Caenorhabditis elegans, a Biological Model for Research in Toxicology

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Contents

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P. de Voogt (ed.), *Reviews of Environmental Contamination and Toxicology Volume 237*, Reviews of Environmental Contamination and Toxicology 237,
DOI 10.1007/978-3-319-23573-8_1

1 Introduction

Caenorhabditis elegans is a non-parasitic nematode which, due to its many convenient features has become an important model in biology research; for example, it was the first animal whose genome was completely sequenced. This nematode was proposed as a model organism by Sydney Brenner in 1965 (Garcia-Sancho 2012). Since then, it has been used in cell biological, genetic and neurobiological studies of higher eukaryotes. Between 1970 and 1980, the complete cell lineage of this worm, from the fertilized egg to adult, was characterized by laser ablation and microscopy (Sulston et al. 1983). Electron microscopy and serial sectioning allowed for the reconstruction of the entire nervous system (White et al. 1986), together with the genetic and genomic data generated in the 1990s (Coulson et al. 1991). This organism has become a powerful tool for the discovery and functional characterization of eukaryotic genes (Dimitriadi and Hart 2010). Many aspects of *C. elegans* as a toxicological model have been reviewed in an excellent paper by Leung et al. (2008). In this state-of-the-art review, the authors present an update on that report, focusing on toxicity end points and assessments for many types of environmental pollutants.

2 Biological Features of *C. elegans*

The body of an adult *C. elegans* is approximately 1 mm long. Its transparency allows viewing of cell types in all stages of development. It has a simple nervous system of 302 neurons as an adult, where each neuron has a unique position (Dimitriadi and Hart 2010; Giles and Rankin 2009). Most organisms are hermaphrodites, with two ovaries, oviducts, a cavity for storing sperm called the spermatheca, and uterus (L'Hernault 2009). Hermaphrodites produce sperm as L4 larvae and oocytes during early adulthood; they reproduce by self-fertilization and therefore cannot fertilize other hermaphrodites. The males, which appear spontaneously with a frequency of less than 0.3 %, are able to fertilize hermaphrodites. The reproductive cycle of *C. elegans* lasts 2.5–4 days at room temperature, and with a usual lifespan of 12–20 days (Giles and Rankin 2009).

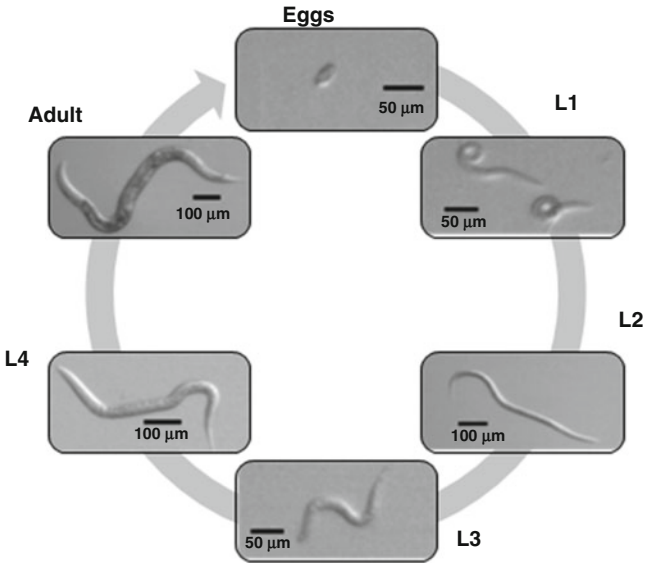

Fig. 1 Life cycle of *C. elegans.* Images were acquired using a dissection microscope Nikon smz 745T with 4× magnification

Embryonic development culminates in the generation of an L1 larva of 550 cells, after 113 cells have died by apoptosis. After four larval stages, the hermaphrodite worm becomes an adult organism with 959 cell nuclei (some syncytial), 302 of which are neurons. Males have 1031 cell nuclei. The mature adult is fertile for 4 days, and it can live between 10 and 15 additional days. Each adult hermaphrodite lays between 200 and 300 eggs, at intervals of about 20 min. Furthermore, the cycle time depends upon the temperature of incubation (Garcia-Sancho 2012). When environmental conditions are adverse, for example, during food shortages, high temperatures, or high population densities, successful reproduction is unlikely. Under such conditions, *C. elegans* can halt its development passing into an alternative L3 stage called *dauer*, which can survive for months. During this stage, the nematode does not feed and its cuticle is tougher. Nematodes can re-enter the reproductive life cycle at L4 when conditions are more favorable (Wang et al. 2010b). Figure 1 shows the complete cycle of *C. elegans.*

3 Advantages of Using *C. elegans* as a Biological Model

C. elegans is used as a model in genetic research because of its convenient features. First, its transparency allows for transgenic proteins fused to fluorescent markers to be visible in living animals in *in vivo* experiments (Giles and Rankin 2009). Its generation time is short (4 days) and it occurs by self-fertilization, ensuring rapid

reproduction in the laboratory (Zhuang et al. 2014) since each adult hermaphrodite produces 200–300 progeny (Megalou and Tavernarakis 2009).

Its excellent performance as a model in genetics has led to the development of many tools and resources, including thousands of characterized mutants and RNA interference libraries, useful for silencing gene expression (Giles and Rankin 2009; Megalou and Tavernarakis 2009). RNA interference (RNAi) with this organism is relatively simple, and therefore gene silencing is often used to dissect signaling pathways (Adam 2009).

C. elegans has been used in toxicological research, from the whole animal level to the level of individual cells (Zhuang et al. 2014). It is cultured in the laboratory in a nematode growth medium (NGM), which contains NaCl, agar, peptone, cholesterol, K_3PO_4, KH_2PO_4, K_2HPO_4 and $MgSO_4$. Another suitable culture medium is K agar, which also contains KCl (Meyer et al. 2010). The worms are maintained in an incubator at 20 °C and the bacteria *Escherichia coli* OP50 is utilized as a food source (Giles and Rankin 2009). The K medium prepared with KCl and NaCl is the liquid used to transfer worms to fresh dishes and to carry out bioassays (Williams and Dusenbery 1990).

4 Applications in Medicine

C. elegans is a model organism that has been important in the studies carried out to identify and understand the functioning of the machinery in nuclear transportation (Adam 2009). It has helped to elucidate biochemical pathways involved in diseases, such as obesity (Finley et al. 2013; MacNeil et al. 2013), diabetes (Estevez et al. 2014; Shi et al. 2012), and Alzheimer's disease (Diomede et al. 2014; Lublin and Link 2013). *C. elegans* is an excellent model to investigate aging because of its short lifespan, its susceptibility to oxidative stress and the similarities with the human aging process (Chatterjee et al. 2013; Pang and Curran 2014). This nematode has also been employed to identify biochemical pathways and mechanisms of action of new drugs, especially antihelmintics (Kumarasingha et al. 2014; Lublin and Link 2013; Wu et al. 2012b).

5 Toxicity Endpoints

Bioassays to assess the effects of a toxicant on *C. elegans* can be carried out through different endpoints. The normal procedure for acute exposure consists of the incubation of young adults in the K medium containing the toxicant at several concentrations, usually without food. In long term exposure assays, worms in the L1 stage are used; in this case *E. coli* OP50 is added as food (Zhuang et al. 2014). When worm reproduction is not required during an experiment, since brood size may affect the results, 5-fluorodesoxiuridine is used to inhibit DNA synthesis (Wu et al. 2012a). Endpoints can be grouped according to their effects on biological parameters, for

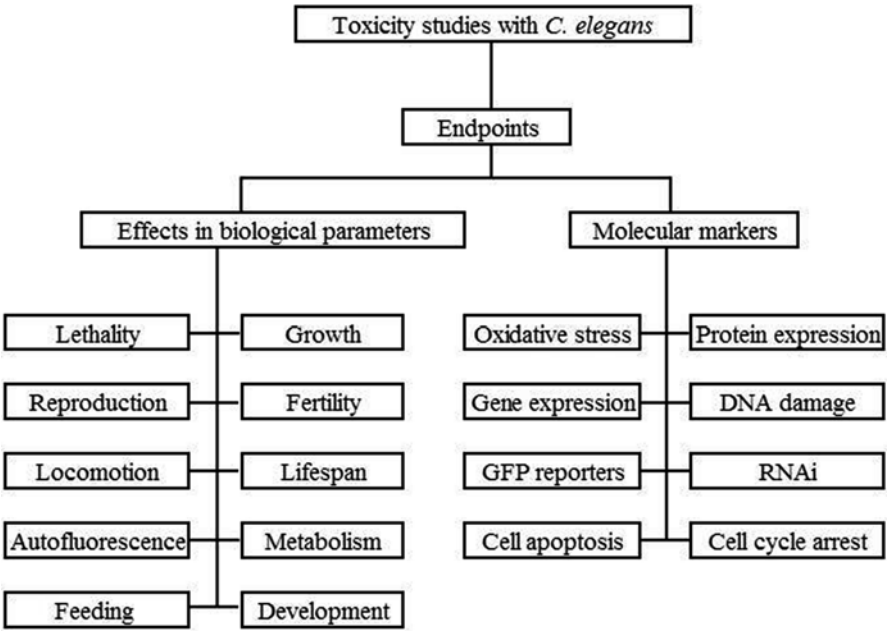

Fig. 2 End points toxicity on *C. elegans*. Toxicity studies with *C. elegans* could be carry out through two kinds of endpoints, those evaluate effects in nematode biology and those use molecular markers

instance, lethality, growth, locomotion, and reproduction. It is also possible to use molecular markers to determine oxidative stress, changes in gene or protein expression, DNA damage, or green fluorescence protein (GFP) expression. A classification of endpoints, commonly utilized in toxicity research using *C. elegans* as model is shown in Fig. 2.

Some of the frequently used endpoints related to toxicity assessment using *C. elegans* are presented below. These assays are usually performed employing concentration-response curves.

5.1 Lethality

This assay is performed to determine the death rate derived from acute toxicity in a concentration-response curve basis. 10 ± 1 young adults are transferred in microplates which contain different concentrations of the toxicant and a negative control. The exposure is carried out at 20 °C during 24 h in the absence of food. Then, the number of live and dead worms is counted through visual inspection using a dissecting microscope (Williams and Dusenbery 1990; Ellegaard et al. 2012; Helmcke and Aschner 2010; Kim et al. 2012; Wu et al. 2012a; Zhuang et al. 2014). Death is assumed when there is no movement during an observation period of 30 s (Rui et al. 2013; Shen et al. 2009; Wang et al. 2009a; Wu et al. 2013).

5.2 Growth

The effect of a toxicant in the development of the nematode can be evaluated by measuring the body length of synchronous worms before and after exposure, then comparing them to a vehicle-control. The bodies of the worms are observed employing a light microscope with 10X magnification and with image analysis software, such as Image-Pro® Express, ImageJ, or Fiji (Boyd et al. 2010; Cha et al. 2012; Höss et al. 2009b; Meyer et al. 2010; Roh and Choi 2011; Shen et al. 2009; Wang et al. 2010a; Yu et al. 2013a, b). Some authors have reported the warming of the worms to 50 °C in order to make them straight and ease the process of measuring their length (Wang et al. 2009a). The immobilization of the worms can also be achieved by using sodium azide (Turner et al. 2013). Growth can also be evaluated by registering the length of a curve, drawn from the tip of the head to the tip of the tail along the dorsal-ventral half of the animal intestine, using the reference line. Width measurements are taken in the vulva, drawing a line on the ventral side of the animal between the front edge and the posterior periphery of the vulva (Rudel et al. 2013). Other authors have proposed measuring the surface area for the flat worm (Rui et al. 2013; Wu et al. 2013; Zhuang et al. 2014). Currently, some laboratories have high-tech equipment, such as COPAS Biosort, which measures the optical density of the worm as an endpoint of growth (Hunt et al. 2012, 2013). The advantage is that the COPAS Biosort can analyze hundreds of nematodes per minute, and it can also evaluate mortality and fluorescence statistics (Hunt et al. 2012; Sprando et al. 2009). For growth assays, some authors perform 24 h exposure periods with *E. coli* OP50 as food (Boyd et al. 2010; Cha et al. 2012; Roh et al. 2009), whereas in other studies, *E. coli* uvrA, previously killed by UVA radiation is used; in this case, the exposure is carried out for 72 h and feeding is re-dosed every 24 h (Turner et al. 2013).

