HHS Public Access

Author manuscript

Toxicol Appl Pharmacol. Author manuscript; available in PMC 2019 September 01.

Published in final edited form as:

Toxicol Appl Pharmacol. 2018 September 01; 354: 126–135. doi:10.1016/j.taap.2018.03.016.

C. elegans as a model in developmental neurotoxicology

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Abstract

Due to many advantages *Caenorhabditis elegans* (*C. elegans*) has become a preferred model of choice in many fields, including neurodevelopmental toxicity studies. This review discusses the benefits of using *C. elegans* as an alternative to mammalian systems and gives examples of the uses of the nematode in evaluating the effects of major known neurodevelopmental toxins, including manganese, mercury, lead, fluoride, arsenic and organophosphorus pesticides. Reviewed data indicates numerous similarities with mammals in response to these toxins. Thus, *C. elegans* studies have the potential to predict possible effects of developmental neurotoxicants in higher animals, and may be used to identify new molecular pathways behind neurodevelopmental disruptions, as well as new toxicants.

Keywords

neurodevelopment; neurotoxicity; *C. elegans*; manganese; mercury; pesticides

1. Neurodevelopmental toxicity studies – a need for alternative models

The developing brain is exceptionally sensitive to toxic chemicals – the levels which lead to adverse effects are often lower than those effective in adults, and even minimal exposures, generally considered as safe, might be harmful. Moreover, only a few chemicals have been studied and identified as toxic to developing brain, and safe dosages have only been estimated. With over 1,000 chemicals currently identified as toxic to animals, and more than 200 recognized as neurotoxic to the adult human, only 12 are characterized as toxic to the developing human brain (Grandjean and Landrigan, 2014). This is mostly due to the fact that

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Conflicts of interest: The authors declare no conflict of interest.

neurotoxicity is usually discovered during incidents of acute poisoning in adults, specifically from occupational exposure or suicide attempts, which provides clear association between exposure and adverse effects. In contrast, in developmental neurotoxicity the exposure occurs sometimes years before any neurological defects might be identified, and therefore the identification of new hazards is often much slower.

As proposed by Grandjean & Landrigan, the current list of developmental neurotoxins includes arsenic (As), lead (Pb), methylmercury (MeHg), manganese (Mn), fluoride (F), ethanol (EtOH), toluene (PhMe), tetrachloroethylene (PERC), polybrominated diphenyl ethers (PBDEs), organophosphate pesticides (chlorpyrifos) and dichlorodiphenyltrichloroethane (DDT)/ dichlorodiphenyldichloro-ethylene (DDE) (Grandjean and Landrigan, 2014). These compounds have been shown to disrupt neurogenesis, brain cell proliferation, migration, synapse formation and myelination during development (Hu *et al.*, 2014), leading to physiological and behavioral deficits which are often permanent and untreatable. Impaired central nervous system (CNS) function might comprise cognitive, motor, language and affective disabilities, which might require constant psychosocial and medical care, thus significantly reducing the quality of life. Some toxicants were identified as contributing factors in neurodevelopmental disorders (NDDs), such as autism spectrum disorder (ASD), intellectual disability (ID), attention deficit/ hyperactivity disorder (ADHD), social communication disorders, as well as motor disorders (Hansen and Rogers, 2013). Currently approximately (approx.) 10–15% of all births are affected by one or a combination of some NDDs and this trend seems to be growing (Bloom *et al.*, 2013). With the contribution of genetic predispositions estimated on maximum 40% of all cases, environmental exposures to toxins (alone or by interaction with genetic factors) are considered as a main cause of NNDs (Grandjean and Landrigan, 2014). Therefore, there is an urgent need to identify and further study these substances.

In developmental neurotoxicity research, the use of mammalian models is still a primary choice. However, the time and expense involved in analyzing a growing number of compounds have recently led to the adaptation of an alternative *in vivo* and *in vitro* models, which provide high-throughput screens much faster and at lower cost (Peterson *et al.*, 2008). This review discusses the benefits of using *Caenorhabditis elegans* (*C. elegans*) as an alternative and complementary model to mammalian systems and gives examples of the uses of *C. elegans* in neurodevelopmental toxicology research.

2. *C. elegans* in neuroscience

2.1. General remarks on *C. elegans* as model organism

The small non-parasitic nematode *C. elegans* is one of the best-established animal models that has contributed greatly to the understanding of many human diseases. It is a very attractive experimental model due to many advantages: small size – adults are approx. 1 mm in length, short life cycle (approx. 3 days at 20°C), ability to self-fertilize and high reproductive rate (>300 offspring per hermaphrodite), which makes its maintainance in the lab relatively easy and inexpensive. *C. elegans* lab assays are usually rapid, of low cost, and amenable to high-throughput analysis. Additionally, the worms' transparency and the ease of making reporter gene fusions (*e.g.* with green fluorescent protein, GFP) enable visualization

of cell morphology and protein expression patterns *in vivo*. The worm is also a powerful genetic tool. Their genome is easy to manipulate -gene knockdowns (KD) can be generated with RNAi and knockouts (KO) can be easily generated by directed mutagenesis. Moreover, the self-fertilizing hermaphrodite allows for homozygosity of mutations and mating with males can produce strains with multiple mutations. The nematode was the first multicellular organism to have its genome fully sequenced (Consortium and T.C.e.S, 1998), revealing a high degree of evolutionary conservation with higher eukaryotes and mammals. Worms express homologues to approx. 80% of human genes, many of which share great sequence identity (Culetto and Sattelle, 2000; Kennedy, 2008). In addition, the basic biological functions and many biochemical pathways are highly conserved with higher organisms, such as apoptosis (Malin and Shaham, 2015), stress response (Rodriguez *et al.*, 2013) and various cell signaling pathways (Blaxter, 2011; Lapierre and Hansen, 2012; Sato *et al.*, 2014), allowing for findings from *C. elegans* to be extrapolated and further confirmed in vertebrate systems.

C. elegans, however, poses several limitations as a model organism, which should not be omitted, when discussing its utility. This simple organism lacks many specific organs, such as kidneys, liver, lung, skin and a circulatory system. The evolutionary distance from humans might be a limitation, especially when studying complex conditions. Self-fertilization and the scarcity of males in wild type (WT) population make it difficult to study sex-specific differences, an approach currently promoted in neuroscience, including developmental neurotoxicology, and in other fields (Brooks and Clayton, 2017).

Nevertheless, the numerous advantages highly outrank these limitations, and the worms have been successfully utilized as model organism in many studies.

