine PLA-MPs (10–100 μg/L), including mitotic cell number, gonad arm length, and gonad arm area (Shao et al., 2024). Besides, studies also suggested that PS-MPs exposure contributed to suppression of gonadal development in various organisms, including marine medaka (*Oryzias melastigma*), zebrafish (*Danio rerio*), and juvenile (*Macrobrachium nipponense*) (Qiang and Cheng, 2021; Wang et al., 2021a; Li et al., 2023). Hence, aPS-MPs exposure resulted in the impairment on gonad development in nematodes than pPS-MPs.

3.4. Comparison of toxicity of between pPS-MPs and aPS-MPs in inducing germline apoptosis

For better elucidating the cellular mechanisms underlying the declination in reproductive capacity exposed to pPS-MPs and aPS-MPs, we next detected germline apoptosis using AO staining in nematodes (Fig. 4A). The numbers of germ cell corpses were used as endpoint and counted by a microscope. As depicted in Fig. 4B, pPS-MPs (10–100 μg/L) exposure induced the noticeable germline apoptosis. Exposure to aPS-MPs (0.1 μg/L) exhibited no obvious changes compared to control;

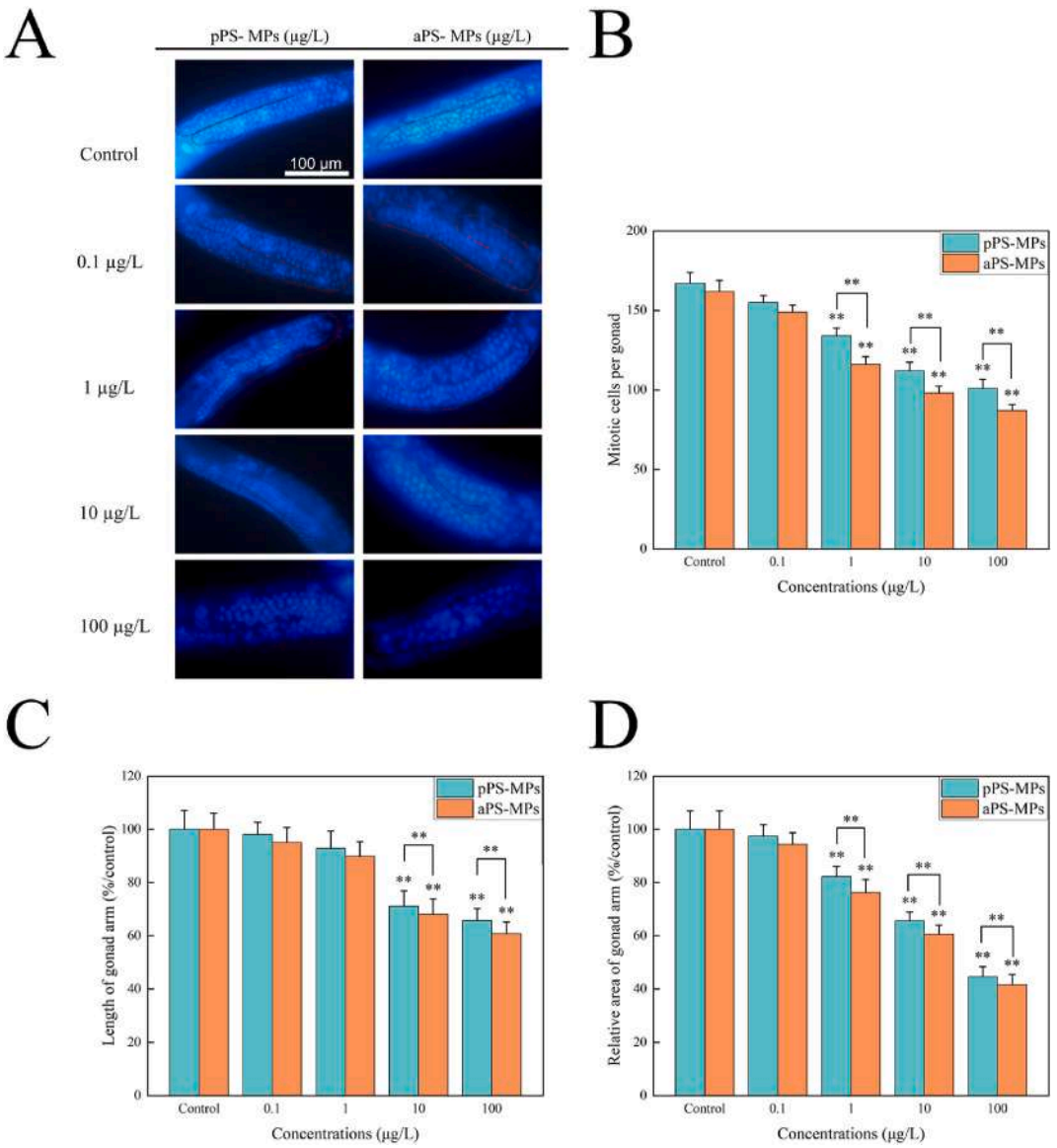


Fig. 3. Effect of pPS-MPs and aPS-MPs with H_2O_2 on gonad development in nematodes. (A) DAPI straining images. (B) Effect of pPS-MPs and aPS-MPs exposure on number of mitotic cells per gonad. (C) Effect of pPS-MPs and aPS-MPs exposure on the length of gonad arm. (D) Effect of pPS-MPs and aPS-MPs exposure on the relative area on gonad arm. $^{*}p < 0.05$, $^{**}p < 0.01$. Bars indicate means ± S.E.M. N = 30. $^{**}p < 0.01$. *vs* control.

whereas, exposure to aPS-MPs (1–100 μg/L) notably triggered a more pronounced instance of germline apoptosis than pPS-MPs (Fig. 4B).

