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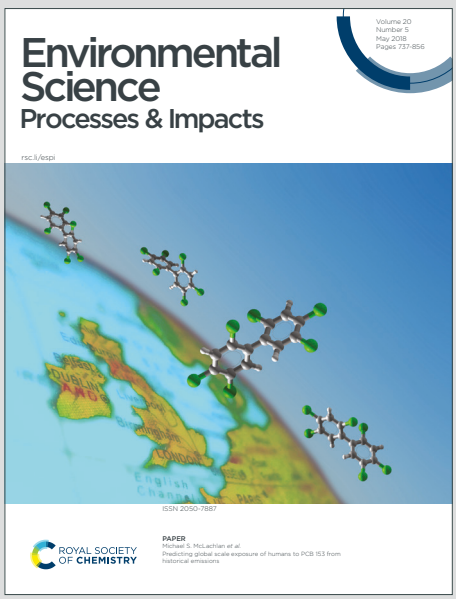

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Environmentally relevant concentrations of 6-PPDQ disrupts vitamin D3 adsorption and receptor function in *Caenorhabditis elegans*

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Abstract

As derivative of antioxidant of *N*-(1,3-dimethylbutyl)-*N'*-phenyl-*p*-phenylenediamine (6-PPD), 6-PPD quinone (6-PPDQ) is frequently found in environments and body of organisms. Vitamin D3 is an important vitamin needing the adsorption from intestine. In *Caenorhabditis elegans*, vitamin D3 content was reduced by 0.1-10 μg/L 6-PPDQ. Meanwhile, 6-PPDQ (0.1-10 μg/L) decreased expressions of *lrp-2*, *scl-12*, *scl-13*, and *ifo-1*, and their RNAi decreased vitamin D3 content in 6-PPDQ exposed animals. 6-PPDQ (0.1-10 μg/L) further decreased *nhr-8* expression and increased *daf-12* expression, and expression of these 2 vitamin D3 receptor genes could be changed by RNAi of *lrp-2*, *scl-12*, *scl-13*, and *ifo-1* after 6-PPDQ exposure. The 6-PPDQ toxicity was increased by RNAi of *lrp-2*, *scl-12*, *scl-13*, *ifo-1*, and *nhr-8* and inhibited by *daf-12* RNAi. Moreover, after 6-PPDQ exposure, SOD-3 and HSP-6 expressions were decreased by *nhr-8* RNAi and increased by *daf-12* RNAi. Therefore, 6-PPDQ potentially caused damage on adsorption of vitamin D3 and function of its receptors, and these effects were related to toxicity induction of 6-PPDQ.

Keywords: vitamin D3 adsorption, receptor function, 6-PPDQ, nematode

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Environmental Significance Statement:

6-PPD quinone (6-PPDQ) can be found in different environments and body of organisms, and further results in toxicity on organism including disruption in metabolisms. Vitamin D is essential for organisms and needs to be obtained from diet sources. In *Caenorhabditis elegans*, we observed reduction in vitamin D3 contents by 6-PPDQ at environmentally relevant concentrations (0.1-10 μg/L). This reduction was related to inhibition in adsorption of vitamin D3 and decrease in expression of vitamin D3 transporter genes. Additionally, 6-PPDQ altered expression of *nhr-8* and *daf-12*, two vitamin D3 receptor genes, and these receptors genes had opposite functions to control 6-PPDQ toxicity by differentially affecting responses of Mn-SOD and mitochondrial unfolded protein response (mt UPR).

1. Introduction

In rubber products, *N*-(1,3-dimethylbutyl)-*N'*-phenyl-*p*-phenylenediamine (6-PPD) is a widely used antioxidant with the aim for effectively delaying material aging and enhancing durability.[1] After release from the tires, 6-PPD can react with ozone to generate 6-PPD quinone (6-PPDQ) through several pathways.[2] Among members of tire wear particles (TWP)-related pollutants, 6-PPDQ initially receives attention due to its cause for acute lethality in coho salmon.[3] PPDQ is widely distributed in environmental media, including urban runoff,[4] rivers,[5] soil,[6] and road dust,[6] which was closely linked to the traffic density. Environmentally relevant concentrations (ERCs) for 6-PPDQ ranged from ng/L to tens of μg/L.[7] 6-PPDQ was further found in some human related biological samples including blood and urine,[8] suggesting its potential exposure risk to the human health. Accompanied with environmental distribution, 6-PPDQ resulted in toxicity on environmental organisms, such as fishes.[9] In mammals, along with organ distribution, 6-PPDQ caused multiple organ injury, including damage on liver and lung.[10-11]

Besides having typical properties of model animal, *Caenorhabditis elegans* is highly sensitive to pollutants.[12-13] Largely because of this, pollutants toxicity at ERCs was frequently observed in nematodes.[14-15] After exposure at ERCs, 6-PPDQ led to intestinal oxidative

damage,[16] suggesting damage on intestine, the primary target organ of pollutants.[17] Besides toxicity on intestine, 6-PPDQ also caused neurotoxicity (such as inhibited locomotion)[18] and reproductive toxicity (such as decreased brood size)[19-20]. In cells, 6-PPDQ further induced mitochondrial dysfunction,[21] which was related to damage on at least mitochondrial complex I-III.[22] In nematodes, recently, we further observed disruption in some metabolisms (such as glucose and glycogen metabolisms) by 6-PPDQ exposure.[23-26]

Vitamin D (such as vitamin D3) is an essential vitamin needing to be obtained from diet source in intestine.[27] Vitamin D deficiency would cause a subset of diseases and further lead to health consequence in the clinical,[28] and this deficiency has become a worldwide question.[29] Pharmacological analysis indicated the role of vitamin D3 in modulating stress response.[30] Thus, we asked whether 6-PPDQ at ERCs could affect vitamin D3 adsorption and function of its receptors in organisms. Considering conserved processes of biochemical metabolisms, *C. elegans* is helpful to elucidate molecular basis of metabolic processes.[31] In the current study, in this model, we first examined possible effect of 6-PPDQ on vitamin D3 adsorption. Moreover, we determined effect of 6-PPDQ on function of vitamin D3 receptors and the association with toxicity induction and underlying mechanism. Three *C. elegans* receptors (NHR-48, NHR-8, and DAF-12) act as orthologs of human vitamin D receptor.[30] Our results highlighted the damage on vitamin adsorption and function of receptors by 6-PPDQ at ERCs, and this was associated with toxicity formation by suppressing certain protective responses, such as Mn-SOD and mitochondrial unfolded protein response (mt UPR).

2. Materials and methods

2.1. Reagents

To prepare a stock solution of 6-PPDQ (1 g/L), 1 mg 6-PPDQ powder (Toronto Chemical Research Co.) was dissolved in 1 mL dimethyl sulfoxide (DMSO). Selection of 6-PPDQ working solutions (0.1, 1, and 10 μg/L) were based on reported ERCs of 6-PPDQ,[7] and they were prepared after dilution of stock solution by K buffer. Vitamin D3 was

purchased from Sangon Biotech. (Shanghai) Co. Working concentration (400 μM) of vitamin D3 was selected as described.[30]

2.2. *C. elegans* maintenance

C. elegans were cultured on nematode growth medium (NGM) plates fed with *E. coli* OP50.[32] Used wild-type strain is N2. To prepare L1-larval population, gravid nematodes were treated by alkaline hypochlorite lysis solution (0.45 M NaOH and 2 % HOCl) to release the eggs.[33] The obtained eggs were transferred to another NGM plate to develop into synchronized L1 larvae.

2.3. Exposure

As previously described, we assessed toxicity of 6-PPDQ by continuously exposing synchronized L1 larvae to 6-PPDQ till to adult day-3 (approximately for 6.5 days).[34] Exposure solution was replaced daily to maintain a consistent concentration of chemical. OP50 was meanwhile added in exposure solutions to satisfy the need of larval development.

2.4. Vitamin D3 content

Vitamin D3 test kit (Sangon Biotech. Co.) was used to quantify vitamin D3 content. *C. elegans* were weighted and homogenized in a tissue crusher. After centrifugation at 5000g for 10 minutes, supernatant was collected for measurement of absorbance at 450 nm. A standard curve was generated, and the vitamin D3 content in each group was calculated. Experiments were repeated three times.

2.5. Endpoints

Intestinal reactive oxygen species (ROS) generation was used to assess oxidative stress induction in intestine. The exposed *C. elegans* were washed with K buffer and centrifuged to

remove the bacteria. *C. elegans* were labeled by 1 μM CM-H_2DCFDA for 2 hours with shaking at 200 rpm.[35] Following incubation, *C. elegans* were added on 2 % agar plates. Intestinal ROS fluorescence signals were analyzed under fluorescence microscopy (excitation/emission wavelength: 488/510 nm). Fluorescence intensity of ROS signals was quantified using ImageJ software. Fifty *C. elegans* were tested per treatment.

