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RESEARCH ARTICLE

Assessing the Impact of Microplastics on DNA Damage in *C. elegans*

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ABSTRACT: DNA damage is a key driver of genetic mutations and disease, including cancer. Microplastics, generated from the degradation of plastic waste, are now widely detected in water, food, and the human body. However, their potential to induce DNA damage remains largely unknown. Here, we examined the effect of polystyrene microplastics on causing DNA damage using *Caenorhabditis elegans* (*C. elegans*) as a model organism. Transgenic *cep-1::GFP* worms, which express green fluorescent protein (GFP) under the control of the *cep-1* promoter in response to DNA damage, were exposed to polystyrene beads of two sizes (0.2 µm or 1 µm) at two concentrations (1×10^7 particles/mL and 1×10^8 particles/mL) for 24 hours. Wild-type and untreated transgenic worms served as controls. GFP expression was quantified by fluorescence microscopy and ImageJ analysis. Microplastic exposure significantly elevated GFP expression in *cep-1::GFP* worms regardless of microplastic size or concentration, suggesting robust induction of DNA damage response, whereas neither control group showed a significant response. Altogether, these results demonstrate that polystyrene microplastics can induce DNA damage in *C. elegans* and suggest that similar genotoxic effects may occur in other organisms, including humans, highlighting a potential link between microplastic exposure and disease pathogenesis.

KEYWORDS: Biology, Cellular and Molecular Biology, DNA Damage, Microplastics, C. elegans.

Introduction

Double-stranded deoxyribonucleic acid (DNA) serves as the genetic blueprint that determines protein and cellular functions in most organisms.[1] Mutations in DNA can disrupt these functions and contribute to a wide range of diseases, including cancer, autism, and degenerative disorders.[2] While some genetic mutations are inherited, most disease-causing mutations are not passed down through familial lines.[3,4] These mutations could result from DNA damage induced by environmental stressors such as chemical exposure, radiation, and pollutants.[3-5] This study investigates the genotoxic potential of microplastics - widespread contaminants found in food and water, and the human body - and their possible contribution to the pathogenesis of cancer and other diseases.

Microplastics are defined as plastic particles less than 5 mm in diameter.[6,7] They are generated from the degradation of plastic waste or intentionally produced for use in paints and personal care products, such as toothpaste, sunscreen, and cosmetics.[8] Among the 50 or more chemically distinct types of plastics, polystyrene, polyethylene, and polypropylene are commonly used through mass production. Over the past century, global plastic production has increased dramatically and now exceeds 400 million tons per year.[9] This has led to the accumulation of a large amount of microplastics in environments.[10] Recent studies have detected microplastics in various ecosystems, including oceans, freshwater bodies, and the air.[11] Microplastics have also been found in food sources, drinking water, and even human breastmilk,[12] placentas,[13] blood,[14] lungs,[15] and brains,[16] raising concerns about their potential toxicity.[16-18] This study examines the ability of polystyrene microplastics, one of the most widely used and potentially toxic plastics,[19] to induce DNA damage in *C. elegans*.

C. elegans is a small nematode, approximately 1mm in length, commonly found in soil where it feeds on bacteria (Figure 1). The species is non-pathogenic and non-parasitic, making it a safe organism for laboratory research. *C. elegans*' unique combination of biological, genetic, and experimental advantages has established it as a widely used model organism.[20,21] The adult worm contains approximately 1,000 somatic cells, facilitating detailed studies of cellular processes. Its transparent body allows direct visualization of these processes by using microscopy. *C. elegans* completes its life cycle in about 3 days, with a total lifespan of only 2-3 weeks, enabling quick experimental turnover. The majority of *C. elegans* adults are hermaphrodites, possessing both male and female reproductive structures, but hermaphrodites cannot mate with each other. Meanwhile, as males can mate with hermaphrodites, a genetic cross can be easily performed in laboratories. The organism is easily maintained on agar plates using *Escherichia coli* OP50 as a food source, reducing both time and cost of experimentation.[20] The fully sequenced *C. elegans* genome consists of roughly 20,000 genes, many of which are conserved with human orthologs. In addition, genetic manipulation in *C. elegans* is straightforward: RNA interference (RNAi) can be performed simply by feeding worms bacteria expressing double-stranded RNA, and CRISPR-Cas9 and other genome-editing tools can be used to generate desired mutants or modify specific genes.[21] Many fundamental cellular and molecular pathways are conserved between *C. elegans* and humans, including programmed cell death, aging, and stress responses, making it a powerful model for studying human biology and disease.[22,23] The small size and