2.2. The nervous system of *C. elegans*

One of the biggest advantages of the model is its nervous system, which is well characterized and considered as structurally and functionally similar to mammals. The nematode nervous system contains 302 neurons in adult hermaphrodites (383 in males), divided into 118 morphologically distinct classes and 56 glia cells, which together form over 7600 synapses (White *et al.*, 1986). *C. elegans* is the only organism whose neuronal wiring diagram has been determined – all neurons and the major interactions between them are well characterized and mapped. The biochemistry of the *C. elegans* nervous system is also highly conserved with mammals – as the worms express similar ion channels, receptors, vesicular transporters, and other synaptic components (Bargmann, 1998). Furthermore, formation, trafficking and release of synaptic vesicles are analogous to mammals (Barclay *et al.*, 2012). The nematode uses classical neurotransmitters such as glutamate (Glu), γ-aminobutyric acid (GABA), dopamine (DA), serotonin (5-hydroxytryptamine; 5-HT) and acetylcholine (ACh) (Brownlee and Fairweather, 1999). However, they seem to lack epinephrine, norepinephrine and histamine signaling, and some other significant differences have been described in sodium-dependent channels (Bargmann, 1998; Goodman *et al.*, 1998). Apart from the cellular and molecular similarity to higher organism, *C. elegans* exhibit variety of behaviors, including social interaction, which might contribute to the understanding of the nervous

system function. Because of its simplicity, investigating worms' behavior may be free from confounding factors, present in higher animals (Williams and Dusenbery, 1990).

2.3. *C. elegans* in neurotoxicology

Structural and functional similarities to the human nervous system, together with other advantages, strengthen the worms' choice as a model in neuroscience. Worms' transparency allows the visualization of neurons and synapses *in vivo* by fluorescent markers (Chalfie *et al.*, 1994), and neuronal excitability can be followed by live calcium imaging (Nguyen *et al.*, 2016), which allows inference on neuronal activity with behavioral phenotypes. Moreover, its ease of genetic manipulations allows the identification of genes significant for neuronal formation, migration, activity, or other functions. Since Sydney Brenner's Nobel Prize-winning studies, using *C. elegans* to investigate development and function of nervous system (Brenner, 1973), the nematode contributed greatly to understanding brain physiology. For instance, worms played a great role in determining the role of synaptic proteins such as neuroligin and neurexin in ASD (Schmeisser and Parker, 2017) and used in studies of neurodegenerative diseases, like Alzheimer 's disease (AD) and Parkinson's disease (PD), shedding light on their causes and progression (Calahorro and Ruiz-Rubio, 2011).

The field of neurotoxicology has also taken advantage of the model. The use *of C. elegans* in toxicological studies has recently escalated, mostly due to the ease of high-throughput chemical-genetic screens, and the ability to analyze the effect of toxic mixtures. Chronic and delayed effects of environmental toxicants are often difficult to monitor in mammalian models due to their long life cycles, therefore the short lifespan of *C. elegans* affords a convenient alternative in assessing this impact (Tejeda-Benitez and Olivero-Verbel, 2016; Honnen, 2017; Hunt, 2017). To date, almost every type of known toxicant has been tested in this animal model (Tejeda-Benitez and Olivero-Verbel, 2016; Hunt, 2017). Many toxicants, including metals and pesticides, have been examined with respect to their effects on the *C. elegans* nervous system and very frequently results from these studies have closely emulated those observed in mammalian systems.

This review focuses specifically on neurodevelopment and aims to highlight the contribution and utility of *C. elegans* to the field of neurodevelopmental toxicology. This review gives examples of the uses of *C. elegans* in neurodevelopmental toxicology research, summarized in Table 1, and discusses relevance to mammalian studies.

3. Neurodevelopmental toxicity studies in *C. elegans*

3.1. Manganese

Manganese (Mn) is one of the most plentiful naturally occurring trace elements. In organisms it exists in all tissues, where at physiological levels it regulates various cellular processes including metabolism of proteins, lipids and carbohydrates, immune system, growth and development. However, at high concentrations, Mn can be toxic, particularly to the brain (Sidoryk-Wegrzynowicz and Aschner, 2013; Chen *et al.*, 2015a). Excessive exposure to Mn may lead to its deposition and accumulation within the brain as it readily crosses the blood brain barrier (BBB). Particularly, dopaminergic (DAergic) neurons of the

nigrostriatal region are compromised leading to a parkinsonian syndrome referred to manganism. The syndrome presents clinical indicators that are similar to PD (Chen *et al.*, 2015a). Mn exposure may also present a risk factor for the development of PD (Guilarte, 2013).

Several studies have reported toxic effects of Mn exposure on children in relation to neurodevelopment (Coetzee *et al.*, 2016; Bjorklund *et al.*, 2017). For example, a study of 7-9-year-old children residing near a ferromanganese industry showed that both high and low concentrations of Mn in blood and hair are associated with lower intelligence quotient (IQ) scores (Haynes *et al.*, 2015). Increased prenatal and early childhood exposure to Mn in drinking water has been shown to elevate the risk of neurobehavioral problems of children at 10 years of age (Rahman *et al.*, 2017). Animal studies have also demonstrated developmental neurotoxicity associated with Mn exposure (Kikuchihara *et al.*, 2015; Peres *et al.*, 2015), however the molecular mechanisms by which Mn imparts neurodevelopment remains unresolved, and in these regards, the use of *C. elegans* in Mn neurotoxicity is applied (Chen *et al.*, 2015b).

Several studies from our lab and others have shown that *C. elegans* treatment with Mn at early life (Larva-1 stage, L1) induces DAergic neurodegeneration in L1, L4 and young adults (Settivari *et al.*, 2009; Benedetto *et al.*, 2010; Settivari *et al.*, 2013; Leyva-Illades *et al.*, 2014; Avila *et al.*, 2016; Ijomone *et al.*, 2016). Additionally, treatment of L1 worms with Mn altered neurobehavioral parameters at a later stage. Mn treatment at L1 increased dauer movement of WT worms at dauer stage (Chen *et al.*, 2015c) and reduced basal slowing response at L4 and young adult stage (Leyva-Illades *et al.*, 2014; Ijomone *et al.*, 2016). Increased dauer movement and reduced basal slowing response are indicative of compromised DAergic neurotransmitter systems. Benedetto et al., (2010) suggested that Mn developmental neurotoxicity is specific to DAergic neurons in *C. elegans*. The authors did not observe any degeneration in cholinergic, GABAergic and chemosensory neurons, at doses that induced DAergic degeneration (Benedetto *et al.*, 2010). However, it is possible that DAergic neurons are most sensitive to Mn developmental neurotoxicity, and higher exposure concentrations and duration may produce analogous degenerating effects in other neurotransmitter systems in *C. elegans*.

