Chapter 3
Exposure Routes of Nanomaterials

Abstract The well-described developmental background and the sensitivity to environmental toxicants of *Caenorhabditis elegans* is helpful for designing suitable exposure routes for different engineered nanomaterials (ENMs) with different research aims. We here introduced different exposure routes (acute exposure, prolonged exposure, chronic exposure, one-generation exposure, and transgenerational exposure) for the toxicity assessment of ENMs in nematodes. Moreover, we discussed the value of some exposure routes, such as prolonged exposure and chronic exposure, in detecting the possible toxicity of ENMs at environmentally relevant concentrations on nematodes.

Keywords Exposure route · Toxicity assessment · Environmentally relevant concentration · *Caenorhabditis elegans*

3.1 Introduction

Selection of suitable exposure routes is very important for toxicity assessment of engineered nanomaterials (ENMs) with different research aims. The well-described developmental background and the sensitivity to different environmental toxicants, including the ENMs, of model animal of *Caenorhabditis elegans* are helpful for our designing different exposure routes so as to satisfy different research aims [1–4]. Most of the designed exposure routes belong to the toxicity assessment of ENMs in one generation in nematodes. Some certain exposure routes have also been raised to apply for the toxicity assessment of ENMs transgenerationally in nematodes.

In this chapter, we introduced several different exposure routes of ENMs in nematodes, and these exposure routes contain acute exposure, prolonged exposure, chronic exposure, one-generation exposure, and transgenerational exposure. Besides this, we also introduced and discussed the toxicity assessment of ENMs at environmentally relevant concentrations in nematodes. The information provided in this chapter will provide cues for design suitable exposure routes for different ENMs with certain research aims in nematodes.

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3.2 Exposure Routes of ENMs

3.2.1 Acute Exposure

Normally, the acute exposure to environmental toxicants or stresses was performed from L4-larvae stage or young adults for 24 h [5, 6]. For the acute exposure to ENMs from L4-larvae for 24 h, the addition of *E. coli* OP50 as the food source is suggested. With the concern of certain research aims, the acute exposure to ENMs could also be designed from other larval stages, such L1-, L2-, or L3-larvae in the presence of OP50 as the food source. Besides these, with the concern of certain research aims, the acute exposure to ENMs could further be designed from the L4-larvae or the young adults for not more than 24 h. Acute exposure is used to assess the short-term effect of ENMs on nematodes.

With the DMSA coated Fe_2O_3-nanoparticle (DMSA coated Fe_2O_3-NPs) as an example, the possible toxicity of acute exposure to DMSA coated Fe_2O_3-NPs from the L4-larvae for 24 h was investigated in nematodes. After acute exposure, all the examined concentrations (0.5–100 mg/L) of DMSA coated Fe_2O_3-NPs did not affect the survival of nematodes (Fig. 3.1) [5]. After acute exposure, only DMSA coated Fe_2O_3-NPs at the concentration of 100 mg/L could significantly decrease the body length, reduce the brood size, suppress the pumping rate, increase the mean defecation cycle length, and induce the intestinal autofluorescence (Fig. 3.1) [5]. In contrast, after acute exposure, DMSA coated Fe_2O_3-NPs at concentrations more than 50 mg/L could significantly decrease the locomotion behavior as reflected by the endpoints of head thrash and body bend (Fig. 3.1) [5]. These results suggest that acute exposure to DMSA coated Fe_2O_3-NPs at concentrations more than 50 mg/L may have adverse effects on nematodes.

3.2.2 Prolonged Exposure

In nematodes, prolonged exposure to environmental toxicants or stresses was normally performed from L1-larvae to young adults or adult day-1 in the presence of OP50 as the food source [5, 7, 8,]. The prolonged exposure is an exposure route used to assess the long-term effect of ENMs on nematodes.