Intestinal lipofuscin accumulation is also an indicator of intestinal oxidative damage.[17] The exposed *C. elegans* were washed with K buffer and centrifuged to remove the bacteria. The *C. elegans* were first fixed for 20 minutes by 4% paraformaldehyde. After fixation, *C. elegans* were analyzed under DAPI filter using fluorescence microscope. Fluorescence intensity was also evaluated using ImageJ software. Fifty *C. elegans* were tested per treatment.

Locomotion was assessed by analyzing body bending and head thrashing frequencies.[36] *C. elegans* were allowed to recover for 1 minute on a fresh NGM plate without OP50 bacteria. Subsequently, locomotion behavior was evaluated. Head thrashing was measured by tracking movement direction along X-axis and changes in direction of posterior (Y-axis), and body bending was quantified by observing direction of bend at mid-body.[37] The frequency was counted within 1 min (head thrashes) or 20 s (body bends). Fifty *C. elegans* were tested per treatment.

Brood size was used to reflect the reproductive capacity of *C. elegans*. Brood size indicates the total number of offspring produced by each *C. elegans*, which was measured until the *C. elegans* ceased egg-laying.[17] Thirty *C. elegans* were tested per treatment.

2.6. Transcriptional expression

C. elegans were homogenized using Trizol and ceramic beads to ensure efficient tissue lysis. cDNA was synthesized using M-MuLV reverse transcriptase. Quantitative real-time polymerase chain reaction (qRT-PCR was performed using a SYBR qPCR Master Mix (Vazyme, China) with a StepOnePlus Real-Time PCR System (Applied Biosystems). The relative expression levels were calculated using the $2^{-\Delta\Delta Ct}$ method. *tba-1* gene is a reference gene.[38] Experiment was conducted in triplicate. Primers are shown in Table S1.

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2.7 RNA interference (RNAi)

To silence expression of candidate genes in *C. elegans*, dsRNA-producing bacterial strains were fed to animals.[39] Monoclones for RNAi feeding were picked to 2 mL LB liquid medium added with ampicillin and tetracycline, and incubated overnight in a shaker at 37 ◦C. The isopropylthiogalactoside was added and further induced for 4 h. The L1 larvae nematodes were cultured on NGM plated fed with RNAi bacteria. The offspring were used for 6-PPDQ exposure. Empty vector L4440 acted as control.[40] Fig. S1 shows the RNAi efficiency.

2.8 Data analysis

Data are represented as means ± SD. Statistical test was analyzed by SPSS v27. Difference among groups was evaluated using one-way or two-way ANOVA (for multi-factor comparison) followed by post-hoc test. The *p*-values of < 0.05 (*) and < 0.01 (**) were deemed statistically significant.

3. Results

3.1. 6-PPDQ affected adsorption of vitamin D3

Vitamin D3 content was reduced by 0.1-10 μg/L 6-PPDQ (Fig. 1A). In *C. elegans*, some transporters including CHUP-1,[41] LRP-2,[42] IFO-1,[43] and SCL-12/13[44] have been identified to control of adsorption of nutrients from intestinal lumen to intestinal cells. Among them, *chup-1* expression was not changed by 0.1-10 μg/L 6-PPDQ (Fig. 1B). In contrast, exposure to 0.1-10 μg/L 6-PPDQ decreased expression of *lrp-2*, *scl-12*, *scl-13*, and *ifo-1* (Fig. 1B). Under normal condition, expression of *lrp-2*, *scl-12*, *scl-13*, and *ifo-1* could be activated by vitamin D3 (400 μM) (Fig. 1C).

We next focused on the concentration of 10 μg/L for 6-PPDQ to examine effects of RNAi of vitamin D3 adsorption related and receptor genes in 6-PPDQ exposed nematodes.

Moreover, after 6-PPDQ exposure, vitamin D3 content could be further reduced by RNAi of *lrp-2*, *scl-12*, *scl-13*, and *ifo-1* (Fig. 1D).

3.2. 6-PPDQ affected expression of receptors genes for vitamin D3

Among *C. elegans* vitamin D3 receptor genes, after 0.1-10 μg/L exposure, *nhr-48* expression was not altered; however, *nhr-8* expression was decreased and *daf-12* expression was increased (Fig. 2A). Under normal condition, vitamin D3 (400 μM) treatment activated *nhr-8* expression and inhibited *daf-12* expression (Fig. 2B). Moreover, after 6-PPDQ exposure, RNAi of *lrp-2*, *scl-12*, *scl-13*, and *ifo-1* cloud inhibit *nhr-8* expression and accelerate *daf-12* expression (Fig. 2C).

3.3. RNAi of genes governing vitamin D3 adsorption and vitamin D3 receptor genes affected intestinal 6-PPDQ toxicity

In intestine, ROS generation and lipofuscin accumulation could be induced by 6-PPDQ.[45] After 6-PPDQ exposure, the induced intestinal ROS generation could be strengthened by *lrp-2*, *scl-12*, *scl-13*, *ifo-1*, and *nhr-8* RNAi, and suppressed by *daf-12* RNAi (Fig. 3A). Similarly, after 6-PPDQ exposure, the induced intestinal lipofuscin accumulation was increased by *lrp-2*, *scl-12*, *scl-13*, *ifo-1*, and *nhr-8* RNAi, and decreased by *daf-12* RNAi (Fig. 3B).

3.4. RNAi of genes governing vitamin D3 adsorption and vitamin D3 receptor genes affected 6-PPDQ toxicity in resulting in inhibited locomotion and reduced brood size

Exposure to 6-PPDQ could induce neurotoxicity (such as inhibited locomotion)[18] and reproductive toxicity (such as reduced brood size)[19]. After 6-PPDQ exposure, the induced locomotion inhibition was enhanced by *lrp-2*, *scl-12*, *scl-13*, *ifo-1*, and *nhr-8* RNAi, and inhibited by *daf-12* RNAi (Fig. 4A). Additionally, after 6-PPDQ exposure, the observed reduction in brood size could be strengthened by *lrp-2*, *scl-12*, *scl-13*, *ifo-1*, and *nhr-8* RNAi,

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and suppressed by *daf-12* RNAi (Fig. 4B).

3.5. RNAi of *nhr-8* and *daf-12* affected response of Mn-SOD and mitochondrial UPR in 6-PPDQ exposed nematodes

Mn-SOD and mitochondrial UPR are important protective responses against 6-PPDQ toxicity.[46-47] SOD-3 is a member of mitochondrial Mn-SODs, and HSP-6 is marker of mitochondrial UPR. Expressions of *sod-3* and *hsp-6*, as well as expressions of SOD-3::GFP and HSP-6::GFP, were decreased by 10 μg/L 6-PPDQ. The 6-PPDQ induced decrease in *sod-3* and SOD-3::GFP expressions were enhanced by *nhr-8* RNAi and suppressed by *daf-12* RNAi (Fig. 5A and 5B). In addition, 6-PPDQ caused reduction in *hsp-6* and HSP-6::GFP expressions were strengthened by *nhr-8* RNAi and inhibited by *daf-12* RNAi (Fig. 5A and 5C).

3.6. Pharmacological effect of treatment with vitamin D3 on 6-PPDQ toxicity in wild-type nematodes

To confirm beneficial role of vitamin D3 against 6-PPDQ toxicity, we performed pharmacological vitamin D3 treatment in 6-PPDQ exposed wild-type. The 6-PPDQ induced intestinal ROS generation and lipofuscin accumulation could be decreased by following vitamin D3 treatment (Fig. 6A ad 6B). Similarly, 6-PPDQ caused inhibition in locomotion and brood size could be suppressed by vitamin D3 treatment (Fig. 6C and 6D). Moreover, the observed decrease in *nhr-8* expression and increase in *daf-12* expression caused by 6-PPDQ were also inhibited by following vitamin D3 treatment (Fig. 6E).

3.7. Pharmacological effect of treatment with vitamin D3 on 6-PPDQ toxicity in *nhr-8(RNAi)* nematodes

Considering the susceptibility of *nhr-8(RNAi)* to 6-PPDQ toxicity, we further carried out pharmacological vitamin D3 treatment in 6-PPDQ exposed *nhr-8(RNAi)* nematodes. Vitamin

D3 treatment could inhibit intestinal ROS generation and lipofuscin accumulation caused by 6-PPDQ in *nhr-8(RNAi)* nematodes (Fig. 7A and 7B). Additionally, vitamin D3 treatment could further increase locomotion and brood size in 6-PPDQ exposed *nhr-8(RNAi)* (Fig. 7C and 7D).

4. Discussion

In organisms, 6-PPDQ caused several aspects of biochemical metabolisms. 6-PPDQ disrupted lipid metabolism in frogs,[48] and affected citric acid cycle in *C. elegans*.[49] In *C. elegans*, both glucose accumulation and glycogen accumulation were induced by 6-PPDQ.[23-24] In adult zebrafish and mice, glucolipid was also affected by 6-PPDQ.[50-51] 6-PPDQ could disrupt amino acid metabolisms (such as arginine biosynthesis) in mice.[52] 6-PPDQ decreased dopamine content by affecting its synthesis and transport in nematodes.[53] We further found the decrease in vitamin D3 content in *C. elegans* (Fig. 1A). These observations suggested that 6-PPDQ can potentially result in disruption in multiple aspects of biochemical metabolisms in various organisms.