In our work, exposure to aPS-MPs induced more obvious germline apoptosis in nematodes than pPS-MPs. Germline apoptosis, as a programmed cell death process, is regarded as an inherent component of the oogenesis program, which is responsible for prevent interference from external stimuli and maintaining genomic stability(Gumienny et al., 1999). Germline cells are optimized for conserving permanent proliferative potential and accurately transmitting the genetic material. Germline apoptosis can be triggered by DNA damage or many stresses in the environment (Salinas et al., 2006). Study indicated that pristine PS-NPs exposure resulted in the induction of germline apoptosis e (Li et al., 2020b). Similarly, exposed to amino modified PS-NPs induced more noticeable germ cell apoptosis (Qu et al., 2019a; Sun et al., 2021). Moreover, the number of germ cell corpses was markedly elevated in the F1–F4 generations after prolonged exposure to PS-NPs in nematodes, which was related to an improvement in transgenerational germline apoptosis (Yu et al., 2021a). Additionally, studies found that acute exposure to aPS-MPs could enhance germline apoptosis compared to pPS-MPs in *C. elegans*. Study indicated that aPS-MPs exposure under UV irradiation significantly elevated the apoptotic germ cell corpses number, suggesting induction of apoptosis in nematodes (Chen et al., 2021). Recently, aged PLA-MPs resulted in more severe induction of cell apoptosis compared with that in pristine PLA-MPs exposed nematodes, indicating that the reproductive toxicity in PLA-MPs was involved in modulating germline apoptosis and affecting potential molecular basis (Shao et al., 2024). Furthermore, studies have been confirmed that PS-MPs exposure leads to abnormal cell apoptosis in various organisms, including mice brain, Chinese mitten crabs (*Eriocheir sinensis*), and human gastric epithelial (GES-1) cells (Kwon et al., 2022; Nan et al., 2022; Qin et al., 2022). Our findings indicated that aPS-MPs exposure induced more severe induction of germline apoptosis than pPS-MPs.

3.5. *Effect of aPS-MPs on DNA damage-induced apoptosis related gene expression*

To comprehensively explore the molecular mechanism of aPS-MPs, the results of apoptosis-related genes (*ced-3*, *-4,* and *-9*) and DNA

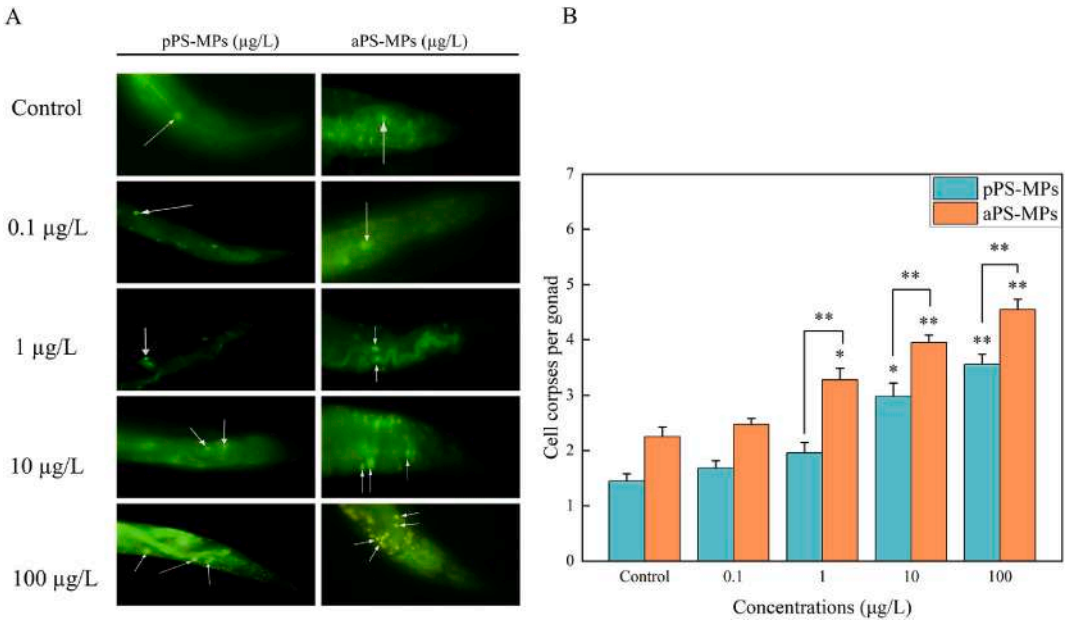


Fig. 4. Effect of pPS-MPs and aPS-MPs with H_2O_2 exposure in inducing germline apoptosis in nematodes. (A) AO staining images. Arrowheads indicate the germline apoptotic signals. (B) Effect of pPS-MPs and aPS-MPs exposure on the cell corpses per gonad in nematodes. $^*p < 0.05$, $^{**}p < 0.01$. Bars indicate means ± S.E.M. N = 30. $^{**}p < 0.01$. *vs* control.

damage-related genes (*cep-1*, *egl-1*, *hus-1*, and *clk-2*) expression were further validated following exposure to aPS-MPs (100 μg/L) in *C. elegans*. As illustrated in Fig. 5, the expression of *cep-1, ced-3, -4, hus-1, egl-1, and clk-2* was significantly upregulated compared to control; in contrast, aPS-MPs exposure was obviously downregulated mRNA expression of *ced-9*. The genetic expression alternation was varying from 0.71-(*ced-3*) to 4.10-(*hus-1*) fold, DNA damage-induced germline apoptosis led to reproductive toxic effects by toxicants in nematodes. *Ced-3* and *-4* (encoding Apaf-1 homolog) as conserved proteins are required for promoting apoptosis induction, which plays a key role in vast cell deaths that occur during nematode development; whereas *ced-9* (encoding homolog of Bcl-2) is responsible for protecting healthy cells to prevent apoptosis (Pourkarimi et al., 2012; Bailly and Gartner, 2013). Programmed death of cells is be regulated by the direct interaction of