Similarly to higher organisms, in worms Mn has been reported to trigger reactive oxygen species (ROS) generation and induced oxidative stress (Settivari *et al.*, 2009; Benedetto *et al.*, 2010; Settivari *et al.*, 2013; Avila *et al.*, 2016), as well as alter activities of apoptotic proteins (Settivari *et al.*, 2013) and PD-associated genes (Chen *et al.*, 2015b). Expression of GST-1 (glutathione transferase 1) and SKN-1, a homologue of human nuclear factor (erythroid-derived 2)-like 2, NRF-2 – a transcription factor regulating antioxidant response involved in antioxidant and detoxification enzymes regulation, has been shown to inhibit Mn-induced developmental neurotoxicity in *C. elegans* (Settivari *et al.*, 2009; Benedetto *et al.*, 2010; Settivari *et al.*, 2013).

Involvement of apoptotic proteins has also been implicated in Mn-induced developmental neurotoxicity. Genetic KD mutations of *jnk-1*, *ced-3*, and *csp-1*, all inhibited DAergic neurodegeneration in young adult following exposure of L1 worms to Mn. JNKs belong to a

class of MAPKs that are triggered by internal or external stressors, and are involved in proapoptotic signaling. CED-3 is homologous to vertebrate proapoptotic caspase-3, while CSP-1 is among three caspase-like proteins (the other two being CSP-2, CSP-3) that show some homology to vertebrate caspase-1-3, and may act as an initiator of apoptosis by upregulating CED-3 (Settivari *et al.*, 2013).

Several transporters have been implicated in Mn-induced developmental neurotoxicity in mammals (Erikson *et al.*, 2005; Au *et al.*, 2008; Tuschl *et al.*, 2012) and studies in worms have helped to understand their role. DAergic degeneration following Mn exposure in early stage of *C. elegans* development has been shown to be dependent on dopamine transporter DAT-1, as deletion mutations of *dat-1* protect against DAergic degeneration following Mn treatment. Nevertheless, *dat-1* mutants as well as mutants with KO of all DA receptors (*dop-1*, *dop-2*, *dop-3*) show overall hypersensitivity to Mn toxicity (decreased survival rate compared to WT controls). In addition, *C. elegans* with tyrosine hydroxylase (*cat-2*) and vesicular monoamine transporter (*cat-1*) loss of function mutations exhibit hyper-resistant to Mn treatment. DAT-1 and DOPs are associated with removal of DA from synapses, while internal DA generation and packaging for release at synapses depends on CAT-2 and CAT-1 respectively. Hence these results suggest extracellular, but not intracellular, DA exacerbates Mn neurotoxicity (Benedetto *et al.*, 2010; Chen *et al.*, 2015b). Contrastingly, Settiviari et al., (2013) showed that Mn-induced DAergic degeneration is not dependent on DAT-1. These authors suggest that differences in the genetic background of the *dat-1* KO strain and WT strain used in the previous study (Benedetto *et al.*, 2010) may have been the likely reason for this contrasting result. Developmental DAergic degeneration has been shown to be partially dependent on divalent metal transporter DMT-1 homologues SMF-1 in DAergic neurons, because *smf-1* deletion mutations partially protected against DAergic degeneration in worms (Benedetto *et al.*, 2010; Settivari *et al.*, 2013). Additionally, SLC30A10, an Mn efflux transporter has been shown to be involved in developmental neurotoxicity on DAergic system. Overexpression of SLC30A10 in worms reduced cellular Mn levels, thereby attenuating against Mn toxicity. Moreover, it significantly attenuated Mn-induced DAergic degeneration in L1 worms, while L89P mutation of SLC30A10 significantly worsened DAergic degeneration upon Mn exposure (Leyva-Illades *et al.*, 2014).

Recent studies identified protein targets that may be involved in developmental neurotoxicity following Mn exposure. They include lipocalin-related protein LPR-5 (Rudgalvyte *et al.,* 2016), telomerase reverse transcriptase TRT-1 (Ijomone *et al.,* 2016), and heat shock proteins HSP-70, HSP-3 and CHN-1 (Avila *et al.,* 2016). *lpr-5* KD resulted in increased animal death in both controls and Mn exposed L1 worms. Although the function of LPR-5 is not clear, it is suggested that it may be involved in Mn induced toxicity *via* endoplasmic reticulum-lipocalin-related pathways (Rudgalvyte *et al.*, 2016). Mutation in TRT-1 (the catalytic subunit of telomerase) in *C. elegans* inhibited DAergic degeneration in L1 worms upon Mn exposure. However, it may be due to the ability of *C. elegans to* survive without functional telomerase (Ijomone *et al.,* 2016). Loss *of hsp-70, hsp-3* and *chn-1* reduced survival rate as well as increased protein oxidation following Mn exposure to L1 worms. Particularly, loss of *hsp-70* worsened Mn-induced DAergic degeneration. This effect may involve the blocking of transcriptional upregulation of *pink-1,* a gene linked to PD (Avila *et al.,* 2016).

PD-associated genes, like *parkin, dj-1* and α-synuclein, have been implicated in Mn-induced DAergic degeneration (Chen *et al.,* 2015a). Overexpression of α-synuclein in worms rescued DAergic degeneration in the background of *parkin* deletion mutation, but not *dj-1* mutation (Chakraborty *et al.,* 2013). However, it should be noted that worms expressing human WT α-synuclein showed increased DAergic degeneration following Mn treatment reinforcing the exacerbating role exerted by α-synuclein in PD (Settivari *et al.,* 2009). *djr-1.2* deletion increased dauer movement following Mn exposure, suggesting *djr-1.2* deletion disrupts DAergic signaling; this effect was exacerbated upon Mn exposure during *C. elegans* development (Chen *et al.,* 2015c).

