Caenorhabditis elegans: A Bridging Model to Assess the Safety of Nanomaterials

11

Nivedita Chatterjee

Abstract

This chapter focuses on the role of *Caenorhabditis elegans* as a bridging model in nanotoxicology and nanosafety research. With its simple multicellular structure, well-characterized genetics, low maintenance costs, short life cycle, and suitability for high-throughput screening, *C. elegans* is effective for evaluating nanoparticle toxicity across various exposure scenarios, including acute and chronic treatments. The chapter examines key physiological endpoints—such as survival rates, growth, reproduction, and behavior—and employs mutant and transgenic strains alongside advanced omics technologies to investigate the molecular pathways affected by nanoparticle exposure, particularly oxidative stress, genotoxicity, and neurotoxicity. By integrating multi-endpoint assessments and behavioral investigations, *C. elegans* provides valuable insights into the safety and potential risks of nanomaterials, contributing to a broader understanding of nanotoxicology in alignment with the 'One Health' framework.

Keywords

Caenorhabditis elegans (*C. elegans*) · Engineered nanomaterial · Nanotoxicology

1 Introduction

The soil-dwelling, non-parasitic nematode *Caenorhabditis elegans* (*C. elegans*) has been a foundational model organism in biological research since the 1970s when it was first was first proposed as a model organism by Sydney Brenner in 1965 and employed to study the genetic regulation of development (Tejeda-Benitez and

N. Chatterjee (✉)
NanoSafety Group, International Iberian Nanotechnology Laboratory, Braga, Portugal
e-mail: nivedita.chatterjee@inl.int

E. Alfaro-Moreno, F. Murphy (eds.), *Nanosafety*,
https://doi.org/10.1007/978-3-031-93871-9_11

Olivero-Verbel 2016; Brenner 2009; Avila et al. 2011). Its popularity has grown significantly due to numerous advantages. These include its small size (approximately 1 mm in length for adults), rapid life cycle (about 3 days at 20 °C to reach adulthood), short lifespan (around 2.5 weeks), self-fertilization capability, large brood size (over 300 offspring per hermaphrodite), and ease of genetic manipulation (Leung et al. 2008). The requirements for maintaining *C. elegans* in the lab are minimal—ambient temperature, humidity, oxygen, and a bacterial food source—making it a cost-effective and accessible model for research (Avila et al. 2011).

1.1 Ecology and Natural Environment

The nematode *C. elegans* thrives in environments rich in decaying organic matter, such as rotting fruits and compost heaps, where it primarily feeds on bacteria. Its population follows a "boom-and-bust" dynamic, increasing when food is abundant. When resources are scarce, it enters the dauer stage, a dormant phase that enables survival under harsh conditions and dispersal to new environments. *C. elegans* demonstrates adaptability and plays a crucial role in nutrient cycling across various habitats, including soil and decomposing plant material (Frézal and Félix 2015).

1.2 Anatomy and Tissues

C. elegans features a simple yet high differentiated anatomical structure. Adult hermaphrodites consist of 959 somatic cells, while males possess 1031. Despite its simplicity, *C. elegans* develops specialized tissues including muscle, hypodermis, intestine, gonads, glands, an excretory system, and a nervous system composed of 302 neurons and their synapses (Sulston 1983; Avila et al. 2011) (Fig. 11.1).

1.2.1 Epidermis

The epidermis consists of a single layer of hypodermal cells, covered by a protective cuticle.

1.2.2 Muscles

Body wall muscles are arranged into four quadrants, enabling the nematode's characteristic sinusoidal movement.

1.2.3 Digestive System

This system includes a pharynx, intestine, and anus, ensuring efficient nutrient absorption.

1.2.4 Nervous System

The hermaphrodite's nervous system contains 302 neurons and 56 glial cells, while the male has 381 neurons. The complete neural network (connectome) has been fully mapped, making *C. elegans* an excellent model for studying neural function, development, and degeneration.

Anatomy of *Caenorhabditis elegans*

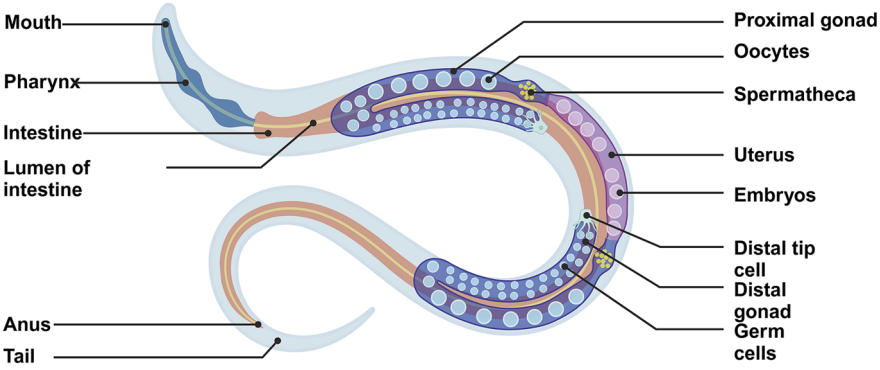

Fig. 11.1 Anatomy of adult *C. elegans* hermaphrodite (schematic). (Figure created with BioRender.com)

1.2.5 Reproductive System

Hermaphrodites have a bilobed gonad, each lobe containing an ovary, oviduct, and spermatheca, while males have a single-lobed gonad with a vas deferens leading to the cloaca.

1.3 Development and Reproduction

Mature oocytes pass through the spermatheca, where they are fertilized by sperm from either the hermaphrodite or a male. The resulting zygote forms a tough chitinous shell and vitelline membrane, rendering it impermeable to most solutes. Eggs are typically retained in the uterus through the first few cleavages before being laid around the time of gastrulation, approximately 3 h after fertilization. During embryogenesis, cell division, organogenesis, and morphogenesis occur, resulting in the first-stage larva. Post-embryonic development sees continuous growth, with somatic cell nuclei increasing from 558 in the first-stage larva to 959 in adult hermaphrodites (Avila et al. 2011; Ferreira et al. 2014).

Larval development proceeds through four stages (L1-L4), with significant cellular differentiation occurring during each phase (Avila et al. 2011; Tejeda-Benitez and Olivero-Verbel 2016). For example, certain proteins such as $Cu^{2+/}Zn^{2+}$ superoxide dismutase and aspartyl proteinase are highly expressed in the L1 stage but decrease as the nematode matures (Mádi et al. 2003). By the L4 stage, gonadogenesis is complete, enabling reproductive capability. The entire life cycle, from egg to reproductive adult, takes just 3.5 days at 20 °C. Under optimal conditions, the lifespan of wild-type *C. elegans* is about 2.5 weeks (~18 days). In response to food scarcity or high population density, an alternative dauer stage can form at the L2/L3

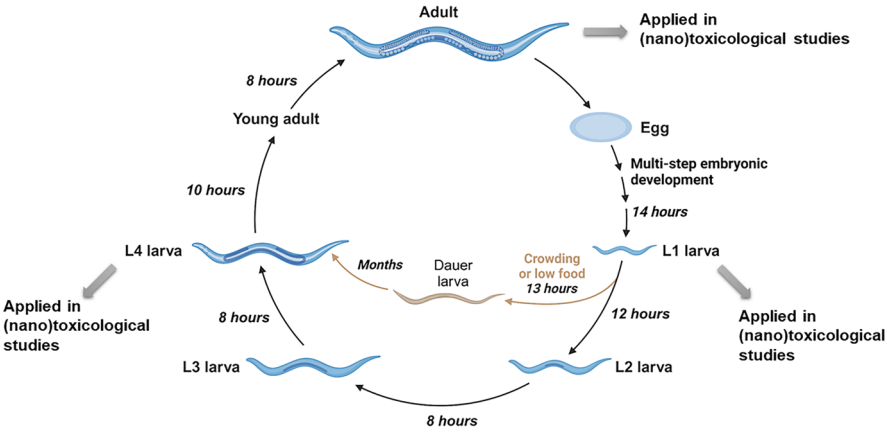

Fig. 11.2 Life cycle of *C. elegans* (schematic representation): relevance of different developmental stages to nanotoxicological study applications. (Created with BioRender.com)

molt. Dauers are resistant to desiccation and can survive up to 3 months without developing further (Avila et al. 2011) (Fig. 11.2).

1.4 Reproduction

C. elegans exists as either hermaphrodite or male. Hermaphrodites can self-fertilize, producing only hermaphrodite offspring, while cross-fertilization between hermaphrodites and males produces both sexes in equal proportions. This unique reproductive strategy is particularly useful for genetic studies. Hermaphrodites possess a bilobed gonad, while males have a single-lobed gonad that connects with the cloaca near the tail. Males also have specialized structures in their tail for mating, including 18 sensory rays and spicules that assist with sperm transfer during copulation (Tejeda-Benitez and Olivero-Verbel 2016; Avila et al. 2011; Ferreira et al. 2014).

1.5 Genome and Genetic Manipulation

The *C. elegans* genome, one of the first multicellular organisms to be fully sequenced, consists of approximately 100 million base pairs and 20,000 genes spread across six chromosomes. This wealth of genetic information is accessible through databases such as WormBase. Various genetic techniques, including mutagenesis, transgenesis, and RNA interference (RNAi), are employed to study *C. elegans*. Knockout mutant libraries and genetic manipulation tools, such as GFP-tagging, have been particularly valuable for in vivo studies of cells and molecular pathways (Avila et al. 2011; Chalfie et al. 1994).

1.6 *C. elegans* as a Model in Biology

C. elegans has been instrumental in advancing biological research since its adoption as a model organism. Key discoveries include the genetic mechanisms behind the development, apoptosis, and neural function, with landmark achievements like the complete mapping of its cell lineage, the sequencing of its genome, and the discovery of RNA interference (RNAi). Its rapid life cycle, transparent body, and self-fertilization simplify genetic studies and cellular observations, while its simple maintenance makes it a cost-effective research tool. Despite its biological simplicity compared to higher organisms, *C. elegans* continues to provide profound insights into fundamental biological processes, driving breakthroughs in science and medicine (Tejeda-Benitez and Olivero-Verbel 2016; Avila et al. 2011).

2 *C. elegans*—The Bridging Model Organism and Its Applications in Toxicity Research

C. elegans became a preferred model for toxicity studies in the late 1990s, owing to its low maintenance cost, short life cycle, and suitability for high-throughput screening (Helmcke et al. 2010; Avila et al. 2011). Unlike isolated cell cultures, *C. elegans* provides a complete multicellular organism to assess whole-system responses to toxicants. It possesses functional nervous, digestive, and reproductive systems, offering insights into the holistic impact of toxin exposure. Its fully sequenced genome allows for easy manipulation through RNA interference (RNAi) and mutagenesis, and researchers can access thousands of transgenic and mutant strains from the Caenorhabditis Genetics Center (Ferreira et al. 2014).

Toxicity assays typically test endpoints such as growth, reproduction, feeding, and movement (Wu et al. 2019; Avila et al. 2011; Tejeda-Benitez and Olivero-Verbel 2016). Growth and reproduction are often more sensitive indicators than lethality for many toxicants like polycyclic aromatic hydrocarbons (Sese et al. 2009). *C. elegans*, especially transgenic strains, is extensively utilized as a bioindicator in ecotoxicology, with a focus on sublethal conditions (Anbalagan et al. 2013; Lagido et al. 2009). Its application is significant across both terrestrial and aquatic environments (Ellegaard-Jensen et al. 2012; Kuhn et al. 2021). Toxicant exposure can be conducted on solid agar plates or in liquid media, providing flexibility in experimental design and distinct advantages for toxicology assays. The transparent nature of the worm's cuticle eliminates the need for dissection, allowing researchers to directly observe a wide range of endpoints and simplifying toxicity assessments. In essence, *C. elegans* enables researchers to collect data on a whole living organism using a methodology often similar to that of cell line monocultures (Ferreira et al. 2014).

To evaluate the toxic effects of chemicals, researchers use various bioassays with *C. elegans*. Typically, young adult worms are exposed to different concentrations of the test substance in a liquid medium. The absence of food during these acute exposures allows for a focused assessment of the chemical's impact. For long-term

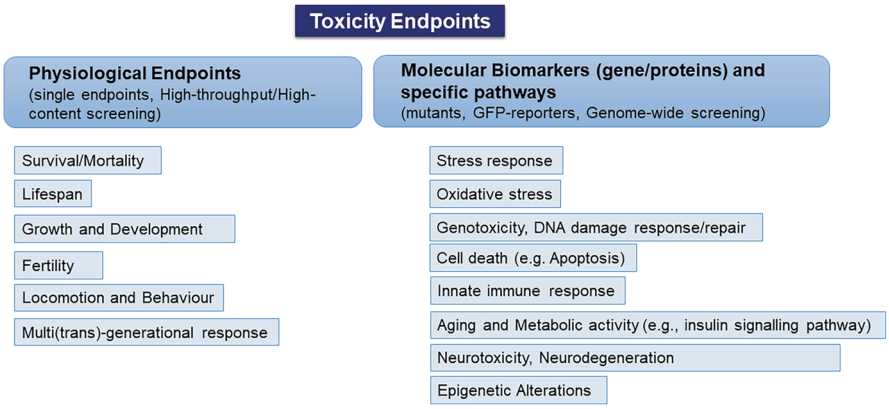

Fig. 11.3 Key endpoints in toxicological assessments with the *C. elegans* model

studies, L1 larvae are exposed to the chemical in the presence of a food source such as *E. coli* OP50. Toxicity endpoints in *C. elegans* encompass a wide range of biological responses, including lethality, growth rate, locomotion, and reproductive capacity. To gain deeper insights into the mechanisms of toxicity, molecular markers like those for oxidative stress, gene expression, DNA damage, or green fluorescent protein (GFP) expression can be employed (Tejeda-Benitez and Olivero-Verbel 2016; Wu et al. 2019). A classification of commonly used endpoints in *C. elegans* toxicity research is presented in Fig. 11.3. This comprehensive approach enables the identification of sensitive endpoints and the characterization of the toxicant's mode of action.