After the exposure, the observed reduction in vitamin D3 content was largely due to inhibition in vitamin D3 adsorption by *C. elegans*. Two lines of evidence supported this. Firstly, 6-PPDQ (0.1-10 μg/L) decreased expression of *lrp-2*, *scl-12*, *scl-13*, and *ifo-1* governing nutrient uptake from intestinal lumen (Fig. 1B). Secondly, after 6-PPDQ exposure, vitamin D3 content could be reduced by RNAi of *lrp-2*, *scl-12*, *scl-13*, and *ifo-1* (Fig. 1D), which confirmed the role of these genes in controlling vitamin D3 adsorption. That is, 6-PPDQ inhibited adsorption of vitamin D3 by suppressing expression and function of *lrp-2*, *scl-12*, *scl-13*, and *ifo-1*. *C. elegans* LRP-2, IFO-1, and SCL-12/13 were also involved in regulating adsorption of other nutrients, such as cholesterol.[42-44] These observations demonstrated that LRP-2, IFO-1, and SCL-12/13 may provide conserved molecular mechanisms for adsorption of nutrients from *C. elegans* intestinal lumen. In addition, under normal condition, expression of *lrp-2*, *scl-12*, *scl-13*, and *ifo-1* could be activated by vitamin D3 treatment (Fig. 1C). This suggested that vitamin D3 treatment could induce the response and activation of *lrp-2*, *scl-12*, *scl-13*, and *ifo-1*, which in turns drives the adsorption of

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vitamin D3 from intestinal lumen.

Besides the effect on vitamin D3 adsorption, 6-PPDQ also affected expression of vitamin D3 receptors. Among candidate receptors, 0.1-10 μg/L 6-PPDQ decreased *nhr-8* expression and increased *daf-12* expression (Fig. 2A), which implied that NHR-8 and DAF-12 had opposite functions in 6-PPDQ exposed nematodes. The *nhr-48* expression was not changed by 0.1-10 μg/L 6-PPDQ (Fig. 2A), suggesting that *nhr-48* expression was not sensitive to 6-PPDQ exposure. Moreover, we provided two lines of evidence to support the role of NHR-8 and DAF-12 as vitamin D3 receptors. Firstly, under normal condition, vitamin D3 treatment could increase *nhr-8* expression and decrease *daf-12* expression (Fig. 2B), indicating the response of *nhr-8* and *daf-12* to vitamin D3. Secondly, after 6-PPDQ exposure, RNAi of *lrp-2*, *scl-12*, *scl-13*, and *ifo-1* could decrease *nhr-8* expression and increase *daf-12* expression (Fig. 2C), which suggested that the inhibited vitamin D3 adsorption by 6-PPDQ would affect expression of these two genes.

After the exposure, RNAi of genes (*lrp-2*, *scl-12*, *scl-13*, and *ifo-1*) governing vitamin D3 adsorption caused susceptibility to 6-PPDQ toxicity in causing intestinal ROS generation and lipofuscin accumulation (Fig. 3), inhibiting locomotion (Fig. 4A), and reducing brood size (Fig. 4B). This indicated that, besides having function to control vitamin D3 adsorption, these genes also participated in controlling stress response, such as the response to 6-PPDQ exposure, which suggested the novel function of these genes. Moreover, this suggested the possible association of reduction in vitamin D3 adsorption with toxicity induction of 6-PPDQ. For the formation of susceptibility to 6-PPDQ toxicity in *lrp-2(RNAi)*, *scl-12(RNAi)*, *scl-13(RNAi)*, and *ifo-1(RNAi)*, we further raised underlying molecular basis. That is, after 6-PPDQ exposure, RNAi of *lrp-2*, *scl-12*, *scl-13*, and *ifo-1* could result in inhibition in protective responses of antioxidation and mitochondrial UPR by suppressing SOD-3 and HSP-6 expressions (Fig. 5). Antioxidation and mitochondrial UPR are two important protective responses for nematodes against toxicity of pollutants and stresses.[54] In *C. elegans*, RNAi of *sod-3* and *hsp-6* strengthened 6-PPDQ toxicity in reducing lifespan.[46] Additionally, *sod-3* RNAi enhanced 6-PPDQ induced ROS generation,[22] and *hsp-6* RNAi enhanced 6-PPDQ toxicity in causing mitochondrial dysfunction and mitochondrial ROS generation.[47]

Besides the genes governing vitamin D3 adsorption, vitamin D3 receptor genes also

participated in controlling 6-PPDQ toxicity. Nevertheless, *nhr-8(RNAi)* showed susceptibility to 6-PPDQ toxicity, whereas *daf-12(RNAi)* exhibited resistance to 6-PPDQ toxicity (Fig. 3 and 4). This suggested that decrease in *nhr-8* expression and increase in *daf-12* expression mediated toxicity induction of 6-PPDQ. Additionally, NHR-8 and DAF-12 had opposite functions in regulating 6-PPDQ toxicity. This is also supported by some of previous studies. RNAi of *nhr-8* also enhanced 6-PPDQ induced immunosenescence.[35] Similarly, *nhr-8(RNAi)* showed susceptibility to toxicity of CPPDQ.[55] In contrast, RNAi of *daf-12* resulted in resistance to toxicity of multi-walled carbon nanotube[56] and nanoplastic[39]. RNAi of *daf-12* further suppressed 6-PPDQ induced immunosenescence.[35] Moreover, opposite functions between NHR-8 and DAF-12 was also reflected by their role in affecting their targets during controlling 6-PPDQ toxicity. The 6-PPDQ exposure caused inhibition in expressions of SOD-3 and HSP-6 could be strengthened by *nhr-8* RNAi and suppressed by *daf-12* RNAi (Fig. 5). Considering the decrease in *nhr-8* expression and increase in *daf-12* expression (Fig. 2A), the altered *nhr-8* and *daf-12* expression after 6-PPDQ exposure both implied inhibition in antioxidation response and mitochondrial UPR. Moreover, using intestinal ROS generation, intestinal lipofuscin accumulation, and locomotion as endpoints, genetic interaction analysis indicated that the resistance of *daf-12(RNAi)* to 6-PPDQ toxicity could be suppressed by RNAi of *nhr-8* (Fig. S2). This implied the possibility that, besides directly affecting SOD-3 and HSP-6 expression, DAF-12 possibly also controlled SOD-3 and HSP-6 expressions and toxicity of 6-PPDQ by inhibiting NHR-8. During control of 6-PPDQ toxicity, SOD-3 and HSP-6 also acted as targets of DAF-16, a FOXO transcriptional factor in insulin signaling pathway.[46] After 6-PPDQ exposure, *daf-16* expression was decreased.[46] Considering the role of DAF-12 and NHR-8 as nuclear hormone receptors (NHRs), after inhibition in NHR-8 and activation of DAF-12 on nuclear membrane in the cells, 6-PPDQ may further induce suppression in SOD-3 and HSP-6 expressions by inhibiting DAF-16. That is, the signaling cascade of DAF-12/NHR-8-DAF-16 may exist to control SOD-3 and HSP-6 expressions in 6-PPDQ exposed nematodes, which needs to be further determined.

Increasing evidence including those from human studies have indicated beneficial effect of vitamin D supplementation.[57] Vitamin D3 treatment could suppress formation of paralysis induced by amyloid-beta in *C. elegans* transgenic AD model.[58] In this study, 6-PPDQ induced

intestinal toxicity (such as ROS generation and lipofuscin accumulation in intestine) could be inhibited by vitamin D3 treatment (Fig. 6A and 6B). Additionally, decreased locomotion and reduced brood size induced by 6-PPDQ could be further inhibited by vitamin D3 treatment (Fig. 6C and 6D). These observations suggested that vitamin D3 treatment can be considered as an intervention strategy against 6-PPDQ induced damage on organisms.

Furthermore, we found that vitamin D3 could show beneficial effect against 6-PPDQ toxicity by affecting its corresponding receptors. After 6-PPDQ exposure, the induced decrease in *nhr-8* expression and increase in *daf-12* expression could be reversed by following vitamin D3 treatment (Fig. 6E). Moreover, vitamin D3 treatment could suppress formation of susceptibility of *nhr-8(RNAi)* nematodes to 6-PPDQ toxicity (Fig. 7). Pharmacological treatment with vitamin D3 could also rescue the short lifespan phenotype of *nhr-8* mutants.[30]

5. Conclusions

Together, we found the reduction in vitamin D3 content by 6-PPDQ exposure in nematodes. This 6-PPDQ induced reduction in vitamin D3 content was due to inhibition in vitamin D3 adsorption mediated by inhibition in *lrp-2*, *scl-12*, *scl-13*, and *ifo-1* expressions. Moreover, 6-PPDQ affected expression of *nhr-8* and *daf-12*, two vitamin D3 receptor genes. RNAi of genes governing vitamin D3 adsorption and receptor genes all could influence 6-PPDQ toxicity. NHR-8 and DAF-12 could regulate 6-PPDQ toxicity by affecting mitochondrial Mn-SOD and mitochondrial UPR responses. The 6-PPDQ induced toxicity could be suppressed by following vitamin D3 treatment, which further confirmed beneficial effect of vitamin D3 against 6-PPDQ toxicity. Our results suggested long-term exposure risk of 6-PPDQ in disrupting vitamin adsorption and function of its receptors in organisms.