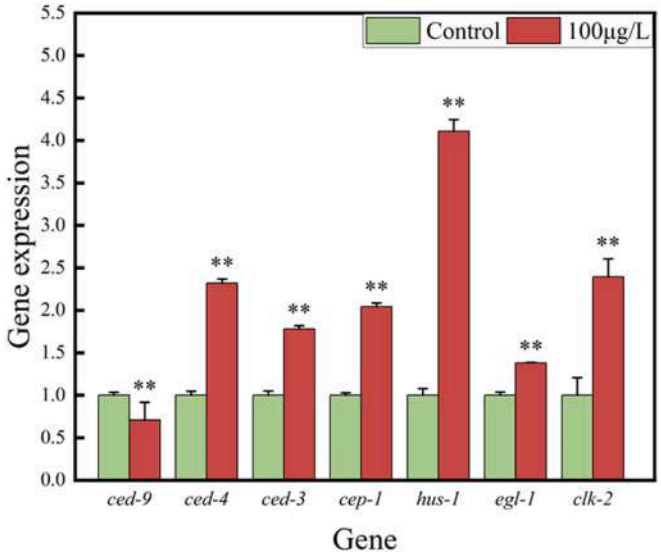


Fig. 5. Effect of aPS-MPs with H_2O_2 at the dose of 100 μg/L on apoptosis-related gene expression in nematodes. Bars indicate means ± S.E.M. $^{**}p < 0.01$. *vs* control.

ced-4 and *-9* in nematodes (Bailly and Gartner, 2013). *Ced-9* is considered as an anti-apoptotic factor which negatively regulate the transcription of *ced-4* by sequestering it at the outer mitochondrial membrane by straight binding. Previous studies have suggested that environmental toxicants exposure induce cell apoptosis by alternations in these genes expression in *C. elegans*, including crude oil, tributyltin, graphene oxide, and UV-filter octyl methoxycinnamate (Polli et al., 2014; Wang et al., 2014; Sivaselvam et al., 2020; Huang et al., 2022a). Analogously, lipopolysaccharide (LPS) exposure changes the expression of *ced-9* (anti-apoptosis-related gene) and *ced-3* (apoptosis-related gene), which is involved in LPS-induced cell apoptosis (Ma et al., 2020). A study reported that prolonged exposure to lindane induces cell apoptosis and significantly upregulates the expression of *ced-3*, *-4,* and *-9* in nematodes (Yu et al., 2021b). Moreover, Qu et al. found that amino-modified PS-NPs elevated the expression of these genes in nematodes, which have an important effect on reproductive toxicity (Qu et al., 2019a). Recently, study indicated that exposure to Tetrachlorobisphenol A (TCBPA) induced cell apoptosis and altered the expression of germline apoptosis pathway-related genes in nematodes (Yu et al., 2022b). Our results suggested that aPS-MPs exposure significantly enhanced the number of germ cell corpses via regulating the expression of apoptosis-related genes, which are responsible for controlling germline apoptosis.

In this study, aPS-MPs exposure notably upregulated the mRNA levels of DNA damage-related genes, which is required for activating germline apoptosis. In nematodes, *cep-1*, *egl-1*, *hus-1*, and *clk-2* genes, as conserved checkpoint proteins, are crucial components of the evolutionarily apoptotic machinery, which play an imperative roles in DNA damage-induced apoptosis (Craig et al., 2012). The *hus-1*, and *clk-2* genes take charge of regulating expression of *cep-1* genes (Kamath, 2001). *Cep-1* (encoding A p53-like protein) is responsible for enhancing DNA damage and inducing germ-cell apoptosis via transcriptionally upregulating *egl-1* expression (Lettre and Hengartner, 2006). *Egl-1* (encoding A BH3-only protein) is seen as an essential activator of the upstream apoptotic pathway and an integrator of cell-death signals which execute the life-versus-death determination and then transduce it to the core apoptotic machinery (Nehme and Conradt, 2008). Prior investigations have demonstrated that DNA damage-induced cell death was attribute to many environmental toxicants, including

Methylmercury, sodium arsenite, and endosulfan (Wang et al., 2007; Du et al., 2015a; Hu et al., 2021). Study indicated that N-(1,3-dimethylbutyl)-N′-phenyl-*p*-phenylenediamine (6-PPD) exposure upregulated the expression of *ced-1*, *-3 -4,* and *egl-1* genes, suggesting that these apoptosis-related genes were related to an enhancement in DNA damage (Hua et al., 2023). Recently, a study reported that the genetic expression level of apoptosis were significantly altered, indicating that DNA damage-induced cell apoptosis contributed to the reproductive impairments of UV-photodegraded aged MPs in nematodes (Chen et al., 2022). Analogously, aged PLA-MPs after 30-day UV-irradiation significantly enhanced germline apoptosis than pristine PLA-MPs via regulating the expression of DNA damage-related genes (*cep-1*, *mrt-2*, *hus-1*, and *clk-2*) (Shao et al., 2024). Our results demonstrated that the enhanced germline apoptosis induced by aPS-MPs exposure were associated with the regulation of DNA damage-related genes. Therefore, reproductive toxicity induced by aPS-MPs could contribute to germline apoptosis activated by DNA damage in *C. elegans*.

3.6. Effect of core apoptotic machinery on cell death

To further determine the underlying molecular mechanisms of reproductive toxicity in aPS-MPs exposed nematodes, germline apoptosis was examined the phenotypes of corresponding mutants. We utilized *egl-1(n487)*, *hus-1(op241)* *cep-1(n1162)*, *ced-3(n717), ced-4 (n1162)*, and *ced-9(n1950)* mutants in aPS-MPs (100 μg/L) exposed nematodes (Fig. 6A). As depicted in Fig. 6 B, *cep-1*, *hus-1*, and *egl-1* mutants did not influence cell death compared to the control group, which suggested that these mutants play a vital role in cell apoptosis. Besides, the number of cell corpses marginally altered in *ced-3* and *-4* strains, which indicated that *ced-3* and *-4* genes are imperative for germline apoptosis caused by aPS-MPs. Furthermore, germ cell corpses were significantly enhanced in *ced-9* mutants, revealing that *ced-9* has negatively apoptotic influence in nematodes.