2.1 Adaptability to High-Throughput, Automated Behaviour System, and Genome-Wide Toxicity Screening

C. elegans is an ideal model organism for high-throughput screening due to its adaptability to both aquatic and terrestrial environments, prolific reproduction, and short life cycles. These features enable the analysis of toxicant effects through various methods (Helmcke et al. 2010). Additionally, multi-endpoint, high-content screening platforms have been developed and applied in various toxicity fields (Wu et al. 2022; Jung et al. 2015). Automated tools such as the Biosort (Union Biometrica, Inc.) and COPAS biosorter can analyze parameters like length, motion, fluorescence, and reproductive endpoints in 96-well plates (Shin et al. 2019; Helmcke et al. 2010). Furthermore, microfluidic devices and robotic systems improve the precision of worm manipulation and immobilization for imaging and microsurgery (Hulme et al. 2007; Mondal et al. 2016; Rohde et al. 2007).

Computer-based assays also offer automated readouts for assessing toxicant impacts on behaviors like thrashing, fluorescence, and developmental endpoints such as egg-laying, dauer formation, and lifespan in wild-type, mutant, and

transgenic worms (Buckingham and Sattelle 2009; Rohde et al. 2007; Leung et al. 2011; Rahman et al. 2020).

Genome-wide screens for molecular contributors to toxicity, using methods such as microarray, RNA sequencing, RNAi screening, and transgenic approaches, have identified genes involved in toxicant responses (McElwee et al. 2013; Chatterjee et al. 2017; Kim et al. 2017a, 2020a, b). Thus, *C. elegans* offers efficient, high-throughput capabilities for studying toxicant effects, supported by advanced genetic tools and automated technologies.

The *C. elegans* model holds promise for connecting in vivo and in vitro approaches (Kaletta and Hengartner 2006; Chakravarty 2022). It addresses the challenges of mammalian models by providing a more affordable, efficient, and ethically favorable alternative. Additionally, *C. elegans* features a fully sequenced genome, the availability of transgenic knock-out mutants, and compatibility with high-throughput automation techniques. Despite its evolutionary distance from humans, *C. elegans* shares many conserved metabolic pathways and gene homologs with humans, enabling in-depth analysis of these shared mechanisms. This makes *C. elegans* a key model for bridging both in vitro and in vivo systems, as well as for advancing research on human and environmental health, aligning with the 'One Health' framework (von Mikecz 2022).

3 *C. elegans* in the Field of Nanotoxicology and Nanosafety

The toxicological potential of engineered nanoparticles (ENPs) has become a growing concern due to their significant release into the environment, positioning them among the group of emerging contaminants. Despite their widespread application in medical and clinical settings, the interactions between these nanomaterials and biological systems are not yet fully understood. This nano-bio interaction knowledge gap has prompted extensive studies using various biological models, including *C. elegans* (Table 11.1). Utilizing *C. elegans* allows researchers to explore the fate and toxicity of NPs within a multicellular organism. *C. elegans* proves to be a valuable model for assessing NP toxicity across different exposure scenarios, including acute, prolonged, and chronic treatments through oral ingestion, topical application, or microinjection. The model supports the evaluation of numerous endpoints, such as physiological effects like average body length and brood size, which indicate developmental and reproductive health. Furthermore, *C. elegans* facilitates the study of molecular mechanisms by employing biological markers like gene expression, green fluorescent protein (GFP) reporters, and application of specific mutant strains which provide insights into the specific pathways and mechanisms affected by NP exposure (Tejeda-Benitez and Olivero-Verbel 2016; Wu et al. 2019; Gonzalez-Moragas et al. 2015a). The efforts are in line with high throughput screening for several nanomaterials with various doses to target several physiological endpoints (Jung et al. 2015).

Table 11.1 Assessment of nanosfaety of manufactured nanomaterials in the bridging model, *C. elegans*, with various toxicological endpoints

Nanomaterials	*C. elegans* (Stage)	Exposure				
Name	Stage	Conc.	Time	Media (food '+')	Endpoints	References
Silver nanoparticles (AgNP)	Young adult (3 days old)	0.05, 0.1, and 0.5 mg/L	24 h and 72 h	K-media	Lethality, growth, reproduction, gene expressions (microarray)	Roh et al. (2009)
Citrate-coated (CIT10), PVP-coated smaller size range (PVPS) and larger size range (PVPL)	L1	0.5, 5, and 50 mg/L	3 days	K-media (+)	Growth, uptake	Meyer et al. (2010)
Silver nanoparticles (AgNP)	Young adult (3 days old)	0.05 mg/L	24 h	EPA water	Lethality, ROS formation, gene/protein expressions	Eom et al. (2013)
Citrate-coated silver nanoparticles (cAgNPs)				NGM (?)	Lethality and reproduction	Kim et al. (2012)
Commercial nano-silver (AgNP1 and PVP coated AgNP28)	L2	0.5–10 mg/L (AgNP1) 0.6–3 mg/L (AgNP28)	24, 48, 72 and 96 h	K-media (+/−)	Lethality	Ellegaard-Jensen et al. (2012)
Bare (AgNP) and two PVP-coated (PVP8 and PVP38) silver nanoparticles	Young adult (3 days old)	0.025, 0.05, 0.075 µg/mL	24 h	EPA water	Lethality, ROS formation, oxidative DNA damage (8OHdG), mitochondrial membrane potential	Ahn et al. (2014)
Citrate-coated (CIT7), small and large PVP -coated (PVPS, PVPL, PVP8 and PVP38). Gum Arabic-coated (GA5 and GA22)	L1	20–46 µM of total silver	3 days	K-media and EPA water (+)	Growth (in COPAS biosort)	Yang et al. (2012)

Silver nanoparticles (AgNP)	Young adult (3 days old)	0.025 mg/L (LC10) and 0.05 mg/L (LC50)	24 h	EPA water	Lethality, oxidative DNA damage (8OHdG), gene expression	Chatterjee et al. (2014a)
Citrate (cit-), PVP, PEG, BPEI-coated silver nanoparticles and silver plates	L1 and adult	0–100 µg/mL	24 h and 3 days	*C. elegans* habitation reagent (CeHR), water, and organic non-fat cows' milk (CeHRM)	Lethality, growth (in COPAS biosort), locomotion, gene expression (microarray), uptake	Hunt et al. (2014)
Silver nanoparticles (as 1 mg/mL in citrate buffer)	L1, L2 and adult	0–50 µg/mL	3 days	CeHRM	Growth (in COPAS biosort), morphology, oxidative DNA damage (several lesions GC/MS/MS) detected in and uptake	Hunt et al. (2013)
Citrate-coated AgNPs (CIT-AgNPs)	L1 and adult	0.1–1.5 mg-Ag/L	24 h and 3 days	EPA water (+)	Lethality, growth	Maurer et al. (2016)
Ag-MNP and sulfidized Ag-MNPs (sAg-MNPs)	L1 and L3	No food: 50–1500 µg/L, with food: 1000–10,000	24 h, 48 h and 50 h	Low chloride MHRW medium	Lethality, growth, reproduction	Starnes et al. (2015)
Silver nanoparticles (NM300K)	L1	0.2–10 mg/L	20 h and 96 h	M9 media (+)	Reproduction, feeding	Kleiven et al. (2018)
PVP-coated silver nanoparticles	L1	0.01–10 µg/mL	24, 48 and 72 h	Semi-fluid nematode growth gelrite media (Dengg and van Meel) (+)	Reproduction, lifespan, growth, ROS formation, mitochondrial membrane potential, apoptosis	Luo et al. (2017)
Silver nanoparticles using PVP as dispersant	L4	0.005–1 mg/L	6 h and 24 h	Multi-well and microfluidic chip-based chamber (+)	Development, growth, protein expression (reporter)	Kim et al. (2017b)

(continued)

Table 11.1 (continued)

Nanomaterials	*C. elegans* (Stage)	Exposure				
Name	Stage	Conc.	Time	Media (food '+')	Endpoints	References
(PVP)-coated (Ag-PVP) and sulfidized (Ag_2S) silver nanoparticles	L1	0.75–24 mg Ag/L (Ag-PVP) and 7.5–240 mg Ag/L (Ag_2S)	24 h and multigenerational	NGM (multigenerational maintenance) and exposure to simulated soil pore water (SSPW) (+)	Reproduction, lifespan, growth, multi-generation	Schultz et al. (2016)
Silver nanoparticles (AgNP)	L1	1–25 mg/mL	24 h and multigenerational	NGM (+) OP50 *E. coli* was treated with AgNP for 12 h and used to fed worms	Uptake & accumulation, reproduction, lifespan	Luo et al. (2016)
Silver nanoparticles (AgNP)	Egg, L4	0.0625–4 mg Ag/L	48 h and 72 h	NGM and simulated soil pore water (SSPW) (+)	Reproduction and growth	Tyne et al. (2015)
Oleic acid coated-silver nanoparticles	L4	1–100 mg Ag/L	24 h	NGM (+)	Reproduction, lifespan, growth, locomotion behaviour	Contreras et al. (2014)
Aluminium nanoparticle (Al_2O_3-NP)	L1, L4 and young adult		3 days, 48 h and 24 h	K-media	Lethality, stress response (reporter strain), intestinal autofluorescence	Wu et al. (2011)
Aluminium nanoparticle (Al_2O_3-NP)	Young adults	0.01–23.1 mg/L	6 h, 24 h and 10 days	K-media and NGM (+)	Locomotion, gene expression, stress response (reporter strain), oxidative stress (ROS, superoxide dismutase (Soddu et al.) activity, carbonylated proteins)	Li et al. (2012)
Gold nanoparticles (AuNP)	L4	100 µg/mL	24 h	NGM (+)	Lethality, reproduction, gene expression	Gonzalez-Moragas et al. (2017a)

Citrate-coated gold nanoparticles (AuNP)	L1, L4	1, 10 and 100 µg/mL	24 h, 3 days	S-media, NGM (+)	Growth, behaviour (feeding, pharyngeal pumping, chemotaxis), morphology of neurons, calcium transit, gene expressions (RNA-seq)	Wang et al. (2023b)
Bare gold (Bare-Au) and different proportions of 11-mercaptoundecanoic acid (MUA)-coated (MUA/Au-0.5 MUA/Au-1 MUA/Au-3)	Eggs	0.1 mg/mL	72 h	S-media (+)	Uptake, growth, locomotion (thrashing), reproduction, primary neuron visualization (isolated and cultured), gene expressions (microarray)	Hu et al. (2018b)
Gold nanocolloids (AuNP)	L1	0, 5, 25, and 50×10^{10} particles/mL (mixed with *E. coli* OP50)	48 h	NGM (+)	Survival, reproduction (number of offspring and reproductive system abnormalities), lipofuscin accumulation, multigenerational effects	Moon et al. (2017)
Gold nanocolloids (AuNP)	L1	5, 25, and 50×10^{10} particles/mL (mixed with *E. coli* OP50)	12 h	NGM (+)	Survival, reproduction, transgenerational effects	Kim et al. (2013)
Gold nanoparticles (AuNP)	L3	2.5, 5.5, 7, 15, and 30 mg/L	12 h	K-media	Gene expressions (microarray ad qPCR) and pathway analysis	Tsyusko et al. (2012)
Two boron nitride nanospheres BN-NS and BN-800-2	L1, L4	1, 10, 100, and 500 µg/mL	24, 48 & 72 h	K-media (+)	Growth, lifespan, reproduction, locomotion, oxidative stress (gene expression, ROS formation), GABA neuron (reporter)	Wang et al. (2017)

(continued)

Table 11.1 (continued)

Nanomaterials	*C. elegans* (Stage)	Exposure				
Name	Stage	Conc.	Time	Media (food '+')	Endpoints	References
CdTe quantum dots (QDs)	L1	2.5–20 mg/L	72 h	NGM (+)	Autofluorescence, locomotion, GFP reporters, gene expression	Wu et al. (2016a)
CdTe quantum dots (QDs)	L1, L2	1–10 μM	12 h, 24 h	NGM (−)	Development, lifespan, autofluorescence	Wang et al. (2016)
CdTe quantum dots (QDs)	L4	400–1600 μg/mL	24 h, 72 h	NGM (+)	Locomotion, behaviour (learning & memory), gene expression, oxidative stress	Wu et al. (2015)
CdTe quantum dots (QDs)	L1, L4	10 nM	24 h	NGM (−)	Autophagy, lifespan	Zhou et al. (2015)
CdTe quantum dots (QDs)	L1	0.1–1 μg/L	3.5, 5 days	NGM (+)	Behaviour (feeding, defecation), development, reproduction, neurodegeneration, oxidative stress	Zhao et al. (2015)
Cerium oxide (CeO_2) nanoparticles	L4	0.17–17.21 μg/mL	24 h	NGM (+)	Growth, reproduction, oxidative stress	Rogers et al. (2015)
Polymer-cationic (diethylaminoethyl dextran; DEAE), anionic (carboxymethyl dextran; CM), and non-ionic (dextran; DEX) polymers-coated cerium oxide nanomaterials	L1, L2, L3	0–5000 mg Ce/L	24 h, 48 h	NGM (±)	Oxidative stress, metabolism, GFP reporters	Arndt et al. (2017)

Citrate- and bovine serum albumine-coated of superparamagnetic iron oxide nanoparticles (C-SPIONs and BSA-SPIONs)	L4	0–500 µg Fe/mL	24 h	Milli-Q water (−)	Lethality, reproduction	Gonzalez-Moragas et al. (2015b)
Zero-valent iron nanoparticles (Fe0 NPs)	L1, L4	5–500 mg/L	48 h, 65 h	NGM (+)	Bioaccumulation, chemical analysis, bioassays (fertility, locomotion, development) biodynamics modelling (TBTK) dose–response-based TD modelling	Yang et al. (2017)
Carboxymethyl cellulose (CMC)-stabilized nZVI, nanoscale iron oxide (nFe_3O_4),	L4	5–100 mg/L	48 h	NGM (+)	Reproduction, oxidative stress	Yang et al. (2016)
Copper oxide nanoparticles (CuONP)	L1	3.8–15.9 mg cu/L	96 h	NGM (+)	Growth, feeding, neurodegeneration, stress response	Mashock et al. (2016)
Copper oxide nanoparticles (CuONP)	L4	150 mg/L	48 h	K-media	Growth, reproduction, transgenerational assays	Wei et al. (2020)
Nickel nanoparticles (NiNP)	L1, L4	1.0, 2.5, and 5.0 µg/cm^2	24 h, 48 h	NGM (+)	Reproduction (fertility, brood size, egg laying rate, spermatogenesis), oxidative stress (ROS formation, SOD, CAT, and GSH level), apoptosis-related gene expressions	Kong et al. (2017)
Silica-nanoparticles (SiO_2)	L4	2.5 mg/mL	24 h	NGM (+)	Behaviour, bag of worm (BOW), protein (aggregome) expression, neurodegeneration (polyQ aggregates, reporter)	Scharf et al. (2016)