5.3 Reproduction

Brood size is the end point used to evaluate whether a toxic environment affects reproduction of the nematodes, placing exposed adult or L4 worms onto fresh plates. The number of offspring at all stages is counted and compared with a control group (Cha et al. 2012; Gomez et al. 2009; Höss et al. 2009b; Höss et al. 2013; Kim et al. 2012; Leelaja and Rajini 2013; Menzel et al. 2009; Li et al. 2012b; Roh et al. 2009; Rui et al. 2013; Smith et al. 2013; Wang et al. 2009a, 2010a, b). Counting is facilitated by heating the worms to 50 °C and staining them with Bengal red (Höss et al. 2013). In several studies the fertility rate is calculated by counting the total number of larvae at the end of the test and dividing by the total progeny recovered to the total parents (Rudel et al. 2013). This assay may also be carried out using the COPAS Biosort by measuring optical density (Boyd et al. 2010). Moreover, the gonad size, obtained by image analysis under a microscope, has been utilized to evaluate the effects on reproductive organs (Wu et al. 2011). Toxic effects may also be seen as

changes in the egg-laying pattern and the number of eggs or larvae at different time intervals (Gomez et al. 2009; Smith et al. 2013). Finally, the rate of egg laying can be estimated by placing adult worms exposed to fresh plates and counting the number of eggs laid in 1 h (Jadhav and Rajini 2009; Shashikumar and Rajini 2010).

5.4 Fertility

Reproductive toxicity can also be assessed by calculating the percentage of L4 larvae that develop fertilized eggs after exposure. Gravid hermaphrodites are considered to have at least one egg inside their bodies (Höss et al. 2009a, b; Roh and Choi 2011; Wang et al. 2009a). To count the number of eggs in the uterus, nematodes can be transferred to a bleach solution, which dissolves the body of the worm, directly exposing the eggs and allowing them to be counted under a light microscope (Wu et al. 2011).

5.5 Lifespan

Healthy worms at the L4 larval stage are exposed to a toxic agent, for example 24 h and then placing them on NGM plates with *E. coli* OP50. To prevent the production of offspring, 5-fluorodeoxyuridine is added. Worms are transferred to new plates every 3 days. The number of survivors is recorded daily until all animals die. The survival rate is calculated by dividing the number of live nematodes by the total number of nematodes, including both live and dead worms. The lifespan is defined as the time period between the L4 larval stage and death (Cha et al. 2012; Li et al. 2009, 2012b; Shen et al. 2009; Wang et al. 2010a; Zhuang et al. 2014).

5.6 Intestinal Autofluorescence

The intestinal lysosomal lipofuscin deposits that accumulate over time in the nematodes generate autofluorescence, feature used as a marker of aging. Treated nematodes are placed on an agar pad on a glass slide, then the fluorescent signals are captured by a fluorescence microscope. A band filter of 525 nm is employed to detect the endogenous intestinal fluorescence, and images are analyzed using software such as Magnafire®. Lipofuscin levels can be measured using the software ImageJ, by determining the mean pixel intensity in the intestine of each animal. Adults need to be photographed on the same day to avoid the light variation related to the intensity of the fluorescence source (Boyd et al. 2010; Helmcke and Aschner 2010; Rui et al. 2013; Shen et al. 2009; Wang et al. 2010a; Wu et al. 2012a, c, 2013; Zhuang et al. 2014).

5.7 Locomotion

Effects on the locomotion of nematodes have been linked to a deterioration of the neural network which can be evaluated based on several criteria, such as head thrash, body bend frequency, and basic movements (Yu et al. 2013a). Each exposed nematode is transferred to a plate containing 60 μL of K medium on the top of the agar. After a recovery period of 1 min, the number of head trashes is counted for 1 min. A head trash is defined as a change in the direction of bending in the body. To test the body bend frequency, nematodes are collected in a second plate, and then the number of times that the body bends in a period of 20 s is recorded. The bend of the body is observed as a change in direction of the upper pharynx along the Y axis, assuming that the nematodes are moved along the X axis. To test the basic movements, the number of sinusoidal forward movements is counted at an interval of 20 s. Locomotion behavior of control and treated nematodes should be analyzed simultaneously to avoid possible influences of the light-darkness cycle (Giles and Rankin 2009; Li et al. 2009, 2012a, b; Matsuura et al. 2013; Roh and Choi 2011; Rui et al. 2013; Wu et al. 2012a, 2013; Xing et al. 2009a; Yu et al. 2013a, b; Zhuang et al. 2014). Alternatively, immobility is determined by counting the number of immobile worms, usually registering a response when touched by platinum wire (Jadhav and Rajini 2009; Leelaja and Rajini 2013; Roh and Choi 2011).

5.8 Metabolism

To assess the state of metabolism, the pharyngeal pumping speed and the average cycle length of defecation can be evaluated. For testing the pumping rate, the nematodes are placed on NGM agar plates with food. After a few minutes, the pumping movement of the pharynx is counted for a minute under a microscope (Jadhav and Rajini 2009). To test the average cycle length of defecation, every nematode is observed individually for a fixed number of cycles. A cycle is defined as the interval between initiation of two successive steps of muscle contraction (Liu et al. 2013; Wu et al. 2012a; Zhao et al. 2014a, b).

5.9 Development

The effects of toxicants on the development of nematodes can be investigated by counting the number of individuals in each stage of their life cycle: egg, L1, L2, L3, L4 and adults, at regular time intervals up to 96 h after treatment (Roh and Choi 2011). The development through the larval stages can be estimated using the following criteria: L1 if they have four or fewer gonadal cells; L2 if they possess over four gonadal cells which have begun to spread along the length of the animal; L3 if there is a further extension of the gonad, and vulval morphogenesis has started; L4 if there is a dorsal rotation of the gonad; and adults if they have observable eggs (Helmcke et al. 2009). Entrance into the *dauer* state can be used to analyze toxicity,

since *dauer* formation is induced by causing starvation in nematodes. Usually, treated nematodes in state of gravidity are placed on agar plates until laying eggs at 20 °C. This progeny is changed to 27 °C, and 72 h later. The organisms in the *dauer* stage are counted (Wang et al. 2010b).

5.10 Feeding Behavior

Some toxics can affect the feeding and foraging behavior in *C. elegans*. Jones and Candido (1999) described a procedure to assess feeding behavior by monitoring the decline in the density of the bacterial food in liquid cultures of nematodes by measuring absorbance at 550 nm. Another method consists of the use of agar with round holes located equidistant from the center of the dish. Each hole is filled with bacterial suspension in K medium. Toxic solutions are placed in different holes, and nematodes are inoculated in the center of the plate. The number of nematodes in the interior of each hole is counted at various intervals of time. This test shows whether the test nematodes try to avoid contaminated food (Monteiro et al. 2014).

5.11 Oxidative Stress

Several markers of oxidative stress can be determined in worms after exposure to the examined agent, both within the organism and the supernatant (Helmcke and Aschner 2010; Leelaja and Rajini 2012; Shashikumar and Rajini 2010). The production of reactive oxygen species (ROS) and oxidative damage may be determined by fluorescence measurements, usually labeling the nematodes with 5-(y-6)-chloromethyl-2′,7′-dichlorodihydrofluorescein diacetate (Eom et al. 2013; Leelaja and Rajini 2013; Li et al. 2012a; Rui et al. 2013; Wu et al. 2012a, 2013; Zhuang et al. 2014), and reading with a laser scanning confocal microscope (Eom et al. 2013; Helmcke and Aschner 2010; Rudgalvyte et al. 2013; Liu et al. 2012; Wang et al. 2010a; Wu et al. 2012a). Oxidative damage on macromolecules has been analyzed by detecting carbonylated proteins (Wang et al. 2010a, b; Wu et al. 2011). Moreover, oxidative stress can be evaluated by quantifying changes in gene expression of oxidative stress-related genes by Real Time PCR or GFP reporters, such as *sod-1, sod-2, sod-3, sod-4, sod-5, gst-4, gst-5, gst-8, gst-24* and *gst-42* (Rui et al. 2013).

5.12 Patterns of Gene Expression

One method used to investigate the change in expression of genes in *C. elegans* exposed to environmental pollutants is the use of DNA microarrays or Real Time PCR (Eom et al. 2013; Menzel et al. 2009; Li et al. 2012a; Roh et al. 2009; Roh and Choi 2011; Rudgalvyte et al. 2013). As reference genes, *act-1* (Zhuang et al. 2014; Wang et al. 2014a) and *ubq-1* (Wu et al. 2011) are commonly used. This method has been applied to evaluate the effect on *C. elegans* gene expression for various

environmental toxicants, such as river sediments (Menzel et al. 2009), veterinary drugs (Zhuang et al. 2014), sodium fluoride (Li et al. 2012b), nanoparticles (Eom et al. 2013), and metals (Roh et al. 2009; Wang et al. 2014b; Rudgalvyte et al. 2013) among others.

5.13 Protein Expression

Induction of proteins by exposure to pollutants can be evaluated by traditional techniques such as ELISA and Western Blot. The ELISA technique was used to determine HSP90 protein expression in wild type *C. elegans* exposure to zinc at different temperatures (Wang and Ezemaduka 2014). Western blots were used to evaluate the effect of lead on HSP90 expression (Wang et al. 2014b).

5.14 DNA Damage

C. elegans has been used to evaluate DNA damage through various techniques. One approach is the use of the qPCR technique to detect damage and DNA repair. This test works on the principle that DNA damage inhibits the progression of the polymerase used in qPCR (Roh et al. 2009; Li et al. 2012b). The amount of long PCR product provides a measure of the frequency of the injury (Leung et al. 2010). Another alternative is the comet assay, which was used to evaluate the genotoxic profile of river sediment (Menzel et al. 2009). More recently, the pathway of base excision repair has been proposed as a mechanism to assess the damage to DNA by specific qPCR (Hunter et al. 2012). Transgenic strains can also be used to assess DNA damage. The strain *xpa-1* is deficient in the mechanism of nucleotide excision repair, and its growth is significantly affected when there is damage to DNA. Therefore, the growth assay on this strain is an indicator of genotoxicity (Leung et al. 2010). The transgenic strain *hus-1::GFP* is utilized to assess DNA damage. HUS-1::GFP foci represents DNA double-strand breaks, allowing quantification by counting the number of bright foci per 20 pachytene gonadal germ cells (Hofmann et al. 2002) which can be observed and counted under a fluorescence microscope (Wang et al. 2014a).

5.15 GFP Reporters

Transgenic nematodes carrying the GFP gene fused to various stress-inducible gene promoters have been developed for the study of various biochemical pathways. GFP strains are placed in wells containing the sample solutions and suitable controls. The plates are incubated at 20 °C, performing fluorescence readings within 4–6, 8–20,

and 24–40 h for short, moderate and long exposures, respectively. GFP expression is quantified using a fluorometer with a wavelength of 485 nm excitation and 525 nm emission (Anbalagan et al. 2012; Anbalagan et al. 2013; De Pomerai et al. 2010; Roh et al. 2010; Roh and Choi 2011). Alternatively, the observation of fluorescence can be achieved under a light microscope, capturing images that are then analyzed by specialized software (Li et al. 2009; Shen et al. 2009; Wang et al. 2010a; Polak et al. 2014). The COPAS Biosort system has also been employed to measure fluorescence (Hunt et al. 2012; Turner et al. 2013). The transgenic strain F25B3.3::GFP with fluorescence expression in neurons has been utilized to study heavy metal (Du and Wang 2009; Helmcke et al. 2009) and pesticide toxicity (Negga et al. 2011).