With the DMSA coated Fe_2O_3-NPs as an example, the possible toxicity of prolonged exposure to DMSA coated Fe_2O_3-NPs from the L1-larvae to adult day-1 was also investigated in nematodes. After prolonged exposure, all the examined concentrations (1–5000 μg/L) of DMSA coated Fe_2O_3-NPs also did not affect the survival of nematodes (Fig. 3.2) [5]. After prolonged exposure, only DMSA coated Fe_2O_3-NPs at the concentration of 5000 μg/L significantly decreased the body length, suppressed the pumping rate, increased the mean defecation cycle length, and induced the intestinal autofluorescence (Fig. 3.2) [5]. In contrast, after prolonged exposure, DMSA coated Fe_2O_3-NPs at concentrations more than 500 μg/L significantly

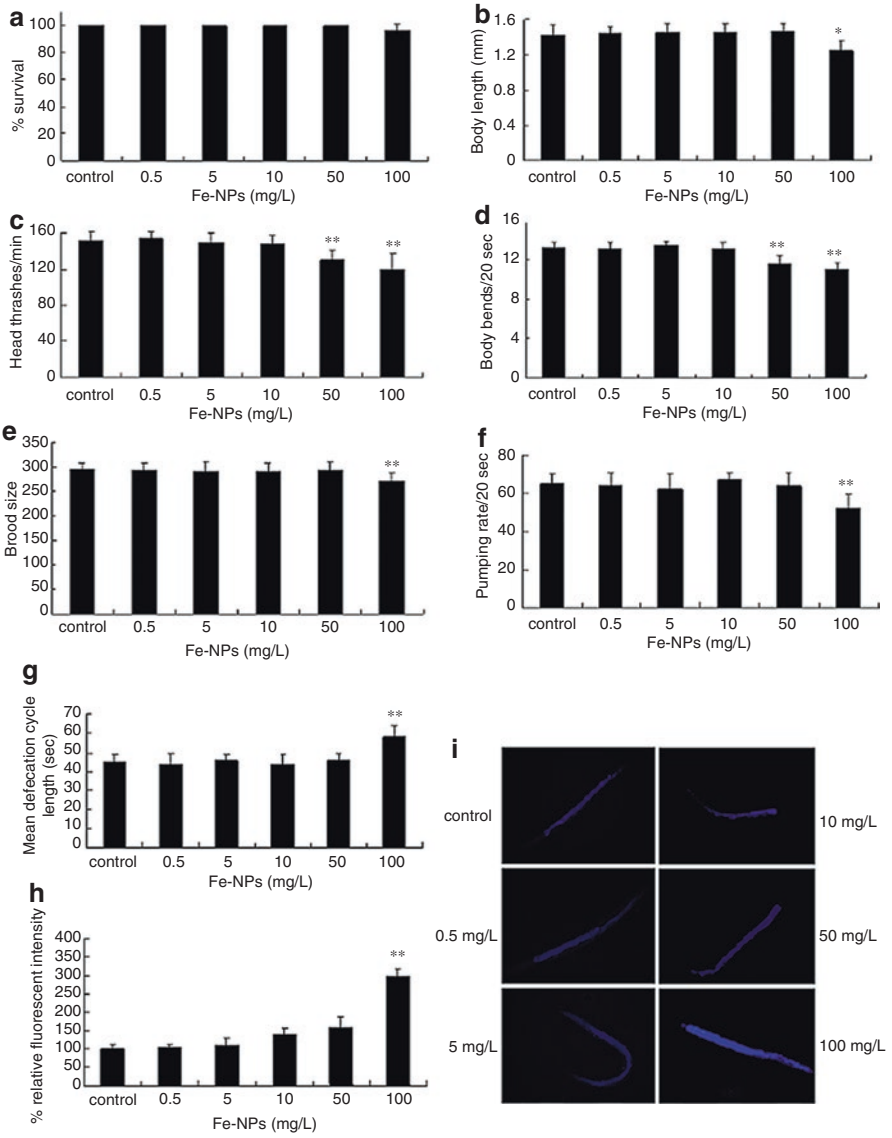

Fig. 3.1 Toxicity assessment of DMSA coated Fe_2O_3-NPs after acute exposure from L4-larvae for 24 h [5]