(continued)

Table 11.1 (continued)

Nanomaterials	*C. elegans* (Stage)	Exposure				
Name	Stage	Conc.	Time	Media (food '+')	Endpoints	References
Four types mesoporous silica particles (bare and functionalized with hydrolyzed starch—M0, M1, N0 and N1)	L1	0.5, 5.0, 50 µg/mL	2 days, 21 days	NGM (+)	Uptake, lifespan, locomotion, reproduction, oxidative stress (defence to H_2O_2 exposure)	Acosta et al. (2018)
Silica nanoparticles (SiNPs)	Young adult	20–500 mg/L	24 h, 48 h. 72 h	K-media (±)	Lethality, reproduction, ROS formation, genome-wide transcriptional changes (microarray), endocytosis (inhibitor, gene expressions)	Eom and Choi (2019)
Amorphous silica nanoparticles (SiNPs)	L4	100 µg/mL	24 h	K-media (+)	Genome-wide transcriptional changes (microarray of mRNA and miRNA)	Liang et al. (2020a)
Mesoporous silica nanoparticles (mSiNPs)	L1, L4	3, 30, 300 and 3000 µg/L	24 h, 48 h	K-media (±)	Intestinal autofluorescence, behaviour (shrinking, foraging, locomotion, pharyngeal pumping, defecation), GABA neurodegeneration (reporter, gene expression), ROS formation	Liang et al. (2020b)
Silica nanoparticles (SiO2 NPs)	L4	0.25–1 mg/mL	24 h	K-media (+)	Lethality, growth, reproduction, locomotion, apoptosis, oxidative stress (ROS formation, GST, MDA level), gene expressions	Zhang et al. (2020)

Fluorescent silicon nanoparticles (SiNPs)	L1, L2, L4, young adult	50 mg/mL	4 h, 24 h, 3 days	NGM (+)	Uptake, growth, lifespan, reproduction, endocytic sorting, stress response, endoplasmic reticulum and mitochondrial stress (reporter), gene expressions (immune response, apoptosis, hypoxia, metal detoxification and aging)	Wang et al. (2022)
TiO2: anatase and rutile	Egg, L1, L4	100–500 mg/mL	72 h	S-media (+)	Uptake and internalization, growth and development, locomotion, reproduction, neuronal function (isolated and cultured), genome-wide transcriptional changes (microarray)	Hu et al. (2018a)
TiO_2	Young adult	2–10 mg/L (±UV)	24 h, 72 h	K-media (±)	Lethality, reproduction, ROS formation, genome-wide transcriptional changes (RNA-seq), global metabolomics, gene expressions	Kim et al. (2017a)
TiO_2	L4	0.01–1 mg/L	2 h	Distilled water (−) followed by NGM (+)	Pharyngeal pumping, reproduction (egg laying and larval growth), development	Iannarelli et al. (2016)
TiO_2	L4	1–500 mg/L	24 h, 72 h	NGM (±)	Locomotion, growth, neurodegeneration, genome-wide transcriptional changes (microarray)	Rocheleau et al. (2015)
TiO_2	L4	7.7–38.5 μg/mL	24 h	NGM (−)	Lethality, oxidative stress, global metabolomics	Ratnasekhar et al. (2015)

(continued)

Table 11.1 (continued)

Nanomaterials	*C. elegans* (Stage)	Exposure				
Name	Stage	Conc.	Time	Media (food '+')	Endpoints	References
TiO_2	L1, L4	1–100 µg/L, 10–100 mg/L	24 h, 72 h		Lethality, reproduction, oxidative stress, gene expression intestinal autofluorescence, oxidative stress, defecation cycle	Zhao et al. (2014)
TiO_2	L1	0.0001–1 µg/L	72 h	NGM (+)	Lethality, reproduction, lifespan, development, growth, oxidative stress, intestinal autofluorescence, behaviour (locomotion, pharyngeal pumping, defecation)	Wu et al. (2014c)
TiO_2	L1	1–100 mg/L	96 h	NGM (+)	Lethality, reproduction, gene expression, oxidative stress	Angelstorf et al. (2014)
TiO_2	Young adult	20 µg/L, 25 mg/L	24 h	K-media (+)	Lethality, growth, reproduction, locomotion, intestinal autofluorescence, ROS formation, gene expression	Rui et al. (2013)
ZnO	L1	0.614–614 µM	72 h	NGM (+)	Reproduction, locomotion	O'Donnell et al. (2017)
ZnO	L4		24 h, 72 h	Milli-Q water (±)	Growth, reproduction, locomotion, gene expression, fertility, oxidative stress	Khare et al. (2015)
ZnO	L4	0.1–2.0 g/L	24 h	NGM (−)	Lethality, oxidative stress	Gupta et al. (2015)
ZnO	L1	5–50 mg/L	48 h, 72 h	NGM (+)	Growth, reproduction, lifespan, ROS formation, gene expression, bacterial growth inhibition	Polak et al. (2014)
TiO_2 and ZnO	L1, L4	20–200 mg/mL (TiO_2) 0.125–0.8 mg/mL (ZnO)	24 h, 72 h	NGM (±)	Lethality, oxidative stress (ROS formation, antioxidant recovery)	Sonane et al. (2017)

AgNP, ZnO and CeO_2	L1/L2	1, 10, 100 µg/mL AgNP; 20, 80, 160 µg/mL for ZnO and CeO_2	~40 days (total life)	S-media (+)	Lifespan, behaviour, bag of worm (BOW), neurodegeneration (reporter)	Piechulek and von Mikecz (2018)
Inorganic nanoparticles (SiO_2, TiO_2, and ZnO)	L4	0.05 mg/mL	~22 days (total life)	NGM (+)	Life span	Ma et al. (2018)
Silver nanoparticles, multiwalled carbon nanotubes (MWCNT) and polyamidoamine dendrimers (PAMAM)	L1	10^{10} particles/mL	24 h, 48 h, 72 h	NGM	Lethality, growth, genome-wide transcriptional changes (microarray)	Walczynska et al. (2018)
Graphene oxide (GO) and reduced graphene oxide (rGO)	Young adult	20–100 mg/L	24 h, 48 h, 72 h	K-media (±)	Lethality, reproduction, gene expression, fluorescence in reporter strains	Chatterjee et al. (2017)
Graphene oxide (GO)	L1	1–100 mg/L	72 h	K-media (+)	Distribution and translocation, behaviour (locomotion, defecation), ROS formation, fluorescence in reporter strains, fat content (lipid and triglyceride level)	Zhi et al. (2016)
Graphene oxide (GO)	L1	1–100 mg/L	72 h	K-media (+)	Lethality, locomotion, oxidative stress	Zhao et al. (2016c)
Graphene oxide (GO)	L1	1–100 mg/L	72 h	K-media (+)	Cell apoptosis, fertility, reproduction, DNA damage, gene expression	Zhao et al. (2016a)
PEG modified graphene oxide (GO-PEG)	L1	1, 10, and 100 mg/L	72 h	K-media (+)	Distribution and translocation, biological permeability, gene expressions	Zhao et al. (2018)
Graphene oxide (GO)	L1	1, 10, and 100 mg/L	72 h	K-media (+)	Genome-wide functional analysis of long-noncoding RNA	Wu et al. (2016b)

(continued)

Table 11.1 (continued)

Nanomaterials	*C. elegans* (Stage)	Exposure				
Name	Stage	Conc.	Time	Media (food '+')	Endpoints	References
Graphene oxide (GO) and PEG modified graphene oxide (GO-PEG)	L1, young adult	0.001–1 mg/L	11 days, 8 days	K-media (+)	Lethality, behaviour (locomotion, defecation), intestinal auto- fluorescence, ROS formation, immune response (OP50 accumulation, related gene expression), AVL and DVB neurons visualization	Wu et al. (2014a)
Graphene oxide (GO)	L1	100 mg/L	3.5 days	K-media (+)	Distribution and translocation, lifespan, reproduction, effects on targeted organs, behaviour (locomotion, defecation), intestinal permeability (lipid level and triglyceride content); oxidative stress (ROS formation, related mutants)	Wu et al. (2014b)
Graphene oxide (GO)	L1	0.1–100 mg/L	3.5 days	K-media (+)	Distribution and translocation, lifespan, locomotion, autofluorescence, ROS formation, global micro-RNA expression (SOLiD sequencing)	Wu et al. (2014d)
Graphite (G), graphite oxide nanoplatelets (GO) and graphene quantum dots (GQDs)	L1	1–100 mg/L	6 days	K-media (+)	Distribution, lethality, behaviour (locomotion), head thrashing, body bending, pharyngeal pumping, neuronal analysis (reporter strains)	Li et al. (2017)
Graphene oxide (GO)	L1, young adult	10–100 mg/L	24 h, 72 h	K-media (+)	Locomotion, ROS formation, gene expression, GFP reporters	Ren et al. (2017)

Graphene oxide (GO)	L4	0.1, 1, 10 mg/L	24 h	K-media (+)	Distribution-translocation, lipid level, lipofuscin	Ren et al. (2018)
Graphene oxide (GO)	L1	10–100 mg/L	72 h	K-media (+)	Lethality, lifespan, autofluorescence, ROS formation, locomotion, lipid content, gene expression	Qu et al. (2017)
Multiwalled carbon nanotubes (MWCNT)	L1	1 mg/L	3.5 days	K-media (+)	Distribution and translocation, lethality, lifespan, locomotion, GFP reporters	Zhuang et al. (2016)
Multiwalled carbon nanotubes (MWCNT)	L1	1 mg/L	72 h	K-media (+)	Global gene (mRNA, miRNA) expressions (RNA-seq), mRNA-miRNA network, reproduction, locomotion, ROS formation, lipid content	Zhao et al. (2016b)
Pristine and hydroxylated (OH)-MWCNTs	L4	1–500 mg/L	4 h, 24 h, 48 h, 72 h	K-media (±)	Lethality, reproduction, feeding, oxidative stress, global gene expressions (microarray) and proteins (proteomics)	Eom et al. (2015)
Pristine and carboxylated single-walled carbon nanotubes (SWCNTs–COOH)	L1, L4	0.001–1000 μg/L	24 h, 48 h	K-media	Lethality, growth, lifespan, reproduction, ROS generation, locomotion, gene expression	Lu et al. (2022)
Pristine and amide-modified single-walled carbon nanotubes (a-SWCNTs)	L1	1–500 μg/mL	48 h, 72 h	NGM (+)	Lifespan, growth, reproduction, feeding, endocytosis, global transcriptomics (microarray), oxygen consumption rate, Daf-16 GFP reporter	Chen et al. (2013)
Fluorescent single-walled carbon nanotubes	Adult	0.1–300 mg/L	24 h, 8 day	NGM (−)	Viability, imaging	Hendler-Neumark et al. (2021)

PVP polyvinylpyrrolidone, *NGM* Nematode growth medium, *ROS* Reactive oxygen species, *BPEI* Branched polyethyleneimine (BPEI), *PEG* Polyethylene glycol

3.1 General Physiological Endpoints Assessment

3.1.1 Survival/Mortality

In *C. elegans*, assessing survival rates is a primary approach for understanding the toxicity of nanoparticles (NPs). Mortality is usually determined by constructing concentration-response curves, which reflect how different doses of NPs influence the death rate of the nematodes (Gonzalez-Moragas et al. 2015a; Tejeda-Benitez and Olivero-Verbel 2016). A critical aspect of this evaluation is distinguishing between lethality and paralysis. While death is indicated by the complete cessation of movement and physiological activity, paralysis refers to immobility where nematodes still exhibit basic life functions, such as normal pharyngeal pumping (Wang 2018). This distinction is crucial to avoid overestimating the toxic effects of NPs based on immobility alone.

3.1.2 Growth and Development

Growth and developmental outcomes in *C. elegans* provide important sublethal endpoints for assessing NP toxicity. One key indicator of developmental progress is the body length of nematodes, as exposure to NPs can delay their growth, especially at early stages like the first and second larval stages (Hu et al. 2018a, b). The inhibition of growth is often associated with disruptions in key biological processes, such as the endocytic process, which plays a significant role in mitigating NP-induced stress. For example, studies suggest that normal lysosomal function is vital for nematode growth under stress from silver nanoparticles (AgNPs) (Maurer et al. 2016). Additionally, NP toxicity may reduce the availability of food or reduces the food sensation which can further hinder growth by limiting nutrient intake (Meyer et al. 2010; Wang et al. 2023b).

3.1.3 Reproduction

Reproductive health is one of the most sensitive indicators of NP toxicity in *C. elegans*, often affected at lower concentrations than those that impair survival or movement. Reproductive toxicity is measured by comparing the reproductive capabilities of NP-exposed nematodes to a control group, focusing on factors such as the number of offspring, brood size, and rate of egg laying (Kong et al. 2017; Zhao et al. 2016a). A decline in reproductive output, often reflected in reduced brood size or increased sterility, is a common observation following NP exposure. In particular, nanoparticles like ZnO and graphene oxide (GO) have been found to induce damage in the gonads of nematodes through mechanisms such as germline apoptosis and cell cycle arrest, which are triggered by DNA damage (Zhao et al. 2016a; O'Donnell et al. 2017). Furthermore, NP-induced damage may not be confined to a single generation; reproductive abnormalities can be passed down to future generations. For instance, after exposure to AuNPs, the F2 generation exhibited significant reproductive system abnormalities, though these effects gradually diminished by the F4 generation, suggesting an adaptive response across generations (Kim et al. 2013). Similar multi-generational studies' impact underscores the need to consider long-term reproductive effects in NP toxicity studies (Contreras et al. 2013; Moon et al. 2017).