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Data availability

The data will be available on request.

Conflicts of interest

There are no conflicts to declare.

References

1 N. Huntink, R. Datta and J. Noordermeer, Addressing durability of rubber compounds. *Rubber Chem. Technol.* 2004, **77,** 476-511.

2 X. Hua and D.-Y. Wang, Tire-rubber related pollutant 6-PPD quinone: a review of its transformation, environmental distribution, bioavailability, and toxicity. *J. Hazard Mater.* 2023, **459,** 132265.

3 Z. Tian, H. Zhao, K.T. Peter, M. Gonzalez, J. Wetzel, C. Wu, X. Hu, J. Prat, E. Mudrock, R. Hettinger, A.E. Cortina, R.G. Biswas, F.V.C. Kock, R. Soong, A. Jenne, B. Du, F. Hou, H. He, R. Lundeen, A. Gilbreath, R. Sutton, N.L. Scholz, J.W. Davis, M.C. Dodd, A. Simpson, J.K. McIntyre and E.P. Kolodziej, A ubiquitous tire rubber-derived chemical induces acute mortality in coho salmon. *Science,* 2021, **371,** 185-189.

4 C. Johannessen, P. Helm, B. Lashuk, V. Yargeau and C.D. Metcalfe, The tire wear compounds 6PPD-quinone and 1,3-diphenylguanidine in an urban watershed. *Arch. Environ. Contam. Toxicol.* 2022, **82,** 171–179.

5 X. Chen, T. He, X. Yang, Y. Gan, X. Qing, J. Wang and Y. Huang, Analysis, environmental occurrence, fate and potential toxicity of tire wear compounds 6PPD and 6PPD-quinone. *J. Hazard Mater.* 2023, **452,** 131245.

6 G. Cao, W. Wang, J. Zhang, P. Wu, X. Zhao, Z. Yang, D. Hu and Z. Cai, New evidence of rubber-derived quinones in water, air, and soil. *Environ. Sci. Technol.* 2022, **56,** 4142-4150.

7 X. Wan, G.-Y. Liang and D.-Y. Wang, Potential human health risk of the emerging environmental contaminant 6-PPD quinone. *Sci. Total Environ.* 2024, **949,** 175057.

8 Y. Jiang, C. Wang, L. Ma, T. Gao and Y. Wang, Environmental profiles, hazard identification, and toxicological hallmarks of emerging tire rubber-related contaminants 6PPD and 6PPD-quinone. *Environ. Int.* 2024, **187,** 108677.

9 S.S.U.H. Kazmi, Q. Xu, M. Tayyab, P. Pastorino, D. Barcelò, Z.M. Yaseen, Z.H. Khan and G. Li, Navigating the environmental dynamics, toxicity to aquatic organisms and human associated risks of an emerging tire wear contaminant 6PPD-quinone. *Environ. Pollut.* 2024, **356,** 124313.

10 Y.Y. Zhang, J.W. Huang, Y.H. Liu, J.N. Zhang, Z. Huang, Y.S. Liu, J.L. Zhao and G.G. Ying, In vitro metabolism of the emerging contaminant 6PPD-quinone in human and rat liver microsomes: Kinetics, pathways, and mechanism. *Environ. Pollut.* 2024, **345,** 123514.

11 W.-M. He, J. Chao, A.-H. Gu and D.-Y. Wang, Evaluation of 6-PPD quinone toxicity on lung of male BALB/c mice by quantitative proteomics. *Sci. Total Environ.* 2024, **922,** 171220.

12 H. Li, L. Zeng, C. Wang, C. Shi, Y. Li, Y. Peng, H. Chen, J. Zhang, B. Cheng, C. Chen, M. Xiang and Y. Huang, Review of the toxicity and potential molecular mechanisms of parental or successive exposure to environmental pollutants in the model organism *Caenorhabditis elegans*. *Environ. Pollut.* 2022, **311,** 119927.

13 J.-W. Wu, Y.-T. Shao, X. Hua, Y.-H. Li and D.-Y. Wang, Photo-aged polylactic acid microplastics causes severe transgenerational decline in reproductive capacity in *C. elegans*: Insight into activation of DNA damage checkpoints affected by multiple germline histone methyltransferases. *Environ. Pollut.* 2025, **382,** 126697.

14 Y.-X. Wang and D.-Y. Wang. Effect of disruption in intestinal barrier function during transgenerational process on nanoplastic toxicity induction in *Caenorhabditis elegans*. *Environ. Sci.: Nano* 2025, **12,** 2741-2749.

15 D.-Y. Wang, Exposure Toxicology in *Caenorhabditis elegans*. Springer Nature Singapore Pte Ltd., 2020.

16 Y.-X. Wang and D.-Y. Wang, Transgenerational intestinal toxicity of 6-PPD quinone in causing ROS production, enhancement in intestinal permeability and suppression in innate immunity in *C. elegans*. *Environ. Pollut.* 2024, **363,** 125208.

17 D.-Y. Wang, Target Organ Toxicology in *Caenorhabditis elegans*. Springer Nature Singapore Pte Ltd., 2019.

18 Z.-J. Liu, Y.-H. Li and D.-Y. Wang, Transgenerational response of glucose metabolism in *Caenorhabditis elegans* exposed to 6-PPD quinone. *Chemosphere* 2024, **367,** 143653.

19 M.-X. Song, Q.-L. Ruan and D.-Y. Wang, Paeoniflorin alleviates toxicity and accumulation of 6-PPD quinone by activating ACS-22 in *Caenorhabditis elegans*. *Ecotoxicol. Environ. Saf.* 2024, **286,** 117226.

20 Z.-Y. Liu, Q. Bian and D.-Y. Wang, 6-PPD quinone induces response of nuclear hormone receptors in the germline associated with formation of reproductive toxicity in *Caenorhabditis elegans*. *J. Hazard. Mater.* 2025, **495,** 138815.

21 X. Hua and D.-Y. Wang, Environmentally relevant concentration of 6-PPD quinone inhibits two types of mitophagy to cause mitochondrial dysfunction and lifespan reduction in *Caenorhabditis elegans*. *Environ. Sci.: Processes & Impacts* 2025, doi: 10.1039/d5em00224a.

22 X. Hua and D.-Y. Wang. 6-PPD quinone causes alteration in ubiquinone-mediated complex III associated with toxicity on mitochondrial function and longevity in *Caenorhabditis elegans*. *J. Environ. Chem. Engineer.* 2025, **13,** 116571.

23 Y.-X. Wang and D.-Y. Wang, Exposure to 6-PPD quinone enhances glycogen accumulation in *Caenorhabditis elegans*. *Environ. Pollut.* 2024, **359,** 124600.

24 Z.-J. Liu, Y.-H. Li and D.-Y. Wang, Exposure to 6-PPD quinone disrupts glucose metabolism associated with lifespan reduction by affecting insulin and AMPK signals in *Caenorhabditis elegans*. *Chemosphere* 2024, **363,** 142975.

25 W. Wang, Y.-H. Li and D.-Y. Wang, Long-term exposure to 6-PPD quinone inhibits glutamate synthesis and glutamate receptor function associated with its toxicity induction in *Caenorhabditis elegans*. *Toxics* 2025, **13,** 434.

26 W. Wang, Y.-H. Li and D.-Y. Wang, Exposure to 6-PPD quinone disrupts adsorption and catabolism of leucine to cause mitochondrial dysfunction in *Caenorhabditis elegans*. *Toxics* 2025, **13,** 544.

27 J. Janoušek, V. Pilařová, K. Macáková, A. Nomura, J. Veiga-Matos, D.D.D. Silva, F. Remião, L. Saso, K. Malá-Ládová, J. Malý, L. Nováková and P. Mladěnka, Vitamin D:

sources, physiological role, biokinetics, deficiency, therapeutic use, toxicity, and overview of analytical methods for detection of vitamin D and its metabolites. *Crit. Rev. Clin. Lab Sci.* 2022, **59,** 517-554.

28 M. Misra, D. Pacaud, A. Petryk, P.F. Collett-Solberg and M. Kappy, Drug and Therapeutics Committee of the Lawson Wilkins Pediatric Endocrine Society., Vitamin D deficiency in children and its management: review of current knowledge and recommendations. *Pediatrics* 2008, **122,** 398-417.

