Physiol. Res. 72: 149-166, 2023 *https://doi.org/10.33549/physiolres.934977*

REVIEW

Applications of a Powerful Model Organism *Caenorhabditis elegans* to Study the Neurotoxicity Induced by Heavy Metals and Pesticides

Kamila MELNIKOV[1], Soňa KUCHARÍKOVÁ[1], Zuzana BÁRDYOVÁ[1], Norbert BOTEK[1,2], Alžbeta KAIGLOVÁ[1]

[1]Department of Laboratory Medicine, Faculty of Health Care and Social Work, Trnava University in Trnava, Trnava, Slovakia, [2]GAMMA-ZA s.r.o., Trenčín, Slovakia

Received September 20, 2022
Accepted February 3, 2023

Summary

The expansion of industry and the use of pesticides in agriculture represent one of the major causes of environmental contamination. Unfortunately, individuals and animals are exposed to these foreign and often toxic substances on a daily basis. Therefore, it is crucial to monitor the impact of such chemicals on human health. Several *in vitro* studies have addressed this issue, but it is difficult to explore the impact of these compounds on living organisms. A nematode *Caenorhabditis elegans* has become a useful alternative to animal models mainly because of its transparent body, fast growth, short life cycle, and easy cultivation. Furthermore, at the molecular level, there are significant similarities between humans and *C. elegans*. These unique features make it an excellent model to complement mammalian models in toxicology research. Heavy metals and pesticides, which are considered environmental contaminants, are known to have affected the locomotion, feeding behavior, brood size, growth, life span, and cell death of *C. elegans*. Today, there are increasing numbers of research articles dedicated to this topic, of which we summarized the most recent findings dedicated to the effect of heavy metals, heavy metal mixtures, and pesticides on the well–characterized nervous system of this nematode.

Key words:

Caenorhabditis elegans • Model organism • Neurotoxicity • Heavy metals • Pesticides

Corresponding author

Soňa Kucharíková, Department of Laboratory Medicine, Faculty of Health Care and Social Work, Trnava University in Trnava, Univerzitné námestie 1, 918 43 Trnava, Slovakia. Email: sona.kucharikova@truni.sk

Introduction

Caenorhabditis elegans is a microscopic nematode measuring a little more than 1 millimeter in length. This nematode mainly feeds on bacteria and fungi found in the soil, as well as rotting fruit. The first evidence on the model organism *C. elegans* brought Sydney Brenner in the 1960-70s, and since then, this nematode has played a critical role in the knowledge of various basic aspects of biology, including apoptosis, RNA interference, and miRNA function [1]. Furthermore, the sequence of its genome is known and contains more than 19,000 genes [2].

The advantage of this worm is that it can reproduce in a large number and at a rapid rate. Around 72 h at 20 °C, development progresses from embryo to adult gravid hermaphrodites through four different larval stages, known as L1, L2, L3 and L4. Due to its transparent body, the interior development of all *C. elegans* cells and organs can be easily observed by light microscopy. In addition, the cultivation of *C. elegans* on plates containing the *Escherichia coli* OP50 strain is simple and affordable in any laboratory [1]. The anatomy and development of this nematode have been described in great detail. We know that adult *C. elegans* consists of basic body systems known in higher animals such as the digestive, excretory, muscular, and nervous system. All these unique features mentioned above make this nematode an excellent model for biomedical and environmental toxicology research [3].

Toxicological studies in animal models are

usually performed at high cost and are often described as time-consuming [4]. Furthermore, meta-analyses showed that rodent models accurately forecasted only particular toxic effects on humans. On the one hand, the employment of several mammalian species can improve predictability, on the other hand, it brings additional costs and reduces output. Predictive toxicology aimed to apply alternative methodologies, mainly non-animal, to forecast the effect of chemical and toxic substances on humans while lowering the expenses, time, and use of mammals in toxicity tests [5,6]. One of these alternatives represents a nematode *C. elegans*, which provides data from a whole animal with fully functional digestive, endocrine, reproductive, sensory, and neuromuscular systems. At the same time, it can be handled under *in vitro* conditions. Moreover, Hunt (2017) described several publications in which *C. elegans* and mammals shared the same pathway of toxic action [7]. *C. elegans* is a unique model that has been widely used in neurotoxicity research.