Prior reports have indicated that hydroxylated fullerene nanoparticles and diesel particulate matter (DPE) plus ultraviolet-A exposure significantly suppressed the synergistic induction of germline apoptosis in *ced-3* and *-4* mutants, which showed that these core pro-apoptotic regulator genes are key components for cell apoptosis (Cha et al., 2012; Guo et al., 2014). Analogously, exposure to environmental toxicants, such as tributyltin, bisphenol A, and copper, altered cell corpses in the apoptosis-related mutants, which unveils that these genes have an essential influence on apoptosis (Wang et al., 2009, 2014, 2017). Additionally, the number of cell corpses of DPE, graphene oxide, and endosulfan were blocked in mutation of *hus-1*, *cep-1*, and *egl-1* genes, which indicated that these genes are indispensable for DNA damage-induced cell death (Du et al., 2015b; Zhao et al., 2016; Wang et al., 2019b). Furthermore, germline apoptosis (CED-3-CED4-CED9 signal transduction) and DNA damage (HUS-1/CEP-1-EGL-1-CLK-2 signal transduction) remarkably caused reproductive toxicity induced by PS-NPs (Qu et al., 2019a, 2019b; Li et al., 2020a). Recently, acute germ cell corpses were altered in these apoptosis-related mutants in UV-photodegraded PS-MPs exposed nematodes, revealing that the signaling pathway of HUS-1/CEP-1-CLK-2/EGL-1-p53-CED-3-CED-4 is responsible for regulating reproductive toxicity induced by DNA damage-induced cell death (Chen et al., 2022). Therefore, we provided a complete signal transduction of EGL-1-CEP-1-HUS-1-CED-3-CED-4-CED-9 to explain the molecular mechanism for aPS-MPs with H_2O_2 induced reproductive toxicity in nematodes.

Our study highlights the effects of aPS-MPs in inducing reproductive toxicity on environmental organisms. Nevertheless, the impacts of prolong aPS-MPs exposure on organisms are proposed for further exploration. The environmental behavior and biological toxicology of actual aPS-MPs in the natural environment are required for further investigated to be close to real environmental exposure in the future.

4. Conclusion

In this study, we employed classical model of nematodes to indicate the reproductive toxicity of aPS-MPs. Firstly, we found that aPS-MPs exposure significantly reduced reproductive capacity than pPS-MPs in

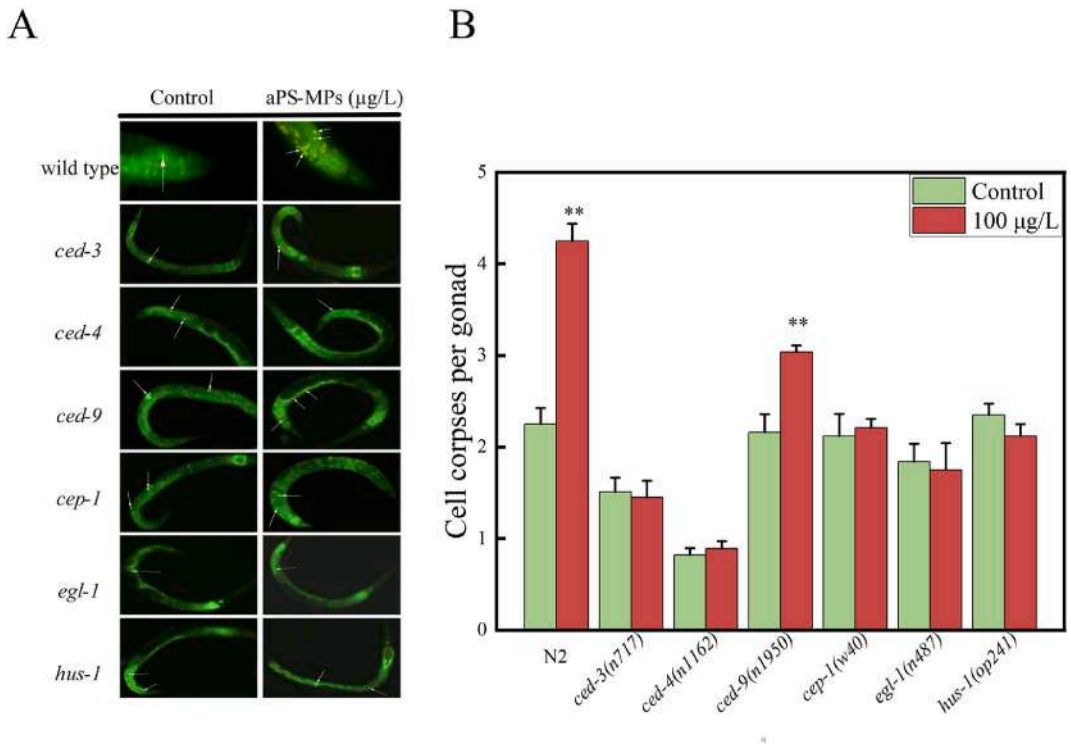


Fig. 6. Role of DNA damage and core apoptotic machinery in germline apoptosis induced by aPS-MPs. (A) AO staining images. Arrowheads indicate the germline apoptotic signals. (B) Effect of 100 μg/L aPS-MPs exposure on the cell corpses per gonad in nematodes. Bars indicate means ± S.E.M. $^{**}p < 0.01$. *vs* control.

nematodes. Additionally, reproductive toxicity on germline apoptosis and gonad development effectively enhanced in aPS-MPs exposed nematodes than pPS-MPs. Moreover, reproductive toxicity may be originated from germline apoptosis and activation of DNA damage in nematodes. For the molecular mechanisms underlying the reproductive toxicity of aPS-MPs-induced germline apoptosis, we detected a signaling cascade of EGL-1-CEP-1-HUS-1-CED-3-CED-4-CED-9 to illustrate the roles of core cell apoptosis signal transduction induced by DNA damage. Our results elucidated the exposure risks of aPS-MPs at environmentally relevant concentrations in inducing toxicity on organisms.