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ease of handling make *C. elegans* suited for high-throughput screening of potential therapeutics and genetic modifiers. As an invertebrate, *C. elegans* also raises fewer ethical concerns than vertebrate model organisms. Therefore, *C. elegans* offers a combination of biological simplicity, ease of genetic analysis, conservation of essential pathways, and cost-effectiveness. These qualities have made it an important model system of research in genetics, toxicology, developmental biology, and aging.[22-24]

The molecular mechanisms underlying the DNA damage response are conserved in *C. elegans*. The *cep-1* gene, the *C. elegans* ortholog of the human tumor suppressor *p53*, plays a central role in stress response and DNA damage repair, and can be activated in response to DNA damage in *C. elegans*.[25] Transgenic *C. elegans* that express GFP under the control of *cep-1* offers a simple, sensitive, and quantitative readout of DNA damage in a living organism.[25] Polyethylene microplastics have been shown to cause reproductive cell toxicity in *C. elegans*.[26] However, the effect of the potentially more toxic plastic, polystyrene, on DNA damage remains unknown.[19] This study leverages the transgenic *cep-1*::GFP *C. elegans*, which expresses GFP in response to DNA damage, to investigate whether polystyrene microplastic beads can induce DNA damage. Microplastics of two different sizes and two concentrations are tested. We choose 0.2 µm because it represents the smallest end of the microplastics spectrum, and 1 µm nears the upper limit of what may enter cells.[7,27] The two concentrations (1×10^7 particles/mL and 1×10^8 particles/mL) resemble the concentration of microplastics found in environmental surface water.[28] Findings from this study will reveal the potential role of polystyrene microplastics in contributing to DNA damage and genotoxicity.

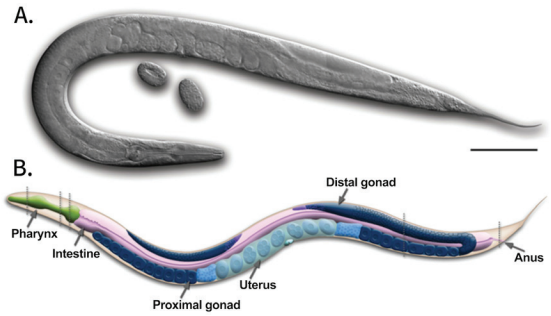

Figure 1: **(A)** Differential interference contrast (DIC) image of an adult hermaphrodite *C. elegans* (left lateral side) and two eggs. Scale bar 0.1 mm. **(B)** Schematic drawing of an adult hermaphrodite *C. elegans* anatomical structures, left lateral side. (Modified from WormAtlas.org)

Methods

Materials:

1. Polystyrene microplastics:

Polystyrene particles with diameters of 1 or 0.2 µm were obtained from Polysciences (#07310, #07304). Prior to use, microplastic beads were washed three times with M9 worm buffer by centrifuging (Eppendorf 5424) at 21,000 g for 5 minutes and discarding the supernatant. They were then suspended in M9 buffer as a 100x stock solution before being used to treat *C. elegans*.