5.16 RNA Interference (RNAi)

This technology has been widely used to study gene function. Bacterial RNAi is introduced for 48 h at room temperature for the expression of dsRNA. Double stranded (ds) RNA expression is induced in HT115 bacteria containing the gene-sequence of interest inserted in the L4440 vector, or else the empty vector as control (Kamath and Ahringer 2003). Approximately ten nematodes in stages L1–L3 are placed on the plate seeded with induced RNAi or vector-control bacteria and incubated at 20 °C. After 36–40 h, the worms are transferred to another plate seeded with the same bacteria and grown to adulthood, at which point cultures are synchronised by egg isolation, and the eggs transferred onto new plates with RNAi bacteria. To evaluate the efficiency of dsRNA feeding over 1000 worms are evaluated by using semiquantitative PCR (Cheng et al. 2014; Kumar et al. 2010; Roh and Choi 2011). RNAi can be used to evaluate genetic pathways involved in toxicant responses. For instance, RNAi has been involved in the transcription of the DAF-16 factor in an unpredicted upregulation of the *cyp-34A9* reporter gene by exposure to high levels of cadmium (De Pomerai et al. 2008). In another case, gene knockdown by RNAi was used to determine the effects on reproduction due to PCB52 exposure; several genes were identified as having a crucial role, being the most remarkable the cytochrome P450s group (Menzel et al. 2007).

5.17 Cell Apoptosis

To assess apoptosis in the cells of the nematode, acridine orange is used. After exposure to the toxicant for 24 h, the nematodes are immersed in mixed medium with acridine orange at 20 °C for 2 h. Then they are placed on top of agar allowing them to recover for 10 min. Finally, they are examined under an inverted fluorescence microscope with an excitation wavelength of 515 and 488 nm absorption. Apoptotic cells appear yellow or yellow-orange showing increased DNA fragmentation, whereas intact cells are uniformly green (Li et al. 2012b; Wang et al. 2009b, 2014a, c).

Another technique involves staining with SYTO 12 for 4 h at room temperature, followed by seeding with food for 30 min, washing with M9 buffer, and final observation under a fluorescence microscope with a red filter (Cha et al. 2012). Alternatively, the transgenic strain *ced-1::GFP* is used for visualization of apoptotic bodies in a fluorescence microscope (Cheng et al. 2014; Kumar et al. 2010).

5.18 Cell Cycle Arrest

To investigate whether the exposure to a toxicant causes cell cycle arrest in the germline, the number of cores of mitotic cells is determined by staining with 4′,6-diamidino-2-phenylindole. The number of mitotic nuclei present at the distal end of the germline is counted under a fluorescence microscope (Cheng et al. 2014; Kumar et al. 2010; Wang et al. 2014a).

5.19 Transgenerational Effects

Sublethal endpoints such as locomotion and growth can be evaluated in the offspring of exposed parents. Wild-type N2 nematodes at the L3 larval stage are exposed during the time when sperm, ova and eggs begin to form, providing a window of prenatal exposure (Yu et al. 2013b). Exposed worms are placed on several plates. Some of them are used for measuring the parents after 24 h of exposure, and the others, for obtaining the generations. This assay has been used to assess the effects of antibiotics (Yu et al. 2011) and heavy metals on the growth and locomotion of exposed parents and their first generation (Yu et al. 2013b).

6 Toxicity Assessments

Most currently known toxicants can be assessed using *C. elegans* as a model. The following are some research studies related to toxicity of environmental matrices, metals, pesticides, nanoparticles, and other chemicals.

6.1 Environmental Samples

C. elegans has been used as a model to assess the toxicity of environmental samples such as soils, sludges, and river sediment. The sediment of the Danube, the Rhine and the Elbe Rivers in Germany were studied by analyzing the changes in gene

expression profiling using DNA microarrays of the entire genome. At the same time, the reproduction and DNA damage were evaluated using the comet assay technique (Menzel et al. 2009). In a study of the toxicity of contaminated soils from Germany, fertility, growth, and reproduction were evaluated using the wild type Bristol N2 strain (Höss et al. 2009b). Organic extracts of contaminated soil from Spain were evaluated using transgenic strains of *C. elegans* carrying GFP reporter genes driven by promoters sequences from five stress-related genes, *hsp-16.2*, *gpx-6*, *hsp-6, gst-1*, and *cyp34A9*; allowing the identification of different mechanisms of toxicity (Anbalagan et al. 2012). Aqueous extracts of the same soils were evaluated using 24 similar GFP transgenic reporter strains, correlating this data with the concentrations of metals present in the soil (Anbalagan et al. 2013). A summary of the results generated from these investigations is shown in Table 1.

6.2 Pesticides

In the environment, *C. elegans* as a free-living nematode, is exposed to various pesticides used in agriculture as well as to persistent organic waste that can contaminate soil for long periods of time (Anbalagan et al. 2013). Some of the most recent studies relating to the toxicity of pesticides in *C. elegans* are summarized in Table 2. As many pesticides are neurotoxic, the well-defined nervous system of *C. elegans* is a suitable tool to assess the neurotoxicity induced by these chemicals (Gomez et al. 2009; Leelaja and Rajini 2012, 2013; Lewis et al. 2013; Negga et al. 2011; Roh and Choi 2008, 2011; Shashikumar and Rajini 2010; Meyer and Williams 2014). Fluorescence expression by GFP reporter genes has been employed to study the toxicity of pesticides such as Glyphosate, Paraquat, 2,4-days, Endosulfan, Cypermethrin, Carbendazim, Chlorpyrifos, Diuron, Rotenone, DDT, Deltamethrin, and Dichlorvos (Anbalagan et al. 2013). In another report, Chlorpyrifos was studied, and although it did not cause severe DNA damage, it inhibited growth of *xpa-1* deficient strain, whose mechanism of nucleotide excision repair is deficient (Leung et al. 2010). The herbicide Glyphosate and the fungicide dithiocarbamate have been studied to assess mortality and neurological damage in *C. elegans*. Neuronal damage by exposure to these pesticides was verified by using the transgenic strain *F25B3.3::GFP* (Negga et al. 2011). The effect of Paraquat, Diquat, and Parathion on brood size was evaluated with COPAS Biosort, with Paraquat showing the highest toxicity (Boyd et al. 2010). Acetylcholinesterase activity of pesticides has also been assessed in nematodes exposed to Fenitrothion and Monocrotophos (Leelaja and Rajini 2013; Roh and Choi 2011). Studies with tributyltin reported that this biocide caused cell apoptosis in *C. elegans* via DNA double-strand breaks (DSBs) (Wang et al. 2014a). Furthermore, tributyltin chloride caused increased sterility and embryonic lethality by DSBs and checkpoint activation in the germline (Cheng et al. 2014). Insecticidal proteins such as Cry, used in transgenic corn, were studied and showed dose-dependent inhibitory effects on *C. elegans* reproduction (Höss et al. 2013).

Table 1 Evaluation of environmental samples using *C. elegans* as a model

Environmental sample	Strain	End point	Result	Reference
Sediments from three rivers in Germany: Danube, Rhine and Elbe	N2	Reproduction, DNA damage, changes in gene expression	Disaccharide and glycogen metabolism and functional pathways were affected	Menzel et al. (2009)
Water and sediment basins affected by coal mining in Virginia (United States)	N2, *gpdh-1, gpdh-2, mtl-2, mtl-2::GFP, pcs-1, smf-2, sod-3*	Growth and GFP expression	Inhibition of growth.	Turner et al. (2013)
Contaminated soils from Germany	N2	Fertility, growth and reproduction	Toxicity was correlated with the organic fraction	Höss et al. (2009a)
Organic extracts of contaminated soils in Southeast Spain	*hsp-16.2::GFP, gpx-6::GFP, hsp-6::GFP, gst-1::GFP, cyp34A9::GFP*	GFP expression	Induction of expression of transgenes	Anbalagan et al. (2013)
Aqueous extracts of soil	*hsp-16.1::GFP:lacZ, cep-1::GFP, hsp-16.2::GFP, sod-3::GFP, cyp-35a2::GFP, daf-16::GFP, gpx-6::GFP, hsp-6::GFP, gpx-4::GFP, cyp34A9::GFP, hsp-3::GFP, mtl-1::GFP, elt-2::GFP, gst-1::GFP, skn-1::GFP, gst-4::GFP, sod-4::GFP, cyp-29A2::GFP, ctl-2::GFP, hsf-1::GFP, mtl-2::GFP, hsp-60::GFP, hsp-70::GFP* y *sod-1::GFP*	GFP expression	Correlation between metals and expressed transgenes	Anbalagan et al. (2012)
Wastewater from recycled paper plant	N2, *daf-2*	Life span, *dauer* formation	Life span decreased	Wang et al. (2010b)
Particulate matter	N2, *unc-47::GFP*	Lethality, lifespan, growth, reproduction, locomotion, intestinal autofluorescence, oxidative stress, defecation behavior, gene expression	Effects on lifespan, reproduction, locomotion behavior, and intestinal development exposed and their progeny	Zhao et al. (2014a,b)
Surface water containing brominated organic compounds	N2	Locomotion, pharyngeal pumping, defecation, mechanical sensory stimulus, chemotaxis, thermotaxis	The surface water was neurostimulatory	Ju et al. (2014)
Dispersed oil crude	N2, (*bcIs39* [*(lim-7)ced-1p::GFP + lin-15(+)]), and TJ1(cep-1(gk138) I*)	Cell apoptosis, gene expression	Increasing of germ cell apoptosis following a CEP-1-dependent pathway	Polli et al. (2014)
Soil-derived Fe oxide colloids	N2, *sod-2(ok1030)*	Uptake of Fe, growth, reproduction	The toxicity of ferrihydrite, goethite and akaganeite depends on aggregate size and specific surface area	Höss et al. (2015)

Table 2 Evaluation of pesticide toxicity

Pesticide	Strain	End point	Result	Reference
Phosphine	N2	Oxidative stress	Delayed development and oxidative stress	Leelaja and Rajini (2012)
Fenitrothion	N2, *cyp35a2*	Paralysis, growth, fertility, development, AC activity	*Cyp35a2* involved in toxicity	Roh and Choi (2011)
Monocrotophos	N2	Fertility, oxidative stress, AC activity, paralysis	Decreased oxidative stress, paralysis and brood size. AC inhibition	Leelaja and Rajini (2013)
Cypermethrin	N2	Expression of *hsp-16*, rate of egg laying, life span, brood size, oxidative stress	Brood size, egg laying and life span decrease in a dose-dependent fashion Increased ROS and carbonylated proteins	Shashikumar and Rajini (2010)
Dithiocarbamate and glyphosate	N2, *F25B3.3::GFP*	Lethality and neuronal damage	Neurotoxicity was verified by fluorescent expression at neuronal level	Negga et al. (2011)
Cry proteins	N2, *bre-5*	Reproduction, gene expression	Reproduction inhibition MAPK pathway upregulated	Höss et al. (2013)
Gliphosate, Paraquat, 2,4-D, Endosulfan, Cypermethrin, Carbendazim, Chlorpyrifos, Diuron, Rotenone, DDT, Deltamethrin, Dichlorvos	*hsp-16.1::GFP:lacZ, cep-1, hsp-16.2, sod-3, cyp-35a2, daf-16, gpx-6, hsp-6, gpx-4::GFP, cyp34A9, hsp-3, mtl-1, elt-2, gst-1, skn-1, gst-4, sod-4, cyp-29A2, ctl-2, hsf-1, mtl-2, hsp-60, hsp-70, and sod-1*	GFP expression	Dichlorvos and Rotenone induced several stress response genes in a dose- dependent manner Endosulfan, DDT, Carbendazim, Deltamethrin, Cypermethrin, 2,4-D and Chlorpyrifos induced few genes	Anbalagan et al. (2013)
Chlorpyrifos	N2, *emb-8, glp-1, xpa-1*	Growth and DNA damage	No DNA damage was detected, but the *xpa-1* mutant was more sensitive than the wild type	Leung et al. (2010)