In summary, *C. elegans'* unique properties contributed greatly to better understanding of mechanism of Mn-induced developmental neurotoxicity. Worms' transparency and ability to visualize neurons allowed to identify DAergic system as a key target for neurodegeneration upon early life Mn exposure (Settivari *et al.*, 2009; Benedetto *et al.*, 2010; Settivari *et al.*, 2013; Leyva-Illades *et al.*, 2014; Avila *et al.*, 2016; Ijomone *et al.*, 2016), which was further confirmed by numerous behavioral tests (Chen *et al.*, 2015c, Leyva-Illades *et al.*, 2014; Ijomone *et al.*, 2016). Moreover, ease of genetic manipulation allowed deeper insight into the mechanism, revealing the important role of extracellular DA and DA transporters in DAergic neurodegeneration (Benedetto *et al.*, 2010; Chen *et al.*, 2015b). Furthermore, genetic modifications allowed to reveal the significance of several antioxidant and detoxification genes (Settivari *et al.*, 2009; Benedetto *et al.*, 2010; Settivari *et al.*, 2013; Avila *et al.*, 2016) and heat-shock proteins (Avila *et al.*, 2016) in Mn resistance, as well as the role of apoptotic proteins (Settivari *et al.*, 2013), metal transporters (Benedetto *et al.*, 2010; Settivari *et al.*, 2013), or PD-associated genes (Chakraborty *et al.*, 2013; Chen *et al.*, 2015c) in the mechanism of Mn-induced neurotoxicity and neurodegeneration during early development.

3.2. Mercury

Mercury (Hg), especially its organic form – methylmercury (MeHg) is one of the most poisonous neurotoxicants found in the environment. Humans are exposed to MeHg mainly through consumption of contaminated fish and plants, but also through occupational exposure and anthropogenic mercury emissions (Clarkson and Magos, 2006; Rice *et al.*, 2014). Acute exposure to MeHg has historically been seen by incidents in Minamata Bay (Japan) and in Barsa (Iraq). Even though over the past few years MeHg exposure has been substantially curbed, one is still affected because it is so persistent in the environment, posing a risk related to low-levels chronic exposures (Grandjean *et al.*, 2010).

MeHg crosses the blood-brain and placental barriers, causing a serious threat to developing brains in fetuses and infants. The neurodevelopmental toxicity of MeHg is well established - numerous studies addressed MeHg developmental neurotoxicity in animals and humans, and linked it to ROS accumulation, oxidative stress, disruption in neurotransmitters or metallothioneins homeostasis (Sanfeliu *et al.*, 2003; Bose-O'Reilly *et al.*, 2010; Llop *et al.*, 2013; Antonelli *et al.*, 2017). However, the mechanism of toxicity is still not fully understood (Llop *et al.*, 2015), and a lack of efficient treatment stimulate further research, in which *C.elegans* has already played a significant role.

To date, several initial studies aimed to characterize the effect of Hg and MeHg on the nematode in general, as well as on its developing nervous system, revealing numerous similarities with mammalian models. MeHg has been shown to be transported in the worm by a conserved mechanism, dependent on amino acid transporters LAT1 (Caito *et al.*, 2013b). MeHg was more toxic to L1 and L4 stage *C. elegans* than $HgCl_2$, when assessing feeding, movement, and reproduction, all of which require neuromuscular activity (McElwee and Freedman, 2011), and these results are consistent with studies in other species. When L4 larval were exposed a to low (2.5 μM) $HgCl_2$ levels for 6 hrs, it caused significant decrease of associative learning behavior, whereas 50 μM caused significant reduction of body bend frequency and thermotaxis to cultivation temperature (Zhang *et al.*, 2010). One of the first studies showed that L1 worms are significantly more sensitive than L4 to acute MeHgCl treatment, likely due to undeveloped detoxification system (Helmcke *et al.*, 2009). Moreover, MeHg treatment inhibited worms' development and pharyngeal pumping, however DAergic and GABAergic neurons morphology was not impaired in animals that survived MeHgCl exposure (up to 72hrs post-treatment), suggesting that *C. elegans* neurons may be less sensitive than mammalian, even though Hg levels found in *C. elegans were* similar to those causing deleterious alterations in mammalian systems (Helmcke *et al.,* 2009). Degeneration of GABAergic neurons has been observed after treatment with $HgCl_2$ (up to 100 μM, 6hrs); of importance, younger larvae (L1-L3) showed greater sensitivity to neurotoxicity (assessed by changes in neuronal survival and synaptic function) than L4 larvae and young adult nematodes (Xing *et al.,* 2009). Martinez-Finley *et al.* has reported a loss of DAergic neurons later in life (96 hrs) following early-life (L1) MeHg exposure (0,5 h, 20 μM). Moreover, loss-of-function of DAergic neurons (basal slowing response) was observed later in life (72 hrs) (Martinez-Finley *et al.,* 2013a). The role of SKN-1 was investigated in this context, showing that DA levels were decreased in *skn-1* KOs compared to WT worms, following early life exposure to MeHg. ROS levels were elevated in *skn-1* KOs and in WT worms following MeHg exposure. SKN-1 expression in the gut have been seen 24 hrs following exposure to 20 μ*M* MeHg (Martinez-Finley *et al.,* 2013a). The upregulation of NRF-2 and genes under its control has been shown in many mammalian models upon exposure to MeHg (Ni *et al.,* 2010; Kumagai *et al.,* 2013; Culbreth *et al.,* 2017) and other developmental neurotoxicants (Lau *et al.,* 2013). A previous study has also shown that reduction in SKN-1 gene expression increases L4 worms vulnerability to MeHg, affects expression of GST, and prevents DAergic degeneration.

The role of mammalian parkin/PARK2 protein was also studied in MeHg neurotoxicity using *C. elegans model.* The *pdr-1* KO exacerbated MeHg toxicity and damage to the DAergic system, however a change in DA-mediated locomotor activity (basal slowing response) was observed in WT worms, but not in *pdr-1* KOs (Martinez-Finley *et al.,* 2013b). The role of metallothioneins in adaptation response to Hg has been also investigated in *C. elegans*. Pre-treatment with mild heat-shock (1 hr at 36°C) at L2 larva stage prevented neurobehavioral defects and the activation of stress response in nematodes exposed (L4, 12hrs) to mercury at lower concentrations (50 and 100 μM), but not at higher levels (200 μM) (Ye *et al.*, 2010).

To date, numerous neurobehavioral studies have confirmed MeHg-induced neurodevelopmental toxicity in young *C. elegans* (Helmcke *et al.*, 2009; Martinez-Finley *et*

al., 2013a; McElwee and Freedman, 2011; Zhang *et al.*, 2010), while neuronal visualization revealed DAergic and GABAergic neurodegeneration later in life (Helmcke *et al.*, 2009; Martinez-Finley *et al.*, 2013a; Xing *et al.*, 2009). Furthermore, genetic engineering allowed better understanding of the protective role of *skn-1* (Martinez-Finley *et al.*, 2013a) and metallothioneins (Ye *et al.*, 2010) in MeHg resistance, as well as the contribution of *pdr-1* (Martinez-Finley *et al.*, 2013b) in the mechanisms of MeHg-induced developmental neurotoxicity.