2. C. elegans worms:

Wild-type N2 and transgenic *cep-1::GFP* (#TG12) worms were obtained from the *Caenorhabditis* Genetics Center (CGC) and maintained using standard protocols, feeding with *E. coli* OP50 on nematode growth medium plates in a 20°C incubator.[5]

3. M9 worm buffer and worm bleach:

M9 buffer preparation: (100mL)	Worm bleach preparation:
1.5 g KH_2PO_4	40 mL H_2O
3.0 g Na_2HPO_4	40 mL bleach (5% hypochlorite)
0.25 g NaCl	20 mL 5N KOH
0.5 g NH_4Cl	Cover bottle with aluminum foil and use within a month of making it.
Bring to 500 mL with H_2O.	

4. Nematode growth medium plates and E. coli OP50:

Nematode growth medium plates are prepared by mixing 3 g NaCl, 17 g agar, 2.5 g peptone, and 975 mL H_2O in a flask. After autoclaving, add 1 mL of 1 M $CaCl_2$, 1 mL of 5 mg/mL cholesterol in ethanol, 1 mL of 1 M $MgSO_4$, and 25 mL of 1 M KPO_4 buffer. Dispense the mixture into sterile petri dishes. *E. coli* OP50 is cultured in standard LB medium at 37°C and stored at 4°C for several months.

Experimental Design and Methods:

1. Cultivation and synchronization of C. elegans:

C. elegans were cultivated on nematode growth medium plates seeded with *E. coli* OP50 as a food source. The health and density of worm populations were routinely monitored throughout the experiment. Wild-type and transgenic strains were synchronized using bleach treatment to minimize variation arising from developmental stage differences. For synchronization, worms were exposed to a worm bleach solution for three minutes. Adult worms were lysed by the bleach, while worm eggs, which are resistant to bleach, remained intact. After five washes, eggs were collected and transferred to fresh nematode growth medium plates, generating a new, synchronous population. This process ensured uniform developmental stages among the worms used for subsequent experiments (Figure 2).

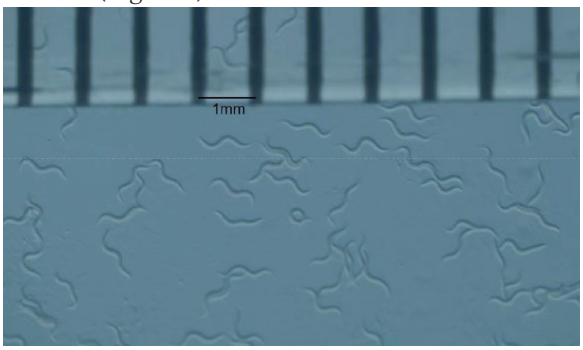

Figure 2: Synchronized wild-type N2 adult worms on a Nematode growth medium plate. Scale *bar 1mm*.

2. Microplastic Treatment:

Synchronized Day 1 adult worms were collected and transferred into 1.5 mL Eppendorf tubes containing M9 worm

buffer. Polystyrene microplastic beads of different sizes were added to the tubes to reach the desired concentration for each treatment group. Worms were incubated with polystyrene microplastic beads at 20°C for 24 hours.

The experiment consists of the following groups:

Group 1: Wild-type worms (no *GFP* transgene) not exposed to microplastics.

Group 2: Wild-type worms exposed to 0.2 µm (size) microplastics at 1 × 10^7 particles/mL.

Group 3: Transgenic *cep-1::GFP* worms not exposed to microplastics.

Group 4: Transgenic *cep-1::GFP* exposed to 0.2 µm (size) microplastics at 1 × 10^7 particles/mL.

Group 5: Transgenic *cep-1::GFP* exposed to 0.2 µm (size) microplastics at 1 × 10^8 particles/mL.

Group 6: Transgenic *cep-1::GFP* exposed to 1 µm (size) microplastics at 1 × 10^7 particles/mL.

3. Worm Mounting and Imaging:

Following experimental treatments, worms were washed three times with saline to remove residual microplastics and subsequently anesthetized using 10 mM (0.7%) sodium azide. The anesthetized worms were embedded in Aquamount mounting medium under coverslips on glass slides for microscopy. GFP expression was imaged using a Keyence BZ-X800 fluorescence microscope, maintaining consistent exposure settings for all samples to ensure reproducibility.