(**a**) Comparison of lethality in nematodes exposed to different concentrations of DMSA coated Fe_2O_3-NPs. (**b**) Comparison of body length in nematodes exposed to different concentrations of DMSA coated Fe_2O_3-NPs. (**c**) Comparison of head thrash in nematodes exposed to different concentrations of DMSA coated Fe_2O_3-NPs. (**d**) Comparison of body bend in nematodes exposed to different concentrations of DMSA coated Fe_2O_3-NPs. (**e**) Comparison of brood size in nematodes exposed to different concentrations of Fe2O3-nanoparticles. (**f**) Comparison of pumping rate in nematodes exposed to different concentrations of DMSA coated Fe_2O_3-NPs. (**g**) Comparison of mean defecation cycle length in nematodes exposed to different concentrations of DMSA coated Fe_2O_3-NPs. (**h**) Comparison of intestinal autofluorescence in nematodes exposed to different concentrations of DMSA coated Fe_2O_3-NPs. (**i**) Pictures showing the intestinal autofluorescence in nematodes exposed to different concentrations of DMSA coated Fe_2O_3-NPs. Bars represent mean ± SEM. $^*p < 0.05$, $^{**}p < 0.01$

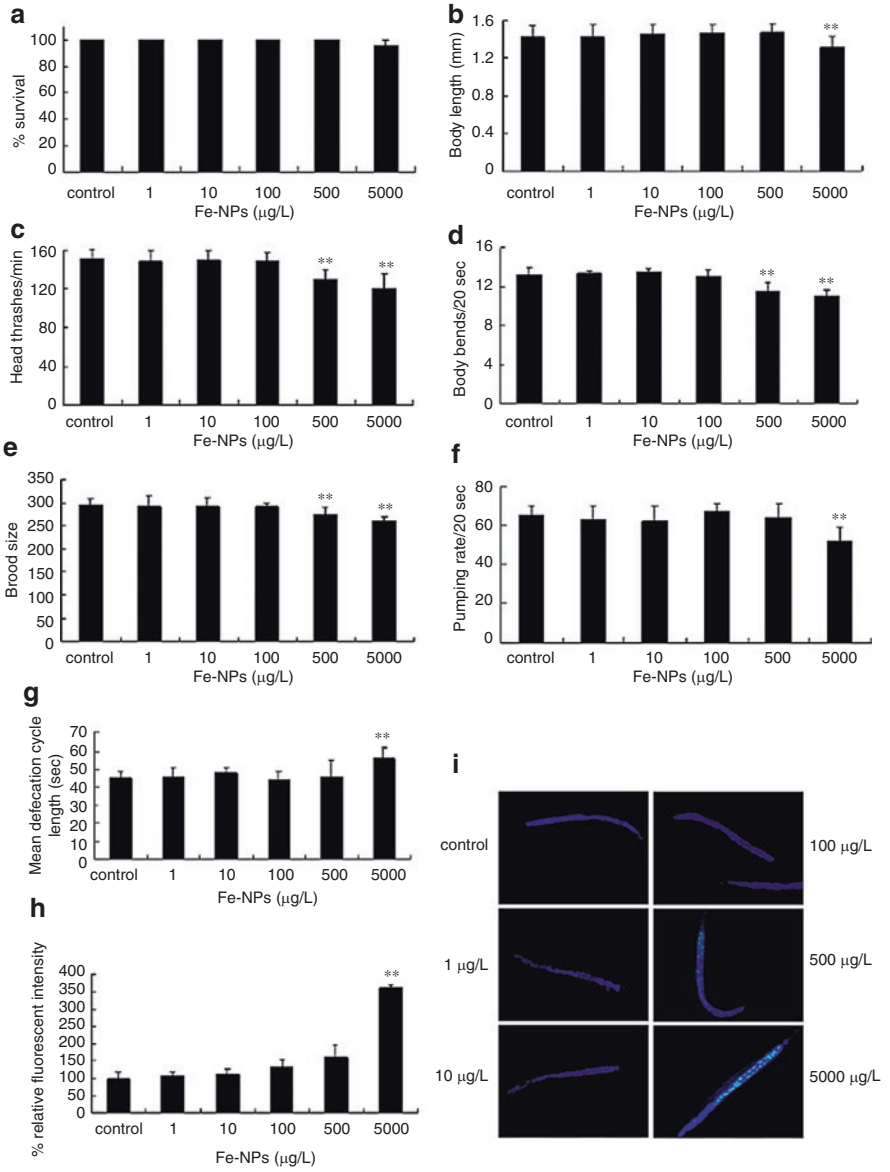

Fig. 3.2 Toxicity assessment of DMSA coated Fe_2O_3-NPs after prolonged exposure from L1-larvae to adult day-1 [5]