3.1.4 Behavioural Alterations

Behavioral changes in response to environmental stressors, including chemicals and pollutants, have long been recognized as critical indicators of organismal and ecological health. Various environmental contaminants can adversely affect an organism's behavior, influencing key activities such as feeding, locomotion, reproduction, and cognitive functions. These behavioral disruptions can cascade into broader ecological consequences, affecting species interactions, predator-prey dynamics, and ecosystem balance. However, behavioral studies have been underrepresented in regulatory ecotoxicology, primarily due to a lack of standardized methods for assessing these effects (Ford et al. 2021). The growing understanding of how environmental stressors alter behavior has emphasized the need for including behavioral metrics in risk assessments to better capture the full scope of toxicity.

When it comes to nanoparticle (NP) exposure, behavioral toxicity has been extensively studied in *C. elegans*, a key model organism. Nanomaterials such as Al_2O_3NPs, CdTe QDs, oleic acid-coated AgNP impair both locomotion and learning abilities in *C. elegans*, indicating neurotoxic effects (Contreras et al. 2014; Wu et al. 2015; Li et al. 2012). Feeding behavior is also disrupted, with nanoparticles like CdTe QDs and Zein-NPs altering pharyngeal pumping speed, RMEs motor neurons, and defecation cycles, which can lead to increased fat storage (Zhao et al. 2015; Lucio et al. 2017). Moreover, chronic exposure to graphene-based NPs causes a significant reduction in crawling distance, mean speed, and bending reversal frequency, all of which indicate a loss of motor coordination and balance (Li et al. 2017).

3.2 Mechanistic Endpoints Evaluations

3.2.1 Application of Mutants and Transgenic *C. elegans* Strains

The use of reverse genetics allows precise manipulation of gene activity in *C. elegans*, enabling researchers to target any gene in the organism. Tools like small interfering RNAs (siRNAs) are valuable for studying the function of single genes in *C. elegans*. Additionally, the extensive library of transgenic, mutant, and reporter strains from the *C. elegans* consortium offers a valuable resource for studying nanoparticle toxicity. Researchers can use these strains to explore molecular pathways, cellular responses, and genetic variations, providing insights into toxicity mechanisms. Various phenotypic effects, including survival, growth, reproduction, and lifespan changes, have been examined in both wild-type and mutant strains in nanotoxicology studies (Rogers et al. 2015; Wang et al. 2017; Qu et al. 2018; Chatterjee et al. 2017). Moreover, transgenic *C. elegans* strains that replicate human molecular disease mechanisms, which are difficult to study in other models, are utilized to assess the toxic effects of nanoparticles (NPs) in organisms affected by chronic conditions like neurodegenerative diseases. For instance, Soria et al. demonstrated that silver nanoparticles (AgNPs) had a more severe impact on movement and oxidative stress in *C. elegans* strains mimicking Alzheimer's disease than in wild-type strains (Soria et al. 2015).

3.2.2 OMICS Platforms for Gene Expression and Toxicity Pathways

Transcriptomics is a powerful tool to study large-scale gene expression changes in *C. elegans* exposed to NPs. Various studies have shown that exposure to NPs affects genes involved in oxidative stress, metal detoxification, DNA damage, endocytosis, and intestinal integrity, with the extent of these effects dependent on the concentration and exposure duration (Starnes et al. 2019; Tsyusko et al. 2012; Hunt et al. 2014; Rocheleau et al. 2015; Gonzalez-Moragas et al. 2017b). Integrating transcriptomics data with proteomics and metabolomics provides a comprehensive understanding of how NPs influence biological processes, contributing to the development of adverse outcomes relevant to risk assessment (Eom et al. 2015; Ratnasekhar et al. 2015). The combination of multiple OMICS techniques allows a more detailed mapping of NP-induced biological changes at various levels of organization, enabling researchers to link molecular alterations with functional outcomes in *C. elegans*.

3.2.3 Oxidative Stress, Innate Immunity, and Signalling Pathway Alterations

Oxidative stress is considered a key mechanism through which NPs cause toxicity in *C. elegans*. The accumulation of reactive oxygen species (ROS) in NP-treated nematodes has been linked to adverse outcomes such as reduced lifespan, impaired growth, and reproductive damage, in a dose- and time-dependent manner (Wu et al. 2012a, b; Ahn et al. 2014; Eom et al. 2013; Lim et al. 2012; Yu et al. 2011; Li et al. 2012). Excessive ROS generation can lead to functional defects even in organs that do not retain NPs, such as reduced locomotion and reproductive issues. Interestingly, pre-treatment with antioxidants like ascorbate or N-acetyl-l-cysteine (NAC) can mitigate these effects (Wu et al. 2013; Lim et al. 2012; Li et al. 2012).

Several signalling pathways, including mitochondrial complex I and MAPK pathways, have been identified as critical regulators in controlling NP-induced oxidative stress and toxicity (Lim et al. 2012; Li et al. 2020; Teng et al. 2024; Eom et al. 2013). Additionally, genes like sod-3, gst-4, and hsp-16, which are associated with stress responses, have been highlighted as sensitive markers for NP toxicity (Li et al. 2012; Zhao et al. 2015; Rui et al. 2013; Wu et al. 2014b).

The exposure of *C. elegans* to nanoparticles (NPs) leads to significant alterations in multiple signalling pathways, which are essential for understanding the mechanisms of NP-induced toxicity. One key pathway is the Wnt signaling pathway, where ligands like CWN-1, CWN-2, and LIN-44 regulate NP toxicity by controlling NP accumulation, with mutations in these genes either increasing resistance or susceptibility (Zhi et al. 2016; Chatterjee et al. 2017). Similarly, the insulin/IGF-1 pathway, particularly through the DAF-2/DAF-16 axis, is involved in longevity and stress resistance, with miRNAs such as mir-355 modulating NP toxicity via insulin signalling (Zhao et al. 2016b). Additionally, the TGF-β pathway is implicated in reproductive toxicity, where disruption by NPs, such as titanium dioxide, causes damage to reproductive capacity and developmental processes (Kim et al. 2017a).

The MAPK signalling pathway is also critical in stress responses. In particular, the p38 MAPK-SKN-1/Nrf cascade is involved in the innate immune response,

offering protection against oxidative stress induced by NPs like graphene oxide (Zhao et al. 2016c). Chronic GO exposure impairs immune function by causing the accumulation of pathogenic microbes like OP50 in the intestine, which disrupts innate immunity. However, surface modification, such as PEG, reduces this toxicity (Wu et al. 2014a). GO also activates the p38 MAPK pathway, with PMK-1 playing a key protective role, while amino-functionalized GO shows less immunotoxicity, highlighting the importance of nanoparticle modification (Rive et al. 2019). AgNPs trigger oxidative stress and activate PMK-1, leading to immune defence responses (Lim et al. 2012). The ERK signalling pathway is also involved in regulating GO toxicity, working in synergy with p38 MAPK to control immune responses (Qu et al. 2017). Additionally, ZnO-NPs suppress innate immunity regulated by SKN-1/Nrf and the p38 MAPK signalling pathway, decreasing survival during infection and downregulating key immune genes (Li et al. 2020).

3.2.4 Neurotoxicity and Neurodegeneration

Nearly all behavioural endpoints in *C. elegans*—such as locomotion, body bending, feeding, defecation, pharyngeal pumping, egg-laying, sensory perception, learning, and memory—are controlled by the nervous system and achieved through muscle contractions. Exposure to nanoparticles (NPs) has been shown to disrupt these behaviours. For instance, a reduction in feeding and defecation behaviours is often linked to NP-induced stress and alterations in pharyngeal pumping and defecation cycles (Wu et al. 2015). CdTe quantum dots (QDs), graphene-based nanomaterials, and copper oxide nanoparticles have been found to cause significant damage to dopamine and glutamatergic neurons in *C. elegans*, leading to abnormal feeding behaviour developmental deficits, neurodegeneration, and abnormalities in the neural network (Zhao et al. 2015; Mashock et al. 2016; Li et al. 2017). Nanoparticles like silver (AgNPs) have been shown to impair a range of neuronal systems, including dopaminergic, GABAergic, and cholinergic neurons, affecting locomotion and sensory perception. The severity of these effects depends on both the dose and duration of exposure (Zhang et al. 2021). Additionally, hybrid nanoparticles such as Fe_3O_4@Ag-NPs have been linked to neurotoxicity by disrupting cholinergic neurons and inducing oxidative stress, leading to behavioural impairments and apoptosis in *C. elegans* (Silva et al. 2023). Graphene oxide (GO) NPs also exhibit considerable neurotoxicity. GO exposure causes damage to AFD sensory neurons, reduces neurotransmitter levels such as dopamine, GABA, and tyramine, and leads to altered locomotion behaviors like reduced speed and coordination (Kim et al. 2020a). Silica (SiO_2) nanoparticles have also shown neurotoxic effects, particularly in disrupting serotonergic neurotransmission. These impairments are associated with neuromuscular defects, notably affecting the egg-laying apparatus in *C. elegans*, which can be mitigated by anti-amyloid compounds (Scharf et al. 2016). This indicates that SiO_2 NPs can interfere with reproductive and muscular systems, compounding their neurotoxic effects. Additionally, exposure to titanium dioxide (TiO_2) NPs has been linked to neuron damage and impaired locomotion, further highlighting the broad toxicological impact of various nanomaterials (Hu et al. 2018a).

3.2.5 Genotoxicity, Mutation, DNA Damage Response, and Apoptosis

Genotoxicity in *C. elegans* can be assessed through several established techniques. Methods like qPCR measure DNA damage by detecting how lesions inhibit polymerase progression, with the extent of damage indicated by the length of the PCR products (Leung et al. 2010). The comet assay has been used to evaluate the genotoxicity of environmental pollutants (Imanikia et al. 2016). Additionally, transgenic strains like hus-1::GFP are utilized to visualize DNA double-strand breaks, where fluorescent foci in gonadal germ cells indicate the extent of damage, allowing precise quantification (Wang et al. 2014; Hofmann et al. 2002). These methods provide a clear understanding of the DNA damage response, involving checkpoint activation that leads to either cell cycle arrest or repair or, in severe cases, apoptosis (Gartner et al. 2004; Craig et al. 2012).

Nanoparticles, particularly silver nanoparticles (AgNPs), graphene oxide (GO), and zinc oxide nanoparticles (ZnO NPs), have been shown to induce significant genotoxicity in *C. elegans*. Smaller, uncoated AgNPs, for example, cause oxidative stress that leads to mitochondrial membrane damage and oxidative DNA damage, such as 8-OHdG lesions (Ahn et al. 2014). This oxidative DNA damage triggers the activation of DNA repair mechanisms, such as DNA glycosylases like NTH-1, which specifically repair oxidative lesions. PMK-1, a p38 MAPK homolog, also plays a protective role in mitigating AgNP-induced DNA damage through repair pathways (Chatterjee et al. 2014a). Similarly, GO nanoparticles activate key components of the apoptosis pathway, such as cep-1 (a homolog of p53), egl-1, ced-4, and ced-3, which either arrest the cell cycle or induce apoptosis when DNA damage becomes too severe to repair, highlighting the role of these pathways in maintaining genomic integrity (Zhao et al. 2016a). Surface modifications, such as coating GO nanoparticles with bovine serum albumin (BSA), have been shown to reduce the activation of DNA damage checkpoints and apoptosis-related genes, thus lowering toxicity (Sivaselvam et al. 2020). Furthermore, prolonged exposure to AgNPs over multiple generations has been linked to the accumulation of DNA damage, insufficient repair activation, and the inheritance of reproductive and developmental defects (Wamucho et al. 2019). Similarly, ZnO NPs disrupt germ cell development, triggering apoptosis through DNA damage checkpoints and causing chromosomal deletions, which impair reproductive capacity (Wang et al. 2023a).

3.2.6 Epigenetic Biomarkers

Emerging research suggests that epigenetic mechanisms, particularly microRNAs (miRNAs), play crucial roles in mediating protective or harmful responses in *C. elegans* exposed to NPs. For example, prolonged exposure to graphene oxide (GO) was shown to significantly affect miRNA-regulated biological processes like development, reproduction, and cell cycle regulation (Wu et al. 2014d). Certain miRNAs, such as mir-259 and mir-360, have been identified as key players in protecting against NP-induced oxidative stress and DNA damage in nematodes (Zhuang et al. 2016; Zhao et al. 2016a). Furthermore, miRNA-mRNA interaction networks, including the regulation of mir-355 with the DAF-2/insulin receptor, have been

linked to the modulation of NP toxicity in *C. elegans* (Zhao et al. 2016b). Similarly, long non-coding RNAs (lncRNAs) have been implicated in controlling NP toxicity, further emphasizing the importance of epigenetic regulation in the organism's response to environmental stressors (Wu et al. 2016b).

3.3 Factors Affecting the Nano-Bio Interaction in *C. elegans*

3.3.1 Exposure

When assessing the toxicity of nanoparticles, it is crucial to consider both exposure concentration and duration. Researchers often use exposure ranges from non-toxic to threshold levels to establish dose-effect relationships. However, creating a precise dose tolerance curve for a specific nanoparticle is challenging due to variations in study conditions. Lower-order developmental stages, such as L1 larvae, are typically more sensitive than later stages, such as young adults. Moreover, longer exposure times generally lead to more severe effects compared to shorter ones, though hormesis effects observed in short-term exposures may diminish with prolonged exposure (Tyne et al. 2015). Additionally, intermittent exposure can sometimes produce more pronounced effects than continuous exposure, highlighting the importance of considering both exposure time and historical exposure in toxicity evaluations (Moon et al. 2017).