(continued)

Table 2 (continued)

Pesticide	Strain	End point	Result	Reference
Chlorpyrifos	N2	Gene expression, changes of free concentration of chlorpyrifos in the medium	The free concentration of chlorpyrifos quickly diminished and the expression of *cyp* genes varied with the volume of exposure medium and the test duration	Roh et al. (2014)
Diquat, Paraquat, Parathion	*N2, myo-2::GFP*	Body length, optical density, GFP expression, reproduction	Parathion > Diquat > Paraquat	Boyd et al. (2010)
Tributyltin	N2, *ced-3*, *ced-4*, *egl-1*, *ced-9*, *cep-1*, *cep-1*, *clk-2*, *hus-1*, *lin-45*,*mek-2*, *ksr-1*, *mpk-1*, *nsy-1*, *mek-1*, *jkk-1*, *mkk-4*, *jnk-1*, *sek-1*, *pmk-1*	Cell apoptosis, Cell cycle arrest, gene expression, DNA damage	Induction of apoptosis in germline via DNA damage	Wang et al. (2014a)
Imidacloprid and Tiacloprid	*N2*	Reproduction	Synergistic effect after exposure to the mixture	Gomez et al. (2009)
Dichlorvos	*N2*	Pharynx pumping, contraction of the nose, paralysis, egg laying rate	Correlation between the biochemical effects and behavior parameters	Jadhav and Rajini (2009)
Monocrotophos	N2	Locomotion, lifespan, egg laying, growth, AC activity	Decreased locomotion, lifespan, egg laying, and brood size	Salim and Rajini (2014)

6.3 Metals

Metals, in particular those named as heavy metals, constitute one of the most important groups of environmental toxicants, and reach the ecosystems from sources such as oil refineries, mining, and industrial effluents, causing severe toxic effects on living systems. This group has been one of the most studied using *C. elegans* as a biological model. Several of the reports related to the effects of heavy metals on *C. elegans* are presented in Table 3. The effects of different metals such as Ag, As, Cr, Cd, Cu, Hg, Mn, Pb, Ni and Zn have been studied for several end points such as lethality (Williams and Dusenbery 1990), lifespan, fertility, growth, intestinal autofluorescence, GFP expression, morphology changes (Shen et al. 2009; Rudel et al. 2013; Hunt et al. 2012), neuronal damage, neurodegeneration, neuronal loss, and axonal degradation (Du and Wang 2009; Xing et al. 2009a, b). On the other hand, exposure to Zn, Cd, Hg, Cu, Fe, Cr, and As has also been monitored using GFP transgenic reporter strains (De Pomerai et al. 2010).

6.4 Nanoparticles

The toxicological potential of nanoparticles (NPs) is receiving increased attention because of their massive release into the environment. Although a number of manufactured NPs are employed for medical and clinical purposes, the interaction between nanomaterials and biological systems remains unknown. For this reason, the NPs have joined the group of Emerging Contaminants and every year more studies on the subject are performed with *C. elegans* (Table 4). It has been considered that the main mechanism of nanotoxicity is oxidative stress (Zhao et al. 2014a). Toxicity of hydroxylated fullerene nanoparticles was studied using *C. elegans*, and it was demonstrated that water-soluble fullerol NPs have a potential for inducing apoptotic cell death (Cha et al. 2012). The study of TiO_2 NPs was carried out by analyzing different toxicity endpoints such as lethality, reproduction, growth, locomotion, intestinal autofluorescence, and oxidative stress. TiO_2 NPs caused severe deficits in gut development, defecation behavior, and changes in gene expression (Rui et al. 2013; Zhao et al. 2014a). The toxicity of TiO_2, ZnO, and SiO_2 NPs has been compared using endpoints including lethality, locomotion, growth, reproduction, and production of ROS. The order of toxicity was $ZnO > TiO_2 > SiO_2$ (Wu et al. 2013). In a study of the intake of silver NPs by image analysis, there was absorption of silver NPs in the body, transgenerational transfer, and inhibition of growth (Meyer et al. 2010). The lethal effects of AgNPs on *C. elegans* are increased if the exposure is through *E. coli OP50* (Ellegaard et al. 2012). In other research, reduction was observed in survival and reproduction and there was interaction of Ag NPs with biological surfaces of *C. elegans*, causing severe edema (Kim et al. 2012).

Table 3 Evaluation of metals and their derivatives

Metal	Strain	End point	Result	Reference
Zn, Cd, Hg, Cu, Fe, Cr, As	*mtl-1::GFP, mtl-2::GFP, hsp-16.1::GFP, hsp-16.2::GFP, hsp-3::GFP, hsp-6::GFP, hsp-60::GFP, hsp-70::GFP, hsf-1::GFP, elt-2::GFP, sod-1::GFP, sod-3::GFP, sod-4::GFP, ctl-2::GFP, skn-1::GFP, gst-1::GFP, gst-4::GFP, cyp-35a2::GFP, cyp34A9::GFP, cyp-29A2::GFP, daf-16::GFP, gpx-6::GFP, gpx-4::GFP, cep-1::GFP*	GFP expression	Pattern of gene expression induced by heavy metals	De Pomerai et al. (2010)
Cd	N2, *age-1,mtl-2, sod-3, daf-21, cyp35a2, skn-1, daf-12, hsp-16.2, daf-18, ctl-2, sod-1, daf-16, cep-1, cdr-2*	LC50, growth, reproduction	*mtl-2* gene expression upregulated	Roh et al. (2009)
Ag, Cr, Cd, Cu, Hg, Mn, Pb, Zn	N2, *hsp-16::2-GFP*	Lethality, life span, intestinal autofluorescence, GFP expression	Ag, Cr, Pb, Cu, Hg and Cd induced a stress response in the intestine; Pb, Hg, Cr, Zn and Mn in the neurons of the head	Shen et al. (2009)
Cd, Cr, Hg, Pb	N2	Brood size, generation time	Brood size was significantly correlated with the concentrations of the Metals. Hg produced severe reproductive toxicity	Guo et al. (2009)
Cd	*N2, myo-2::GFP*	Body length, optical density, GFP expression, reproduction	Brood size decreased in a concentration-dependent manner	Boyd et al. (2010)
Cr (VI)	N2	Lethality, growth, gene expression	Alterations in growth and modified gene expression were observed	Saikia et al. (2014)
Cr (VI)	N2	Lethality, locomotion, metabolism, intestinal autofluorescence, oxidative stress	Lethality, locomotion, metabolism, intestinal autofluorescence and ROS production were severely altered	Wu et al. (2012a)
Ca, Cd	*N2, mev-1*, *daf-16*, *daf-2*, *daf-16, hsp-16.2::GFP*	Life span, autofluorescence, GFP expression, oxidative stress, brood size, body length	The combined exposure to Cd and Ca increased *hsp-16.2::GFP* expression and oxidative damage	Wang et al. (2010a, b)

Metal	Strain	End point	Result	Reference
$H_3C\text{-}Hg^{(+)}$	*N2, F25B3.3*::*GFP, unc-17*::*GFP, unc-25*::*GFP, unc-47*::*GFP, cat-1*::*GFP, tph-1*::*GFP, eat-4*::*GFP, dat-1*::*GFP, F49H12.4*::*GFP*	Lethality, life span, growth, reproduction, development, pharynx pumping, GFP expression	Induction of developmental delay and reduced pharynx pumping	Helmcke et al. (2009)
$H_3C\text{-}Hg^{(+)}$	N2, *gst-4::GFP, hsp-4::GFP, mtl-1::GFP, mtl-2::GFP, gst-4, mtl-2, mtl-1*	Lethality, intestinal autofluorescence, GFP expression, oxidative stress	The methyl mercury induced hormesis.	Helmcke and Aschner (2010)
Cd, Pb, Cu, Zn	N2	Locomotion, growth, generational effects	Inhibition of growth and locomotion was more severe in the offspring than in exposed parents	Yu et al. (2013b)
Pb, Zn, Ni	N2	Reproduction, growth, feeding behavior	In reproduction Pb > Ni > Zn. In growth Ni > Pb > Zn	Monteiro et al. (2014)
Mn	N2, *hsp12.2::GFP*	Life span, growth, reproduction, GFP expression, oxidative stress	Defects in life span, development and reproduction. Increase in GFP expression and genes responsive to oxidative stress	Xiao et al. (2009)
Ni	N2, JK574	Growth, life span, fertility	Decreased larval survival and adult longevity depending upon concentration. Aqueous Ni inhibits fertility	Rudel et al. (2013)
$NiCl_2$	N2, MD701, KX84, DJR1	DNA damage, gene expression, fluorescence microscopy, apoptosis	Apoptosis is a result of exposure at high concentrations	Huffnagle et al. (2014)
Cd, Cu, Hg, K, As	N2	Lethality, fluorescence using COPAS Biosort	Changes in the morphology of the vulva and gonad, the thickness and internal intestinal integrity and retained eggs	Hunt et al. (2012)
$CdCl_2$, $CrCl_2$, $HgCl_2$, $Pb(NO_3)_2$	N2	Locomotion	Younger larvae showed more severe deficits in neurobehavioral phenotypes	Xing et al. (2009a)
$CrCl_2$, $CuSO_4$, $HgCl_2$, $AgNO_3$	N2, *lin-15, gcy-8::GFP*	Thermotaxis, GFP expression, gene expression	Severe deficits in thermotaxis	Xing et al. (2009b)

(continued)

Table 3 (continued)

Metal	Strain	End point	Result	Reference
Zn	N2, *daf-21*	Lethality, protein expression	High temperatures increase the toxicity of zinc. *daf-21* was more sensitive.	Wang and Ezemaduka 2014
Hg	N2, *hsp-16.2::GFP*	Reproduction, fertility, gonad size, GFP expression	Altered expression patterns of genes regulating oxidative stress causing deficiencies in the reproduction	Wu et al. 2011
Pb, Hg, Cu, Cd	N2, *unc-47*::*GFP*	Neuronal loss and neuronal degradation by fluorescence	Axonal degeneration and loss of neurons	Du and Wang (2009)
Cu	N2, *ced-3*, *ced-4*, *egl-1*, *ced-9*, *cep-1*, *clk-2*, *hus-1*, *abl-1*, *lin-45*, *mek-2*, *mpk-1*, *nsy-1*, *mek-1*, *jkk-1*, *mkk-4*, *jnk-1*, *sek-1*, *pmk-1*, *pmk-3*	Cell apoptosis	Caspases and Apaf-1 are required for the apoptosis germline	Wang et al. (2009b)
Cu	N2, *unc-2*	Changes in cuticle, chemotaxis, reproduction, development, and development	Body surface from vulva to tail was wrinkled and folded. Vulva size decreased. Development was delayed, egg-laying was reduced	Song et al. (2014)
Pb	N2, *daf*-2	Lethality, HSP 90 expression, pharyngeal pumping, reproduction, longevity	Temperatures affected lead toxicity	Wang et al. (2014b)