29 M.F. Holick and T.C. Chen, Vitamin D deficiency: a worldwide problem with health consequences. *Am. J. Clin. Nutr.* 2008, **87,** 1080S-1086S.

30 B. Huggins and M. Farris, Vitamin D3 promotes longevity in *Caenorhabditis elegans*. *GeroScience* 2023, **45,** 345-358.

31 J.L. Watts and M. Ristow, Lipid and carbohydrate metabolism in *Caenorhabditis elegans*. *Genetics* 2017, **207,** 413-446.

32 S. Brenner, The genetics of *Caenorhabditis elegans*. *Genetics* 1974, **77,** 71-94.

33 J.-W. Wu, Y.-T. Shao, X. Hua, Y.-X. Wang and D.-Y. Wang, Nanoplastic at environmentally relevant concentrations induces toxicity across multiple generations associated with inhibition in germline G protein-coupled receptor CED-1 in *Caenorhabditis elegans*. *Chemosphere* 2024, **364,** 143011.

34 Y.-X. Wang, J.-W. Wu and D.-Y. Wang, 6-PPD quinone causes lipid accumulation across multiple generations differentially affected by metabolic sensors and components of COMPASS complex in *Caenorhabditis elegans*. *Environ. Pollut.* 2025, **366,** 125539.

35 J.-W. Wu, S.-H. Shen and D.-Y. Wang, 6-PPD quinone at environmentally relevant concentrations induces immunosenescenece by causing immunosuppression during the aging process. *Chemosphere* 2024, **368,** 143719.

36 X. Hua, L. Zhang and D.-Y. Wang, Nanoplastic at environmentally relevant concentrations activates germline *mir-240-rab-5* signaling cascade to affect secreted ligands associated with transgenerational toxicity induction in *C. elegans*. *Environ. Sci.: Nano* 2024, **11,** 3615.

37 T.-S. Wu, J. He, Z.-J. Ye, J.-Y. Xia, M. Chen, S.-Y. Chen, K.-H. Liu, P.-C. Xing, J.-F. Yang, Y.-J. Qian and D.-Y. Wang, Aged biodegradable nanoplastics enhance body

accumulation associated with worse neuronal damage in *Caenorhabditis elegans*. *Environ. Sci. Technol.* 2025, **59,** 4352-4363.

38 X. Hua and D.-Y. Wang, Transgenerational response of germline histone acetyltransferases and deacetylases to nanoplastics at predicted environmental doses in *Caenorhabditis elegans*. *Sci. Total Environ.* 2024, **952,** 175903.

39 J.-W. Wu, Y.-T. Shao, X. Hua and D.-Y. Wang, Activated hedgehog and insulin ligands by decreased transcriptional factor DAF-16 mediate transgenerational nanoplastic toxicity in *Caenorhabditis elegans*. *J. Hazard. Mater*. 2024, **480,** 135909.

40 M.-X. Song, Q.-L. Ruan and D.-Y. Wang, Comparison of transgenerational toxicity on locomotion behavior and development of D-type motor neurons between pristine and amino modified nanoplastics at environmentally relevant doses in *C. elegans*. *Toxics* 2024, **12,** 555.

41 V.J. Valdes, A. Athie, L.S. Salinas, R.E. Navarro and L. Vaca, CUP-1 is a novel protein involved in dietary cholesterol uptake in *Caenorhabditis elegans*. *PLoS One* 2012, **7,** e33962.

42 S. Zhang, S.A. Glukhova, K.A. Caldwell and G.A. Caldwell, NCEH-1 modulates cholesterol metabolism and protects against α-synuclein toxicity in a *C. elegans* model of Parkinson's disease. *Hum. Mol. Genet.* 2017, **26,** 3823-3836.

43 R.A. Coch, F. Geisler, A. Annibal, A. Antebi and R.E. Leube, Identification of a novel link between the intermediate filament organizer IFO-1 and cholesterol metabolism in the *Caenorhabditis elegans* intestine. *Int. J. Mol. Sci.* 2020, **21,** 8219.

44 K. Schmeisser, D. Kaptan, B.K. Raghuraman, A. Shevchenko, J. Rodenfels, S. Penkov and T.V. Kurzchalia, Mobilization of cholesterol induces the transition from quiescence to growth in *Caenorhabditis elegans* through steroid hormone and mTOR signaling. *Commun. Biol.* 2024, **7,** 121.

45 X. Hua, X. Feng, G.-Y. Liang, J. Chao and D.-Y. Wang, Long-term exposure to tire-derived 6-PPD quinone causes intestinal toxicity by affecting functional state of intestinal barrier in *Caenorhabditis elegans*. *Sci. Total Environ.* 2023, **861,** 160591.

46 X. Hua and D.-Y. Wang, Exposure to 6-PPD quinone at environmentally relevant concentrations inhibits both lifespan and healthspan in *C. elegans*. *Environ. Sci. Technol.*

View Article Online
DOI: 10.1039/D5EM00358J

2023, **57,** 19295-19303.

47 X. Hua and D.-Y. Wang, 6-PPD quinone at environmentally relevant concentrations induced damage on longevity in *C. elegans*: Mechanistic insight from inhibition in mitochondrial UPR response. *Sci. Total Environ.* 2024, **954,** 176275.

48 Z. Liu, Y. Feng, W. Sun, B. Wang, C. Shi, R. Ran, Y. Zhang, L. Lu and H. Zhang, Environmental concentrations of 6PPD and 6PPD-quinone induce hepatic lipid metabolism disorders in male black-spotted frogs. *J. Hazard. Mater.* 2024, **480,** 136400.

49 X. Wan, G.-Y. Liang, D.-Y. Wang. 6-PPD quinone at environmentally relevant concentrations disrupts citric acid cycle in Caenorhabditis elegans: Role of reduction in acetyl CoA and pyruvate contents. *Environ. Chem. Ecotoxicol.* 2025, **7,** 1119-1129.

50 L. Fang, C. Fang, S. Di, Y. Yu, C. Wang, X. Wang and Y. Jin, Oral exposure to tire rubber-derived contaminant 6PPD and 6PPD-quinone induce hepatotoxicity in mice. *Sci. Total Environ.* 2023, **869,** 161836.

51 Z. Wang, M. Zhu, Y. Hu, J. Liu, X. Ma and H. Zhou, Comparative effects of 6PPD and 6PPD-Quinone at environmentally relevant concentrations on hepatotoxicity, glucolipid metabolism and ferroptotic response in adult zebrafish. *Environ. Res.* 2025, **275,** 121386.

52 K. Yao, Q. Kang, W. Liu, D. Chen, L. Wang and S. Li, Chronic exposure to tire rubber-derived contaminant 6PPD-quinone impairs sperm quality and induces the damage of reproductive capacity in male mice. *J. Hazard. Mater.* 2024, **470,** 134165.

53 X. Hua and D.-Y. Wang, Disruption of dopamine metabolism by exposure to 6-PPD quinone in *Caenorhabditis elegans*. *Environ. Pollut.* 2023, **337,** 122649.

54 D.-Y. Wang, Molecular Toxicology in *Caenorhabditis elegans*. Springer Nature Singapore Pte Ltd., 2019.

55 X. Wan, G.-Y. Liang and D.-Y. Wang, Neurotoxicity and accumulation of CPPD quinone at environmentally relevant concentrations in *Caenorhabditis elegans*. *Chemosphere* 2024, **361,** 142499.

56 Y. Zhao, X. Hua, Q. Rui and D. Wang, Exposure to multi-walled carbon nanotubes causes suppression in octopamine signal associated with transgenerational toxicity induction in *C. elegans*. *Chemosphere* 2023, **318,** 137986.

57 R. Bouillon, D. Manousaki, C. Rosen, K. Trajanoska, F. Rivadeneira and J.B. Richards,

The health effects of vitamin D supplementation: evidence from human studies. *Nat. Rev. Endocrinol.* 2022, **18,** 96-110.

58 A. Leiteritz, T. Schmiedl, S. Baumanns and U. Wenzel, Amyloid-beta induced paralysis is reduced by cholecalciferol through inhibition of the steroid-signaling pathway in an Alzheimer model of *Caenorhabditis elegans*. *Nutr. Neurosci.* 2021, **24,** 82-89.

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DOI: 10.1039/D5EM00358J

Figure legends:

Fig. 1 Effect of 6-PPDQ exposure on vitamin D3 content and adsorption. (A) Effect of 6-PPDQ exposure on vitamin D3 content. Relative vitamin D3 content is shown after normalization to control. $^{*}P < 0.05$ and $^{**}P < 0.01$ vs control. (B) Effect of 6-PPDQ exposure on expression of *clup-1*, *lrp-2*, *scl-12*, *scl-13*, and *ifo-1*. $^{**}P < 0.01$ vs control. (C) Effect of vitamin D3 treatment at 400 μM on expression of *clup-1*, *lrp-2*, *scl-12*, *scl-13*, and *ifo-1*. Young adults were treated with 400 μM vitamin D3 for 24-h. Relative vitamin D3 content is shown after normalization to control. $^{**}P < 0.01$ vs control. (D) Effect of RNAi of *clup-1*, *lrp-2*, *scl-12*, *scl-13*, and *ifo-1* on vitamin D3 content in 6-PPDQ exposed nematodes. Exposure concentration of 6-PPDQ was 10 μg/L. Relative vitamin D3 content is shown after normalization to control. $^{**}P < 0.01$ vs wild-type(L4440).