Nanoparticles often exhibit unstable behaviour in liquid media, such as K-medium and S-medium, where they can aggregate to sizes over 100 times their original dimensions and precipitate, thus reducing the effective exposure dose to organisms. Additionally, some metal nanoparticles in liquid media may partially dissolve or release ions due to hydration kinetics, complicating toxicity assessments. While the release of metallic ions is believed to contribute to observed toxicity, it remains unclear whether the effects are due to the particles themselves or the ions. Researchers suggest that simulated soil pore water (SSPW) provides a more realistic testing environment for metal nanoparticle toxicity in *C. elegans* due to its low ionic strength and organic content, which stabilize the nanoparticles (Tyne et al. 2013). In contrast, applying nanomaterials to whole NGM agar media can affect the effective exposure dose because the worms interact only with the solid surface of the NGM, not with the entire medium. Mixing nanoparticles with viable E. coli OP50, used as food, can alter nanoparticle transformation and toxicity evaluations. Applying a mixture of deactivated E. coli and selected nanomaterials spread over the surface of solid NGM plates as a lawn provides a more reliable exposure medium by minimizing biotransformation and enhancing nano-bio interactions, as demonstrated for diesel exhaust particles (Chatterjee et al. 2024). Additionally, semi-fluid nematode growth gelrite medium (Dengg and van Meel 2004) is suitable for nanoparticle toxicity evaluation compared to standard nematode growth medium (NGM) and K-medium, with Ag-NPs demonstrating stability in NGG without increased dissolution of Ag ions over time (Luo et al. 2017). Therefore, the choice of exposure medium—liquid, solid, or bacterial suspension—plays a crucial role in determining the effective concentration and toxicity of nanoparticles, underscoring the need for standardized testing protocols.

3.3.2 Physiochemical Properties of Nanomaterials

The physicochemical properties of nanomaterials, such as size, shape, surface modification, and charge, significantly influence their toxicity and biological interactions. These properties can affect how nanomaterials are absorbed, distributed, and accumulated within organisms, ultimately impacting their potential health risks and environmental effects.

Size

The correlation between nanoparticle size and toxicity is significant, with smaller nanoparticles generally causing more severe effects in *C. elegans* compared to larger ones. Smaller particles can penetrate more easily, leading to increased toxicity (Khare et al. 2015; Roh et al. 2010), possibly through mechanisms such as alterations in metabolic pathways (Ratnasekhar et al. 2015) or the formation of aggregates that limit food availability (Luo et al. 2016). However, the same study suggests that larger particles may accumulate more within the body, potentially causing long-term effects such as reduced lifespan, while impaired reproductive capacity was observed with smaller particle exposure (Contreras et al. 2014). The impact of nanoparticle size on toxicity is complex and may depend on factors such as agglomeration state and particle-specific effects (Jung et al. 2015).

Coating and Surface Modification

Surface modifications and coatings can significantly influence the toxicity of nanoparticles. Sulfidized silver nanoparticles (AgNPs), for example, exhibit reduced toxicity compared to uncoated AgNPs due to decreased solubility and limited silver ion release, which lowers their bioavailability and particle-specific toxicity (Starnes et al. 2015). Similarly, citrate coatings on AgNPs reduce silver ion availability, although they are less effective than BSA coatings (Yang et al. 2012; Hunt et al. 2014; Meyer et al. 2010). CdTe quantum dots (QDs) with ZnS coatings, unlike bare CdTe QDs, did not translocate into motor neurons, thereby avoiding neurotoxicity (Zhao et al. 2015). Surface modifications such as hydroxylation, carboxylation, and amination have also reduced the reproductive toxicity of multi-walled carbon nanotubes (MWCNTs), especially carboxylation, which might facilitate the elimination of functionalized MWCNTs than the pristine one (Chatterjee et al. 2014b). Additionally, PEG modification, commonly used in nanoparticles, effectively mitigates the negative effects of graphene oxide (GO) on both primary and secondary target organs (Wu et al. 2016b). However, some coatings, like gum arabic, can increase nanoparticle toxicity, while others, such as polyvinylpyrrolidone (PVP), show conflicting results, with studies reporting both higher and lower toxicity compared to uncoated nanoparticles (Bone et al. 2015; Yang et al. 2012; Ellegaard-Jensen et al. 2012; Ahn et al. 2014).

Charge

Positively charged nanoparticles tend to be more toxic to *C. elegans* and accumulate more than neutral or negatively charged particles. This increased toxicity and bioaccumulation are observed in most cases, highlighting the importance of particle charge in toxicity assessments (Collin et al. 2014; Arndt et al. 2017).

Shape

The shape of nanoparticles can influence their toxic effects in *C. elegans*. For example, different shapes of TiO_2 nanoparticles exhibit varying effects on pharyngeal function, reproduction, and larval growth (Iannarelli et al. 2016). Anatase-TiO_2 had a stronger impact on metabolic pathways compared to rutile, while rutile-TiO_2 influenced developmental processes more significantly (Rocheleau et al. 2015). Silver nanocubes generally show lower toxicity compared to quasi-spherical silver nanoparticles and silver nanowires, indicating that shape engineering can optimize nanoparticle properties while minimizing adverse effects (Gorka et al. 2015). Additionally, the crystalline structure could explain the differences in agglomeration behaviour observed in the intestine, which in turn influenced the reproductive toxicity of the TiO_2 material (Angelstorf et al. 2014).

3.3.3 Other Factors

Environmental factors such as UV irradiation can enhance the toxicity of metal oxide nanoparticles like ZnO and TiO_2 through mechanisms such as photocatalytic ROS generation and photo-enhanced dissolution (Ma et al. 2011, 2014; Lee and An 2013). Moreover, the stability and toxicity of nanoparticles are influenced by dissolved organic matter and the physiological properties of the test organism, such as pH and biomolecular interactions within the intestinal lumen (Gonzalez-Moragas et al. 2017a). Variations in toxicity may also result from differences in material formulation, nematode life stage, and testing procedures (Ma et al. 2013).

4 Conclusion and Perspectives

C. elegans has proven to be an effective and versatile model in nanotoxicology studies, particularly for initial biological screenings of nanoparticles (NPs). Its small size, low cost, and short lifespan facilitate large-scale, long-term toxicity assessments under controlled conditions, making it ideal for chronic exposure studies (Leung et al. 2008). Additionally, the transparency of *C. elegans* enables straightforward observation of NPs at both molecular and cellular levels, especially when using transgenic strains that express fluorescent markers (Scharf et al. 2013). Advances such as microfluidic chip platforms further enhance its utility, offering a high-throughput, on-site system for rapidly assessing NP uptake and toxicity while reducing labor and time requirements (Mondal et al. 2016; Rohde et al. 2007). These features, along with the nematode's genetic tractability and the conservation of many molecular pathways with humans (Kaletta and Hengartner 2006; Markaki and Tavernarakis 2020), make *C. elegans* a robust platform for nanotoxicology research (Wu et al. 2019).

Nevertheless, *C. elegans* has inherent limitations when used in nanotoxicology studies, particularly in comparison to mammalian models. For example, it lacks key mammalian organs such as the heart, kidneys, bones, and eyes, rendering it unsuitable for evaluating NP toxicity in these organ-specific systems. Additionally, the absence of a circulatory system restricts its ability to mimic intravenous NP exposure scenarios (Tejeda-Benitez and Olivero-Verbel 2016).

Despite these limitations, *C. elegans* continues to excel as a bridging model between ecological and human health risk assessments, aligning well with the 3R principles (Replacement, Reduction, and Refinement) and New Approach Methodologies (NAMs). By connecting in vitro and in vivo assessments, it supports more ethical, cost-effective, and efficient toxicity testing. Its capacity to evaluate a range of endpoints—including lethality, growth, reproduction, fertility, and locomotion—makes it invaluable for early-stage evaluations of nanomaterials. Furthermore, as a fully sequenced organism with high genetic tractability, *C. elegans* offers the added benefit of creating transgenic strains to study gene expression changes in response to toxicants and nanomaterials. This capability allows researchers to gain mechanistic insights into gene regulation and biochemical pathways affected by pollutants, toxicants, and nanoparticles. By observing direct molecular responses, such as changes in gene expression, *C. elegans* helps uncover the biological mechanisms underlying toxicity at various levels—from single-cell interactions to whole-organism responses. Consequently, *C. elegans* remains a highly effective and versatile model for advancing nanotoxicology, providing critical data that can enhance the safety and regulation of emerging nanomaterials.

Acknowledgments This study was funded by the European Union's H2020 projects, Sinfonia (N.857253), LEARN (N.101057510), iCare (N.101092971) and SbDToolBox, with reference NORTE-01-0145-FEDER-000047, supported by Norte Portugal Regional Operational Programme (NORTE 2020), under the PORTUGAL 2020 Partnership Agreement, through the European Regional Development Fund.

Declaration of Competing Interest None.

References

Acosta C, Barat JM, Martínez-Máñez R, Sancenón F, Llopis S, González N, Genovés S, Ramón D, Martorell P (2018) Toxicological assessment of mesoporous silica particles in the nematode Caenorhabditis elegans. Environ Res 166:61–70. https://doi.org/10.1016/j.envres.2018.05.018

Ahn JM, Eom HJ, Yang X, Meyer JN, Choi J (2014) Comparative toxicity of silver nanoparticles on oxidative stress and DNA damage in the nematode Caenorhabditis elegans. Chemosphere 108:343–352. https://doi.org/10.1016/j.chemosphere.2014.01.078

Anbalagan C, Lafayette I, Antoniou-Kourounioti M, Gutierrez C, Martin JR, Chowdhuri DK, De Pomerai DI (2013) Use of transgenic GFP reporter strains of the nematode Caenorhabditis elegans to investigate the patterns of stress responses induced by pesticides and by organic extracts from agricultural soils. Ecotoxicology 22:72–85. https://doi.org/10.1007/s10646-012-1004-2

Angelstorf JS, Ahlf W, Von Der Kammer F, Heise S (2014) Impact of particle size and light exposure on the effects of TiO2 nanoparticles on Caenorhabditis elegans. Environ Toxicol Chem 33:2288–2296. https://doi.org/10.1002/etc.2674

Arndt DA, Oostveen EK, Triplett J, Butterfield DA, Tsyusko OV, Collin B, Starnes DL, Cai J, Klein JB, Nass R, Unrine JM (2017) The role of charge in the toxicity of polymer-coated cerium oxide nanomaterials to Caenorhabditis elegans. Comp Biochem Physiol C Toxicol Pharmacol 201:1–10. https://doi.org/10.1016/j.cbpc.2017.08.009

Avila DS, Adams MR, Chakraborty S, Aschner M (2011) Chapter 16—Caenorhabditis elegans as a model to assess reproductive and developmental toxicity. In: Gupta RC (ed) Reproductive and developmental toxicology. Academic, San Diego. https://doi.org/10.1016/B978-0-12-382032-7.10016-5

Bone AJ, Matson CW, Colman BP, Yang X, Meyer JN, Di Giulio RT (2015) Silver nanoparticle toxicity to Atlantic killifish (Fundulus heteroclitus) and Caenorhabditis elegans: a comparison of mesocosm, microcosm, and conventional laboratory studies. Environ Toxicol Chem 34:275–282. https://doi.org/10.1002/etc.2806

Brenner S (2009) In the beginning was the worm. Genetics 182:413–415. https://doi.org/10.1534/genetics.109.104976

Buckingham SD, Sattelle DB (2009) Fast, automated measurement of nematode swimming (thrashing) without morphometry. BMC Neurosci 10:84. https://doi.org/10.1186/1471-2202-10-84

Chakravarty B (2022) The evolving role of the Caenorhabditis elegans model as a tool to advance studies in nutrition and health. Nutr Res 106:47–59. https://doi.org/10.1016/j.nutres.2022.05.006

Chalfie M, Tu Y, Euskirchen G, Ward WW, Prasher DC (1994) Green fluorescent protein as a marker for gene expression. Science 263:802–805. https://doi.org/10.1126/science.8303295

Chatterjee N, Eom HJ, Choi J (2014a) Effects of silver nanoparticles on oxidative DNA damage-repair as a function of p38 MAPK status: a comparative approach using human Jurkat T cells and the nematode Caenorhabditis elegans. Environ Mol Mutagen 55:122–133. https://doi.org/10.1002/em.21844

Chatterjee N, Yang J, Kim HM, Jo E, Kim PJ, Choi K, Choi J (2014b) Potential toxicity of differential functionalized multiwalled carbon nanotubes (MWCNT) in human cell line (BEAS2B) and Caenorhabditis elegans. J Toxicol Environ Health A 77:1399–1408. https://doi.org/10.1080/15287394.2014.951756

Chatterjee N, Kim Y, Yang J, Roca CP, Joo SW, Choi J (2017) A systems toxicology approach reveals the Wnt-MAPK crosstalk pathway mediated reproductive failure in Caenorhabditis elegans exposed to graphene oxide (GO) but not to reduced graphene oxide (RGO). Nanotoxicology 11:76–86. https://doi.org/10.1080/17435390.2016.1267273

Chatterjee N, González-Durruthy M, Costa MD, Ribeiro AR, Vilas-Boas V, Vilasboas-Campos D, Maciel P, Alfaro-Moreno E (2024) Differential impact of diesel exhaust particles on glutamatergic and dopaminergic neurons in Caenorhabditis elegans: a neurodegenerative perspective. Environ Int 186:108597. https://doi.org/10.1016/j.envint.2024.108597

Chen PH, Hsiao KM, Chou CC (2013) Molecular characterization of toxicity mechanism of single-walled carbon nanotubes. Biomaterials 34:5661–5669. https://doi.org/10.1016/j.biomaterials.2013.03.093

Collin B, Oostveen E, Tsyusko OV, Unrine JM (2014) Influence of natural organic matter and surface charge on the toxicity and bioaccumulation of functionalized ceria nanoparticles in Caenorhabditis elegans. Environ Sci Technol 48:1280–1289. https://doi.org/10.1021/es404503c

Contreras EQ, Cho M, Zhu H, Puppala HL, Escalera G, Zhong W, Colvin VL (2013) Toxicity of quantum dots and cadmium salt to Caenorhabditis elegans after multigenerational exposure. Environ Sci Technol 47:1148–1154. https://doi.org/10.1021/es3036785