CRediT authorship contribution statement

Tiantian Xu: Writing – original draft, Investigation, Data curation. **Haibo Chen:** Writing – review & editing, Writing – original draft, Formal analysis. **Luohong Zhang:** Validation. **Dongli Xie:** Validation. **Shihui Tan:** Investigation. **Hongzhi Guo:** Investigation. **Mingdeng Xiang:** Writing – review & editing. **Yunjiang Yu:** Writing – review & editing, Resources, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgements

Financial support was received from the Key Program of National Natural Science Foundation of China (41931298), and National Key R&D Program of China (2019YFC1803403).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.chemosphere.2024.142519.

References

Abbasi, S., Turner, A., 2021. Human exposure to microplastics: a study in Iran. J. Hazard Mater. 403, 123799.
Al-Sid-Cheikh, M., Rowland, S.J., Stevenson, K., Rouleau, C., Henry, T.B., Thompson, R. C., 2018. Uptake, whole-body distribution, and depuration of nanoplastics by the scallop pecten maximus at environmentally realistic concentrations. Environ. Sci. Technol. 52, 14480–14486.
Bailly, A., Gartner, A., 2013. Germ cell apoptosis and DNA damage responses. In: Schedl, T. (Ed.), Germ Cell Development in C. elegans. Springer, New York, New York, NY, pp. 249–276.
Cha, Y.J., Lee, J., Choi, S.S., 2012. Apoptosis-mediated in vivo toxicity of hydroxylated fullerene nanoparticles in soil nematode Caenorhabditis elegans. Chemosphere 87, 49–54.
Chae, Y., An, Y.-J., 2018. Current research trends on plastic pollution and ecological impacts on the soil ecosystem: a review. Environ. Pollut. 240, 387–395.
Chen, H., Hua, X., Yang, Y., Wang, C., Jin, L., Dong, C., Chang, Z., Ding, P., Xiang, M., Li, H., Yu, Y., 2021. Chronic exposure to UV-aged microplastics induces neurotoxicity by affecting dopamine, glutamate, and serotonin neurotransmission in Caenorhabditis elegans. J. Hazard Mater. 419, 126482.
Chen, H., Yang, Y., Wang, C., Hua, X., Li, H., Xie, D., Xiang, M., Yu, Y., 2022. Reproductive toxicity of UV-photodegraded polystyrene microplastics induced by DNA damage-dependent cell apoptosis in Caenorhabditis elegans. Sci. Total Environ. 811, 152350.
Craig, A.L., Moser, S.C., Bailly, A.P., Gartner, A., 2012. Methods for studying the DNA damage response in the caenorhabdatis elegans germ line. In: Rothman, J.H., Singson, A. (Eds.), Methods in Cell Biology. Academic Press, pp. 321–352.
Cui, H., Yang, W., Cui, Y., Qi, L., Jiang, X., Li, M., 2023. Adverse effects of pristine and aged polystyrene microplastics in mice and their Nrf2-mediated defense mechanisms with tissue specificity. Environ. Sci. Pollut. Control Ser. 30, 39894–39906.
Du, H., Wang, M., Dai, H., Hong, W., Wang, M., Wang, J., Weng, N., Nie, Y., Xu, A., 2015a. Endosulfan isomers and sulfate metabolite induced reproductive toxicity in Caenorhabditis elegans involves genotoxic response genes. Environ. Sci. Technol. 49, 2460–2468.
Du, H., Wang, M., Dai, H., Hong, W., Wang, M.D., Wang, J.J., Weng, N.Y., Nie, Y.G., Xu, A., 2015b. Endosulfan isomers and sulfate metabolite induced reproductive toxicity in Caenorhabditis elegans involves genotoxic response genes. Environ. Sci. Technol. 49, 2460–2468.
Esplugas, S., Bila, D.M., Krause, L.G.T., Dezotti, M., 2007. Ozonation and advanced oxidation technologies to remove endocrine disrupting chemicals (EDCs) and pharmaceuticals and personal care products (PPCPs) in water effluents. J. Hazard Mater. 149, 631–642.
Gumienny, T.L., Lambie, E., Hartwieg, E., Horvitz, H.R., Hengartner, M.O., 1999. Genetic control of programmed cell death in the Caenorhabditis elegans hermaphrodite germline. Development 126, 1011–1022.
Guo, X.Y., Bian, P., Liang, J.T., Wang, Y.C., Li, L.Z., Wang, J., Yuan, H., Chen, S.P., Xu, A., Wu, L.J., 2014. Synergistic effects induced by a low dose of diesel particulate extract and ultraviolet-A in Caenorhabditis elegans: DNA damage-triggered germ cell apoptosis. Chem. Res. Toxicol. 27, 990–1001.
Hanna, S.K., Bustos, A.R.M., Peterson, A.W., Reipa, V., Scanlan, L.D., Coskun, S.H., Cho, T.J., Johnson, M.E., Hackley, V.A., Nelson, B.C., Winchester, M.R., Elliott, J.T., Petersen, E.J., 2018. Agglomeration of Escherichia coli with positively charged nanoparticles can lead to artifacts in a standard Caenorhabditis elegans toxicity assay. Environ. Sci. Technol. 52, 5968–5978.