Despite the fact that nematodes are very distant from humans in terms of evolution, *C. elegans* has many anatomical features and organs found in higher-level animals, such as those related to its nervous, reproductive, muscular, and digestive systems. However, it lacks many physiologically important organs that exist in humans, such as the organs of the circulatory and respiratory system. Additionally, *C. elegans* does not possess an adaptive immune system and specialized immune cells, which are essential for mammalian immunity. Another disadvantage is that the number of compounds that can be screened is currently limited because all substances examined in *C. elegans* must be water soluble. Furthermore, small changes in temperature, nutrition, or salt concentration trigger adaptive responses that, sometimes over numerous generations of the species, can drastically alter analytical results. Good culture practice and particular handling procedures are crucial for this reason, as well [8,9,10].

In the following paragraphs of this review, we discuss the applications of *C. elegans* in biomedical toxicology with a focus on the neurotoxicity of selected chemical substances. For a better understanding of the effect of chemicals on this living organism, we first describe the nervous system of this nematode. Furthermore, we focus on the effect of heavy metals and pesticides, which have been directly linked to several neurodegenerative diseases.

C. elegans nervous system

C. elegans is the only genetic model organism with a completely mapped and well-defined nervous system. Worms have almost the same number of genes as humans, approximately 20 000, and almost all mammalian neurotransmitters, making them the simplest model to study the mechanisms underlying neural activities, as well as global brain states, such as sleep and wakefulness [11].

The nervous system of this nematode consists of cells organized into ganglia, which are located in the head and tail. Most neurons are located in the area around the pharynx, which forms the brain of *C. elegans*. Additional neurons are found in the area of the centre axis of the body in a continuous row adjacent to the ventral hypoderme. Moreover, there are two other lateral ganglia, as well as several neurons scattered throughout the body. The nervous system of *C. elegans* hermafrodite consists of 302 neurons and 56 glial cells, each of which is unique in morphology and location. Males have a larger nervous system consisting of 473 cells with additional 79 neurons and 36 glial cells. Most of these extra cells are involved in the mating process and are located at the back of the body [12]. Neurons with similar morphologies and connections are grouped into 118 classes. All neurons are interconnected through synapses. An adult hermaphrodite has two independent nervous systems, namely the large somatic nervous system and the small pharyngeal nervous system. These systems communicate with each other through interneurons, which provide the transmission of nerve impulses [13]. In the case of the somatic nervous system, neurons are located between the hypodermis and the muscles of the body wall. Neurons of the small pharyngeal system are situated right in the middle of the pharyngeal muscles and are not separated by a basal lamina [14].

The *C. elegans* nematode contains 4 types of chemosensory organs: amphid, phasmid, external, and internal labial organs. Chemosensory organs consist of cells that form the capsule and cells that create pores [15]. In addition to connecting individual neurons, synapses are the main places of information processing. Synapses consist of presynaptic and postsynaptic cells. Individual information is transmitted through neurotransmitters released by pro-synaptic cells. *C. elegans* neurons were divided into 3 functional groups: a) motor neurons b) sensory neurons and c) interneurons. In *C. elegans*, the connections of those neurons have been mapped into

a complete wiring diagram, also known as connectome [16].

C. elegans moves on solid surfaces by "crawling" and in liquid media by "swimming". Approximately 113 of the total 302 neurons are responsible for this movement, but also for intestinal peristalsis. Seventy-nine neurons out of 113 innervate the muscles in the head and neck. These neurons fall into 8 classes: AS, DA, DB, DD, VA, VB, VCa VD. Type A and B motor neurons are cholinergic, which use acetylcholine to transmit nerve impulses. Type D motor neurons release a neurotransmitter called γ-aminobutyric acid (GABA) [17].

C. elegans responds to environmental stimuli through chemotaxis and thermotaxis and therefore avoids harmful substances or, conversely, seeks food through these properties. This worm senses mechanical stimuli, temperature, water-soluble substances, volatile chemicals, osmolarity, oxygen levels, pH, and light through sensory neurons. A chemosensory neuron contains two types of glial cells, namely sheath and socket cells. Interneurons act as information processors, receiving a signal from one or more classes of neurons and sending that signal to other neurons. In most cases, the worms could detect attractive compounds using the AWA and AWC olfactory neurons, whereas the AWB neurons were responsible for sensing repulsive volatile compounds [18].