3.3. Arsenic

Arsenic (As) and arsenic compounds are known carcinogens that are widely present in the environment and are particularly elevated in groundwater in a variety of nations around the world due to both natural and anthropogenic sources. The World Health Organization has set 10-50 ppb as provisional permissible concentration limits for As in groundwater, yet many areas around the world have populations that are exposed to ten to hundreds of times those limits (Shankar *et al.*, 2014). General consequences of As exposure include, but are not limited to, arsenicosis, skin melanosis, cancers of various organs, and IQ declines (O'Bryant *et al.*, 2011; Hunt *et al.*, 2014; Tsuji *et al.*, 2015). Though extensively studied in the context of cancers, much remains to be understood in terms of the long-term effects of early life arsenic exposure on neurodevelopment and how early life toxicant insults or low level chronic exposures may contribute to neurological diseases.

The role of *abts-1* (bicarbonate transporter) in As neurotoxicity was evaluated in *C. elegans*. The experimental model utilized WT and *abts-1* mutants grown on plates containing 1 mM As from egg stage to adult (approx. 2.5 days). The *abts-1* expression was increased in neurons of transgenic worms expressing *Pabts-1::GFP* growing on As-containing plates. As-treated worms showed increased sensitivity in a paralysis assay performed with exposure to levamisole and aldicarb - the finding alludes to the involvement of *abts-1* and arsenic toxicity in alterations of the cholinergic system (Liao *et al.*, 2010).

When L4 worms were treated with As (10–200 μM) for 24 hrs, it resulted in robust changes in a variety of locomotor behaviors. Worms tagged with GFP in AFD thermosensory neurons showed decreases in cell body size and fluorescence intensity when comparing 100 μM As treated worms to control (Yu and Liao, 2014). The AFD neurons are two ciliated neurons responsible for a variety of functions related to thermotactic behaviors and interacts with the AIY interneuron (Clark *et al.*, 2006). These visible changes in the neurons also reflected functional change as As-treated worms exhibited a significant decrease in isothermal tracking (IT) behavior. Though the paper focuses on neurotoxic effects in AFD neurons, the locomotor behavior deficits suggest impairments of other neurons. Changes in the body bends and head thrashing allude to possible direct changes to motor neurons; loss of the AIZ interneuron has been found to lead to hyporeversal effects, similar to what was observed in worms treated with As, suggesting another neuron might be affected (Tsalik and Hobert, 2003). Reactive oxygen species (ROS) levels were also increased in L4 worms treated with As, and the As- induced toxicity effects were ameliorated in a variety of neurobehavioral assays when worms were pretreated with curcumin, a natural antioxidant,

further highlighting that the mechanism of neurotoxicity involves oxidative processes (Yu and Liao, 2014).

Other literature utilizing the *C. elegans* model for As research has built on the observation of increased ROS levels post-As treatment at various life stages of the worm and connected it to acceleration of aging and mitochondrial dysfunction (Luz *et al.*, 2016; Yu *et al.*, 2016; Luz *et al.*, 2017). These findings were whole body effects and were not evaluated specifically in neurons, and did not take advantage of potentially exposing worms at earlier life stages in development that could have given further insight into the effects of chronic arsenic exposure on neurodevelopment. Sahu et al. (2013) treated early development L1 stage worms with As, and although they did not look directly at neuronal changes, they did examine changes in gene expression - of particular interest were changes in ferroportin (responsible for iron export out of the cell) and zinc ion binding gene classes after As treatment (Sahu *et al.*, 2013). Changes in iron and zinc homeostasis have been linked to a variety of neurological diseases including, but not limited to, AD, PD, ADHD, amyotrophic lateral sclerosis (ALS), and depression. Thus, results from this paper suggest that early-life As exposure may contribute to neurological disease development by affecting the homeostasis of other biologically pertinent metals in the body (Hare *et al.*, 2013; Szewczyk, 2013).

To date, early life exposure to As in *C. elegans* has been linked to increased ROS production and neurodegeneration, especially of AFD neurons, followed by neurobehavioral changes, like impaired thermosensory function (Yu and Liao, 2014). Moreover, the involvement of *abts-1* in As developmental neurotoxicity has been demonstrated (Liao *et al.*, 2010). These studies employed a key advantages of worm model – ease of neuronal visualization, conduction of behavioral assays and genetic manipulations.

3.4. Lead

Lead (Pb) is a naturally occurring heavy metal in the Earth's crust and a non-essential metal in human biology. Human exposure to Pb is mostly due to its industrial use in a variety of alloys and compounds. Lead and Pb-alloys can be found in radiation shielding, pipes, weights, ammunition and batteries, moreover, Pb compounds can be found in dyes, paints, and gasoline, though the amounts have been greatly reduced or eliminated in recent years with increased awareness of the detrimental effects of Pb poisoning and exposure. These effects include kidney function impairment, thyroid function impairment, cardiovascular disease, decreased reproductive health, encephalopathy, neurodevelopmental defects in children, preterm birth, and more. Delving into neurodevelopmental effects of Pb exposure, the literature notes changes and impairment in IQ, memory, attention, language comprehension, processing speed, motor function and affect (Abadin *et al.*, 2007; Mason *et al.*, 2014). Molecular mechanism of Pb neurotoxicity is complex and include oxidative stress, membrane disruptions, deregulation of cell signaling and impaired neurotransmission (Sanders *et al.*, 2009).

The *C. elegans* model has been extensively used to examine Pb toxicity in general, but has not been specifically taken advantage of in the field of neurotoxicity and neurodevelopment, as noted by the scarcity of literature in this subfield. In one study, worms were pre-treated

with selenium (0.01 μM Se(IV)) for 40 hrs starting from L1, and L4 worms were then exposed to 100 μM $Pb(NO_3)_2$ for 24 hrs. The study showed decreased number of body bends, head thrashing, and reversal frequency after Pb exposure, which might be mitigated by pretreatment with Se (Li *et al.*, 2013). Changes in locomotor assays could be attributed to changes in AFD neuron function, as stated before. Indeed, morphological changes in AFD neurons were observed after Pb-treatment, namely decreased cell body size. Moreover, a significant reduction in mRNA levels of tax-2, tax-4, and ceh-14 were also observed after Pb exposure; these genes are required for proper AFD differentiation and function (Li *et al.*, 2013). Decreased locomotor activity (body bends and head thrashes) was also shown when L1 worms were chronically treated with 1.45 mg/l Pb until young adult life stage (Sun *et al.*, 2016).