Hu, K., Xu, Y., Xu, S., Cheng, L., Zhou, T., Xie, A., Xu, A., Wu, L., Chen, S., 2021. Ecotoxicity risk of low-dose Methylmercury exposure to Caenorhabditis elegans: multigenerational toxicity and population discrepancy. Chem. Res. Toxicol. 34, 1114–1123.
Hua, X., Feng, X., Liang, G., Chao, J., Wang, D., 2023. Long-term exposure to 6-PPD quinone reduces reproductive capacity by enhancing germline apoptosis associated with activation of both DNA damage and cell corpse engulfment in Caenorhabditis elegans. J. Hazard Mater. 454, 131495.
Huang, C.-W., Kung, Z.-Y., Wei, C.-C., 2022a. UV-filter octyl methoxycinnamate causes reproductive toxicity associated with germline apoptosis and vitellogenin decrease in Caenorhabditis elegans. Aquat. Toxicol. 247, 106149.
Huang, C.-W., Yen, P.-L., Kuo, Y.-H., Chang, C.-H., Liao, V.H.-C., 2022b. Nanoplastic exposure in soil compromises the energy budget of the soil nematode C. elegans and decreases reproductive fitness. Environ. Pollut. 312, 120071.
Huang, Y., Ding, J., Zhang, G., Liu, S., Zou, H., Wang, Z., Zhu, W., Geng, J., 2021. Interactive effects of microplastics and selected pharmaceuticals on red tilapia: role of microplastic aging. Sci. Total Environ. 752, 142256.
Hüffer, T., Weniger, A.-K., Hofmann, T., 2018. Sorption of organic compounds by aged polystyrene microplastic particles. Environ. Pollut. 236, 218–225.
Jaikumar, G., Brun, N.R., Vijver, M.G., Bosker, T., 2019. Reproductive toxicity of primary and secondary microplastics to three cladocerans during chronic exposure. Environ. Pollut. 249, 638–646.
Jia, H., Zhao, S., Zhu, K., Huang, D., Wu, L., Guo, X., 2018. Activate persulfate for catalytic degradation of adsorbed anthracene on coking residues: role of persistent free radicals. Chem. Eng. J. 351, 631–640.
Jin, H., Ma, T., Sha, X., Liu, Z., Zhou, Y., Meng, X., Chen, Y., Han, X., Ding, J., 2021. Polystyrene microplastics induced male reproductive toxicity in mice. J. Hazard Mater. 401, 123430.
Kamath, C., 2001. On mining scientific datasets. In: Grossman, R.L., Kamath, C., Kegelmeyer, P., Kumar, V., Namburu, R.R. (Eds.), Data Mining for Scientific and Engineering Applications. Springer US, Boston, MA, pp. 1–21.
Kik, K., Bukowska, B., Sicińska, P., 2020. Polystyrene nanoparticles: sources, occurrence in the environment, distribution in tissues, accumulation and toxicity to various organisms. Environ. Pollut. 262, 114297.
Kwon, W., Kim, D., Kim, H.-Y., Jeong, S.W., Lee, S.-G., Kim, H.-C., Lee, Y.-J., Kwon, M.K., Hwang, J.-S., Han, J.E., Park, J.-K., Lee, S.-J., Choi, S.-K., 2022. Microglial phagocytosis of polystyrene microplastics results in immune alteration and apoptosis in vitro and in vivo. Sci. Total Environ. 807, 150817.
Lambert, S., Wagner, M., 2016. Characterisation of nanoplastics during the degradation of polystyrene. Chemosphere 145, 265–268.
Lang, M., Yu, X., Liu, J., Xia, T., Wang, T., Jia, H., Guo, X., 2020. Fenton aging significantly affects the heavy metal adsorption capacity of polystyrene microplastics. Sci. Total Environ. 722, 137762.
Lant, B., Derry, W.B., 2014. Immunostaining for markers of apoptosis in the Caenorhabditis elegans germline. Cold Spring Harb. Protoc., 2014
Lei, L.L., Wu, S.Y., Lu, S.B., Liu, M.T., Song, Y., Fu, Z.H., Shi, H.H., Raley-Susman, K.M., He, D.F., 2018. Microplastic particles cause intestinal damage and other adverse effects in zebrafish Danio rerio and nematode Caenorhabditis elegans. Sci. Total Environ. 619, 1–8.
Lenz, R., Enders, K., Nielsen, T.G., 2016. Microplastic exposure studies should be environmentally realistic. Proc. Natl. Acad. Sci. U.S.A. 113, E4121–E4122.
Lettre, G., Hengartner, M.O., 2006. Developmental apoptosis in C. elegans: a complex CEDnario. Nat. Rev. Mol. Cell Biol. 7, 97–108.
Li, D., Ji, J., Yuan, Y., Wang, D., 2020a. Toxicity comparison of nanopolystyrene with three metal oxide nanoparticles in nematode Caenorhabditis elegans. Chemosphere 245, 125625.
Li, D., Ji, J., Yuan, Y.J., Wang, D.Y., 2020b. Toxicity comparison of nanopolystyrene with three metal oxide nanoparticles in nematode Caenorhabditis elegans. Chemosphere 245, 125625.
Li, Y., Ye, Y., Rihan, N., Jiang, Q., Liu, X., Zhao, Y., Che, X., 2023. Polystyrene nanoplastics decrease nutrient accumulation, disturb sex hormones, and inhibit reproductive development in juvenile Macrobrachium nipponense. Sci. Total Environ. 891, 164481.