Application of *C. elegans* in neurotoxicology research

The use of worms as model organisms to study neurotoxicity is strengthened by many advantages. One of the most important benefits is the fact that the anatomical and functional components of the *C. elegans* nervous system are mapped and known, and, moreover, they share homology with the one in humans. Nobel prize winning experiments that used *C. elegans* to explore the nervous system, its development, and function, helped to substantially understand brain physiology [1]. The effects of many toxic substances, including metals and pesticides, have been studied in the nervous system of *C. elegans*. The results of these studies demonstrated similar adverse effects of chemicals that are often seen in mammalian systems [19,20,21].

In Table 1, we summarize the effect of selected pesticides and heavy metals on *C. elegans*. The following paragraphs are dedicated to the neurotoxicity of *C. elegans* caused by these substances in detail.

Table 1. The effect of selected pesticides and heavy metals on *Caenorhabditis elegans*.

Compound		Effect
Heavy metals	nickel	• reproductive toxicity generated through oxidative stress and apoptosis [26] • toxic effects on locomotion, growth, brood size and feeding [24] • decreased cholinergic and dopaminergic neuronal function [25]
	manganese	• dopaminergic neurodegeneration [31,32,33] • reduction of olfactory adaptive learning and memory [34,35,36]
	mercury	• neurodevelopmental toxicity • DAergic and GABAergic neurodegeneration [45,46,47]
	arsenic	• changes in behavior and movement [50] • increased ROS production [52] • neurodegeneration [51,53]
	lead	• degeneration of AFD, DAergic and GABAergic neurons [45] • formation of different neurobehavioral abnormalities [64] • changes in locomotion [65] • reduction in body length [66]
	cadmium	• affected feeding and movement [74, 70] • decreased growth, life span and reproduction [72] • loss of superoxide dismutase 1 enzymatic activity [75,73]

Table 1. (continued)

Pesticides	paraquat	• impact on reproduction, longevity, gene expression, and mitochondrial function [95,98,99,100]
	rotenone	• impact on mitochondrial DNA replication and gene expression [95,96,97]
	dichlorvos	• changes in several neural growth/repair-related gene expressions [104]
	malathion	• decreased survival, reproduction, feeding, and locomotion [109, 111]
	monocrotophos	• aggravated the dopaminergic neuronal dysfunction, changes in locomotion, significant reduction in AChE activity [115,116,137]
	glyphosate	• increased levels of ROS and hydrogen peroxide production [122,123] • an increase in the oxidative stress response [124]
	thiocarbamate	• neurodegeneration and specific dopaminergic cell dysfunction [130]
	dithiocarbamate (mancozeb)	• neurodegeneration of GABAergic and dopaminergic neurons [132, 133]

Table 2. Comparison of *C. elegans* aquatic lethality to *Daphnia* and other invertebrate data. Adapted according to Williams and Dusenbery (1990) [23].

	96-h LC_{50} (µg/l)		
Metal	***C. elegans* (95 % C.I.)**	***Daphnia* (range)**	**Avg. of all invertebrates**
Cr	59	6,400	16,326
Cd	61	55	3,091
Pb	62	1,158	7,745
As	173,000	2,687	7.690
Be	Data not available	Data not available	Data not available

Neurotoxicity induced by heavy metals

C. elegans has been used successfully to explore the toxicity and toxicological pathways of the following heavy metals: aluminium, arsenic, barium, cadmium, copper, lead, manganese, mercury, nickel, uranium, and zinc. The main end points of these experiments included changes in *C. elegans* lethality, reproduction, life span, and protein expression. Additionally, behavioral, reporter expression, and neuronal morphology analyses were used to investigate the effect of these metals on the nervous system of this worm [22].