Fig. 2 Effect of 6-PPDQ exposure on expression of vitamin D3 receptor genes. (A) Effect of 6-PPDQ exposure on expressions of *nhr-8*, *nhr-48*, and *daf-12*. $^{**}P < 0.01$ vs control. (B) Effect of vitamin D3 treatment at 400 μM on expression of *nhr-8* and *daf-12*. Young adults were treated with 400 μM vitamin D3 for 24-h. $^{**}P < 0.01$ vs control. (C) Effect of RNAi of *clup-1*, *lrp-2*, *scl-12*, *scl-13*, and *ifo-1* on expression of *nhr-8* and *daf-12* in 6-PPDQ exposed nematodes. Exposure concentration of 6-PPDQ was 10 μg/L. $^{**}P < 0.01$ vs wild-type(L4440).

Fig. 3 Effect of RNAi of *lrp-2*, *scl-12*, *scl-13*, *ifo-1*, *nhr-8*, and *daf-12* on 6-PPDQ toxicity in causing intestinal ROS generation (A) and intestinal lipofuscin accumulation (B). Exposure concentration of 6-PPDQ was 10 μg/L. $^{**}P < 0.01$ vs wild-type(L4440).

Fig. 4 Effect of RNAi of *lrp-2*, *scl-12*, *scl-13*, *ifo-1*, *nhr-8*, and *daf-12* on 6-PPDQ toxicity in causing inhibition in locomotion (A) and reduction in brood size (B). Exposure concentration of 6-PPDQ was 10 μg/L. $^{**}P < 0.01$ vs wild-type(L4440).

Fig. 5 Effect of RNAi of *nhr-8* and *daf-12* on expression of SOD-3 and HSP-6 in 6-PPDQ exposed nematodes. (A) Effect of RNAi of *nhr-8* and *daf-12* on expression of *sod-3* and *hsp-6*

in 6-PPDQ exposed nematodes. (B) Effect of RNAi of *nhr-8* and *daf-12* on expression of SOD-3::GFP in 6-PPDQ exposed nematodes. (C) Effect of RNAi of *nhr-8* and *daf-12* on expression of HSP-6::GFP in 6-PPDQ exposed nematodes. Exposure concentration of 6-PPDQ was 10 μg/L. $^{**}P < 0.01$.

Fig. 6 Pharmacological effect of treatment with vitamin D3 on 6-PPDQ toxicity in wild-type nematodes. (A) Pharmacological effect of treatment with vitamin D3 on 6-PPDQ toxicity in inducing intestinal ROS generation in wild-type nematodes. (B) Pharmacological effect of treatment with vitamin D3 on 6-PPDQ toxicity in inducing intestinal lipofuscin accumulation in wild-type nematodes. (C) Pharmacological effect of treatment with vitamin D3 on 6-PPDQ toxicity in causing inhibition in locomotion in wild-type nematodes. (D) Pharmacological effect of treatment with vitamin D3 on 6-PPDQ toxicity in causing reduction in brood size in wild-type nematodes. (E) Pharmacological effect of treatment with vitamin D3 on expression of *nhr-8* and *daf-12* in 6-PPDQ exposed wild-type nematodes. After exposure to 10 μg/L 6-PPDQ from L1-larvae for 6.5 days, the nematodes were treated with 400 μM vitamin D3 for 24-h. $^{**}P < 0.01$.

Fig. 7 Pharmacological effect of treatment with vitamin D3 on 6-PPDQ toxicity in *nhr-8(RNAi)* nematodes. (A) Pharmacological effect of treatment with vitamin D3 on 6-PPDQ toxicity in inducing intestinal ROS generation in *nhr-8(RNAi)* nematodes. (B) Pharmacological effect of treatment with vitamin D3 on 6-PPDQ toxicity in inducing intestinal lipofuscin accumulation in *nhr-8(RNAi)* nematodes. (C) Pharmacological effect of treatment with vitamin D3 on 6-PPDQ toxicity in causing inhibition in locomotion in *nhr-8(RNAi)* nematodes. (D) Pharmacological effect of treatment with vitamin D3 on 6-PPDQ toxicity in causing reduction in brood size in *nhr-8(RNAi)* nematodes. After exposure to 10 μg/L 6-PPDQ from L1-larvae for 6.5 days, the nematodes were treated with 400 μM vitamin D3 for 24-h. $^{**}P < 0.01$.

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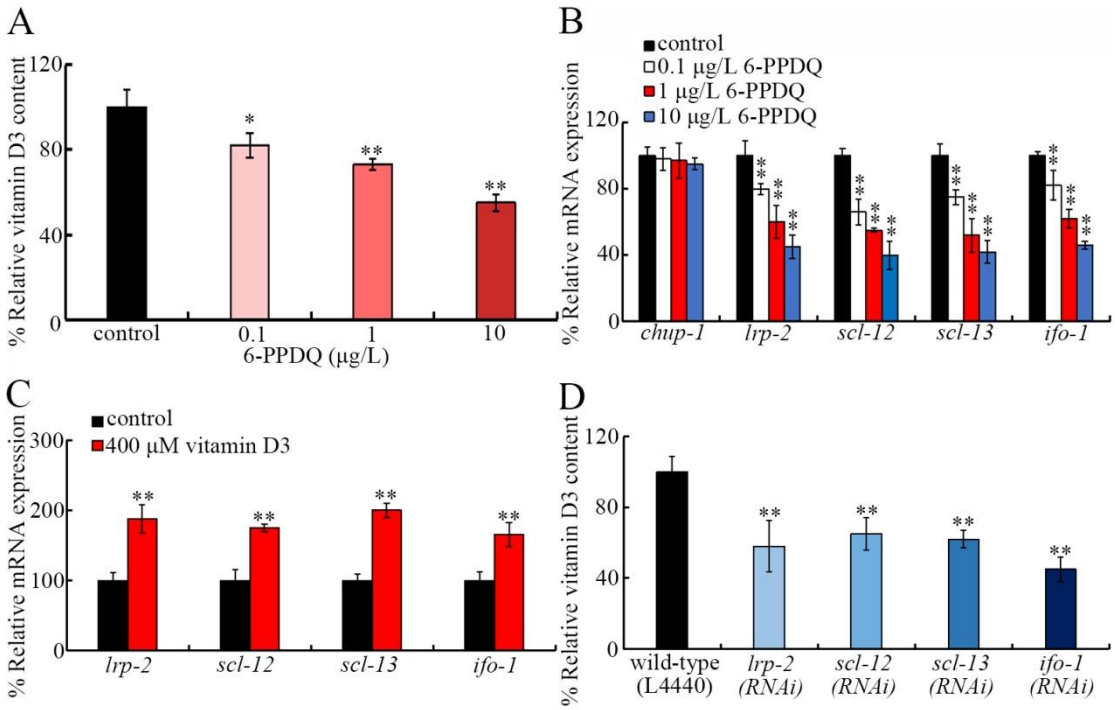
Figure 1:



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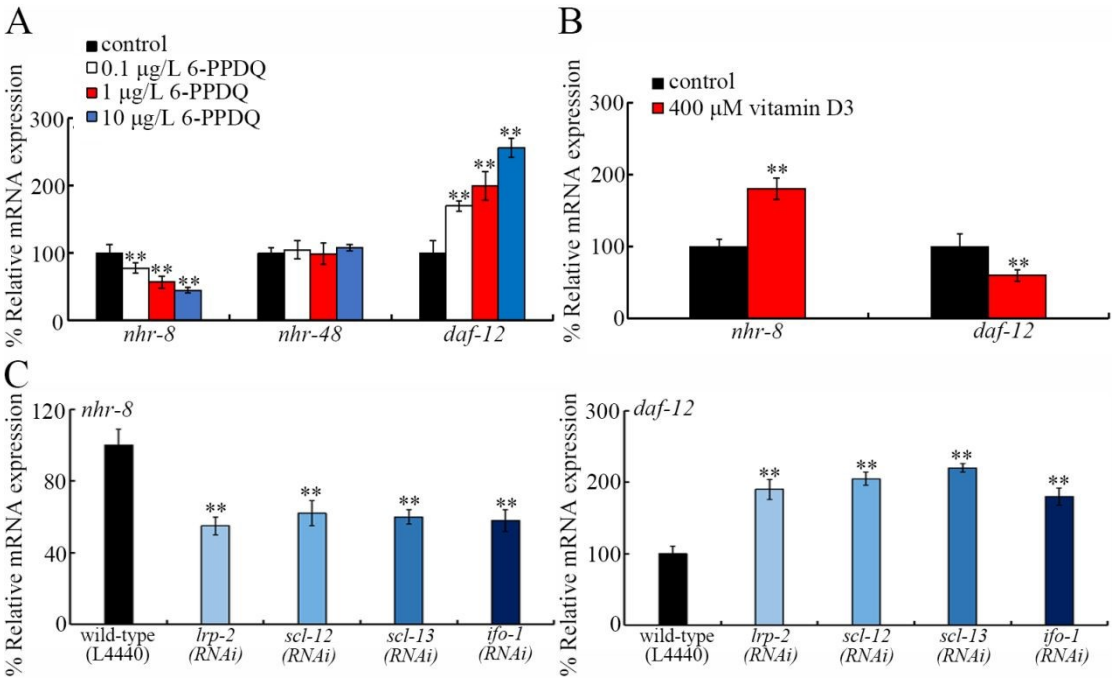