Contreras EQ, Puppala HL, Escalera G, Zhong W, Colvin VL (2014) Size-dependent impacts of silver nanoparticles on the lifespan, fertility, growth, and locomotion of Caenorhabditis elegans. Environ Toxicol Chem 33:2716–2723. https://doi.org/10.1002/etc.2705

Craig AL, Moser SC, Bailly AP, Gartner A (2012) Methods for studying the DNA damage response in the Caenorhabditis elegans germ line. In: Rothman JH, Singson A (eds) Methods in cell biology. Academic. https://doi.org/10.1016/B978-0-12-394620-1.00011-4

Dengg M, Van Meel JC (2004) Caenorhabditis elegans as model system for rapid toxicity assessment of pharmaceutical compounds. J Pharmacol Toxicol Methods 50:209–214. https://doi.org/10.1016/j.vascn.2004.04.002

Ellegaard-Jensen L, Jensen KA, Johansen A (2012) Nano-silver induces dose-response effects on the nematode Caenorhabditis elegans. Ecotoxicol Environ Saf 80:216–223. https://doi.org/10.1016/j.ecoenv.2012.03.003

Eom HJ, Choi J (2019) Clathrin-mediated endocytosis is involved in uptake and toxicity of silica nanoparticles in Caenorhabditis elegans. Chem Biol Interact 311:108774. https://doi.org/10.1016/j.cbi.2019.108774

Eom HJ, Ahn JM, Kim Y, Choi J (2013) Hypoxia inducible factor-1 (HIF-1)–flavin containing monooxygenase-2 (FMO-2) signaling acts in silver nanoparticles and silver ion toxicity in the nematode, Caenorhabditis elegans. Toxicol Appl Pharmacol 270:106–113. https://doi.org/10.1016/j.taap.2013.03.028

Eom HJ, Roca CP, Roh JY, Chatterjee N, Jeong JS, Shim I, Kim HM, Kim PJ, Choi K, Giralt F, Choi J (2015) A systems toxicology approach on the mechanism of uptake and toxicity of MWCNT in Caenorhabditis elegans. Chem Biol Interact 239:153–163. https://doi.org/10.1016/j.cbi.2015.06.031

Ferreira DW, Chen Y, Allard P (2014) Using the alternative model C. elegans in reproductive and developmental toxicology studies. In: Faqi AS (ed) Developmental and reproductive toxicology. Springer New York, New York. https://doi.org/10.1007/7653_2014_27

Ford AT, Ågerstrand M, Brooks BW, Allen J, Bertram MG, Brodin T, Dang Z, Duquesne S, Sahm R, Hoffmann F, Hollert H, Jacob S, Klüver N, Lazorchak JM, Ledesma M, Melvin SD, Mohr S, Padilla S, Pyle GG, Scholz S, Saaristo M, Smit E, Steevens JA, Van Den Berg S, Kloas W, Wong BBM, Ziegler M, Maack G (2021) The role of behavioral ecotoxicology in environmental protection. Environ Sci Technol 55:5620–5628. https://doi.org/10.1021/acs.est.0c06493

Frézal L, Félix MA (2015) C. elegans outside the Petri dish. elife 4:e05849. https://doi.org/10.7554/eLife.05849

Gartner A, Macqueen AJ, Villeneuve AM (2004) Methods for analyzing checkpoint responses in Caenorhabditis elegans. Methods Mol Biol 280:257–274. https://doi.org/10.1385/1-59259-788-2:257

Gonzalez-Moragas L, Roig A, Laromaine A (2015a) C. elegans as a tool for in vivo nanoparticle assessment. Adv Colloid Interf Sci 219:10–26. https://doi.org/10.1016/j.cis.2015.02.001

Gonzalez-Moragas L, Yu SM, Carenza E, Laromaine A, Roig A (2015b) Protective effects of bovine serum albumin on superparamagnetic iron oxide nanoparticles evaluated in the nematode Caenorhabditis elegans. ACS Biomater Sci Eng 1:1129–1138. https://doi.org/10.1021/acsbiomaterials.5b00253

Gonzalez-Moragas L, Berto P, Vilches C, Quidant R, Kolovou A, Santarella-Mellwig R, Schwab Y, Stürzenbaum S, Roig A, Laromaine A (2017a) In vivo testing of gold nanoparticles using the Caenorhabditis elegans model organism. Acta Biomater 53:598–609. https://doi.org/10.1016/j.actbio.2017.01.080

Gonzalez-Moragas L, Yu S-M, Benseny-Cases N, Stürzenbaum S, Roig A, Laromaine A (2017b) Toxicogenomics of iron oxide nanoparticles in the nematode C. elegans. Nanotoxicology 11:647–657. https://doi.org/10.1080/17435390.2017.1342011

Gorka DE, Osterberg JS, Gwin CA, Colman BP, Meyer JN, Bernhardt ES, Gunsch CK, Di Giulio RT, Liu J (2015) Reducing environmental toxicity of silver nanoparticles through shape control. Environ Sci Technol 49:10093–10098. https://doi.org/10.1021/acs.est.5b01711

Gupta S, Kushwah T, Vishwakarma A, Yadav S (2015) Optimization of ZnO-NPs to investigate their safe application by assessing their effect on soil nematode Caenorhabditis elegans. Nanoscale Res Lett 10:303. https://doi.org/10.1186/s11671-015-1010-4

Helmcke KJ, Avila DS, Aschner M (2010) Utility of Caenorhabditis elegans in high throughput neurotoxicological research. Neurotoxicol Teratol 32:62–67. https://doi.org/10.1016/j.ntt.2008.11.005

Hendler-Neumark A, Wulf V, Bisker G (2021) In vivo imaging of fluorescent single-walled carbon nanotubes within C. elegans nematodes in the near-infrared window. Mater Today Bio 12:100175. https://doi.org/10.1016/j.mtbio.2021.100175

Hofmann ER, Milstein S, Boulton SJ, Ye M, Hofmann JJ, Stergiou L, Gartner A, Vidal M, Hengartner MO (2002) Caenorhabditis elegans hus-1 is a DNA damage checkpoint protein required for genome stability and egl-1-mediated apoptosis. Curr Biol 12:1908–1918. https://doi.org/10.1016/s0960-9822(02)01262-9

Hu CC, Wu GH, Hua TE, Wagner OI, Yen TJ (2018a) Uptake of TiO2 nanoparticles into C. elegans neurons negatively affects axonal growth and worm locomotion behavior. ACS Appl Mater Interfaces 10:8485–8495. https://doi.org/10.1021/acsami.7b18818

Hu CC, Wu GH, Lai SF, Muthaiyan Shanmugam M, Hwu Y, Wagner OI, Yen TJ (2018b) Toxic effects of size-tunable gold nanoparticles on Caenorhabditis elegans development and gene regulation. Sci Rep 8:15245. https://doi.org/10.1038/s41598-018-33585-7

Hulme SE, Shevkoplyas SS, Apfeld J, Fontana W, Whitesides GM (2007) A microfabricated array of clamps for immobilizing and imaging C. elegans. Lab Chip 7:1515–1523. https://doi.org/10.1039/B707861G

Hunt PR, Marquis BJ, Tyner KM, Conklin S, Olejnik N, Nelson BC, Sprando RL (2013) Nanosilver suppresses growth and induces oxidative damage to DNA in Caenorhabditis elegans. J Appl Toxicol 33:1131–1142. https://doi.org/10.1002/jat.2872

Hunt PR, Keltner Z, Gao X, Oldenburg SJ, Bushana P, Olejnik N, Sprando RL (2014) Bioactivity of nanosilver in Caenorhabditis elegans: effects of size, coat, and shape. Toxicol Rep 1:923–944. https://doi.org/10.1016/j.toxrep.2014.10.020

Iannarelli L, Giovannozzi AM, Morelli F, Viscotti F, Bigini P, Maurino V, Spoto G, Martra G, Ortel E, Hodoroaba VD, Rossi AM, Diomede L (2016) Shape engineered TiO2 nanoparticles in Caenorhabditis elegans: a Raman imaging-based approach to assist tissue-specific toxicological studies. RSC Adv 6:70501–70509. https://doi.org/10.1039/C6RA09686G

Imanikia S, Galea F, Nagy E, Phillips DH, Stürzenbaum SR, Arlt VM (2016) The application of the comet assay to assess the genotoxicity of environmental pollutants in the nematode Caenorhabditis elegans. Environ Toxicol Pharmacol 45:356–361. https://doi.org/10.1016/j.etap.2016.06.020

Jung SK, Qu X, Aleman-Meza B, Wang T, Riepe C, Liu Z, Li Q, Zhong W (2015) Multi-endpoint, high-throughput study of nanomaterial toxicity in Caenorhabditis elegans. Environ Sci Technol 49:2477–2485. https://doi.org/10.1021/es5056462

Kaletta T, Hengartner MO (2006) Finding function in novel targets: C. elegans as a model organism. Nat Rev Drug Discov 5:387–398. https://doi.org/10.1038/nrd2031

Khare P, Sonane M, Nagar Y, Moin N, Ali S, Gupta KC, Satish A (2015) Size dependent toxicity of zinc oxide nanoparticles in soil nematode Caenorhabditis elegans. Nanotoxicology 9:423–432. https://doi.org/10.3109/17435390.2014.940403

Kim SW, Nam SH, An YJ (2012) Interaction of silver nanoparticles with biological surfaces of Caenorhabditis elegans. Ecotoxicol Environ Saf 77:64–70. https://doi.org/10.1016/j.ecoenv.2011.10.023

Kim SW, Kwak JI, An YJ (2013) Multigenerational study of gold nanoparticles in Caenorhabditis elegans: transgenerational effect of maternal exposure. Environ Sci Technol 47:5393–5399. https://doi.org/10.1021/es304511z

Kim H, Jeong J, Chatterjee N, Roca CP, Yoon D, Kim S, Kim Y, Choi J (2017a) Jak/Stat and TGF-β activation as potential adverse outcome pathway of TiO2 nanoparticles phototoxicity in Caenorhabditis elegans. Sci Rep 7:17833. https://doi.org/10.1038/s41598-017-17495-8

Kim JH, Lee SH, Cha YJ, Hong SJ, Chung SK, Park TH, Choi SS (2017b) C. elegans-on-a-chip for in situ and in vivo Ag nanoparticles' uptake and toxicity assay. Sci Rep 7:40225. https://doi.org/10.1038/srep40225

Kim M, Eom HJ, Choi I, Hong J, Choi J (2020a) Graphene oxide-induced neurotoxicity on neurotransmitters, AFD neurons and locomotive behavior in Caenorhabditis elegans. Neurotoxicology 77:30–39. https://doi.org/10.1016/j.neuro.2019.12.011

Kim Y, Jeong J, Lee S, Choi I, Choi J (2020b) Identification of adverse outcome pathway related to high-density polyethylene microplastics exposure: Caenorhabditis elegans transcription factor RNAi screening and zebrafish study. J Hazard Mater 388:121725. https://doi.org/10.1016/j.jhazmat.2019.121725

Kleiven M, Rossbach LM, Gallego-Urrea JA, Brede DA, Oughton DH, Coutris C (2018) Characterizing the behavior, uptake, and toxicity of NM300K silver nanoparticles in Caenorhabditis elegans. Environ Toxicol Chem 37:1799–1810

Kong L, Gao X, Zhu J, Zhang T, Xue Y, Tang M (2017) Reproductive toxicity induced by nickel nanoparticles in Caenorhabditis elegans. Environ Toxicol 32:1530–1538. https://doi.org/10.1002/tox.22373

Kuhn EC, Jacques MT, Teixeira D, Meyer S, Gralha T, Roehrs R, Camargo S, Schwerdtle T, Bornhorst J, Ávila DS (2021) Ecotoxicological assessment of Uruguay River and affluents pre- and post-pesticides' application using Caenorhabditis elegans for biomonitoring. Environ Sci Pollut Res Int 28:21730–21741. https://doi.org/10.1007/s11356-020-11986-4

Lagido C, McLaggan D, Flett A, Pettitt J, Glover LA (2009) Rapid sublethal toxicity assessment using bioluminescent *Caenorhabditis elegans*, a novel whole-animal metabolic biosensor. Toxicol Sci 109(1):88–95. https://doi.org/10.1093/toxsci/kfp058

Lee WM, An YJ (2013) Effects of zinc oxide and titanium dioxide nanoparticles on green algae under visible, UVA, and UVB irradiations: no evidence of enhanced algal toxicity under UV pre-irradiation. Chemosphere 91(4):536–544. https://doi.org/10.1016/j.chemosphere.2012.12.033

Leung MC, Williams PL, Benedetto A, Au C, Helmcke KJ, Aschner M, Meyer JN (2008) *Caenorhabditis elegans*: an emerging model in biomedical and environmental toxicology. Toxicol Sci 106(1):5–28. https://doi.org/10.1093/toxsci/kfn121

Leung MC, Goldstone JV, Boyd WA, Freedman JH, Meyer JN (2010) *Caenorhabditis elegans* generates biologically relevant levels of genotoxic metabolites from aflatoxin B1 but not benzo[a]pyrene in vivo. Toxicol Sci 118(2):444–453. https://doi.org/10.1093/toxsci/kfq295

Leung CK, Deonarine A, Strange K, Choe KP (2011) High-throughput screening and biosensing with fluorescent *C. elegans* strains. J Vis Exp 51:e2745. https://doi.org/10.3791/2745

Li Y, Yu S, Wu Q, Tang M, Pu Y, Wang D (2012) Chronic Al2O3-nanoparticle exposure causes neurotoxic effects on locomotion behaviors by inducing severe ROS production and disruption of ROS defense mechanisms in nematode *Caenorhabditis elegans*. J Hazard Mater 219–220:221–230. https://doi.org/10.1016/j.jhazmat.2012.03.083

Li P, Xu T, Wu S, Lei L, He D (2017) Chronic exposure to graphene-based nanomaterials induces behavioral deficits and neural damage in *Caenorhabditis elegans*. J Appl Toxicol 37(10):1140–1150. https://doi.org/10.1002/jat.3468