The first evidence on the effect of soluble forms of silver, mercury, copper, beryllium, aluminium, lead, chromium, arsenic, thallium, zinc, cadmium, nickel, strontium, and antimony on *C. elegans* was described by Williams and Dusenbery in 1990 [23]. During these experiments, the nematodes were exposed to heavy metals from day 1 to day 4. The lowest lethal concentration of metal that killed 50 % of the worms (LC_{50}) after 96 h was documented for lead, chromium and cadmium, while the highest LC_{50} was demonstrated for strontium, compared to data retrieved from studies in invertebrates (Table 2). Furthermore, *C. elegans* was more sensitive to lead, chromium, and beryllium and less susceptible to arsenic than any of the other invertebrates examined [23]. In the following section, we describe that acute and chronic exposure of *C. elegans* to selected heavy metals caused toxic effects on the central nervous system (CNS).

Nickel

Nickel (Ni), which is a common metal found in the environment, has been increasingly used in industry. Although Ni compounds cause toxicity in various organs, such as the kidney, lungs, liver, skin, and gonads, their neurotoxic consequences have not yet been thoroughly explored. Ijomone *et al.* (2020) described a particular neuronal susceptibility in the *C. elegans* model of acute Ni neurotoxicity [24]. In these experiments, *C. elegans*

wild type and worms expressing green fluorescence protein (GFP) in both cholinergic, dopaminergic (DAergic), or GABAergic neurons at the first larval (L1) stage, were exposed to nickel chloride ($NiCl_2$) for 1 hour. Analyses of the morphology of GFP-expressing worms revealed a considerable increase in the degeneration of cholinergic, DAergic and GABAergic neurons. Next, significant functional alterations in locomotion and baseline slowing response experiments showed that Ni administration decreased cholinergic and DAergic neuronal function. Surprisingly, their data demonstrated that exposure to Ni during development caused oxidative stress, which was directly related to brain damage and altered behavior [24]. Similar findings were observed by Tang *et al.* (2020). They demonstrated that Ni caused multiple toxic effects on locomotion, growth, brood size, and feeding of *C. elegans* [25]. Moreover, Kong *et al.* (2017), discovered that nickel nanoparticle-induced reproductive toxicity in *C. elegans* was generated by oxidative stress and apoptosis [26]. Several scientific studies demonstrated that exposure of human blood lymphocytes to nickel chloride *in vitro* manifested increased levels of intracellular reactive oxygen species (ROS), hydroxyl radicals, and lipid peroxidation, supporting the idea that inorganic nickel induced oxidative stress [27,28,29].

Manganese

Manganese (Mn) is one of the most abundant trace elements found in nature, as well as in all tissues of living organisms. It regulates a variety of cellular activities at physiological levels, including lipid, protein, and glucose metabolism, the immune system, development, and growth. However, at high doses, it can be toxic to the brain [30]. The acute and long-term exposure to Mn has been used to elucidate Mn neurotoxicity in *C. elegans*. Several studies described that Mn treatment of *C. elegans* in the early stage of development (L1) caused DAergic neurodegeneration in L1, L4 and young adults [31,32,33,34]. Additional important findings published by Raj *et al.* (2021) demonstrated that exposure of *C. elegans* larvae to Mn significantly reduced adaptive olfactory learning and memory [35]. Despite the fact that the fundamental mechanism of Mn neurotoxicity is still unknown, Neumann *et al.* (2020) showed, while using genetically manipulated worms, that Mn-induced oxidative stress contributed to the pathogenesis of several neurological diseases [36]. Together, all of these data point to the toxic effect of Mn on the central nervous system of this nematode. Furthermore, in humans, overexposure to Mn negatively affected both higher-order cognitive functions and motor function. Motor control was particularly impaired by disruption of DAergic function [37]. This phenomenom was observed mainly in workers who experienced occupational exposure to manganese [38] [39]. Moreover, the adverse effects of occupational exposure to Mn have also been associated with neurodegeneration and learning deficiencies [40].