Table 4 Assessment of nanotoxicity

Nanoparticle	Strain	End point	Result	Reference
AgNPs	N2, *nth-1, sod-2, xpa-1, mtl-2, mev-1*	Growth, intake of silver NPs by image analysis	There was absorption of silver NPs in the body, transgenerational transfer and inhibition of growth	Meyer et al. (2010)
AgNPs	N2, *pmk-1, hif-1, egl-9, vhl-1, fmo-2, cyp35a2*	Oxidative stress and gene expression	Toxicity depends on size of NPs and ions in solution	Eom et al. (2013)
AgNPs	N2	Lethality and reproduction.	Reduction in survival and reproduction. Interaction of Ag NPs with biological surfaces	Kim et al. (2012)
AgNPs	*pha-1*	Lethality	Lethality is increased if the exposure is through food	Ellegaard et al. (2012)
AgNPs	N2, *cep-1*	Lethality, oxidative stress, DNA damage, mitochondrial membrane potential	Induction of oxidative stress-related mitochondrial DNA damage	Ahn et al. (2014)
AgNPs	N2	Lethality, hiperspectral image analysis	The presence of natural organic matter decreased toxicity and intestinal matter	Yang et al. (2014)
AgNPs	N2, pmk-1, ndx-4, nth-1.	RNAi, gene expression, DNA damage, survival	p38 MAPK/PMK-1 plays an important protective role in AgNP-induced oxidative DNA damage-repair	Chatterjee et al. (2014a)
AgNPs	N2	Growth, locomotion, gene expression, uptake of AgNPs	Results with *C. elegans* correlated with outcomes in rodents for AgNP size vs. uptake and toxicity	Hunt et al. (2014)
AgNPs	N2	Lethality, growth, reproduction	Sulfidation of AgNPs decreased toxicity. Reproduction was the most sensitive endpoint	Starnes et al. (2015)
Al_2O_3 and AgNPs	N2	Growth, survival, reproduction	In NPs-treated soils no acute toxic effects were found	Fajardo et al. (2014)
Al_2O_3NPs	*N2, sod-3*, *sod-2*, *hsp-16.2::GFP*	Locomotion, gene expression, oxidative stress, oxidative damage	Effects on locomotion by induction of ROS production	Li et al. (2012a)
CeO_2 and TiO_2NPs	N2	Lethality, Growth, fertility, gene expression, RNAi	Induction of *cyp35a2* gene expression, reduction of fertility and life span	Roh et al. (2010)
TiO_2NPs	N2, *mtl-1, mtl-2, sod-1, sod-2, sod-3, sod-4, sod-5, mev-1, aak-2, xpa-1, pcm-1, hsp-16.48, hsp-16.2, gst-4, gst-8, gst-24, gst-5, gst-42, isp-1*	Lethality, growth, reproduction, locomotion, intestinal autofluorescence, oxidative stress, gene expression	*sod-2*, *sod-3*, *mtl-2*, and *hsp-16.48* genes were susceptible to exposure.	Rui et al. (2013)
TiO_2NPs	N2	Lethality, growth, locomotion, autofluorescence intestinal, oxidative stress	Toxicity in oversized NPs was detected in the range of mg L^{-1} and small sizes were in the range of ng L^{-1}	Wu et al. (2012c)

(continued)

Table 4 (continued)

Nanoparticle	Strain	End point	Result	Reference
TiO_2 NPs	*mtl-1*, *mtl-2*, *sod-1*, *sod-2*, *sod-3*, *sod-4*, *sod-5*, *mev-1*, *aak-2*, *xpa-1*, *pcm-1*, *hsp-16.48*, *hsp-16.2, gst-4*, *gst-8*, *gst-24*, *gst-5*, *gst-42*, *isp-1*	Lethality, reproduction, locomotion, lifespan, development, growth, oxidative stress, intestinal autofluorescence, pharyngeal pumping and defecation	*sod-2*, *sod-3*, *mtl-2*, and *hsp-16.48* were susceptible strains for reproduction and locomotion, *sod-2*, *sod-3* and *mtl-2* for survival and *mtl-2* for development	Wu et al. (2014b)
TiO_2NPs	N2	Intestinal autofluorescence, oxidative stress, defecation cycle	Severe deficits in gut development and defecation behavior	Zhao et al. (2014a)
ZnONPs	N2	Lethality	Fototoxicity of NPs was correlated with ROS generation	Ma et al. (2011)
ZnO, Al_2O_3 and TiO_2NPs	N2	Lethality, fertility, growth and reproduction	Inhibition of growth and reproduction	Wang et al. (2009a)
TiO_2, ZnO and SiO_2NPs	N2	Lethality, locomotion, growth, reproduction and oxidative stress	ZnO > TiO_2 > SiO_2	Wu et al. (2013)
CeNPs	N2	Lethality, synchrotron X-ray analysis	The positively charged CeO_2-NPs were more toxic. The addition of humic acid decreased the toxicity	Collin et al. (2014)
Carbon nanotubes	N2	Reproduction, growth, life span, gene expression	Retarded growth, lower life span and defective embryogenesis	Chen et al. (2013)
Graphene oxide	N2, *mir-231,mir-235,mir-244, mir-73, mir-74,mir-247, mir-797,mir-246, mir-81-82,mir-360,mir-259*	Lifespan, oxidative stress, intestinal autofluorescence, locomotion, miRNA expression	23 up-regulated and eight down-regulated miRNAs, and correlation with lifespan	Wu et al. (2014a)
Nanosized zero-valent iron	N2	Growth, survival and reproduction	There were toxic effects at 5 and 10 mg mL^{-1}	Sacca et al. (2014)
Hydroxylated fullerene	N2, *ced-3, ced-4, elt-2::GFP*	Life span, reproduction, body length, cell apoptosis	Induction of death by cell apoptosis	Cha et al. (2012)
CdTe quantum dots	N2, *oxIs12[Is(*P*unc-47::GFP)], Ex(*P*unc-25-unc-30), Ex(*P*unc-25-sod-2),* and *Ex(*P*unc-25-sod-3)*	Foraging behavior, defecation behavior, reproduction, fluorescence	Neurotoxicity in the range of µg/L via oxidative stress and cell identity	Zhao et al. (2015)

6.5 Drugs

Drugs and their metabolites have been classified within the group of emerging contaminants because of increased concentrations in the environment. The impact of several drugs on organisms has been evaluated using *C. elegans* as a model (Table 5).

Table 5 Evaluation of the toxicity of drugs

Drug	Strain	End point	Result	Reference
Sulphamethoxazol	N2	Generational effects on locomotion and growth	Defects in locomotion of exposed parents and first generation were dose dependent	Yu et al. (2011)
Sulphamethoxazol	N2	Reproduction, growth, life span, pharynx pumping, lipid peroxidation and gene expression	Reduction in reproduction	Liu et al. (2013)
Sulfonamides	N2	Lethality, life span, growth, behavior inhibition	Concentration-dependent toxicity interactions	Yu et al. (2015)
Ethyl methanesulfonate	N2, *unc-58(e665)*	Mutagenesis, gene expression, DNA damage	Elevated mutation frequencies because embryonic cell cycles are rapid and DNA damage checkpoints are muted in embryos	Hartman et al. (2014)
Clenbuterol and Ractopamin	N2, *daf-2, daf-15, daf-16, sgk-1, skn-1, aak-1, age-1, sod-2, pdk-1, rict-1, act-1*	Lethality, reproduction, growth, locomotion, intestinal autofluorescence, oxidative stress, life span, gene expression	Toxicity by different mechanisms	Zhuang et al. (2014)
Caffeine and methadone	*N2, myo-2::GFP*	Growth, optical density, fluorescence and reproduction	Alteration in reproduction	Boyd et al. (2010)
Nicotine	N2	MicroRNA expression	There was alteration of microRNA expression profiles during post-embryonic stages	Taki et al. (2014)

(continued)

Table 5 (continued)

Drug	Strain	End point	Result	Reference
Nicotine	N2	Reproduction and gene expression	Loss of response to stimuli, early egg laying and alterations in genes related to reproduction and neuronal development	Smith et al. (2013)
Nicotine	N2, *lev-1*, *unc-29*, *bas-1*, *cat-2*, *tph-1*	Nicotine preference, taste plasticity, locomotion	Nicotine preference increased and taste plasticity inhibited	Matsuura et al. (2013)
Nicotine	N2	Locomotion	Reduction in velocity of basic movements and paralysis	Sobkowiak et al. (2011)
5-Fluorouracil	N2, *rrf-3*	Cell cycle arrest, apoptosis, RNAi, growth, development, gene expression	Induction of cell cycle arrest and germline apoptosis. Alteration in vulva development and egg laying	Kumar et al. (2010)
Genkwa Flos (traditional Chinese medicine)	N2, *daf-16*, *skn-1*, *mdt-15*, *oxIs12*	Lethality, growth, reproduction, locomotion, oxidative stress, defecation, gene expression	Toxicity effects on lifespan, development, reproduction, and locomotion. There was formation of abnormal vulva	Qiao et al. (2014)
Acrylamide	*gst-4::GFP::NLS, dop-3::RFP*	GFP and RFP expression	GSTs and other phase II enzymes were down regulated by XREP-1	Leung et al. (2011)

For instance, the effects of nicotine in plasticity and locomotion (Matsuura et al. 2013; Sobkowiak et al. 2011), changes in gene expression (Smith et al. 2013), and changes in microRNA expression (Taki et al. 2014); other studies show the effects of caffeine and methadone on reproduction (Boyd et al. 2010); and the effects of sulphamethoxazol on locomotion and growth of offspring of exposed parents (Yu et al. 2011), and on reproduction, growth, lifespan, pharynx pumping, lipid peroxidation, and gene expression (Liu et al. 2013); and the effect of 5-fluorouracil on reproduction and development (Kumar et al. 2010) among others.

6.6 Toxins

Natural toxins have also been studied using *C. elegans* as a biological model (Table 6). Microcystin, a toxin produced by toxic blooms of cyanobacteria on eutrophic waters, produced changes in the behavior of locomotion and GFP expression in *C. elegans* (Moore et al. 2014; Li et al. 2009; Saul et al. 2014). Aflatoxin β1, generated by *Aspergillus fungi*, inhibited growth and formed adducts with DNA by activation of the Cytochrome P system (Leung et al. 2010).

6.7 Other Chemicals

In addition to the above groups, *C. elegans* has been reported as a biological model for assessing the toxicity induced by other chemicals (Table 7) such as sodium fluoride (Li et al. 2012b), vinyl chloride (Nam and An 2010), benzo pyrene [a] and β-naphthoflavone (Leung et al. 2010), ethyl methanesulfonate and DMSO (Boyd et al. 2010), $NaAsO_2$, NaF, $Na_2B_4O_7$, valproic acid, caffeine and DMSO (Sprando et al. 2009); and acrylamide which has been shown to induce a *gst-4::GFP* transgene (Leung et al. 2011).