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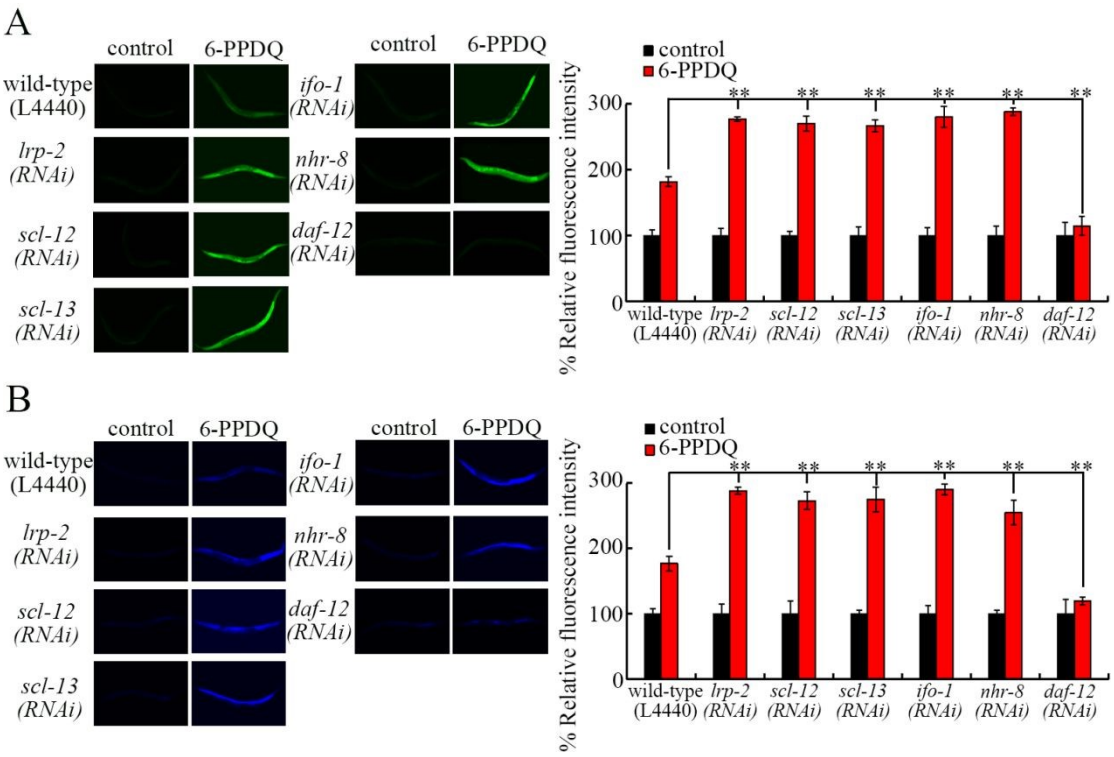

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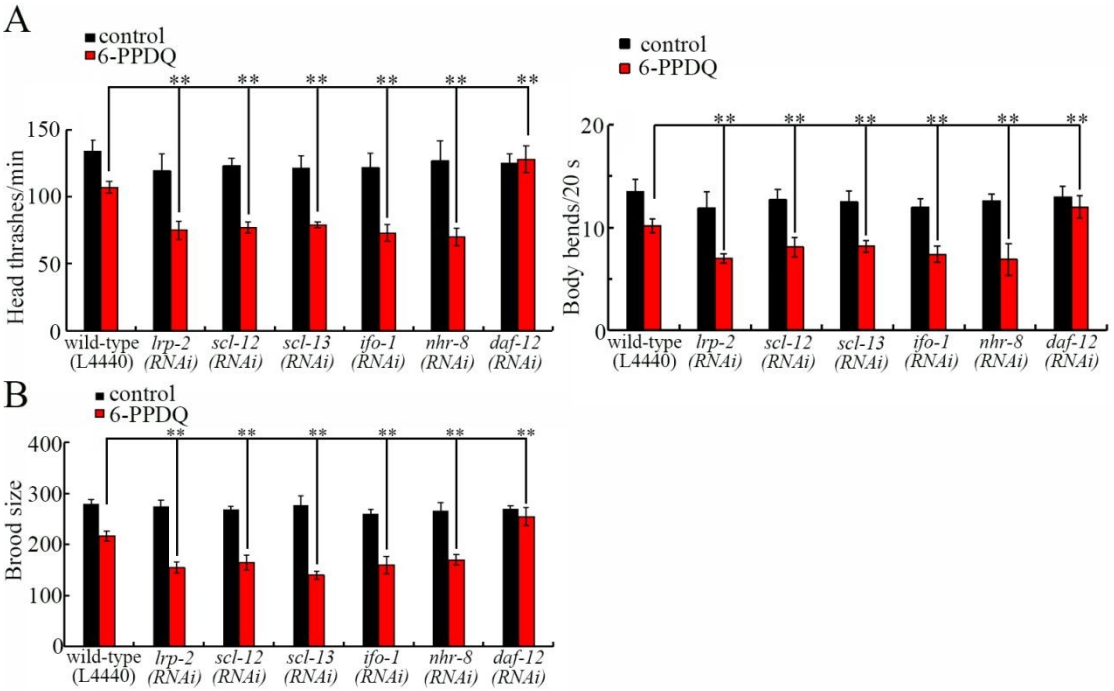

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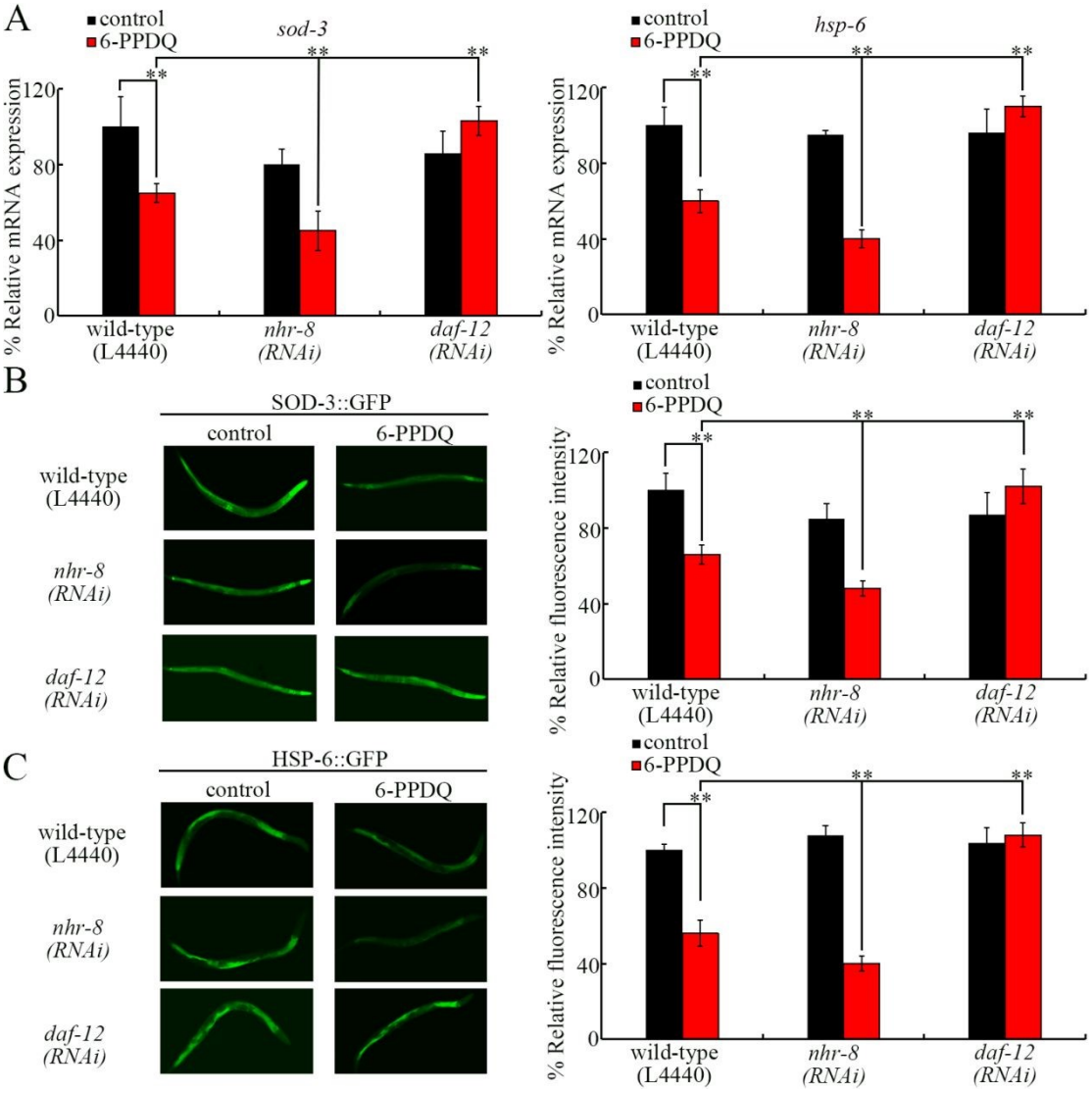