Mercury

Mercury (Hg) is a dangerous pollutant, which occurs in different chemical forms, namely elemental Hg (Hg°), inorganic Hg compounds (Hg^{2+}) and organic Hg forms, also known as methylmercury (MeHg). In particular, MeHg is considered one of the most dangerous neurotoxins found in the environment. By direct contact with thiols and other indirect mechanisms, MeHg can change the oxidation state of -SH groups in proteins, modifying their oxidation state and hence their activities [41]. Human exposure to MeHg is primarily due to eating contaminated fish and vegetables, but also by occupational exposure and anthropogenic mercury emissions [42]. Some extensive research has been dedicated to the developmental neurotoxicity of MeHg in humans and animals. While chronic exposure to MeHg during the developmental stage caused long-term effects on neurobehavioral functions, acute exposure at high doses permanently damaged neurons. MeHg posed a major threat to the developing brains of fetuses and newborns because it could pass through the placental barriers [43]. These data indicated ROS accumulation, oxidative stress, disturbance of neurotransmitters, or metallothioneins homeostasis after exposure to MeHg [44]. Numerous neurobehavioral studies conducted in *C. elegans* confirmed MeHg-induced neurodevelopmental toxicity, while neuronal visualization revealed DAergic and GABAergic neurodegeneration later in their life [45,46,47].

Arsenic

Arsenic (As) and its compounds are well-known carcinogens that are abundant in the environment. Elevated concentrations of these heavy metals were found notably in groundwater from different countries throughout the world. Exposure to As is known to cause various neurological disorders through diverse molecular mechanisms such as chromosomal aberrations, increased

ROS, cell DNA damage, and cytotoxicity [48,49]. Arsenic-induced neurotoxicity in *C. elegans* has been extensively documented. For example, significant changes in numerous locomotor activities of these nematodes were documented after exposure of *C. elegans* worms (stage L4) to different concentrations of As (10-200 μM) for 24 h [50]. Chronic exposure of *C. elegans* to As caused changes in behavior and movement of nematodes in a variety of ways that depended on the dose of this metal [51]. With elevated concentrations of As, the severity of the impairments increased. For instance, head thrash, body bend, track length, forward speed, reversal speed, mean wavelength, and mean amplitude all started to decrease after 24 h of exposure to As and this phenomenon persisted throughout the observation periods [51]. In addition, GFP-tagged worms in amphid finger neuron D (AFD) showed reductions in cell body size and fluorescence intensity compared to a control group. Early life As exposure of *C. elegans* was associated with increased ROS production, neurodegeneration, particularly of AFD neurons, and neurobehavioral alterations, such as decreased thermosensory function [50]. Additional studies also documented elevated ROS levels at different stages of *C. elegans* development after exposure to As. These changes were associated with mitochondrial dysfunction and accelerated aging [52,53]. According to epidemiological and toxicological research, As caused intellectual and cognitive deficits in humans [54,55]. In addition, a review by Tolins *et al.* (2014) described that chronic exposure of children, from different countries, to low concentrations of As (<10 μg/l) reduced their IQ and memory performance [56].

Although the toxicity of As has been extensively documented in *C. elegans*, studies dedicated to the toxicity of arsenolipids, which are lipid-soluble forms composed of As and a long aliphatic chain, are rare. Recently, Bornhorst *et al.* (2020) investigated the effects of three types of arsenolipids, such as arsenic-containing hydrocarbons (AsHC), a saturated arsenic-containing fatty acid (AsFA) and an arsenic-containing triacylglyceride (AsTAG), on *C. elegans* toxicity, bioavailability and metabolism [57]. Despite the fact that all arsenolipids were highly bioavailable in *C. elegans*, only AsHC was significantly metabolized to thioxylated or reduced metabolic products and caused severe toxicity that negatively affected survival and development. Furthermore, AsHCs were much more toxic than the hazardous reference arsenite [57]. Except Bornhorst *et al.* (2020) who used *C. elegans* to study arsenolipids, there are available data on the biotransformation of AsFA and AsHC studied using human liver cells [58,59]. It is known that AsFAs and AsHC interfered with the citric acid cycle, but the consequences of this process are not yet understood [59,60]. Therefore, further research on these pathways is still required to fully understand the harmful mechanisms of action of AsFAs, AsHCs, and their metabolites.