When L4 worms were treated with 2.5 or 10 μM $Pb(NO_3)_2$ for 6 hrs, they showed significant decrease in association learning behavior, as compared to control in a thermotaxis assay paradigm (Zhang *et al.*, 2010). In another study, the same group utilized L1–L4 worms expressing GFP in GABAergic neurons and treated them with $Pb(NO_3)_2$ solutions (2.5, 50, or 100 μM) for 6 hrs. The study demonstrated that the Pb exposures led to significant dose-dependent increases in dorsal and ventral cord gaps and increases in loss of neuronal cell bodies for L1-L3 treated worms, reflecting axonal and cell body degeneration post-Pb treatment (Xing *et al.*, 2009). Worms were also treated with aldicarb and levamisole with the same paradigm, to determine if Pb treatment impacted cholinergic neurons. Normally, worms are paralyzed in these assays and continued motility upon exposure to aldicarb or levamisole suggest pre- or post-synaptic changes in cholinergic neurons, respectively. Increased motility was observed for all three concentrations of Pb for L1–L3 worms, as well as for 50 and 100 μM for L4 worms, and 100 μM Pb treatment for young adult, suggesting deficits in cholinergic transmission. Throughout the paper, there is a consistent theme of increased maturity in life stage leading to greater resistance against the impacts of lead exposure (Xing *et al.*, 2009). Again, major advantages of the model application in neuroscience – neuronal GFP labeling and relative simplicity of behavioral testing, have been employed to reveal the effects of Pb on *C. elegans* larvae, which were degeneration of AFD neurons (Li *et al.*, 2013) and GABAergic neurons (Xing *et al.*, 2009), as well as various neurobehavioral changes (Li *et al.*, 2013; Sun *et al.*, 2016; Xing *et al.*, 2009; Zhang *et al.*, 2010).

3.5. Fluoride

Fluoride (F) is an essential trace element ubiquitous in the environment (Whitford, 1983). Additionally, due to its positive effect on teeth and bones, one of fluoride compounds – sodium fluoride (NaF)- is supplemented worldwide in public drinking water. In 2012, 74.6% of people with access to public water systems, or nearly 211 million people in the United States, drank artificially fluoridated water (Chaudhary *et al.*, 2010). Thus, human exposure to substantial amounts of this element occurs on regular basis. Although consumption of drinking water and usage of NaF-containing dental hygiene products is considered the major source of human intake, potential for bioaccumulation of fluoride ion in living organisms and environment results from additional sources of exposure, like consumption of seafood or plants grown in high-fluoride waters or soils (Ozsvath, 2009; Chaudhary *et al.*, 2010).

Despite its positive effects, some studies suggest that prolonged ingestion of fluoride produces deleterious effects, especially on the CNS, which poses serious global health concern (Gui *et al.*, 2010). Sodium fluoride has been shown to induce neurological impairment particularly in the hippocampus and cerebral cortex in experimental animals (Bhatnagar *et al.*, 2002; Sharma *et al.*, 2014). Several epidemiological and experimental studies have highlighted closed relationship between fluoride ingestion and neurodevelopmental delays (Chioca *et al.*, 2008; Choi *et al.*, 2012), alike decreased IQ of children (Aravind *et al.*, 2016).

Previous studies have demonstrated that the toxic effect of fluoride exposure on the CNS could be attributed to their ability to cross the BBB and lead to oxidative stress, DNA damage and decrease in nicotinic acetylcholine receptors (Ranpariya *et al.*, 2011; Sharma *et al.*, 2014), however the mechanisms underlying its developmental neurotoxicity are not fully understood.

Recently, Li *et al.* reported that NaF exposure can induce multiple biological toxicities to *C. elegans* in a concentration- dependent manner at physiological, biochemical, and molecular levels. In this study *C. elegans* (L4 stage) were exposed to three concentrations of NaF (0.038, 0.38, 3.8 mM) for 24 hrs. Furthermore, NaF induced behavioral deficits – decreased head thrashes and body bend frequency, and increased ROS levels and expression of some oxidative stress-related genes: *hsp-16.1*, *sod-3*, *ctl-2*, *dhs-28*, *gst-1*, and *cep-1* (Li *et al.*, 2012). These results are consistent with some observations in higher organisms (Dec *et al.*, 2017).

3.6. Pesticides

Dichlorvos, an organophosphorus (OP) pesticide, is widely used globally due to its high potency and low price (Zhang *et al.*, 2017). As an OP, it inhibits acetylcholinesterase (AChE) – the enzyme which removes acetylcholine (ACh) from the synapse – leading to an excess of ACh in the synapse and impaired nerve function (Kobayashi *et al.*, 1986). Dichlorvos is classified by the World Health Organization (WHO) as highly hazardous and its detrimental effects on humans are likely related to continuous dietary uptake, including some nervous system diseases at relatively low exposure levels (Mackenzie Ross *et al.*, 2010; Velmurugan *et al.*, 2013; Du *et al.*, 2014).

Lewis et al. studied the effects of developmental exposure to dichlorvos in the *C. elegans* model; following exposure of L4 worms to dichlorvos, several neuronal growth/repair - related gene expressions were altered: *dlk-1* (MAPKKK) and *pmk-3* (p38 MAPK), genes related to axon regeneration, were upregulated; expression level of *unc-14, unc-129, eva-1, klc-2,* and *pak-1,* all related to axonal guidance, was also increased; finally, the components of the SMA TGF-β pathway – which is related to sensory neuronal development – were also upregulated. The suspected underlying mechanism for these findings is, according to the authors, mitochondrial dysfunction, which stems from the changes in metabolic processes found following dichlorvos exposure (Lewis *et al.,* 2013). Similar observations were true for rodents (Masoud *et al.,* 2009; Wani *et al.,* 2011).