(**a**) Comparison of lethality in nematodes exposed to different concentrations of DMSA coated Fe_2O_3-NPs. (**b**) Comparison of body length in nematodes exposed to different concentrations of DMSA coated Fe_2O_3-NPs. (**c**) Comparison of head thrash in nematodes exposed to different concentrations of DMSA coated Fe_2O_3-NPs. (**d**) Comparison of body bend in nematodes exposed to different concentrations of DMSA coated Fe_2O_3-NPs. (**e**) Comparison of brood size in nematodes exposed to different concentrations of DMSA coated Fe_2O_3-NPs. (**f**) Comparison of pumping rate in nematodes exposed to different concentrations of DMSA coated Fe_2O_3-NPs. (**g**) Comparison of mean defecation cycle length in nematodes exposed to different concentrations of DMSA coated Fe_2O_3-NPs. (**h**) Comparison of intestinal autofluorescence in nematodes exposed to different concentrations of DMSA coated Fe_2O_3-NPs. (**i**) Pictures showing the intestinal autofluorescence in nematodes exposed to different concentrations of DMSA coated Fe_2O_3-NPs. Bars represent mean ± SEM. $*p < 0.05$, $**p < 0.01$

decreased the locomotion behavior and reduced the brood size (Fig. 3.2) [5]. Therefore, prolonged exposure to DMSA coated Fe_2O_3-NPs at concentrations more than 500 μg/L may have adverse effects on nematodes.

3.2.3 Chronic Exposure

Chronic exposure to environmental toxicants or stresses can be performed from young adults for 10 days or from adult day-1 to adult day-8 in the presence of OP50 as the food source in nematodes [9, 10]. In nematodes, chronic exposure to environmental toxicants, such as the ENMs, can be further performed from L1-larvae to adult day-8 in the presence of OP50 as the food source [5, 9]. Besides the prolonged exposure, chronic exposure is another usually used exposure route to assess the long-term effect of ENMs on nematodes.

With the DMSA coated Fe_2O_3-NPs as an example, the possible toxicity of chronic exposure to DMSA coated Fe_2O_3-NPs from the L1-larvae to adult day-8 was further investigated in nematodes. After chronic exposure, only DMSA coated Fe_2O_3-NPs at the concentration of 5000 μg/L significantly decreased the survival of nematodes (Fig. 3.3) [5]. In contrast, after chronic exposure, DMSA coated Fe_2O_3-NPs at concentrations more than 500 μg/L could significantly decrease the body length, suppress the pumping rate, and increase the mean defecation cycle length (Fig. 3.3) [5]. Moreover, after chronic exposure, DMSA coated Fe_2O_3-NPs at concentrations more than 100 μg/L significantly decreased the locomotion behavior, reduced the brood size, and induced the intestinal autofluorescence (Fig. 3.3) [5]. Therefore, chronic exposure to DMSA coated Fe_2O_3-NPs at concentrations more than 100 μg/L may have adverse effects on nematodes.

3.2.4 One-Generation Exposure

In nematodes, in order to determine the long-term effects of ENMs or their possible hormesis effects, one-generation exposure can also be designed from L1-larvae till the end of the examined generation in the presence of OP50 as the food source.

With graphene oxide (GO) as an example, the effect of one-generation exposure to GO at different concentrations on lifespan was investigated in nematodes. After one-generation exposure, GO at concentrations of 5–20 mg/L did not significantly alter the lifespan of nematodes (Fig. 3.4) [12], suggesting that the GO at the examined concentrations may exert no adverse effects on the aging process of nematodes under normal conditions.