Lead

In human biology, lead (Pb) is described as a non-essential and naturally occurring heavy metal that can be found in the Earth's crust. Humans are often exposed to Pb as a result of its industrial use in numerous alloys and compounds. Examination of the impact of Pb on neurodevelopment discovered alterations and deficits in IQ, memory, attention, language comprehension, processing speed, motor function, and affect [61]. The complicated molecular mechanisms underlying Pb neurotoxicity have been shown to include membrane abnormalities, oxidative stress, dysregulation of cell signaling, and decreased neurotransmission [62]. Although *C. elegans* has been used to study the toxicity caused by Pb in general, the lack of literature in this area indicates that this model organism has not been explicitly utilized to study neurotoxicity and neurodevelopment. However, neuronal GFP labeling and very simple behavioral tests showed adverse effects of Pb on *C. elegans* larvae [63]. As documented by several researchers, exposure of *C. elegans* to Pb led to the degeneration of AFD neurons and GABAergic neurons, as well as to different neurobehavioral abnormalities [45, 64]. Additionally, Xing *et al.* (2009) demonstrated that younger *C. elegans* larvae (stage L1-L3) exhibited greater vulnerability to Pb-induced neurotoxicity, in terms of neuronal survival and synaptic function, compared to larvae in stage L4 and young adult nematodes [45]. DAergic dysfunction observed in *C. elegans* after exposure to Pb^{2+} for 1 h was also documented in another study [65]. Furthermore, the results of Tiwari *et al.* (2020) demonstrated that *C. elegans* exposed to lead nitrate ($Pb(NO_3)_2$) at three sublethal doses (3 μM, 15 μM and 30 μM) for 24 h manifested significant changes in locomotion and successive reductions in body length [66]. Similarities between the same pathways and cellular functions affected by lead exposure found in humans and *C. elegans* may be helpful in extrapolating the effects of

lead exposure on living organisms [66].

Cadmium

Cadmium (Cd) is a non-essential transition heavy metal and an environmental pollutant that has been classified as a category 1 human carcinogen. Exposure to Cd was directly linked to mutagenic and teratogenic consequences. It was previously shown that occupational exposure to Cd was directly associated with lung cancer, as well as other cancers such as prostate, kidney, liver, hematopoietic system, urinary bladder, pancreatic, testes, and stomach [67]. In addition to these findings, Cd was also found to cause detrimental neuronal changes in humans. For example, the nervous system of children was more vulnerable to the negative effects of chronic exposure to Cd compared to adults [68]. These findings indicated that these neurotoxic effects could play a substantial role in the systemic toxic effects of exposure to Cd, especially during long-term exposure [69]. Research dedicated to the effect of Cd on *C. elegans* showed altered behavior, as well as decreased growth, life span, and reproduction [70]. Although previous studies have shown that Cd can have a negative impact on reproduction in *C. elegans*, rats, and mice [71,72], the specific molecular pathways involved in this process are still unknown. Based on transcriptome sequencing, Qu *et al.* (2022) found that Cd can influence reproductive, neurological, and aging processes in *C. elegans* by controlling the expression of circRNAs and lncRNAs [73]. Additional research demonstrated that Cd affected the feeding and movement of nematodes [74]. Furthermore, Bovio *et al.* (2021) demonstrated that Cd caused a loss of superoxide dismutase 1 (SOD1) enzymatic activity in all biological models, including *C. elegans, E. coli* BL21 cells, and the human SH-SY5Y neuronal cell line, without affecting protein expression [75]. A study by Gonzalez-Hunt *et al.* (2014) showed that exposure of *C. elegans* to cadmium chloride ($CdCl_2$) caused more mitochondrial than nuclear DNA damage [76].

Neurotoxicity of heavy metal mixtures

Humans are exposed to many neurotoxic agents simultaneously and hence studies dedicated to combined exposures to these compounds remain crucial. Cedergreen published one of the first systematic reviews describing mixture toxicity studies within environmental toxicology in 2014 [77]. She concluded that despite the large number of studies conducted on metal mixtures, well-documented synergistic metal-metal interactions seem to be rare [77]. Although some metals are considered "*essential elements*" (manganese, selenium, and copper), at higher concentrations they can interfere with normal biological processes and trigger cellular stress responses, which can lead to the development of diseases [78]. Therefore, the following paragraph of this review is dedicated to the most recent findings on the effect of metal mixtures on *C. elegans*.