Monocrotophos (MCP), an OP insecticide, also works by inhibiting AChE; it is highly toxic, fast acting and non-organism specific, which is why its use is banned in most developed countries (Kavitha and Venkateswara Rao, 2007; Maniyar, 2011; Leelaja and Rajini, 2013). As a high glucose diet has already been associated with the exacerbation of OP toxicity (Olivier *et al.,* 2001; Liu *et al.,* 2007), Salim and Rajini (Salim and Rajini, 2014) used the *C. elegans* model to study the developmental neurotoxicity of MCP and the effects of a high-glucose diet on such toxicity. Following exposure of L4 worms to MCP, a dose-dependent decrease in AChE was reported for both control and high-glucose worm groups, with a lower mean activity in the high glucose group, suggesting an increased nervous-system vulnerability under the high glucose conditions.

Glyphosate, a glycine analogue, is the active ingredient in some of the most common herbicides globally; its popularity led to the development of genetically modified crops which are resistant to its effects (Woodburn, 2000; Gardner, 2008; Dewar, 2009). Recent studies show that while glyphosate alone is relatively non-toxic, exposure to glyphosate-containing products is related to mitochondrial dysfunction (Bababunmi *et al.*, 1979; Olorunsogo *et al.*, 1979; Olorunsogo and Bababunmi, 1980; Peixoto, 2005). Mancozeb, a manganese (Mn) containing fungicide, acts by inhibiting enzymes and is massively used worldwide – approximately 3.6 million kilograms annually (University, 1987; Giannesse, 2000; Gianessi, 2006). Exposure to mancozeb-containing products has been associated with apoptosis, neurodegeneration and inhibition of mitochondrial respiration (Soleo *et al.*, 1996; Calviello *et al.*, 2006; Domico *et al.*, 2006; Domico *et al.*, 2007; Zhang *et al.*, 2017). In order to study the potential developmental neurotoxicity of TouchDown (TD; a glyphosate-containing herbicide) and Mancozed (MZ; a Mn-containing fungicide), the *C. elegans* model was used, specifically the NW1229 strain, which has all neurons tagged with GFP: L2 worms were exposed, acutely or chronically, to either product or both. Fluorescent microscopy analyses revealed dose-dependent neurodegeneration for all parameters, and to the highest extent following MZ exposure (Negga *et al.*, 2011). In a follow-up study, using the same design, it was found that exposure to TD and MZ is associated with neurodegeneration of GABAergic and DAergic neurons, suggesting a more specific effect for these compounds in the nervous system (Negga *et al.*, 2012). Further, impaired neuronal development and axon growth was observed in rat primary cultures of hippocampal pyramidal cells exposed to this herbicide (Coullery *et al.*, 2016).

Thiocarbamate and dithiocarbamate pesticides are among the most commonly used pesticides in the US (Grube, 2011); their mechanism of action involves the metal-dependant and sulfhydryl enzyme systems in fungi, bacteria, plants, insects and mammals (Miller, 1982). A major concern regarding exposure to these compounds is their metabolites, which can lead to protein modification and enzyme inhibition associated with mammalian neurotoxicity (Savolainen and Hervonen, 1985; Pentyala and Chetty, 1993; Staub *et al.*, 1995; Tonkin *et al.*, 2004; Viquez *et al.*, 2012). Caito *et al.* (Caito *et al.*, 2013a) studied the effects of S-ethyl N,N-dipropylthiocarbamate (EPTC), molinate, and S-methyl-N,N-diethylthiocarbamate (MeDETC) – two thiocarbamate pesticides and one reactive intermediate of their metabolism, respectively – on the development and function of the nervous system in *C. elegans*. Following acute exposure during the L1 phase the following parameters were studied in the worms: neuronal morphology, neurotransmitter content and

basal-slowing response behavior. Pesticide exposure was associated with a DAergic deficit, including loss of cell morphology, decreased DA content and impaired DA-related behavior. Similar findings were not found in other neurotransmitter systems (cholinergic, glutamatergic and GABAergic), suggesting a potential role these compounds may have in the development of PD.

4. Conclusions and perspectives

C. elegans is a model organism which allows to combine the study of neuronal development, connectivity, physiology and behavior, changed by environmental and genetic factors in a simplified, *in vivo* context. Numerous advantages favor its use in toxicology and make it a recent model of choice in studying neurodevelopmental disorders. From the reviewed data, it emerges that *C. elegans* can be a useful tool in evaluating the effects of toxins on developing nervous system, advancing our understanding of the molecular mechanisms underlying toxicity. With effects frequently analogous to those observed in mammals, there is a strong premise, that *C. elegans* findings can be extrapolated to higher organisms, including humans. Therefore, its experimental popularity will probably continue to grow and hopefully result in identification of new hazardous chemicals, as well as better insight into pathomechanisms of known neurotoxicants.

C. elegans has already been successfully implemented in general toxicity studies of other developmental neurotoxins, like ethanol (Davis *et al.*, 2008) toluene (Davies *et al.*, 2012), polychlorinated biphenyls (Menzel *et al.*, 2007; Schafer *et al.*, 2009), various flame retardants (Behl *et al.*, 2016) and chlorpyrifos (Ruan *et al.*, 2012), resulting in characteristic, pathological phenotypes; however, their neurodevelopmental effects in worms have yet to be addressed.

Acknowledgments

This work has been supported by the National Institutes of Health to MA [grant numbers NIEHS R01ES07331, NIEHS R01ES10563 and NIEHS R01ES020852. OMI acknowledges the support of IBRO-ISN research fellowship.

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Highlights

- Due to many experimental advantages C. elegans has become a model of choice in numerous neurodevelopmental toxicity studies.
- C.elegans is a valuable tool in both identification of new chemicals and explanation of molecular pathways behind developmental disruptions caused by known neurotoxicants.
- Reviewed data indicates numerous similarities with mammalian and human response to developmental neurotoxicants.