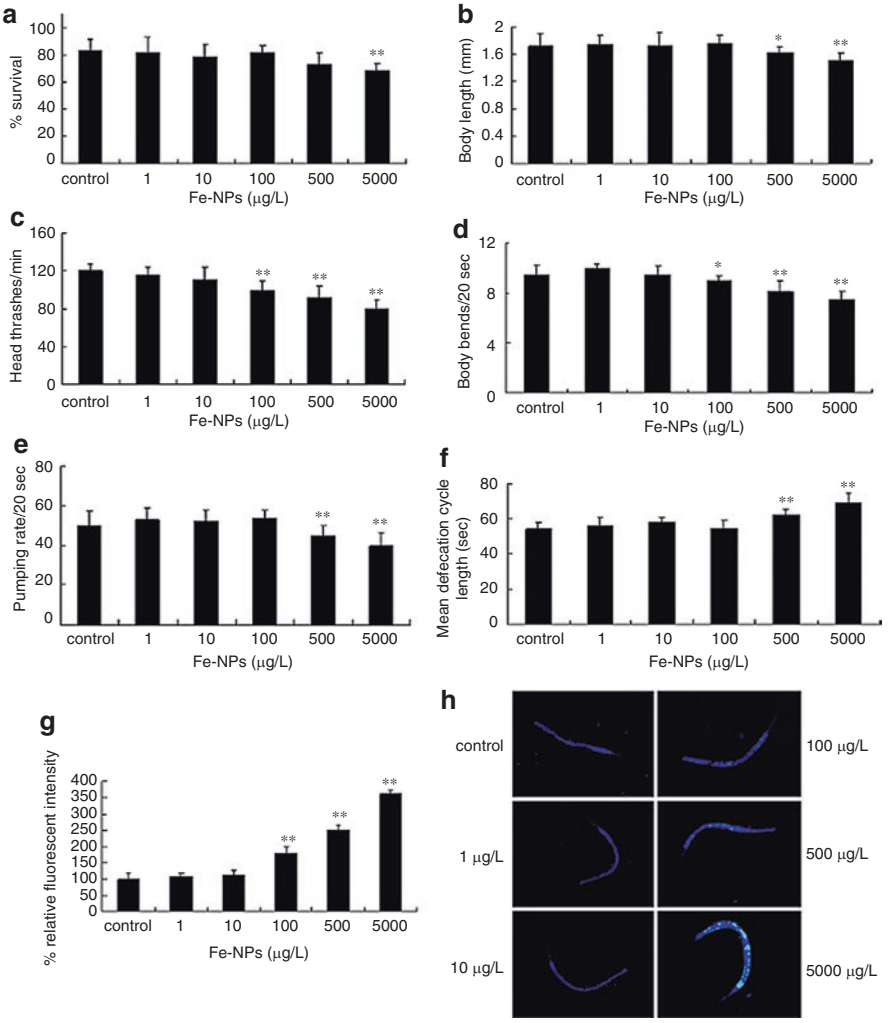

Fig. 3.3 Toxicity assessment of DMSA coated Fe_2O_3-NPs after chronic exposure from L1-larvae to adult day-8 [5]

(**a**) Comparison of lethality in nematodes exposed to different concentrations of DMSA coated Fe_2O_3-NPs. (**b**) Comparison of body length in nematodes exposed to different concentrations of DMSA coated Fe_2O_3-NPs. (**c**) Comparison of head thrash in nematodes exposed to different concentrations of DMSA coated Fe_2O_3-NPs. (**d**) Comparison of body bend in nematodes exposed to different concentrations of DMSA coated Fe_2O_3-NPs. (**e**) Comparison of pumping rate in nematodes exposed to different concentrations of DMSA coated Fe_2O_3-NPs. (**f**) Comparison of mean defecation cycle length in nematodes exposed to different concentrations of DMSA coated Fe_2O_3-NPs. (**g**) Comparison of intestinal autofluorescence in nematodes exposed to different concentrations of DMSA coated Fe_2O_3-NPs. (**h**) Pictures showing the intestinal autofluorescence in nematodes exposed to different concentrations of DMSA coated Fe_2O_3-NPs. Bars represent mean ± SEM. $*p < 0.05$, $**p < 0.01$

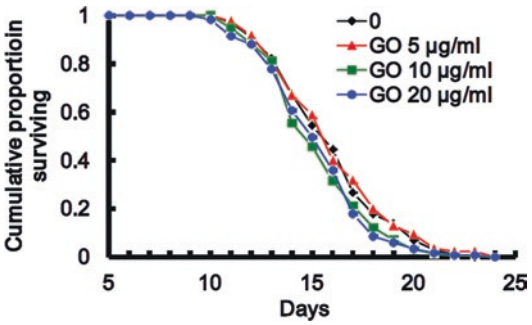

Fig. 3.4 Effect of one-generation exposure to GO on lifespan of nematodes [12]

3.2.5 Transgenerational Exposure

To examine the transgenerational effect of certain ENMs on nematodes, the exposure is usually performed in the first generation in the presence of OP50 as the food source, and then the nematodes are transferred to normal medium without the addition of ENMs to obtain the progeny of exposed nematodes after the exposure. In the F1 generation, the transgenerational effects of certain ENMs on progeny of exposed nematodes would be examined.