Liu, P., Shi, Y., Wu, X., Wang, H., Huang, H., Guo, X., Gao, S., 2021. Review of the artificially-accelerated aging technology and ecological risk of microplastics. Sci. Total Environ. 768, 144969.
Liu, Z., Zhuan, Q., Zhang, L., Meng, L., Fu, X., Hou, Y., 2022. Polystyrene microplastics induced female reproductive toxicity in mice. J. Hazard Mater. 424, 127629.
Luo, H., Liu, C., He, D., Xu, J., Sun, J., Li, J., Pan, X., 2022. Environmental behaviors of microplastics in aquatic systems: a systematic review on degradation, adsorption, toxicity and biofilm under aging conditions. J. Hazard Mater. 423, 126915.
Ma, J., Xu, X., Wang, R., Yan, H., Yao, H., Zhang, H., Jiang, S., Xu, A., 2020. Lipopolysaccharide exposure induces oxidative damage in Caenorhabditis elegans: protective effects of carnosine. BMC Pharmacology and Toxicology 21, 85.
Mueller, M.-T., Fueser, H., Trac, L.N., Mayer, P., Traunspurger, W., Höss, S., 2020. Surface-related toxicity of polystyrene beads to nematodes and the role of food availability. Environ. Sci. Technol. 54, 1790–1798.
Nan, X., Jin, X., Song, Y., Zhou, K., Qin, Y., Wang, Q., Li, W., 2022. Effect of polystyrene nanoplastics on cell apoptosis, glucose metabolism, and antibacterial immunity of Eriocheir sinensis. Environ. Pollut. 311, 119960.
Nehme, R., Conradt, B., 2008. egl-1: a key activator of apoptotic cell death in C. elegans. Oncogene 27, S30–S40.
Ni, Z., Tan, L., Wang, J., Chen, Y., Zhang, N., Meng, F., Wang, J., 2023. Toxic effects of pristine and aged polystyrene and their leachate on marine microalgae Skeletonema costatum. Sci. Total Environ. 857, 159614.
Pelegrini, K., Pereira, T.C.B., Maraschin, T.G., Teodoro, L.D.S., Basso, N.R.D.S., De Galland, G.L.B., Ligabue, R.A., Bogo, M.R., 2023. Micro- and nanoplastic toxicity: a review on size, type, source, and test-organism implications. Sci. Total Environ. 878, 162954.
Polli, J.R., Zhang, Y.Q., Pan, X.P., 2014. Dispersed crude oil amplifies germ cell apoptosis in Caenorhabditis elegans, followed a CEP-1-dependent pathway. Arch. Toxicol. 88, 543–551.
Pourkarimi, E., Greiss, S., Gartner, A., 2012. Evidence that CED-9/Bcl2 and CED-4/Apaf-1 localization is not consistent with the current model for C. elegans apoptosis induction. Cell Death Differ. 19, 406–415.
Qiang, L., Cheng, J., 2021. Exposure to polystyrene microplastics impairs gonads of zebrafish (Danio rerio). Chemosphere 263, 128161.
Qin, J., Xia, P.-F., Yuan, X.-Z., Wang, S.-G., 2022. Chlorine disinfection elevates the toxicity of polystyrene microplastics to human cells by inducing mitochondria-dependent apoptosis. J. Hazard Mater. 425, 127842.
Qu, M., Qiu, Y., Kong, Y., Wang, D., 2019a. Amino modification enhances reproductive toxicity of nanopolystyrene on gonad development and reproductive capacity in nematode Caenorhabditis elegans. Environ. Pollut. 254, 112978.
Qu, M., Qiu, Y., Lv, R., Yue, Y., Liu, R., Yang, F., Wang, D., Li, Y., 2019b. Exposure to MPA-capped CdTe quantum dots causes reproductive toxicity effects by affecting oogenesis in nematode Caenorhabditis elegans. Ecotoxicol. Environ. Saf. 173, 54–62.
Ragusa, A., Svelato, A., Santacroce, C., Catalano, P., Notarstefano, V., Carnevali, O., Papa, F., Rongioletti, M.C.A., Baiocco, F., Draghi, S., D'Amore, E., Rinaldo, D., Matta, M., Giorgini, E., 2021. Plasticenta: first evidence of microplastics in human placenta. Environ. Int. 146, 106274.
Salinas, L.S., Maldonado, E., Navarro, R.E., 2006. Stress-induced germ cell apoptosis by a p53 independent pathway in Caenorhabditis elegans. Cell Death Differ. 13, 2129–2139.
Schultz, C.L., Bart, S., Lahive, E., Spurgeon, D.J., 2021. What is on the outside matters—surface charge and dissolve organic matter association affect the toxicity and physiological mode of action of polystyrene nanoplastics to C. elegans. Environ. Sci. Technol. 55, 6065–6075.
Schur, C., Weil, C., Baum, M., Wallraff, J., Schreier, M., Oehlmann, J., Wagner, M., 2021. Incubation in wastewater reduces the multigenerational effects of microplastics in Daphnia magna. Environ. Sci. Technol. 55, 2491–2499.
Shao, Y., Hua, X., Li, Y., Wang, D., 2024. Comparison of reproductive toxicity between pristine and aged polylactic acid microplastics in Caenorhabditis elegans. J. Hazard Mater. 466, 133545.
Shi, Q., Tang, J., Liu, X., Liu, R., 2021. Ultraviolet-induced photodegradation elevated the toxicity of polystyrene nanoplastics on human lung epithelial A549 cells. Environ. Sci.: Nano 8, 2660–2675.
Siddiqui, S.A., Singh, S., Bahmid, N.A., Shyu, D.J.H., Domínguez, R., Lorenzo, J.M., Pereira, J.A.M., Câmara, J.S., 2023. Polystyrene microplastic particles in the food chain: characteristics and toxicity - a review. Sci. Total Environ. 892, 164531.
Sivaselvam, S., Mohankumar, A., Thiruppathi, G., Sundararaj, P., Viswanathan, C., Ponpandian, N., 2020. Engineering the surface of graphene oxide with bovine serum albumin for improved biocompatibility in Caenorhabditis elegans. Nanoscale Adv. 2, 5219–5230.
Song, J.A., Choi, C.Y., Park, H.-S., 2020. Exposure of bay scallop Argopecten irradians to micro-polystyrene: bioaccumulation and toxicity. Comp. Biochem. Physiol. C Toxicol. Pharmacol. 236, 108801.