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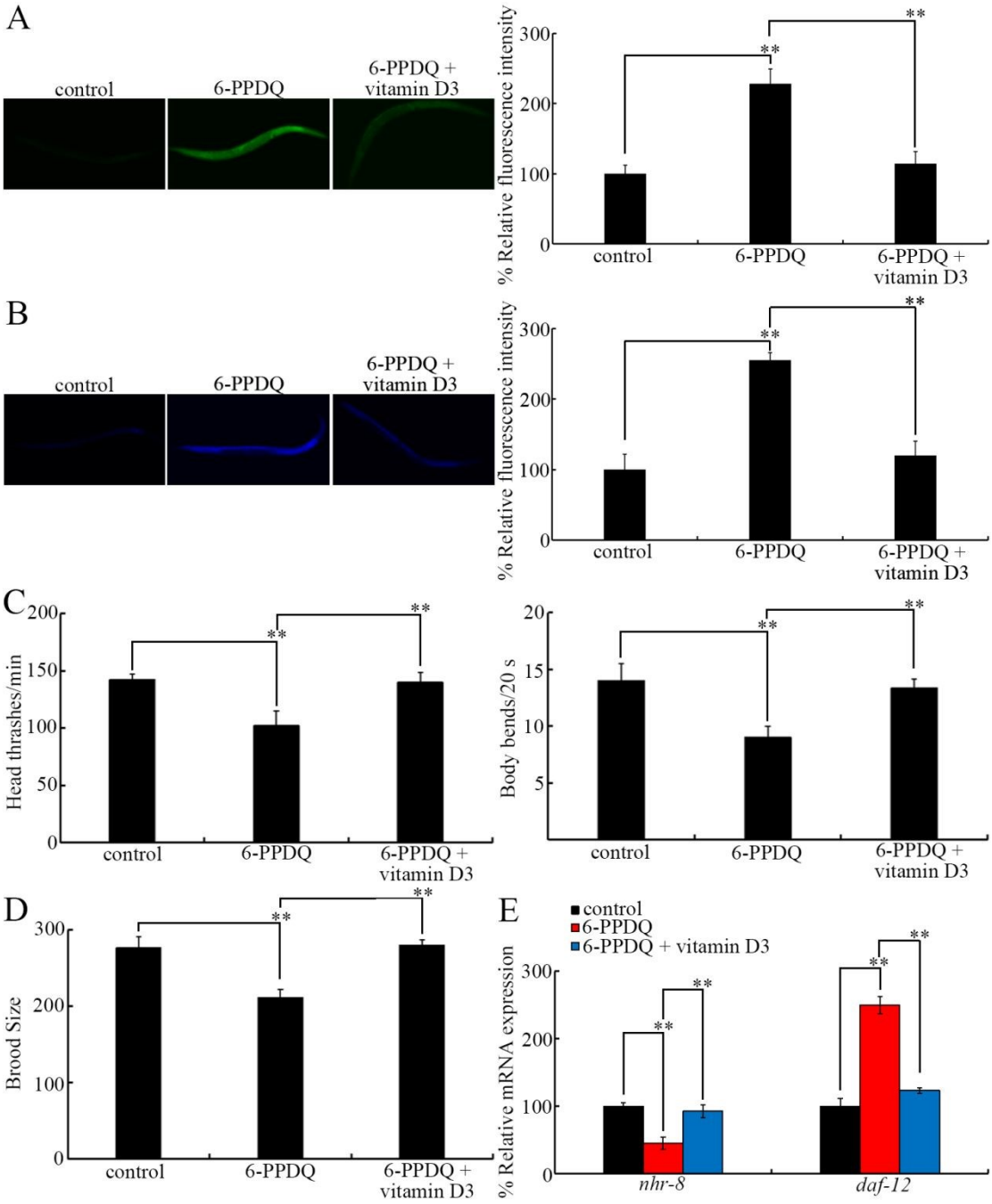

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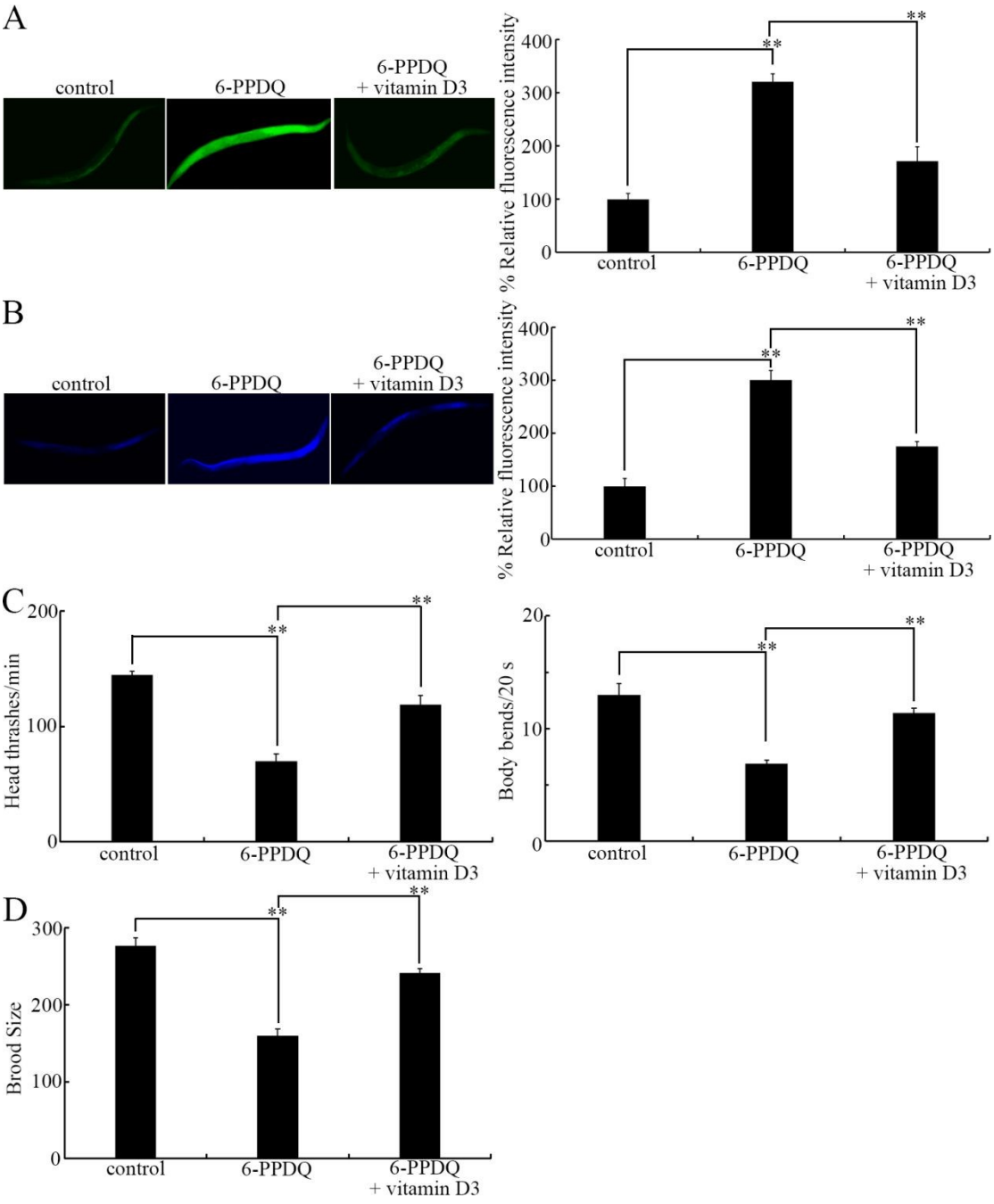

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Data availability statement:

The data will be available on request.