With nitrogen-doped graphene quantum dots (N-GQDs) as an example, the transgenerational effect of N-GQDs was investigated in nematodes after the prolonged exposure from L1-larvae to adult day-1 in the first generation. In the first generation, N-GQDs at concentrations of 0.1–100 mg/L could not significantly affect the lifespan, brood size, and locomotion behavior in nematodes [13]. Additionally, N-GQDs at concentrations of 0.1–100 mg/L could not induce the significant intestinal ROS production in the first generation in nematodes [13]. Moreover, in the second generation, the normal lifespan, brood size, and locomotion behavior were observed, and no significant intestinal ROS production was detected (Fig. 3.5) [13], indicating that no obvious transgenerational toxicity of N-GQDs will be formed in nematodes. Different from the transgenerational effects of N-GQDs, prolonged exposure to nanopolystyrene particles at concentrations more than 10 μg/L significantly decreased the locomotion behavior and reduced the brood size and induced the significant induction of intestinal ROS production in the first generation in nematodes [14]. Moreover, nanopolystyrene particles at concentrations more than 100 μg/L induced the obvious transgenerational toxicity as reflected by the decreased locomotion behavior, the reduced brood size, and the induced significant induction of intestinal ROS production in the second generation in nematodes [14], demonstrating the formation of transgenerational toxicity of nanopolystyrene particles in nematodes.

In nematodes, with the concern of certain research aims, the exposure can be further performed in multiple generations, such as up to six generations [11]. With the $Gd@C_{82}(OH)_{22}$ nanoparticles as an example, $Gd@C_{82}(OH)_{22}$ at concentrations of 0.01–10 mg/L did not obviously affect the lifespan of nematodes after successive exposure to $Gd@C_{82}(OH)_{22}$ for six generations under the normal conditions [11].