Table 6 Evaluation of toxicity of toxins

Toxin	Strain	End point	Result	Reference
Microcystin	N2, *hsp-16-2::GFP*	Life span, development, generation time, brood size, locomotion and gene expression	Life span reduced, development retarded, generation time lengthened, brood size decreased, locomotion inhibited	Li et al. (2009)
Microcystin	N2	Chemotaxis	Alteration of chemotactic behavior	Moore et al. (2014)
Microcystin	N2	Life span, reproduction, growth, gene expression	Deficiencies in lifespan, reproduction and growth. Changes in gene expression were dominated by neuromodulation	Saul et al. (2014)
Aflatoxin β1	N2, *emb-8, glp-1, xpa-1*	DNA damage and growth	DNA damage, DNA adducts by CYP activation	Leung et al. (2010)
Bioactive and probiotic marine bacteria	N2	Survival	*V. coralliilyticus* S2052 caused decreased survival after 72 h	Neu et al. (2014)

Table 7 Evaluation of the toxicity of other chemicals

Compound	Strain	End point	Result	Reference
Vinyl chloride	N2	Lethality, *cyp35s* expression.	Alterations in reproduction and gene expression	Nam and An (2010)
NaF	N2	Growth, life span, locomotion, cell apoptosis oxidative stress, gene expression	Growth and locomotion inhibited, life span decreased, ROS production increased in a dose-dependent relationship	Li et al. (2012b)
$NaAsO_2$, NaF, $Na_2B_4O_7$, valproic acid, caffeine and DMSO	N2	Growth and development	$NaAsO_2$ > NaF > $Na_2B_4O_7$ > valproic acid > caffeine > DMSO	Sprando et al. (2009)
PCB 52	N2, *cyp-14A2, cyp-14A5, cyp-23A1, cyp-34A9*	RNAi, gene expression	CYP-14A and CYP-34A6 contribute to the metabolism of PCB52	Schäfer et al. (2009)
Benzo[a]piren and β-naftoflavona	N2, *emb-8, glp-1, xpa-1*	DNA damage and growth	There was no DNA damage, but there was inhibited growth	Leung et al. (2010)
Ethyl methanesulfonate and DMSO	*N2, myo-2::GFP*	Body length, optical density, GFP expression, reproduction	DMSO inhibited brood size in a concentration-dependent manner	Boyd et al. (2010)
Dibromoacetic acid and tetrabromobisphenol-A	N2	Locomotion, pharyngeal pumping, defecation, mechanical sensory stimulus, chemotaxis, thermotaxis	TBBP-A had an impact on locomotion DBAA led to neurostimulation	Ju et al. (2014)
$NaAsO_2$	N2, *daf-16*, *daf-2*, *age-1*, *sqt-1*, *dpy-10*, *unc-52*, *akt-1*, *pdk-1*, *akt-2*, *sgk-1*	Cell apoptosis, oxidative stress	DAF-2, AGE-1 and AKT-1 negatively regulate arsenite-induced apoptosis	Wang et al. (2014c)

Se	N2	Growth, lifespan, reproduction, and hatching rate	Survival decreased significantly at all stages. Growth in the L1 stage declined	Li et al. (2014)
Se	N2	Gene expression	Increase in ROS and stress responses, visible by amplified expression of oxidoreductases and reduced expression of cuticle-associated genes	Boehler et al. (2014)
7-Ketocholesterol	N2	Lethality, growth, lifespan, thermotolerance, reproduction, oxidative stress, germline apoptosis	Reduction in reproduction, decrease in lifespan in a concentration-dependent manner, impairment in thermotolerance, induction of germline apoptosis and increase in ROS	Zong et al. (2014)
Na_2SiF_6, H_2SiF_6 and NaF	N2	Growth, feeding behavior, reproduction	Silicofluorides showed similar toxicity to NaF	Rice et al. (2014)
4-(Methylnitrosamino)-1-(3-pyridyl)-1-butanone	N2, PE255	Growth, mitochondrial and nuclear DNA damage, ATP level, O_2 consumption	Reduced oxygen consumption. Damage to nuclear and mitochondrial genomes	Bodhicharla et al. (2014)
Bio oils from biomass pyrolysis	N2, *pmk-1, daf-16, sir-2.1, hif-1, ctl-2, cyp35a2, mtl-2, sod-3, hsp16.2, ced-3, cep-1*	Survival	Dose-dependent decrease in survival	Chatterjee et al. (2014b)

7 Conclusion

C. elegans is a powerful, suitable and robust model for toxicological studies due to the transparency of its body, its short life cycle, easy fertilization, economical maintenance in the laboratory, large numbers of offspring and easy genetic manipulation. The use of this nematode has enabled the understanding of many biochemical pathways activated by environmental toxicants, allowing the study of multiple endpoints including lethality, growth, reproduction, fertility, and locomotion among others. Additionally, the ease of obtaining transgenic nematodes allows the possibility of studying direct changes in gene expression induced by toxicants or mixtures. Finally, this model may be used in the assessment of toxicity of several pollutants such as environmental samples, metals, pesticides, nanoparticles, drugs, and toxins, among others.

8 Summary

Caenorhabditis elegans is a nematode of microscopic size which, due to its biological characteristics, has been used since the 1970s as a model for research in molecular biology, medicine, pharmacology, and toxicology. It was the first animal whose genome was completely sequenced and has played a key role in the understanding of apoptosis and RNA interference. The transparency of its body, short lifespan, ability to self-fertilize and ease of culture are advantages that make it ideal as a model in toxicology. Due to the fact that some of its biochemical pathways are similar to those of humans, it has been employed in research in several fields.

C. elegans' use as a biological model in environmental toxicological assessments allows the determination of multiple endpoints. Some of these utilize the effects on the biological functions of the nematode and others use molecular markers. Endpoints such as lethality, growth, reproduction, and locomotion are the most studied, and usually employ the wild type Bristol N2 strain. Other endpoints use reporter genes, such as green fluorescence protein, driven by regulatory sequences from other genes related to different mechanisms of toxicity, such as heat shock, oxidative stress, CYP system, and metallothioneins among others, allowing the study of gene expression in a manner both rapid and easy. These transgenic strains of *C. elegans* represent a powerful tool to assess toxicity pathways for mixtures and environmental samples, and their numbers are growing in diversity and selectivity. However, other molecular biology techniques, including DNA microarrays and MicroRNAs have been explored to assess the effects of different toxicants and samples.

C. elegans has allowed the assessment of neurotoxic effects for heavy metals and pesticides, among those more frequently studied, as the nematode has a very well defined nervous system. More recently, nanoparticles are emergent pollutants whose toxicity can be explored using this nematode. Overall, almost every type of known toxicant has been tested with this animal model. In the near future, the available knowledge on the life cycle of *C. elegans* should allow more studies on reproduction

and transgenerational toxicity for newly developed chemicals and materials, facilitating their introduction in the market. The great diversity of endpoints and possibilities of this animal makes it an easy first-choice for rapid toxicity screening or to detail signaling pathways involved in mechanisms of toxicity.

Acknowledgements The authors thank the Vice-Rectory for Research of the University of Cartagena for its financial aid and to Carson Ward, visitor student from Purdue University, for his technical support.

References

Adam SA (2009) The nuclear transport machinery in *Caenorhabditis elegans:* a central role in morphogenesis. Semin Cell Dev Biol 20(5):576–581

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

Anbalagan C, Lafayette I, Antoniou-Kourounioti M, Gutierrez C, Martin JR, Chowdhuri DK, De Pomerai DI (2012) Transgenic nematodes as biosensors for metal stress in soil pore water samples. Ecotoxicology 21(2):439–455

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 *C. elegans* to investigate the patterns of stress responses induced by pesticides and by organic extracts from agricultural soils. Ecotoxicology 22(1):72–85

Bodhicharla R, Ryde I, Prasad G, Meyer J (2014) The tobacco-specific nitrosamine 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) induces mitochondrial and nuclear DNA damage in *Caenorhabditis elegans*. Environ Mol Mutagen 55(1):43–50

Boehler C, Raines A, Sunde R (2014) Toxic-selenium and low-selenium transcriptomes in *Caenorhabditis elegans*: toxic selenium up-regulates oxidoreductase and down-regulates cuticle-associated genes. PLoS One 9(6), e101408

Boyd W, McBride S, Rice J, Snyder D, Freedman J (2010) A high-throughput method for assessing chemical toxicity using a *C. elegans* reproduction assay. Toxicol Appl Pharmacol 245(2):153–159

Cha Y, Lee J, Choi S (2012) Apoptosis-mediated *in vivo* toxicity of hydroxylated fullerene nanoparticles in soil nematode *Caenorhabditis elegans*. Chemosphere 87(1):49–54

Chatterjee I, Ibanez C, Vijay P, Singaravelu G, Baldi C, Bair J, Ng S, Smolyanskaya A, Driscoll M, Singson A (2013) Dramatic fertility decline in aging *C. elegans* males is associated with mating execution deficits rather than diminished sperm quality. Exp Gerontol 48(1):1156–1166

Chatterjee N, Eom H, 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(2):122–133

Chatterjee N, Eom H, Jung S, Kim J, Choi J (2014b) Toxic potentiality of bio-oils, from biomass pyrolysis, in cultured cells and *Caenorhabditis elegans*. Environ Toxicol 29(12):1409–1419

Chen P, Hsiao K, Chou C (2013) Molecular characterization of toxicity mechanism of single-walled carbon nanotubes. Biomaterials 34(22):5661–5669

Cheng Z, Tian H, Chu H, Wu J, Li Y, Wang Y (2014) The effect of tributyltin chloride on *Caenorhabditis elegans* germline is mediated by a conserved DNA damage checkpoint pathway. Toxicol Lett 225(3):413–421

Collin B, Oostveen E, Tsyusko O, Unrine J (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(2):1280–1289

Coulson A, Kozono Y, Lutterbach B, Shownkeen R, Sulston J, Waterston R (1991) YACs and the *C. elegans* genome. Bioessays 13:413–417

De Pomerai D, Madhamshettiwar P, Anbalagan C, Loose M, Haque M, King J, Chowdhuri D, Sinha P, Johnsen B, Baillie D (2008) The stress-response network in animals: proposals to develop a predictive mathematical model. Open Toxicol J 2:71–76

De Pomerai D, Anbalagan C, Lafayette I, Rajagopalan D, Loose M, Haque M, King J (2010) High-throughput analysis of multiple stress pathways using GFP reporters in *C. elegans*. Environ Toxicol 132:177–187

Dimitriadi M, Hart A (2010) Neurodegenerative disorders: insights from the nematode *Caenorhabditis elegans*. Neurobiol Dis 40(1):4–11

Diomede L, Di Fede G, Romeo M, Bagnati R, Ghidoni R, Fiordaliso F, Salio M, Rossi A, Catania M, Paterlini A, Benussi L, Bastone A, Stravalaci M, Gobbi M, Tagliavini F, Salmona M (2014) Expression of A2V-mutated Aβ in *Caenorhabditis elegans* results in oligomer formation and toxicity. Neurobiol Dis 62:521–532

Du M, Wang D (2009) The neurotoxic effects of heavy metal exposure on GABAergic nervous system in nematode *Caenorhabditis elegans*. Environ Toxicol Pharmacol 27(3):314–320

Ellegaard L, Jensen K, Johansen A (2012) Nano-silver induces dose–response effects on the nematode *Caenorhabditis elegans*. Ecotoxicol Environ Saf 80(1):216–223

Eom H, Ahn J, 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(2):106–113

Estevez AO, Morgan KL, Szewczyk NJ, Gems D, Estevez M (2014) The neurodegenerative effects of selenium are inhibited by FOXO and PINK1/PTEN regulation of insulin/insulin-like growth factor signaling in *Caenorhabditis elegans*. NeuroToxicology 41:28–43

Fajardo C, Sacca M, Costa G, Nande M, Martin M (2014) Impact of Ag and Al2O3 nanoparticles on soil organisms: *in vitro* and soil experiments. Sci Total Environ 473–474:254–261

Finley J, Sandlin C, Holliday D, Keenan M, Prinyawiwatkul W, Zheng J (2013) Legumes reduced intestinal fat deposition in the *Caenorhabditis elegans* model system. J Funct Foods 5(3): 1487–1493

Garcia-Sancho M (2012) From the genetic to the computer program: the historicity of 'data' and 'computation' in the investigations on the nematode worm *C. elegans* (1963–1998). Stud Hist Philos Biol Biomed Sci C 43(1):16–28

Giles A, Rankin C (2009) Behavioral and genetic characterization of habituation using *Caenorhabditis elegans*. Neurobiol Learn Mem 92(2):139–146

Gomez J, Svendsen C, Lister L, Martin H, Hodson M, Spurgeon D (2009) Measuring and modelling mixture toxicity of imidacloprid and thiacloprid on *C. elegans* and *Eisenia fetida*. Ecotoxicol Environ Saf 72(1):71–79