Table 1
C.elegans as a model organism in neurodevelopmental toxicity studies

Compound	Experimental design	Effect	Similar effects in mammals	Reference
Manganese	L1 0.5 hr 50 mM in dH_2O	Increased DAergic degeneration, partially dependent on expression of SMF-1.	Yes	(Settivari *et al.*, 2009)
	L1 0.5 hr 0.001–100 mM in 85 mM NaCl	Increased DAergic, but not cholinergic, GABAergic degeneration; effect dependent on DAT-1, DOPs and SMF-1 and inhibited by SKN-1 overexpression.	Yes	(Benedetto *et al.*, 2010)
	L1 0.5r h 50 mM in dH_2O	Increased DAergic degeneration; dependent on GST-1 and SKN-1, but not DAT-1 expression; the effect inhibited by KD of *jnk-1*, *ced-3* and *csp-1*.	Yes	(Settivari *et al.*, 2013)
	L1 1 hr 10, 25 mM in 85 mM NaCl	Increased DAergic degeneration and reduced basal slowing response. SLC30A10 overexpression attenuated DAergic injury, while KO worsened the effects of Mn.	Yes	(Leyva-Illades *et al.*, 2014)
	L1 0.5 hr 50 mM in 85 mM NaCl	Increased dauer movement due to *djr-1.2*-dependent disruption of DAergic signaling.	ND	(Chen *et al.*, 2015c)
	L1 1 hr 5, 10 mM in 85 mM NaCl	Increased DAergic degeneration and reduced basal slowing response; mutation in *trt-1* resisted compromised DAergic systems.	Yes	(Ijomone *et al.*, 2016)
	L1 0.5 hr 10, 50 mM in 85 mM NaCl	Increased DAergic degeneration; effect aggravated by loss of *hsp-1*.	Yes	(Avila *et al.*, 2016)
Mercury	L1, L4 0.5, 6, 15 hrs 0.1–10 mM MeHgCl in M9	Unchanged DA and GABA neurons morphology.	No	(Helmcke *et al.*, 2009)
	L1 0,5 hr 20 μM MeHgCl in M9	Degeneration of DAergic neurons later in life (96 hrs), increased ROS levels, upregulation of SKN-1.	Yes	(Martinez-Finley *et al.*, 2013a)
	L1 0,5 hr 10, 20 μM MeHgCl in M9	Decreased DA-mediated locomotor activity.	Yes	(Martinez-Finley *et al.*, 2013b)
	L4 48, 72, 96 hrs 0.5–125 μM MeHgCl in NGM plates	Degeneration of DA neurons, increased ROS levels and GST mRNA, developmental defects, protective role of SKN-1.	Yes	(Vanduyn *et al.*, 2010)
	L1, L4 5, 48 hrs 0.3–200 μM $HgCl_2$ 0.03–50 μM MeHgCl in K medium	MeHgCl-induced neurotoxic effects at concentrations lower than for HgCl2.	Yes	(McElwee and Freedman, 2011)
	L2, L4 12 hrs 50, 100, 200 μM MeHgCl in K medium	Neurobehavioral defects partially prevented by preconditioning with mild heat shock.	ND	(Ye *et al.*, 2010)
	L4 6 hrs	Decreased associative learning behavior, thermotaxis and locomotion behavior.	Yes	(Zhang *et al.*, 2010)

Compound	Experimental design	Effect	Similar effects in mammals	Reference
	2.5–50 μM $HgCl_2$			
	L1–L4 6 hrs 2.5–50 μM $HgCl_2$ in K medium	Degeneration of GABAergic neurons.	Yes	(Xing *et al.*, 2009)
Arsenic (As)	L4 24 hrs 100 μM As (III) in K-medium	Deficits in AFD sensory neurons, decrease in isothermal tracking behavior, altered locomotor behaviors.	Yes	(Yu and Liao, 2014)
	Egg to adult Chronic exposure 1 mM As (III) in NGM plates	Increased sensitivity in paralysis assays suggesting alterations in cholinergic system; the role of *abts-1* in this process.	Yes	(Liao *et al.*, 2010)
Lead (Pb)	L1 worms pre-treated with 0.01 μM Se(IV) 40 hrs L4 24 hrs 100 μM $Pb(NO_3)_2$ in K-medium	Decreased locomotor behaviors: body bends, head thrashing, and reversal frequency; deficits in AFD sensory neurons; effects mitigated by Se pre-treatment.	Yes	(Li *et al.*, 2013)
	L1-young adult 2.5 days 1.45 mg/l Pb in K-medium	Decreased locomotor behaviors: body bends and head thrashes.	Yes	(Sun *et al.*, 2016)
	L4 6 hrs 2.5, 10 μM $Pb(NO_3)_2$ in K-medium	Impaired association learning behavior.	Yes	(Zhang *et al.*, 2010)
	L1-L4 6 hrs 2.5, 50, 100 μM $Pb(NO_3)_2$ in K-medium	Dose-dependent increase in dorsal and ventral cord gaps and increased loss of neuronal cell bodies in L1-L3 worms; deficits in cholinergic transmission in L1-L4 worms.	Yes	(Xing *et al.*, 2009)
Sodium fluoride (NaF)	L4 24 hrs 0.038, 0.38, 3.8 mM in M9	Defects in locomotion behavior, increased ROS levels and upregulation of oxidative stress-related genes.	Yes	(Li *et al.*, 2012)
Dichlorvos	L4 2, 8, 26 hrs 6, 15 μ*M* in M9	Altered gene expression of several neuronal growth/repair-related genes.	Yes	(Lewis *et al.*, 2013)
Monocrotophos	L4 24 hrs 0.5, 0.75, 1.5 mM in K medium	Decreased AChE, enhancing effects for a high glucose diet.	Yes	(Salim and Rajini, 2014)
TouchDown/ Mancozed	L2 0.5 hr 0.22 – 12.5% in M9	Neurodegeneration of DA and GABA systems.	ND	(Negga *et al.*, 2011; Negga *et al.*, 2012)
EPTC/ molinate/ MeDETC	L1 1 hr 0.2, 0.4, 0.1 mM in M9	Changed DAergic cell morphology, decreased DA content and impaired DA-related behavior.	ND	(Caito *et al.*, 2013a)

Abbreviations: *abts-1:* bicarbonate transporter; ACh: acetylcholine; AChE: acetylcholinesterase; *ced-3:* cell death protein 3 subunit 2; *csp-1:* caspase A subunit p16; DA: dopamine; DAT-1: sodium-dependent dopamine transporter; dH_2O : distilled water, *djr-1.2:* glutathione-independent glyoxalase; DMT-1 divalent metal transporter 1; DOPs: dopamine receptors; EPTC: S-ethyl N,N-dipropylthiocarbamate; ERM: medaka embryo-rearing medium; GABA: γ-aminobutyric acid; GST: glutathione transferase; *hsp-1:* heat shock protein A; *jnk-1:* stress-activated protein kinase; M9: M9 minimal medium; MeDETC: S-methyl-N,N-diethylthiocarbamate; MeHgCl: methylmercury chloride; mRNA: messenger ribonucleic acid; ND: no data; NGM: nematode growth medium; NPs: nanoparticles; ROS: reactive oxygen species; Se: selenium; SKN-1: homologue of nuclear factor (erythroid-derived 2)-like 2; SLC30A10: solute carrier family 30 member 10; SMF-1: NRAMP-like transporter ; *trt-1*: telomerase reverse transcriptase.