4. Utilize ImageJ to quantify fluorescence intensity:

Images were converted to 8-bit black and white mode using the free ImageJ software prior to analysis. To minimize background signals, a fixed threshold was applied consistently across all images. The rectangle tool was used to select the region containing each worm, and the total pixel intensity within the selected area was measured and recorded in arbitrary units as the GFP expression level. For each experimental group, fluorescence levels were quantified in at least fifteen worms or images, with analysis conducted in a blinded manner to reduce bias.

5. Statistical analysis:

Statistical analysis of GFP expression was conducted to assess the effects of polystyrene microplastic treatment. One-way ANOVA was performed to compare GFP expression levels among four groups: wild type, wild type with microplastics, *cep-1::GFP*, and *cep-1::GFP* with microplastics, with microplastic treatment serving as the independent variable. Statistical significance was determined based on group comparisons. Furthermore, Student's t-test was used to evaluate whether significant differences in GFP expression existed between *cep-1::GFP* worms treated with different concentrations of 0.2 µm polystyrene microplastic beads (Group 4 vs. Group 5) and between *cep-1::GFP* worms exposed to microplastic beads of varying sizes at the same concentration of 1 × 10^7 particles/mL (Group 4 vs. Group 6), considering microplastic concentration or size as the independent variables. The complete experimental procedure is illustrated in Figure 3.

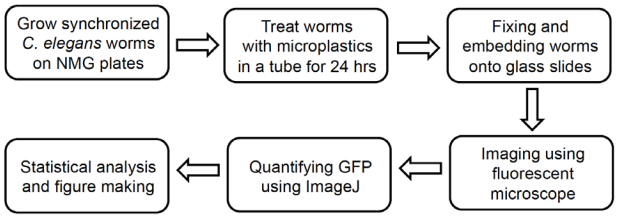

Figure 3: Flow chart for the experimental procedure.

Results and Discussion

The Effect of Microplastic Treatment:

Imaging and quantification of GFP expression revealed weak fluorescence in wild-type worms, both in untreated controls (Figure 4A, B) and in those exposed to 1 × 10^7 particles/mL 0.2 µm polystyrene microplastics (Figure 4C, D), showing background level fluorescence. Similarly, untreated *cep-1::GFP* transgenic worms displayed low GFP expression (Figure 4E, F), serving as a negative control. In contrast, a marked increase in GFP expression was observed in *cep-1::GFP* worms exposed to 1 × 10^7 particles/mL 0.2 µm microplastics (P<0.0001) (Figure 4G, H; Figure 5). These results indicate that microplastic exposure activates *cep-1* and DNA damage response pathways, demonstrating that 0.2 µm polystyrene microplastics at 1 × 10^7 particles/mL induce DNA damage in *C. elegans*.

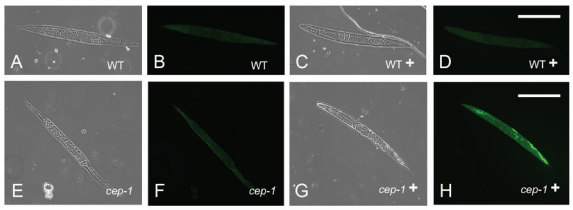

Figure 4: Microplastic treatment activates *cep-1* and DNA damage response pathways in *C. elegans*. **(A, B)** Bright field (A) and fluorescent (B) images of a wild type (WT) worm unexposed to microplastics, showing background level fluorescence. **(C, D)** WT worm exposed (+) to 0.2 µm polystyrene microplastics at 1 × 10^7 particles/mL, showing background level fluorescence. **(E, F)** Untreated *cep-1::GFP* transgenic worm showing baseline GFP expression. **(G, H)** *cep-1::GFP* transgenic worm exposed to 0.2 µm polystyrene microplastics at 1.66 x 10^{-14} M, showing strongly elevated GFP expression indicative of DNA damage response activation. Scale bar: 0.5 mm.