Guo Y, Yang Y, Wang D (2009) Induction of reproductive deficits in nematode *C. elegans* exposed to metals at different developmental stages. Reprod Toxicol 28(1):90–95

Hartman P, Barry J, Finstad W, Khan N, Tanaka M, Yasuda K, Ishii N (2014) Ethyl methanesulfonate induces mutations in *Caenorhabditis elegans* embryos at a high frequency. Mutat Res Fund Mol M 766–767:44–48

Helmcke K, Aschner M (2010) Hormetic effect of methylmercury on *Caenorhabditis elegans*. Toxicol Appl Pharmacol 248(2):156–164

Helmcke K, Syversen T, Miller D, Aschner M (2009) Characterization of the effects of methylmercury on *Caenorhabditis elegans*. Toxicol Appl Pharmacol 240(2):265–272

Hofmann E, Milstein S, Boulton S, Ye M, Hofmann J, Stergiou L, Gartner A, Vidal M, Hengartner M (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

Höss S, Jänsch S, Moser T, Junker T, Römbke J (2009a) Assessing the toxicity of contaminated soils using the nematode *C. elegans* as test organism. Ecotoxicol Environ Saf 72(7): 1811–1818

Höss S, Haitzer M, Traunspurger W, Steinberg C (2009b) Growth and fertility of *C. elegans* (nematoda) in unpolluted freshwater sediments: response to particle size distribution and organic content. Environ Toxicol Chem 18(12):2921–2925

Höss S, Menzel R, Gessler F, Nguyen H, Jehle J, Traunspurger W (2013) Effects of insecticidal crystal proteins (cry proteins) produced by genetically modified maize (Bt maize) on the nematode *Caenorhabditis elegans*. Environ Pollut 178:147–151

Höss S, Fritzsche A, Meyer C, Bosch J, Meckenstock RU, Totsche KU (2015) Size- and composition-dependent toxicity of synthetic and soil-derived Fe oxide colloids for the nematode *Caenorhabditis elegans*. Environ Sci Technol 49(1):544–552

Huffnagle I, Joyner A, Rumble B, Hysa S, Rudel D, Hvastkovs E (2014) Dual electrochemical and physiological apoptosis assay detection of *in vivo* generated nickel chloride induced DNA damage in *Caenorhabditis elegans*. Anal Chem 86(16):8418–8424

Hunt P, Olejnik N, Sprando R (2012) Toxicity ranking of heavy metals with screening method using adult *Caenorhabditis elegans* and propidium iodide replicates toxicity ranking in rat. Food Chem Toxicol 50(9):3280–3290

Hunt P, Keltner Z, Gao X, Oldenburg S, Bushana P, Olejnik N, Sprando R (2014) Bioactivity of nanosilver in *Caenorhabditis elegans*: effects of size, coat, and shape. Toxicol Rep 1:923–944

Hunter SE, Gustafson MA, Margillo KM, Lee SA, Ryde IT, Meyer JN (2012) In vivo repair of alkylating and oxidative DNA damage in the mitochondrial and nuclear genomes of wild-type and glycosylase-deficient *Caenorhabditis elegans*. DNA Repair (Amst) 11(11):857–863

Jadhav K, Rajini P (2009) Neurophysiological alterations in *Caenorhabditis elegans* exposed to dichlorvos, an organophosphorus insecticide. Pest Biochem Physiol 94(2–3):79–85

Jones D, Candido E (1999) Feeding is inhibited by sublethal concentrations of toxicants and by heat stress in the nematode *Caenorhabditis elegans:* relationship to the cellular stress response. J Exp Zool 284:147–157

Ju J, Lieke T, Saul N, Pu Y, Yin L, Kochan C, Putschew A, Baberschke N, Steinberg C (2014) Neurotoxic evaluation of two organobromine model compounds and natural AOBr-containing surface water samples by a *Caenorhabditis elegans* test. Ecotoxicol Environ Saf 104:194–201

Kamath R, Ahringer J (2003) Genome-wide RNAi screening in *Caenorhabditis elegans*. Methods 30(4):313–321

Kim S, Nam S, An Y (2012) Interaction of silver nanoparticles with biological surfaces of *Caenorhabditis elegans*. Ecotoxicol Environ Saf 77(1):64–70

Kumar S, Aninat C, Michaux G, Morel F (2010) Anticancer drug 5-fluorouracil induces reproductive and developmental defects in *Caenorhabditis elegans*. Reprod Toxicol 29(4):415–420

Kumarasingha R, Palombo EA, Bhave M, Yeo TC, Lim DS, Tu CL, Shaw JM, Boag PR (2014) Enhancing a search for traditional medicinal plants with anthelmintic action by using wild type and stress reporter *Caenorhabditis elegans* strains as screening tools. Int J Parasitol 44(5): 291–298

L'Hernault S (2009) The genetics and cell biology of spermatogenesis in the nematode *Caenorhabditis elegans*. Mol Cell Endocrinol 306(1–2):59–65

Leelaja B, Rajini P (2012) Impact of phosphine exposure on development in *Caenorhabditis elegans:* involvement of oxidative stress and the role of glutathione. Pest Biochem Physiol 104(1):38–43

Leelaja BC, Rajini PS (2013) Biochemical and physiological responses in *C. elegans* exposed to sublethal concentrations of the organophosphorus insecticide, monocrotophos. Ecotoxicol Environ Saf 94(1):8–13

Leung M, Williams P, Benedetto A, Au C, Helmcke K, Aschner M, Meyer J (2008) *Caenorhabditis elegans*: an emerging model in biomedical and environmental toxicology. Toxicol Sci 106(1):5–28

Leung M, Goldstone J, Boyd W, Freedman J, Meyer J (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

Leung C, Deonarine A, Strange K, Choe K (2011) High-throughput screening and biosensing with fluorescent *C. elegans* strains. J Vis Exp 19(51):2745

Lewis J, Gehman E, Baer C, Jackson D (2013) Alterations in gene expression in *C. elegans* associated with organophosphate pesticide intoxication and recovery. BMC Genomics 14:291

Li Y, Wang Y, Yin L, Pu Y, Wang D (2009) Using the nematode *C. elegans* as a model animal for assessing the toxicity induced by microcystin-LR. J Environ Sci 21(3):395–401

Li Y, Yu S, Wu Q, Tang M, Pu Y, Wang D (2012a) Chronic Al_2O_3-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

Li Q, Zhang S, Yu Y, Wang L, Guan S, Li P (2012b) Toxicity of sodium fluoride to *Caenorhabditis elegans*. Biomed Environ Sci 25(2):216–223

Li W, Ju Y, Liao C, Liao V (2014) Assessment of selenium toxicity on the life cycle of *Caenorhabditis elegans*. Ecotoxicology 23(7):1245–1253

Liu P, He K, Li Y, Wu Q, Yang P, Wang D (2012) Exposure to mercury causes formation of male-specific structural deficits by inducing oxidative damage in nematodes. Ecotoxicol Environ Saf 79:90–100

Liu S, Saul N, Pan B, Menzel R, Steinberg C (2013) The non-target organism *Caenorhabditis elegans* withstands the impact of sulfamethoxazole. Chemosphere 93(10):2373–2380

Lublin AL, Link CD (2013) Alzheimer's disease drug discovery: *in vivo* screening using *Caenorhabditis elegans* as a model for β-amyloid peptide-induced toxicity. Drug Discov Today Technol 10(1):e115–e119

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(6):1473–1480

MacNeil L, Watson E, Arda HF, Zhu LJ, Walhout A (2013) Diet-induced developmental acceleration independent of TOR and insulin in *C. elegans*. Cell 153(1):240–252

Matsuura T, Miura H, Nishino A (2013) Inhibition of gustatory plasticity due to acute nicotine exposure in the nematode *Caenorhabditis elegans*. Neurosci Res 77(3):155–161

Megalou E, Tavernarakis N (2009) Autophagy in *Caenorhabditis elegans*. BBA Mol Cell Res 1793(9):1444–1451

Menzel R, Yeo H, Rienau S, Li S, Steinberg C, Stürzenbaum S (2007) Cytochrome P450s and short-chain dehydrogenases mediate the toxicogenomic response of PCB52 in the nematode *Caenorhabditis elegans*. J Mol Biol 370(1):1–13

Menzel R, Swain SC, Hoess S, Claus E, Menzel S, Steinberg S, Reifferscheid G, Stürzenbaum S (2009) Gene expression profiling to characterize sediment toxicity – a pilot study using *C. elegans* whole genome microarrays. BMC Genomics 10(160):1–15

Meyer D, Williams P (2014) Toxicity testing of neurotoxic pesticides in *Caenorhabditis elegans*. J Toxicol Environ Health B Crit Rev 17(5):284–306

Meyer J, Lord C, Yang X, Turner E, Badireddy A, Marinakos S, Chilkoti A, Wiesner M, Auffan M (2010) Intracellular uptake and associated toxicity of silver nanoparticles in *Caenorhabditis elegans*. Aquat Toxicol 100(2):140–150

Monteiro L, Brinke M, dos Santos G, Traunspurger W, Moens T (2014) Effects of heavy metals on free-living nematodes: a multifaceted approach using growth, reproduction and behavioural assays. Eur J Soil Biol 62:1–7

Moore C, Lein P, Puschner B (2014) Microcystins alter chemotactic behavior in *Caenorhabditis elegans* by selectively targeting the AWA sensory neuron. Toxins (Basel) 6(6):1813–1836

Nam S, An Y (2010) Assessing the ecotoxicity of vinyl chloride using green alga *P. subcapitata*, nematode *C. elegans*, and the SOS chromotest in a closed system without headspace. Sci Total Environ 408(15):3148–3152

Negga R, Rudd D, Davis N, Justice A, Hatfield H, Valente A, Fields A, Fitsanakis V (2011) Exposure to Mn/Zn ethylene-bis-dithiocarbamate and glyphosate pesticides leads to neurodegeneration in *Caenorhabditis elegans*. NeuroToxicology 32(3):331–341

Neu A, Mansson M, Gram L, Prol-García M (2014) Toxicity of bioactive and probiotic marine bacteria and their secondary metabolites in *Artemia sp*. and *Caenorhabditis elegans* as eukaryotic model organisms. Appl Environ Microbiol 80(1):146–153

Pang S, Curran S (2014) Adaptive capacity to bacterial diet modulates aging in *C. elegans*. Cell Metab 19(2):221–231

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

Polli J, Zhang Y, Pan X (2014) Dispersed crude oil amplifies germ cell apoptosis in *Caenorhabditis elegans*, followed a CEP-1-dependent pathway. Arch Toxicol 88(3):543–551

Qiao Y, Zhao Y, Wu Q, Sun L, Ruan Q, Chen Y, Wang M, Duan J, Wang D (2014) Full toxicity assessment of Genkwa Flos and the underlying mechanism in nematode *Caenorhabditis elegans*. PLoS One 9(3), e91825

Rice J, Boyd W, Chandra D, Smith M, Den Besten P, Freedman J (2014) Comparison of the toxicity of fluoridation compounds in the nematode *Caenorhabditis elegans*. Environ Toxicol Chem 33(1):82–88

Roh J, Choi J (2008) Ecotoxicological evaluation of chlorpyrifos exposure on the nematode *Caenorhabditis elegans*. Ecotoxicol Environ Saf 71(2):483–489

Roh J, Choi J (2011) *Cyp35a2* gene expression is involved in toxicity of fenitrothion in the soil nematode *Caenorhabditis elegans*. Chemosphere 84(10):1356–1361

Roh J, Park Y, Choi J (2009) A cadmium toxicity assay using stress responsive *C. elegans* mutant strains. Environ Toxicol Pharmacol 28(3):409–413