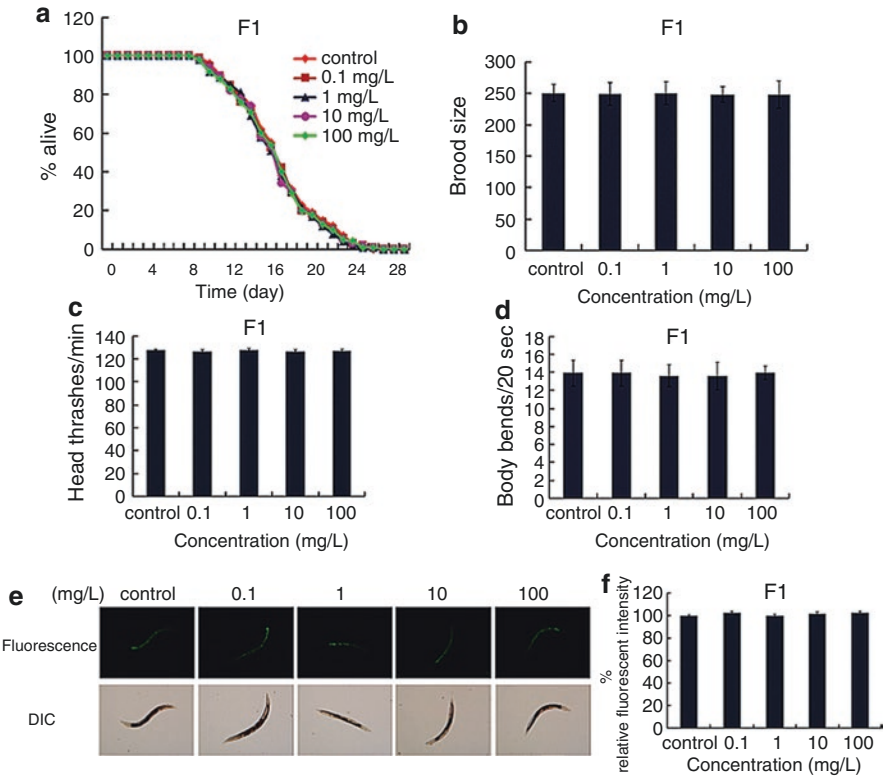

Fig. 3.5 Phenotypic analysis in progeny (F1 generation) of nematodes exposed to N-GQDs [13] (**a**) Lifespan in progeny of nematodes exposed to N-GQDs. (**b**) Brood size in progeny of nematodes exposed to N-GQDs. (**c**) Locomotion behavior in progeny of nematodes exposed to N-GQDs. (**d**) Intestinal ROS production in progeny of nematodes exposed to N-GQDs. Prolonged exposure was performed from L1-larvae to adult day-1. Bars represent means ± SEM

Moreover, under the heat-shock stress, $Gd@C_{82}(OH)_{22}$ at concentrations of 0.01–10 mg/L also could not noticeably alter the lifespan of nematodes after successive exposure to $Gd@C_{82}(OH)_{22}$ for six generations [11].

3.3 Toxicity Assessment of ENMs at Environmentally Relevant Concentrations

The concentrations of ENMs in the real environment are usually in the range of μg/L or ng/L [15–17]. Due to the sensitivity of nematodes to environmental toxicants, several exposure routes have been used to assess the potential toxicity of ENMs at environmentally relevant concentrations [18–22].

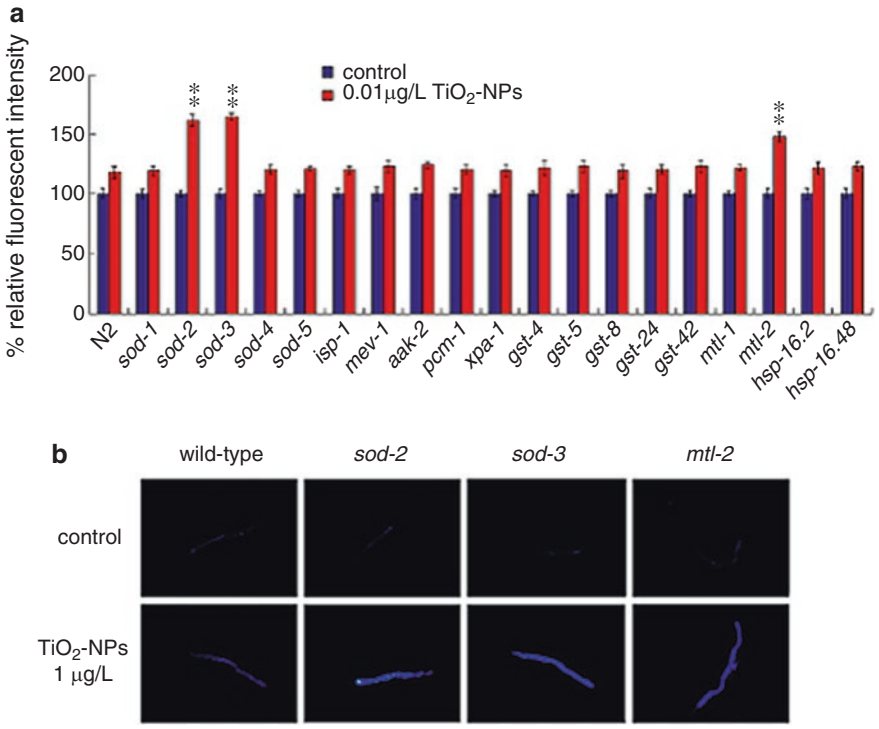

Fig. 3.6 Comparison of intestinal autofluorescence between wild-type and mutants exposed to 1 μg/L of TiO_2-NPs [23]
(**a**) Comparison of intestinal autofluorescence between wild-type and mutant nematodes exposed to TiO_2-NPs. (**b**) Pictures showing the intestinal autofluorescence in wild-type and mutant nematodes. Bars represent means ± SEM. $^{**}P < 0.01$