Li SW, Huang CW, Liao VH (2020) Early-life long-term exposure to ZnO nanoparticles suppresses innate immunity regulated by SKN-1/Nrf and the p38 MAPK signaling pathway in *Caenorhabditis elegans*. Environ Pollut 256:113382. https://doi.org/10.1016/j.envpol.2019.113382

Liang S, Duan J, Hu H, Zhang J, Gao S, Jing H, Li G, Sun Z (2020a) Comprehensive analysis of SiNPs on the genome-wide transcriptional changes in *Caenorhabditis elegans*. Int J Nanomedicine 15:5227–5237. https://doi.org/10.2147/ijn.S251269

Liang X, Wang Y, Cheng J, Ji Q, Wang Y, Wu T, Tang M (2020b) Mesoporous silica nanoparticles at predicted environmentally relevant concentrations cause impairments in GABAergic motor neurons of nematode *Caenorhabditis elegans*. Chem Res Toxicol 33(6):1665–1676. https://doi.org/10.1021/acs.chemrestox.9b00477

Lim D, Roh JY, Eom HJ, Choi JY, Hyun J, Choi J (2012) Oxidative stress-related PMK-1 p38 MAPK activation as a mechanism for toxicity of silver nanoparticles to reproduction in the nematode *Caenorhabditis elegans*. Environ Toxicol Chem 31(3):585–592. https://doi.org/10.1002/etc.1706

Lu JH, Hou WC, Tsai MH, Chang YT, Chao HR (2022) The impact of background-level carboxylated single-walled carbon nanotubes (SWCNTs-COOH) on induced toxicity in *Caenorhabditis elegans* and human cells. Int J Environ Res Public Health 19(12):7525. https://doi.org/10.3390/ijerph19031218

Lucio D, Martínez-Ohárriz MC, Jaras G, Aranaz P, González-Navarro CJ, Radulescu A, Irache JM (2017) Optimization and evaluation of zein nanoparticles to improve the oral delivery of glibenclamide: in vivo study using *C. elegans*. Eur J Pharm Biopharm 121:104–112. https://doi.org/10.1016/j.ejpb.2017.09.018

Luo X, Xu S, Yang Y, Li L, Chen S, Xu A, Wu L (2016) Insights into the ecotoxicity of silver nanoparticles transferred from *Escherichia coli* to *Caenorhabditis elegans*. Sci Rep 6:36465. https://doi.org/10.1038/srep36465

Luo X, Xu S, Yang Y, Zhang Y, Wang S, Chen S, Xu A, Wu L (2017) A novel method for assessing the toxicity of silver nanoparticles in *Caenorhabditis elegans*. Chemosphere 168:648–657. https://doi.org/10.1016/j.chemosphere.2016.11.011

Ma H, Kabengi NJ, Bertsch PM, Unrine JM, Glenn TC, Williams PL (2011) Comparative phototoxicity of nanoparticulate and bulk ZnO to a free-living nematode *Caenorhabditis elegans*: the importance of illumination mode and primary particle size. Environ Pollut 159(5):1473–1480. https://doi.org/10.1016/j.envpol.2011.03.013

Ma H, Williams PL, Diamond SA (2013) Ecotoxicity of manufactured ZnO nanoparticles—a review. Environ Pollut 172:76–85. https://doi.org/10.1016/j.envpol.2012.08.011

Ma H, Wallis LK, Diamond S, Li S, Cañas-Carrell J, Parra A (2014) Impact of solar UV radiation on toxicity of ZnO nanoparticles through photocatalytic reactive oxygen species (ROS) generation and photo-induced dissolution. Environ Pollut 193:165–172. https://doi.org/10.1016/j.envpol.2014.06.027

Ma Z, Garrido-Maestu A, Lee C, Chon J, Jeong D, Yue Y, Sung K, Park Y, Jeong KC (2018) Comprehensive in vitro and in vivo risk assessments of chitosan microparticles using human epithelial cells and *Caenorhabditis elegans*. J Hazard Mater 341:248–256. https://doi.org/10.1016/j.jhazmat.2017.07.071

Mádi A, Mikkat S, Ringel B, Thiesen HJ, Glocker MO (2003) Profiling stage-dependent changes of protein expression in *Caenorhabditis elegans* by mass spectrometric proteome analysis leads to the identification of stage-specific marker proteins. Electrophoresis 24(10):1809–1817. https://doi.org/10.1002/elps.200305390

Markaki M, Tavernarakis N (2020) *Caenorhabditis elegans* as a model system for human diseases. Curr Opin Biotechnol 63:118–125. https://doi.org/10.1016/j.copbio.2019.12.011

Mashock MJ, Zanon T, Kappell AD, Petrella LN, Andersen EC, Hristova KR (2016) Copper oxide nanoparticles impact several toxicological endpoints and cause neurodegeneration in *Caenorhabditis elegans*. PLoS One 11(1):e0167613. https://doi.org/10.1371/journal.pone.0167613

Maurer LL, Yang X, Schindler AJ, Taggart RK, Jiang C, Hsu-Kim H, Sherwood DR, Meyer JN (2016) Intracellular trafficking pathways in silver nanoparticle uptake and toxicity in *Caenorhabditis elegans*. Nanotoxicology 10(8):831–835. https://doi.org/10.3109/17435390.2015.1110759

McElwee MK, Ho LA, Chou JW, Smith MV, Freedman JH (2013) Comparative toxicogenomic responses of mercuric and methyl-mercury. BMC Genomics 14:698. https://doi.org/10.1186/1471-2164-14-698

Meyer JN, Lord CA, Yang XY, Turner EA, Badireddy AR, Marinakos SM, Chilkoti A, Wiesner MR, Auffan M (2010) Intracellular uptake and associated toxicity of silver nanoparticles in *Caenorhabditis elegans*. Aquat Toxicol 100(2):140–150. https://doi.org/10.1016/j.aquatox.2010.07.016

Mondal S, Hegarty E, Martin C, Gökçe SK, Ghorashian N, Ben-Yakar A (2016) Large-scale microfluidics providing high-resolution and high-throughput screening of Caenorhabditis elegans poly-glutamine aggregation model. Nat Commun 7:13023. https://doi.org/10.1038/ncomms13023

Moon J, Kwak JI, Kim SW, An YJ (2017) Multigenerational effects of gold nanoparticles in Caenorhabditis elegans: continuous versus intermittent exposures. Environ Pollut 220:46–52. https://doi.org/10.1016/j.envpol.2016.09.021

O'Donnell B, Huo L, Polli JR, Qiu L, Collier DN, Zhang B, Pan X (2017) From the cover: ZnO nanoparticles enhanced germ cell apoptosis in Caenorhabditis elegans, in comparison with ZnCl2. Toxicol Sci 156:336–343. https://doi.org/10.1093/toxsci/kfw258

Piechulek A, Von Mikecz A (2018) Life span-resolved nanotoxicology enables identification of age-associated neuromuscular vulnerabilities in the nematode Caenorhabditis elegans. Environ Pollut 233:1095–1103. https://doi.org/10.1016/j.envpol.2017.10.012

Polak N, Read DS, Jurkschat K, Matzke M, Kelly FJ, Spurgeon DJ, Stürzenbaum SR (2014) Metalloproteins and phytochelatin synthase may confer protection against zinc oxide nanoparticle induced toxicity in Caenorhabditis elegans. Comp Biochem Physiol C: Toxicol Pharmacol 160:75–85. https://doi.org/10.1016/j.cbpc.2013.12.001

Qu M, Li Y, Wu Q, Xia Y, Wang D (2017) Neuronal ERK signaling in response to graphene oxide in nematode Caenorhabditis elegans. Nanotoxicology 11:520–533. https://doi.org/10.1080/17435390.2017.1315190

Qu M, Xu K, Li Y, Wong G, Wang D (2018) Using ACS-22 mutant Caenorhabditis elegans to detect the toxicity of nanopolystyrene particles. Sci Total Environ 643:119–126. https://doi.org/10.1016/j.scitotenv.2018.06.173

Rahman M, Edwards H, Birze N, Gabrilska R, Rumbaugh KP, Blawzdziewicz J, Szewczyk NJ, Driscoll M, Vanapalli SA (2020) Nemalife chip: a micropillar-based microfluidic culture device optimized for aging studies in crawling C. elegans. Sci Rep 10:16190. https://doi.org/10.1038/s41598-020-73002-6

Ratnasekhar C, Sonane M, Satish A, Mudiam MK (2015) Metabolomics reveals the perturbations in the metabolome of Caenorhabditis elegans exposed to titanium dioxide nanoparticles. Nanotoxicology 9:994–1004. https://doi.org/10.3109/17435390.2014.993345

Ren M, Zhao L, Lv X, Wang D (2017) Antimicrobial proteins in the response to graphene oxide in Caenorhabditis elegans. Nanotoxicology 11:578–590. https://doi.org/10.1080/17435390.2017.1329954

Ren M, Zhao L, Ding X, Krasteva N, Rui Q, Wang D (2018) Developmental basis for intestinal barrier against the toxicity of graphene oxide. Part Fibre Toxicol 15:26. https://doi.org/10.1186/s12989-018-0262-4

Rive C, Reina G, Wagle P, Treossi E, Palermo V, Bianco A, Delogu LG, Rieckher M, Schumacher B (2019) Improved biocompatibility of amino-functionalized graphene oxide in Caenorhabditis elegans. Small 15:e1902699. https://doi.org/10.1002/smll.201902699

Rocheleau S, Arbour M, Elias M, Sunahara GI, Masson L (2015) Toxicogenomic effects of nano- and bulk-TiO2 particles in the soil nematode Caenorhabditis elegans. Nanotoxicology 9:502–512. https://doi.org/10.3109/17435390.2014.948941

Rogers S, Rice KM, Manne ND, Shokuhfar T, He K, Selvaraj V, Blough ER (2015) Cerium oxide nanoparticle aggregates affect stress response and function in Caenorhabditis elegans. SAGE Open Med 3:2050312115575387. https://doi.org/10.1177/2050312115575387

Roh JY, Sim SJ, Yi J, Park K, Chung KH, Ryu DY, Choi J (2009) Ecotoxicity of silver nanoparticles on the soil nematode Caenorhabditis elegans using functional ecotoxicogenomics. Environ Sci Technol 43:3933–3940. https://doi.org/10.1021/es803477u

Roh JY, Park YK, Park K, Choi J (2010) Ecotoxicological investigation of CeO2 and TiO2 nanoparticles on the soil nematode Caenorhabditis elegans using gene expression, growth, fertility, and survival as endpoints. Environ Toxicol Pharmacol 29:167–172. https://doi.org/10.1016/j.etap.2009.12.003

Rohde CB, Zeng F, Gonzalez-Rubio R, Angel M, Yanik MF (2007) Microfluidic system for on-chip high-throughput whole-animal sorting and screening at subcellular resolution. Proc Natl Acad Sci 104:13891–13895. https://doi.org/10.1073/pnas.0706513104

Rui Q, Zhao Y, Wu Q, Tang M, Wang D (2013) Biosafety assessment of titanium dioxide nanoparticles in acutely exposed nematode Caenorhabditis elegans with mutations of genes required for oxidative stress or stress response. Chemosphere 93:2289–2296. https://doi.org/10.1016/j.chemosphere.2013.08.007

Scharf A, Piechulek A, Von Mikecz A (2013) Effect of nanoparticles on the biochemical and behavioral aging phenotype of the nematode Caenorhabditis elegans. ACS Nano 7:10695–10703. https://doi.org/10.1021/nn403443r

Scharf A, Gührs KH, Von Mikecz A (2016) Anti-amyloid compounds protect from silica nanoparticle-induced neurotoxicity in the nematode C. elegans. Nanotoxicology 10:426–435. https://doi.org/10.3109/17435390.2015.1073399

Schultz CL, Wamucho A, Tsyusko OV, Unrine JM, Crossley A, Svendsen C, Spurgeon DJ (2016) Multigenerational exposure to silver ions and silver nanoparticles reveals heightened sensitivity and epigenetic memory in Caenorhabditis elegans. Proc R Soc B Biol Sci 283:20152911. https://doi.org/10.1098/rspb.2015.2911

Sese BT, Grant A, Reid BJ (2009) Toxicity of polycyclic aromatic hydrocarbons to the nematode Caenorhabditis elegans. J Toxicol Environ Health A 72:1168–1180. https://doi.org/10.1080/15287390903091814

Shin N, Cuenca L, Karthikraj R, Kannan K, Colaiácovo MP (2019) Assessing effects of germline exposure to environmental toxicants by high-throughput screening in C. elegans. PLoS Genet 15:e1007975. https://doi.org/10.1371/journal.pgen.1007975

Silva AC, Dos Santos AGR, Pieretti JC, Rolim WR, Seabra AB, Ávila DS (2023) Iron oxide/silver hybrid nanoparticles impair the cholinergic system and cause reprotoxicity in Caenorhabditis elegans. Food Chem Toxicol 179:113945. https://doi.org/10.1016/j.fct.2023.113945

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. https://doi.org/10.1039/d0na00574f

Sonane M, Moin N, Satish A (2017) The role of antioxidants in attenuation of Caenorhabditis elegans lethality on exposure to TiO2 and ZnO nanoparticles. Chemosphere 187:240–247. https://doi.org/10.1016/j.chemosphere.2017.08.080

Soria C, Coccini T, De Simone U, Marchese L, Zorzoli I, Giorgetti S, Raimondi S, Mangione PP, Ramat S, Bellotti V, Manzo L, Stoppini M (2015) Enhanced toxicity of silver nanoparticles in transgenic Caenorhabditis elegans expressing amyloidogenic proteins. Amyloid 22:221–228. https://doi.org/10.3109/13506129.2015.1077216

Starnes DL, Unrine JM, Starnes CP, Collin BE, Oostveen EK, Ma R, Lowry GV, Bertsch PM, Tsyusko OV (2015) Impact of sulfidation on the bioavailability and toxicity of silver nanoparticles to Caenorhabditis elegans. Environ Pollut 196:239–246. https://doi.org/10.1016/j.envpol.2014.10.009