Roh J, Park Y, Park K, Choi J (2010) Ecotoxicological investigation of CeO_2 and TiO_2 nanoparticles on the soil nematode *C. elegans* using gene expression, growth, fertility, and survival as endpoints. Environ Toxicol Pharmacol 29(2):167–172

Roh J, Lee H, Kwon J (2014) Changes in the expression of *cyp35a* family genes in the soil nematode *Caenorhabditis elegans* under controlled exposure to chlorpyrifos using passive dosing. Environ Sci Technol 48(17):10475–10481

Rudel D, Douglas CD, Huffnagle IM, Besser JM, Ingersoll CG (2013) Assaying environmental nickel toxicity using model nematodes. PLoS One 8(10), e77079

Rudgalvyte M, VanDuyn N, Arnaio V, Heikkinen L, Peltonen J, Lakso M, Nass R, Wong G (2013) Methylmercury exposure increases lipocalin related (lpr) and decreases activated in blocked unfolded protein response (abu) genes and specific miRNAs in *Caenorhabditis elegans*. Toxicol Lett 222(2):189–196

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(10):2289–2296

Sacca M, Fajardo C, Costa G, Lobo C, Nande M, Martin M (2014) Integrating classical and molecular approaches to evaluate the impact of nanosized zero-valent iron (nZVI) on soil organisms. Chemosphere 104:184–189

Saikia S, Gupta R, Pant A, Pandey R (2014) Genetic revelation of hexavalent chromium toxicity using *Caenorhabditis elegans* as a biosensor. J Expo Sci Environ Epidemiol 24(2):180–184

Salim C, Rajini P (2014) Glucose feeding during development aggravates the toxicity of the organophosphorus insecticide monocrotophos in the nematode, *Caenorhabditis elegans*. Physiol Behav 131:142–148

Saul N, Chakrabarti S, Stürzenbaum S, Menzel R, Steinberg C (2014) Neurotoxic action of microcystin-LR is reflected in the transcriptional stress response of Caenorhabditis elegans. Chem Biol Interact 223:51–57

Schäfer P, Müller M, Krüger A, Steinberg CE, Menzel R (2009) Cytochrome P450-dependent metabolism of PCB52 in the nematode *Caenorhabditis elegans*. Arch Biochem Biophys 488(1):60–68

Shashikumar S, Rajini P (2010) Cypermethrin elicited responses in heat shock protein and feeding in *Caenorhabditis elegans*. Ecotoxicol Environ Saf 73(5):1057–1062

Shen L, Xiao J, Ye H, Wang D (2009) Toxicity evaluation in nematode *C. elegans* after chronic metal exposure. Environ Toxicol Pharmacol 28(1):125–132

Shi Y, Liao V, Pan T (2012) Monascin from red mold dioscorea as a novel antidiabetic and antioxidative stress agent in rats and *Caenorhabditis elegans*. Free Radic Biol Med 52(1):109–117

Smith M, Zhang Y, Polli J, Wu H, Zhang B, Xiao P, Farwell M, Pan X (2013) Impacts of chronic low-level nicotine exposure on *Caenorhabditis elegans* reproduction: identification of novel gene targets. Reprod Toxicol 40:69–75

Sobkowiak R, Kowalski M, Lesicki A (2011) Concentration- and time-dependent behavioral changes in *Caenorhabditis elegans* after exposure to nicotine. Pharmacol Biochem Behav 99(3):365–370

Song S, Guo Y, Zhang X, Zhang X, Zhang J, Ma E (2014) Changes to cuticle surface ultrastructure and some biological functions in the nematode *Caenorhabditis elegans* exposed to excessive copper. Arch Environ Contam Toxicol 66(3):390–399

Sprando R, Olejnik N, Nese H, Ferguson M (2009) A method to rank order water soluble compounds according to their toxicity using *Caenorhabditis elegans*, a complex object parametric analyzer and sorter, and axenic liquid media. Food Chem Toxicol 47(4):722–728

Starnes D, Unrine J, Starnes C, Collin B, Oostveen E, Ma R, Lowry G, Bertsch P, Tsyusko O (2015) Impact of sulfidation on the bioavailability and toxicity of silver nanoparticles to *Caenorhabditis elegans*. Environ Pollut 196:239–246

Sulston J, Schierenberg E, White J, Thomson J (1983) The embryonic cell lineage of the nematode *Caenorhabditis elegans*. Dev Biol 100:64–119

Taki F, Pan X, Zhang B (2014) Chronic nicotine exposure systemically alters microRNA expression profiles during post-embryonic stages in *Caenorhabditis elegans*. J Cell Physiol 229(1):79–89

Turner E, Kroeger G, Arnold M, Thornton B, Di Giulio R, Meyer J (2013) Assessing different mechanisms of toxicity in mountaintop removal/valley fill coal mining-affected watershed samples using *Caenorhabditis elegans*. PLoS One 8(9), e75329

Wang Y, Ezemaduka AN (2014) Combined effect of temperature and zinc on *Caenorhabditis elegans* wild type and daf-21 mutant strains. J Therm Biol 41:16–20

Wang H, Wick R, Xing B (2009a) Toxicity of nanoparticulate and bulk ZnO, Al_2O_3 and TiO_2 to the nematode *C elegans*. Environ Pollut 157(4):1171–1177

Wang S, Wu L, Wang Y, Luo X, Lu X (2009b) Copper-induced germline apoptosis in *Caenorhabditis elegans*: the independent roles of DNA damage response signaling and the dependent roles of MAPK cascades. Chem Biol Interact 180(2):151–157

Wang D, Liu P, Yang Y, Shen L (2010a) Formation of a combined Ca/Cd toxicity on lifespan of nematode *Caenorhabditis elegans*. Ecotoxicol Environ Saf 73(6):1221–1230

Wang D, Wang Y, Shen L (2010b) Confirmation of combinational effects of calcium with other metals in a paper recycling mill effluent on nematode lifespan with toxicity identification evaluation method. J Environ Sci 22(5):731–737

Wang Y, Wang S, Luo X, Yang Y, Jian F, Wang X, Xie L (2014a) The roles of DNA damage-dependent signals and MAPK cascades in tributyltin-induced germline apoptosis in *Caenorhabditis elegans*. Chemosphere 108:231–238

Wang Y, Xu S, Liu J, Zhang Y, Guo T (2014b) Regulation of lead toxicity by heat shock protein 90 (*daf-21*) is affected by temperature in *Caenorhabditis elegans*. Ecotoxicol Environ Saf 104:317–322

Wang S, Teng X, Wang Y, Yu H, Luo X, Xu A, Wu L (2014c) Molecular control of arsenite-induced apoptosis in *Caenorhabditis elegans*: roles of insulin-like growth factor-1 signaling pathway. Chemosphere 112:248–255

White JG, Southgate E, Thomson JN, Brenner S (1986) The structure of the nervous system of the nematode *Caenorhabditis elegans*. Philos Trans R Soc Lond B Biol Sci 314(1165):1–340

Williams P, Dusenbery D (1990) Aquatic toxicity testing using the nematode, Caenorhabditis elegans. Environ Toxicol Chem 9(10):1285–1290

Wu Q, He K, Liu P, Li X, Wang D (2011) Association of oxidative stress with the formation of reproductive toxicity from mercury exposure on hermaphrodite nematode *Caenorhabditis elegans*. Environ Toxicol Pharmacol 32(2):175–184

Wu Q, Qu Y, Li X, Wang D (2012a) Chromium exhibits adverse effects at environmental relevant concentrations in chronic toxicity assay system of nematode *Caenorhabditis elegans*. Chemosphere 87(11):1281–1287

Wu H, Zhao Y, Guo Y, Xu L, Zhao B (2012b) Significant longevity-extending effects of a tetrapeptide from maize on *C. elegans* under stress. Food Chem 130(2):254–260

Wu Q, Wang W, Li Y, Ye B, Tang M, Wang D (2012c) Small sizes of TiO_2-NPs exhibit adverse effects at predicted environmental relevant concentrations on nematodes in a modified chronic toxicity assay system. J Hazard Mater 243:161–168

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 environmental relevant concentrations in nematode *Caenorhabditis elegans*. Chemosphere 90(3):1123–1131

Wu Q, Zhao Y, Zhao G, Wang D (2014a) microRNAs control of *in vivo* toxicity from graphene oxide in *Caenorhabditis elegans*. Nanomedicine 10(7):1401–1410

Wu Q, Zhao Y, Zhao G, Wang D (2014b) Susceptible genes regulate the adverse effects of TiO_2-NPs at predicted environmental relevant concentrations on nematode *Caenorhabditis elegans*. Nanomedicine 10(6):1263–1271

Xiao J, Rui R, Guo Y, Chang X, Wang D (2009) Prolonged manganese exposure induces severe deficits in lifespan, development and reproduction possibly by altering oxidative stress response in *Caenorhabditis elegans*. J Environ Sci 21(6):842–848

Xing X, Guo Y, Wang D (2009a) Using the larvae nematode *C. elegans* to evaluate neurobehavioral toxicity to metallic salts. Ecotoxicol Environ Saf 72(7):1819–1823

Xing X, Du M, Xu M, Rui Q, Wang D (2009b) Exposure to metals induces morphological and functional alteration of AFD neurons in nematode *Caenorhabditis elegans*. Environ Toxicol Pharmacol 28(1):104–110

Y H, Wang Y, Ye B, Wang D (2008) Phenotypic and behavioral defects induced by iron exposure can be transferred to progeny in *Caenorhabditis elegans*. Biomed Environ Sci 21(6):467–473

Yang X, Jiang C, Hsu-Kim H, Badireddy A, Dykstra M, Wiesner M, Hinton D, Meyer J (2014) Silver nanoparticle behavior, uptake, and toxicity in *Caenorhabditis elegans*: effects of natural organic matter. Environ Sci Technol 48(6):3486–3495

Yu Z, Jiang L, Yin D (2011) Behavior toxicity to *C. elegans* transferred to the progeny after exposure to sulfamethoxazole at environmentally relevant concentrations. J Environ Sci 23(2):294–300

Yu Z, Chen X, Zhang J, Wang R, Yin D (2013a) Transgenerational effects of heavy metals on L3 larva of *C. elegans* with greater behavior and growth inhibitions in the progeny. Ecotoxicol Environ Saf 88(1):78–184

Yu Z, Chen X, Zhang J, Yin D, Deng H (2013b) Inhibitions on the behavior and growth of the nematode progeny after prenatal exposure to sulfonamides at micromolar concentrations. J Hazard Mater 250–251:198–203

Yu Z, Yin D, Deng H (2015) The combinational effects between sulfonamides and metals on nematode *Caenorhabditis elegans*. Ecotoxicol Environ Safe 111:66–71

Zhao Y, Wu Q, Tang M, Wang D (2014a) The *in vivo* underlying mechanism for recovery response formation in nano-titanium dioxide exposed *Caenorhabditis elegans* after transfer to the normal condition. Nanomedicine 10(1):89–98

Zhao Y, Lin Z, Jia R, Li G, Xi Z, Wang D (2014b) Transgenerational effects of traffic-related fine particulate matter (PM2.5) on nematode *Caenorhabditis elegans*. J Hazard Mater 274:106–114

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

Zhuang Z, Zhao Y, Wu Q, Li M, Liu H, Sun L, Gao W, Wang D (2014) Adverse effects from clenbuterol and ractopamine on nematode *Caenorhabditis elegans* and the underlying mechanism. PLoS One 9(1):e85482

Zong Y, Gao J, Feng H, Cheng B, Zhang X (2014) Toxicity of 7-ketocholesterol on lethality, growth, reproduction, and germline apoptosis in the nematode *Caenorhabditis elegans*. J Toxicol Environ Health A 77(12):716–723