Current investigations suggest the role of Mn and MeHg in the harmful effects on the nervous system. It should be noted that exposure to MeHg and Mn in combination resulted in detrimental effects compared to exposure to either metal alone. According to the study by Schetinger *et al.* (2019), co-exposure of *C. elegans* to these metals was associated with developmental delays in worms, an increase in antioxidant system-related enzymes, and cholinergic degeneration [79]. This study described exposure of *C. elegans* (stage L1) to these metals separately and simultaneously for 24 h. The nematodes accumulated Mn and MeHg in their bodies, and at the highest doses, the interaction between MeHg (trace metal) and Mn (biometal) did not increase the levels of Mn in worms, whereas Hg levels were elevated. In addition to the reduced survival of *C. elegans*, the combination of these metals also induced a delay in the development of the worm. Co-exposure of nematodes to both metals dramatically decreased the size of the brood. Furthermore, catalase and superoxide dismutase, two enzymes of the antioxidant system, were both up-regulated, probably demonstrating an adaptive response to resist an increase in ROS generation [79]. Additional research carried out by Lu *et al.* (2018) used *C. elegans* as a model organism to investigate the acute lethal toxicity of Mn, Cd, and Pb alone, as well as mixtures, namely Mn+Cd or Mn+Pb [80]. The acute toxicity (LC_{50}) of the individual metals was consistent with previous reports [81, 82], with Pb being the most toxic and Mn the least [80]. Exposure to metal mixtures can have effects independent of each other, additive to the dose, interactive (synergistic or antagonistic), or both [83]. Using the toxic unit model, Lu *et al.* (2018) found that the Mn+Pb mixture showed synergistic lethal toxicity to *C. elegans*, while the Mn+Cd mixture demonstrated an antagonistic effect [80]. Interestingly, Moyson and her colleagues (2018) came to the same conclusion, studying the toxicity of zinc (Zn), copper (Cu), and cadmium (Cd) alone and in mixtures on the size and body length of the *C. elegans* population [84].

Although single metals were less hazardous than mixtures, populations exposed to lethal concentrations of Cd, Zn+Cu, Cu+Cd, and Zn+Cu+Cd that killed 20 % of worms (LC_{20}) decreased population growth, while all lethal levels of heavy metals and their combinations that killed 5 % of worms (LC_5) showed population growth [84]. In addition, the very recent publication of Pei *et al.* (2022) investigated the combined toxicity of these metals in *C. elegans* compared to exposure to metals alone at low concentrations [85]. They demonstrated a synergistic effect between Cd and As at non-toxic doses on the development and reproduction of *C. elegans*. Furthermore, Cd stimulated *C. elegans* to absorb As, leading to increased accumulation of As in the gonad and intestine [85].

Neurotoxicity caused by pesticides

Pesticides are widely used in agriculture around the world. These substances are known for their high resistance to natural biodegradation and their increased tendency to accumulate in the environment. The main concern about their usage is constantly rising mainly due to their negative impact on both target and non-target species. Clinicians and environmental health specialists try to investigate the mode of action of these substances, as well as to assess their concentration and duration. Exposure to some environmental toxins, which are mentioned below, can have negative consequences on the nervous system and can cause neurodegenerative diseases [86]. Currently, there are more than 100 different pesticides available. Paraquat, rotenone, organophosphates and carbamates, which target the cholinergic system, were among the pesticides tested using *C. elegans*, as a model organism. In most organisms, including the nematode, acetylcholine is the main neurotransmitter involved in motor activity and inhibition of acetylcholinesterase (AChE) activity is the main toxic effect of these substances [87].