Starnes D, Unrine J, Chen C, Lichtenberg S, Starnes C, Svendsen C, Kille P, Morgan J, Baddar ZE, Spear A, Bertsch P, Chen KC, Tsyusko O (2019) Toxicogenomic responses of Caenorhabditis elegans to pristine and transformed zinc oxide nanoparticles. Environ Pollut 247:917–926. https://doi.org/10.1016/j.envpol.2019.01.077

Sulston JE (1983) Neuronal cell lineages in the nematode Caenorhabditis elegans. Cold Spring Harb Symp Quant Biol 48(Pt 2):443–452. https://doi.org/10.1101/sqb.1983.048.01.049

Tejeda-Benitez L, Olivero-Verbel J (2016) Caenorhabditis elegans, a biological model for research in toxicology. In: De Voogt WP (ed) Reviews of environmental contamination and toxicology 237. Springer, Cham. https://doi.org/10.1007/978-3-319-23573-8_1

Teng J, Yu T, Yan F (2024) GABA attenuates neurotoxicity of zinc oxide nanoparticles due to oxidative stress via DAF-16/FOXO and SKN-1/Nrf2 pathways. Sci Total Environ 934:173214. https://doi.org/10.1016/j.scitotenv.2024.173214

Tsyusko OV, Unrine JM, Spurgeon D, Blalock E, Starnes D, Tseng M, Joice G, Bertsch PM (2012) Toxicogenomic responses of the model organism Caenorhabditis elegans to gold nanoparticles. Environ Sci Technol 46:4115–4124. https://doi.org/10.1021/es2033108

Tyne W, Lofts S, Spurgeon DJ, Jurkschat K, Svendsen C (2013) A new medium for Caenorhabditis elegans toxicology and nanotoxicology studies designed to better reflect natural soil solution conditions. Environ Toxicol Chem 32:1711–1717. https://doi.org/10.1002/etc.2247

Tyne W, Little S, Spurgeon DJ, Svendsen C (2015) Hormesis depends upon the life-stage and duration of exposure: examples for a pesticide and a nanomaterial. Ecotoxicol Environ Saf 120:117–123. https://doi.org/10.1016/j.ecoenv.2015.05.024

Von Mikecz A (2022) Exposome, molecular pathways and one health: the invertebrate Caenorhabditis elegans. Int J Mol Sci 23:9084. https://doi.org/10.3390/ijms23169084

Walczynska M, Jakubowski W, Wasiak T, Kadziola K, Bartoszek N, Kotarba S, Siatkowska M, Komorowski P, Walkowiak B (2018) Toxicity of silver nanoparticles, multiwalled carbon nanotubes, and dendrimers assessed with multicellular organism Caenorhabditis elegans. Toxicol Mech Methods 28:432–439. https://doi.org/10.1080/15376516.2018.1449277

Wamucho A, Unrine JM, Kieran TJ, Glenn TC, Schultz CL, Farman M, Svendsen C, Spurgeon DJ, Tsyusko OV (2019) Genomic mutations after multigenerational exposure of Caenorhabditis elegans to pristine and sulfidized silver nanoparticles. Environ Pollut 254:113078. https://doi.org/10.1016/j.envpol.2019.113078

Wang D (2018) Endpoints for toxicity assessment of nanomaterials. In: Nanotoxicology in Caenorhabditis elegans. Springer Singapore, Singapore. https://doi.org/10.1007/978-981-13-0233-6_2

Wang Y, Wang S, Luo X, Yang Y, Jian F, Wang X, Xie L (2014) The roles of DNA damage-dependent signals and MAPK cascades in tributyltin-induced germline apoptosis in Caenorhabditis elegans. Chemosphere 108:231–238. https://doi.org/10.1016/j.chemosphere.2014.01.045

Wang Q, Zhou Y, Song B, Zhong Y, Wu S, Cui R, Cong H, Su Y, Zhang H, He Y (2016) Linking subcellular disturbance to physiological behavior and toxicity induced by quantum dots in Caenorhabditis elegans. Small 12:3143–3154. https://doi.org/10.1002/smll.201600766

Wang N, Wang H, Tang C, Lei S, Shen W, Wang C, Wang G, Wang Z, Wang L (2017) Toxicity evaluation of boron nitride nanospheres and water-soluble boron nitride in Caenorhabditis elegans. Int J Nanomedicine 12:5941–5957. https://doi.org/10.2147/ijn.S130960

Wang Q, Zhu Y, Song B, Fu R, Zhou Y (2022) The in vivo toxicity assessments of water-dispersed fluorescent silicon nanoparticles in Caenorhabditis elegans. Int J Environ Res Public Health 19:4101. https://doi.org/10.3390/ijerph19074101

Wang M, Feng Y, Cao Z, Yu N, Wang J, Wang X, Kang D, Su M, Hu J, Du H (2023a) Multiple generation exposure to ZnO nanoparticles induces loss of genomic integrity in Caenorhabditis elegans. Ecotoxicol Environ Saf 249:114383. https://doi.org/10.1016/j.ecoenv.2022.114383

Wang M, Zhang Z, Sun N, Yang B, Mo J, Wang D, Su M, Hu J, Wang M, Wang L (2023b) Gold nanoparticles reduce food sensation in Caenorhabditis elegans via the voltage-gated channel EGL-19. Int J Nanomedicine 18:1659–1676. https://doi.org/10.2147/ijn.S394666

Wei CC, Yen PL, Chaikritsadakarn A, Huang CW, Chang CH, Liao VHC (2020) Parental CuO nanoparticles exposure results in transgenerational toxicity in Caenorhabditis elegans associated with possible epigenetic regulation. Ecotoxicol Environ Saf 203:111001. https://doi.org/10.1016/j.ecoenv.2020.111001

Wu S, Lu J, Rui Q, Yu S, Cai T, Wang D (2011) Aluminum nanoparticle exposure in L1 larvae results in more severe lethality toxicity than in L4 larvae or young adults by strengthening the formation of stress response and intestinal lipofuscin accumulation in nematodes. Environ Toxicol Pharmacol 31:179–188. https://doi.org/10.1016/j.etap.2010.10.005

Wu Q, Li Y, Tang M, Wang D (2012a) Evaluation of environmental safety concentrations of DMSA coated Fe2O3-NPs using different assay systems in nematode Caenorhabditis elegans. PLoS One 7:e43729. https://doi.org/10.1371/journal.pone.0043729

Wu Q, Wang W, Li Y, Li Y, Ye B, Tang M, Wang D (2012b) Small sizes of TiO2-NPs exhibit adverse effects at predicted environmental relevant concentrations on nematodes in a modified chronic toxicity assay system. J Hazard Mater 243:161–168. https://doi.org/10.1016/j.jhazmat.2012.10.013

Wu Q, Nouara A, Li Y, Zhang M, Wang W, Tang M, Ye B, Ding J, Wang D (2013) Comparison of toxicities from three metal oxide nanoparticles at environmentally relevant concentrations in nematode Caenorhabditis elegans. Chemosphere 90:1123–1131. https://doi.org/10.1016/j.chemosphere.2012.09.019

Wu Q, Zhao Y, Fang J, Wang D (2014a) Immune response is required for the control of in vivo translocation and chronic toxicity of graphene oxide. Nanoscale 6:5894–5906. https://doi.org/10.1039/C4NR00699B

Wu Q, Zhao Y, Li Y, Wang D (2014b) Molecular signals regulating translocation and toxicity of graphene oxide in the nematode Caenorhabditis elegans. Nanoscale 6:11204–11212. https://doi.org/10.1039/c4nr02688h

Wu Q, Zhao Y, Li Y, Wang D (2014c) Susceptible genes regulate the adverse effects of TiO2-NPs at predicted environmental relevant concentrations on nematode Caenorhabditis elegans. Nanomedicine 10:1263–1271. https://doi.org/10.1016/j.nano.2014.03.010

Wu Q, Zhao Y, Zhao G, Wang D (2014d) MicroRNAs control of in vivo toxicity from graphene oxide in Caenorhabditis elegans. Nanomedicine 10:1401–1410. https://doi.org/10.1016/j.nano.2014.04.005

Wu T, He K, Zhan Q, Ang S, Ying J, Zhang S, Zhang T, Xue Y, Tang M (2015) MPA-capped CdTe quantum dots exposure causes neurotoxic effects in nematode Caenorhabditis elegans by affecting the transporters and receptors of glutamate, serotonin and dopamine at the genetic level, or by increasing ROS, or both. Nanoscale 7:20460–20473. https://doi.org/10.1039/C5NR05914C

Wu Q, Zhi L, Qu Y, Wang D (2016a) Quantum dots increased fat storage in intestine of Caenorhabditis elegans by influencing molecular basis for fatty acid metabolism. Nanomedicine 12:1175–1184. https://doi.org/10.1016/j.nano.2016.01.016

Wu Q, Zhou X, Han X, Zhuo Y, Zhu S, Zhao Y, Wang D (2016b) Genome-wide identification and functional analysis of long noncoding RNAs involved in the response to graphene oxide. Biomaterials 102:277–291. https://doi.org/10.1016/j.biomaterials.2016.06.041

Wu T, Xu H, Liang X, Tang M (2019) Caenorhabditis elegans as a complete model organism for biosafety assessments of nanoparticles. Chemosphere 221:708–726. https://doi.org/10.1016/j.chemosphere.2019.01.021

Wu J, Gao Y, Xi J, You X, Zhang X, Zhang X, Cao Y, Liu P, Chen X, Luan Y (2022) A high-throughput microplate toxicity screening platform based on Caenorhabditis elegans. Ecotoxicol Environ Saf 245:114089. https://doi.org/10.1016/j.ecoenv.2022.114089

Yang X, Gondikas AP, Marinakos SM, Auffan M, Liu J, Hsu-Kim H, Meyer JN (2012) Mechanism of silver nanoparticle toxicity is dependent on dissolved silver and surface coating in Caenorhabditis elegans. Environ Sci Technol 46:1119–1127. https://doi.org/10.1021/es202417t

Yang YF, Chen PJ, Liao VH (2016) Nanoscale zerovalent iron (Nzvi) at environmentally relevant concentrations induced multigenerational reproductive toxicity in Caenorhabditis elegans. Chemosphere 150:615–623. https://doi.org/10.1016/j.chemosphere.2016.01.068

Yang YF, Lin YJ, Liao CM (2017) Toxicity-based toxicokinetic/toxicodynamic assessment of bioaccumulation and nanotoxicity of zerovalent iron nanoparticles in Caenorhabditis elegans. Int J Nanomedicine 12:4607–4621. https://doi.org/10.2147/ijn.S138790

Yu S, Rui Q, Cai T, Wu Q, Li Y, Wang D (2011) Close association of intestinal autofluorescence with the formation of severe oxidative damage in intestine of nematodes chronically exposed to Al(2)O(3)-nanoparticle. Environ Toxicol Pharmacol 32:233–241. https://doi.org/10.1016/j.etap.2011.05.008

Zhang F, You X, Zhu T, Gao S, Wang Y, Wang R, Yu H, Qian B (2020) Silica nanoparticles enhance germ cell apoptosis by inducing reactive oxygen species (Ros) formation in Caenorhabditis elegans. J Toxicol Sci 45:117–129. https://doi.org/10.2131/jts.45.117

Zhang W, Li W, Li J, Chang X, Niu S, Wu T, Kong L, Zhang T, Tang M, Xue Y (2021) Neurobehavior and neuron damage following prolonged exposure of silver nanoparticles with/without polyvinylpyrrolidone coating in Caenorhabditis elegans. J Appl Toxicol 41:2055–2067. https://doi.org/10.1002/jat.4197

Zhao Y, Wu Q, Tang M, Wang D (2014) The in vivo underlying mechanism for recovery response formation in nano-titanium dioxide exposed Caenorhabditis elegans after transfer to the normal condition. Nanomedicine 10:89–98. https://doi.org/10.1016/j.nano.2013.07.004

Zhao Y, Wang X, Wu Q, Li Y, Wang D (2015) Translocation and neurotoxicity of CdTe quantum dots in RMEs motor neurons in nematode Caenorhabditis elegans. J Hazard Mater 283:480–489. https://doi.org/10.1016/j.jhazmat.2014.09.063

Zhao Y, Wu Q, Wang D (2016a) An epigenetic signal encoded protection mechanism is activated by graphene oxide to inhibit its induced reproductive toxicity in Caenorhabditis elegans. Biomaterials 79:15–24. https://doi.org/10.1016/j.biomaterials.2015.11.052

Zhao Y, Yang J, Wang D (2016b) A microRNA-mediated insulin signaling pathway regulates the toxicity of multi-walled carbon nanotubes in nematode Caenorhabditis elegans. Sci Rep 6:23234. https://doi.org/10.1038/srep23234

Zhao Y, Zhi L, Wu Q, Yu Y, Sun Q, Wang D (2016c) P38 MAPK-SKN-1/Nrf signaling cascade is required for intestinal barrier against graphene oxide toxicity in Caenorhabditis elegans. Nanotoxicology 10:1469–1479. https://doi.org/10.1080/17435390.2016.1235738

Zhao L, Kong J, Krasteva N, Wang D (2018) Deficit in the epidermal barrier induces toxicity and translocation of PEG modified graphene oxide in nematodes. Toxicol Res (Camb) 7:1061–1070. https://doi.org/10.1039/c8tx00136g

Zhi L, Ren M, Qu M, Zhang H, Wang D (2016) Wnt ligands differentially regulate toxicity and translocation of graphene oxide through different mechanisms in Caenorhabditis elegans. Sci Rep 6:39261. https://doi.org/10.1038/srep39261

Zhou Y, Wang Q, Song B, Wu S, Su Y, Zhang H, He Y (2015) A real-time documentation and mechanistic investigation of quantum dots-induced autophagy in live Caenorhabditis elegans. Biomaterials 72:38–48. https://doi.org/10.1016/j.biomaterials.2015.08.044

Zhuang Z, Li M, Liu H, Luo L, Gu W, Wu Q, Wang D (2016) Function of RSKS-1-AAK-2-DAF-16 signaling cascade in enhancing toxicity of multi-walled carbon nanotubes can be suppressed by mir-259 activation in Caenorhabditis elegans. Sci Rep 6:32409. https://doi.org/10.1038/srep32409