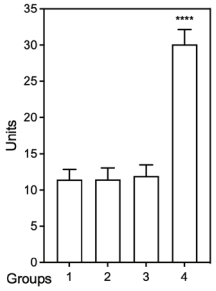

Figure 5: Quantification of GFP expression showing that polystyrene microplastic exposure induces robust GFP expression in *cep-1::GFP* transgenic worms (Group 4), but not in any other control groups. Group 1: WT worms unexposed to microplastics, 11.44 ± 1.40 Units, n = 20; Group 2: WT worms exposed to 0.2 µm polystyrene microplastics at 1 × 10^7 particles/mL, 11.45 ± 1.59 Units, n = 20; Group 3: *cep-1::GFP* worms unexposed to microplastics, 11.92 ± 1.54 Units, n = 20; Group 4: *cep-1::GFP* worms exposed to 0.2 µm microplastics at 1 × 10^7 particles/mL, 30.09 ± 2.05 Units, n = 20. Units: arbitrary units of GFP expression. Data are presented as mean ± SD. **** P<0.0001, one-way ANOVA with Tukey's HSD *post hoc* test was performed on Groups 1-4. Data are from four independent biological/experimental replicates.

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The Effects of Microplastic Concentration and Particle Size:

To evaluate the impact of microplastic concentration, *cep-1::GFP* transgenic worms were exposed to 0.2 μm microplastics at two concentrations: 1×10^7 particles/mL and 1×10^8 particles/mL. Both concentrations led to robust *cep-1* activation and GFP expression. Importantly, both concentrations produced a significant rise in GFP expression compared to the control. These findings indicate that the DNA damage response triggered by microplastic exposure operates in a threshold-dependent, binary manner; once this threshold is exceeded, increasing microplastic concentration does not further enhance the magnitude of the DNA damage response.

To examine the effect of microplastic size on DNA damage, *cep-1::GFP* transgenic worms were exposed to polystyrene microplastic beads of two sizes (0.2 μm or 1 μm; both at 1×10^7 particles/mL; Group 4 vs. Group 6). Both sizes induced robust cep-1 activation and GFP expression. However, worms exposed to smaller (0.2 μm) microplastic beads exhibited stronger GFP expression than those exposed to larger (1 μm) beads (P = 0.02). This is likely because smaller microplastic particles may be easier to enter *C. elegans* and its cells, or smaller particles have different surface chemistry or physics that make them more toxic.

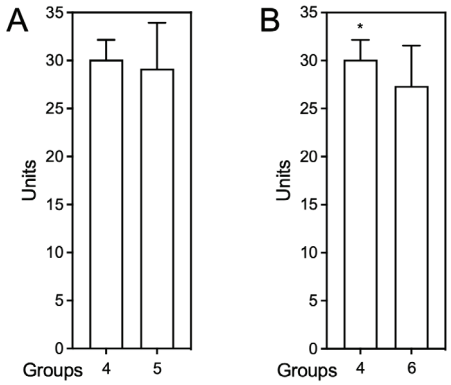

Figure 6: Both concentrations of microplastics induce robust *cep-1* activation and GFP expression, with smaller microplastics showing greater efficiency. **A.** Quantification of GFP expression showing no significant difference between two distinct concentrations of 0.2 μm polystyrene microplastics in inducing GFP expression in *cep-1::GFP* transgenic worms. Group 4: *cep-1::GFP* worms exposed to 0.2 μm polystyrene microplastic beads at 1×10^7 particles/mL, 30.09 ± 2.05 Units, n = 20; Group 5: *cep-1::GFP* worms exposed to 0.2 μm microplastics at 1×10^8 particles/mL, 29.17 ± 4.78 Units, n = 15. Units: arbitrary units of GFP expression quantified by Image J. Data are presented as mean ± SD. P = 0.44, Student's t-test. **B.** Quantification showing stronger GFP expression in *cep-1::GFP* worms exposed to 0.2 μm (size) microplastic beads than worms exposed to 1 μm microplastic beads, both at 1×10^7 particles/mL concentration. Group 4: *cep-1::GFP* worms exposed to 0.2 μm microplastics at 1×10^7 particles/mL, 30.09 ± 2.05 Units, n = 20; Group 6: *cep-1::GFP* worms exposed to 1 μm microplastics at 1×10^7 particles/mL, 27.36 ± 4.19 Units, n = 15. Data are presented as mean ± SD. * P=0.02, Student's t-test. Data are from four independent biological/experimental replicates.