Sun, L., Liao, K., Wang, D., 2021. Comparison of transgenerational reproductive toxicity induced by pristine and amino modified nanoplastics in Caenorhabditis elegans. Sci. Total Environ. 768, 144362.
Sun, S., Jin, Y., Luo, P., Shi, X., 2022. Polystyrene microplastics induced male reproductive toxicity and transgenerational effects in freshwater prawn. Sci. Total Environ. 842, 156820.
Tsitonaki, A., Petri, B., Crimi, M., MosbÆK, H., Siegrist, R.L., Bjerg, P.L., 2010. In situ chemical oxidation of contaminated soil and groundwater using persulfate: a review. Crit. Rev. Environ. Sci. Technol. 40, 55–91.
Wang, J., Li, Y., Lu, L., Zheng, M., Zhang, X., Tian, H., Wang, W., Ru, S., 2019a. Polystyrene microplastics cause tissue damages, sex-specific reproductive disruption and transgenerational effects in marine medaka (Oryzias melastigma). Environ. Pollut. 254, 113024.
Wang, J., Zheng, M., Lu, L., Li, X., Zhang, Z., Ru, S., 2021a. Adaptation of life-history traits and trade-offs in marine medaka (Oryzias melastigma) after whole life-cycle exposure to polystyrene microplastics. J. Hazard Mater. 414, 125537.
Wang, M., Nie, Y., Liu, Y., Dai, H., Wang, J., Si, B., Yang, Z., Cheng, L., Liu, Y., Chen, S., Xu, A., 2019b. Transgenerational effects of diesel particulate matter on Caenorhabditis elegans through maternal and multigenerational exposure. Ecotox Environ Safe 170, 635–643.
Wang, Q., Wangjin, X., Zhang, Y., Wang, N., Wang, Y., Meng, G., Chen, Y., 2020a. The toxicity of virgin and UV-aged PVC microplastics on the growth of freshwater algae Chlamydomonas reinhardtii. Sci. Total Environ. 749, 141603.
Wang, S., Zhao, Y., Wu, L., Tang, M., Su, C., Hei, T.K., Yu, Z., 2007. Induction of germline cell cycle arrest and apoptosis by sodium arsenite in Caenorhabditis elegans. Chem. Res. Toxicol. 20, 181–186.
Wang, S.C., Wu, L.J., Wang, Y., Luo, X., Lu, Y., 2009. Copper-induced germline apoptosis in Caenorhabditis elegans: the independent roles of DNA damage response signaling and the dependent roles of MAPK cascades. Chem. Biol. Interact. 180, 151–157.
Wang, X., Zheng, H., Zhao, J., Luo, X.X., Zhenyu, W., Xing, B.S., 2020b. Photodegradation elevated the toxicity of polystyrene microplastics to grouper (Epinephelus moara) through disrupting hepatic lipid homeostasis. Environ. Sci. Technol. 54, 6202–6212.
Wang, Y., Wang, S.C., Luo, X., Yang, Y.A., Jian, F.L., Wang, X.M., Xie, L.C., 2014. The roles of DNA damage-dependent signals and MAPK cascades in tributyltin-induced germline apoptosis in Caenorhabditis elegans. Chemosphere 108, 231–238.
Wang, Y., Zhang, L.F., Luo, X., Wang, S.C., Wang, Y.Y., 2017. Bisphenol A exposure triggers apoptosis via three signaling pathways in Caenorhabditis elegans. Rsc Adv 7, 32624–32631.
Wang, Z., Dong, H., Wang, Y., Ren, R., Qin, X., Wang, S., 2020c. Effects of microplastics and their adsorption of cadmium as vectors on the cladoceran Moina monogolica Daday: implications for plastic-ingesting organisms. J. Hazard Mater. 400, 123239.
Wang, Z.Z., Fu, D.D., Gao, L., Qi, H.Y., Su, Y.Y., Peng, L.C., 2021b. Aged microplastics decrease the bioavailability of coexisting heavy metals to microalga Chlorella vulgaris. Ecotoxicol. Environ. Saf. 217, 112199.
Williams, P.L., 1990. Aquatic toxicity testing using the nematode, Caenorhabditis elegans. Environ. Toxicol. Chem. 9, 1285.
Youssef, K., Archonta, D., Kubiseski, T.J., Tandon, A., Rezai, P., 2021. Microfluidic electric parallel egg-laying assay and application to in-vivo toxicity screening of microplastics using C. elegans. Sci. Total Environ. 783, 147055.
Yu, C.-W., Luk, T.C., Liao, V.H.-C., 2021a. Long-term nanoplastics exposure results in multi and trans-generational reproduction decline associated with germline toxicity and epigenetic regulation in Caenorhabditis elegans. J. Hazard Mater. 412, 125173.
Yu, Y., Chen, H., Hua, X., Dang, Y., Han, Y., Yu, Z., Chen, X., Ding, P., Li, H., 2020. Polystyrene microplastics (PS-MPs) toxicity induced oxidative stress and intestinal injury in nematode Caenorhabditis elegans. Sci. Total Environ. 726, 138679.
Yu, Y., Chen, H., Hua, X., Wang, C., Dong, C., Xie, D., Tan, S., Xiang, M., Li, H., 2022a. A review of the reproductive toxicity of environmental contaminants in Caenorhabditis elegans. Hygiene and Environmental Health Advances 3, 100007.
Yu, Y., Chen, H., Hua, X., Wang, Z., Li, L., Li, Z., Xiang, M., Ding, P., 2021b. Long-term toxicity of lindane through oxidative stress and cell apoptosis in Caenorhabditis elegans. Environ. Pollut. 272, 116036.
Yu, Y., Hua, X., Chen, H., Yang, Y., Dang, Y., Xiang, M., 2022b. Tetrachlorobisphenol A mediates reproductive toxicity in Caenorhabditis elegans via DNA damage-induced apoptosis. Chemosphere 300, 134588.
Zhang, X., Xia, M., Su, X., Yuan, P., Li, X., Zhou, C., Wan, Z., Zou, W., 2021. Photolytic degradation elevated the toxicity of polylactic acid microplastics to developing zebrafish by triggering mitochondrial dysfunction and apoptosis. J. Hazard Mater. 413, 125321.
Zhao, Y.L., Wu, Q.L., Wang, D.Y., 2016. An epigenetic signal encoded protection mechanism is activated by graphene oxide to inhibit its induced reproductive toxicity in Caenorhabditis elegans. Biomaterials 79, 15–24.