With the TiO_2-NPs as an example, prolonged exposure from L1-larvae to adult day-1 was employed to determine the long-term effects of TiO_2-NPs at environmentally relevant concentrations on nematodes. After prolonged exposure, TiO_2-NPs at the concentration of 0.01 μg/L could cause the significant reduction in brood size, decrease in locomotion behavior, and induction of intestinal autofluorescence in nematodes (Fig. 3.6) [23]. Using intestinal autofluorescence as the toxicity assessment endpoint, the toxicity of TiO_2-NPs at environmentally relevant concentrations could be even enhanced by *sod-2*, *sod-3*, or *mtl-2* mutation in nematodes (Fig. 3.6) [23].

Further with the TiO_2-NPs as an example, chronic exposure from adult day-1 to adult day-8 was also employed to determine the long-term effects of TiO_2-NPs at environmentally relevant concentrations on nematodes. Using the locomotion behavior as the toxicity assessment endpoint, TiO_2-NPs (4 and 10 nm) at concentrations more than 0.01 μg/L could already significantly decrease the locomotion behavior of nematodes after chronic exposure (Fig. 3.7) [24]. In contrast, after acute

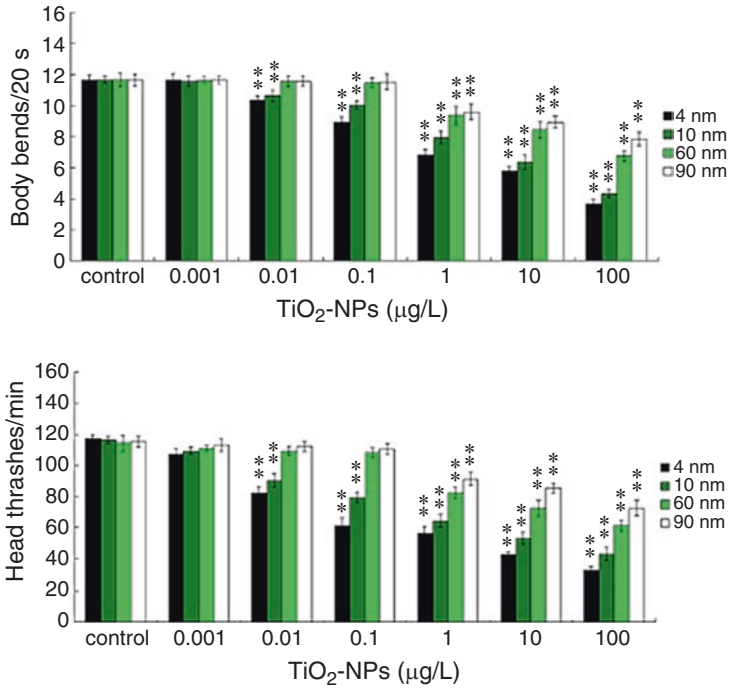

Fig. 3.7 Comparison of locomotion behavior in nematodes chronically exposed to different sizes of TiO_2-NPs [24]
Bars represent means ± SEM. $^{**}P < 0.01$

exposure from adult day-1 for 24 h, only TiO_2-NPs (4 and 10 nm) at concentrations more than 1 mg/L could significantly decrease the locomotion behavior of nematodes [24].

3.4 Perspectives

We here introduced some useful exposure routes for toxicity assessment of ENMs in nematodes, including acute exposure, prolonged exposure, chronic exposure, one-generation exposure, and transgenerational exposure. Nevertheless, with the concerns on certain research aims, the further modifications of these raised exposure routes are still needed. Additionally, new exposure routes are also welcomed to be further designed in order to satisfy new research aims in nematodes.

In this chapter, we only discussed the value of prolonged exposure and chronic exposure for the toxicity assessment of ENMs at environmentally relevant concentrations in nematodes. Besides these, the possible value of one-generation exposure and multiple-generation exposure for the toxicity assessment of ENMs at

environmentally relevant concentrations is also needed to be carefully examined and judged in nematodes. For the use of one-generation exposure or multiple-generation exposure in assessing the possible toxicity of ENMs at environmentally relevant concentrations, both the hormesis effect and the transgenerational effect of ENMs should be carefully considered in nematodes.

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