Discussions:

This study examined polystyrene microplastics of two different sizes (0.2 μm or 1 μm) and at two concentrations (1×10^7 particles/mL and 1×10^8 particles/mL). These particle sizes were chosen because 0.2 μm is close to the smallest end of the microplastics spectrum, bordering on nanoplastics, and 1 μm is near the upper limit of what may enter cells.[7,27] The two concentrations were tested because they resemble the concentration of microplastics found in environmental surface water.[28] Findings from this study reveal no significant difference between the two concentrations of polystyrene microplastics in their abilities to induce DNA damage in *C. elegans* (Figure 6A). This suggests that the relationship between DNA damage and microplastic concentration is not linear – once exposure exceeds the threshold required to elicit a damage response, increasing concentration does not proportionally increase the severity of DNA damage. This study also showed that smaller microplastic beads (0.2 μm) can induce a stronger DNA damage response in *C. elegans* than bigger microplastic beads (1 μm) (Figure 6B), likely because smaller microplastic particles are easier to get into *C. elegans* and their cells to induce DNA damage.

Future directions:

This study measured DNA damage indirectly using the transgenic *cep-1::GFP C. elegans*, which expresses GFP in response to DNA damage.[27] To directly quantify DNA damage at the single-cell level, future studies could employ the comet assay, which visualizes and quantifies DNA fragmentation and damage.[29] In addition, the long-term effects of microplastic exposure on DNA integrity and health outcomes in *C. elegans* beyond the 24-hour window used in this study warrant further investigation. Future studies could also extend this work to microplastics derived from other widely used plastic polymers, such as polyethylene and polypropylene. Finally, to assess the relevance of these findings to human health, human cell lines could be used to investigate whether microplastics induce DNA damage in a mammalian system.

Underlying mechanisms:

How do microplastics cause DNA damage? Microplastics may enter cells through endocytosis or engulfment. The accumulation of microplastics in cells can lead to DNA damage through several potential mechanisms. First, microplastics can trigger oxidative stress within cells by stimulating the excessive generation of reactive oxygen species (ROS) in response to foreign materials (microplastics). ROS can damage a wide range of cellular components, including DNA, leading to mutations or strand breaks.[30,31] Second, microplastics can initiate inflammatory responses. Inflammatory cells produce ROS and other reactive molecules that can cause DNA damage in surrounding cells. Third, microplastics can disrupt cellular membranes, leading to cytotoxicity. This disruption can lead to the release of cellular contents or the entry of harmful substances, which can cause direct DNA damage or interfere with DNA repair processes. Finally, microplastics might interfere with the cellular machinery responsible for DNA replication, potentially causing replication errors, chromosomal aberrations, or double-stranded breaks. Collectively, the mechanisms by which microplastics induce DNA damage are likely multifactorial and may vary depending on particle size, chemical composition, concentration, exposure duration, and cellular context.

Further research is needed to fully elucidate these underlying mechanisms and their relative contributions to genotoxicity.

Conclusion

This study investigates the effects of polystyrene microplastics on causing DNA damage response in the multicellular model organism *C. elegans*. We show that polystyrene microplastics induce a strong DNA damage response in *cep-1::GFP* transgenic *C. elegans*, suggesting potentially similar mechanisms may operate in other multicellular organisms, including humans. Given that DNA damage underlies the genetic mutations that drive cancer and other diseases, these preliminary findings in a model organism suggest a potential link between microplastic exposure and disease pathogenesis and open new avenues for future investigation.

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