Paraquat (methyl viologen) and rotenone

Paraquat is a non-selective contact herbicide, which is a known environmental contributor to the etiology of Parkinson´s disease (PD) [88]. The toxicity mechanism involves cyclic reduction–oxidation reactions, which produce ROS and depletion of reduced nicotinamide adenine dinucleotide phosphate (NADPH) [89]. The toxicity of paraquat to the neuronal system was tested in frogs a few decades ago [90]. The insecticide rotenone is a well-established inhibitor of mitochondrial complex I, which was directly linked to the development of PD after acute and long-term exposures [91]. Until now, the adverse effects of these chemicals and their role in the development of PD have been studied in neurotoxin-induced models, including *C. elegans* [92]. In these models, the pathophysiological mechanisms of PD caused by the activity of paraquat and rotenone were characterized by mitochondrial dysfunction and oxidative stress [20]. Furthermore, in experimental models, paraquat and rotenone caused loss of nigral DAergic neurons and behavioral modification linked to human PD [93]. Human epidemiological studies indicate that exposure to heavy metals and pesticides, including paraquat and rotenone, increased the risk of PD [20] [94]. The research findings of Wu *et al.* (2018) confirmed the role of paraquat and rotenone in PD. More specifically, they showed that exposure of *C. elegans* to different concentrations of paraquat (0.5–10.0 μM) and rotenone (0.2–1.6 mM) for 3 days induced signs similar to Parkinsonism, including motor deficits and DAergic degeneration [95]. An additional study by Zhou *et al.* (2013) demonstrated that chronic exposure of *C. elegans* to low concentrations of rotenone (2 or 4 μM) over time acquired loss of dopamine neurons, which is a sign of Parkinsonism [96]. It should be noted that this phenomenon was accompanied by a significant change in mitochondrial biogenesis. In the *C. elegans* model, rotenone caused an early and permanent drop in mitochondrial DNA content and suppressed mitochondrial gene expression [96]. Very recently, Mello *et al.* (2022) utilized *C. elegans* to investigate the role of mitochondrial disruption in modulating conserved immuno-metabolic molecular pathways and disease susceptibility [97]. They found that rotenone disrupted nematode growth and mitochondrial bioenergetics, as expected, and modulated the expression of genes and pathways involved primarily in detoxification, energy metabolism, and defense of pathogens [97]. A recent study by Bora *et al.* (2021) demonstrated that paraquat had an impact on *C. elegans* reproduction, longevity, gene expression, and mitochondrial function [98]. According to these results, an acute exposure of the wild-type strain of *C. elegans* (N2) to paraquat led to the response of mitochondrial unfolded protein (mtUPR), decreased mitochondrial membrane potential, increased expression of mitochondrial superoxide dismutase, a dose-dependent progression from linear to fragmented mitochondria and dose-dependent changes in longevity.

Furthermore, multiple generations of N2 exposed to a modest dose of paraquat (0.035 mM) demonstrated decreased fertility, eventually leading to the complete loss of viable embryo development in the third generation [98]. Mitochondrial dysfunction, as well as decreased survival of *C. elegans* after exposure to paraquat, was also documented by Lu *et al.* (2021) [99]. However, they found that the adverse effects of paraquat could be reduced by pretreating worms with peptides isolated from the sea cucumber *Apostichopus japonicus*. Due to their antioxidant activity at both the cellular and organism levels, sea cucumber peptides may be appealing as dietary supplements for healthy aging [99]. Very recently, Ji *et al.* (2022) confirmed that *C. elegans* can be used as a suitable model to search for the harmful effects of paraquat [100]. They evaluated ROS and malondialdehyde (MDA) levels, mitochondrial morphology, and the survival rate of worms after exposure to paraquat in solid media. Paraquat poisoning of nematodes resulted in elevated levels of ROS, MDA content, and mitochondrial fragments, significantly reducing life. These data were confirmed by experiments performed in mice [100].

Organophosphates

Organophosphates (Ops) represent an additional group of pesticides that have been associated with neurobehavioral changes described in the *C. elegans* model organism. In the following paragraph, we review some of the organophosphate insecticides.

Dichlorvos, also known as DDVP, DDVF, denkavepon, and vapona, is widely utilized insecticide and acaricide throughout the world. This organophosphorus agent is often used mainly due to its high efficacy and inexpensive price. However, the World Health Organisation (WHO) classified dichlorvos as an extremely dangerous substance, which is highly poisonous to mammals with significant bioaccumulation potential [101]. In addition, the use of this agent in the environment displayed moderate to high toxicity to most biodiversity, including honeybees [102]. As a consequence of its mutagenic and neurotoxic potential, it could harm reproduction and development. Furthermore, its negative effects in humans were directly associated with several nervous system diseases even at relatively low doses [103]. In connection with these findings, the research of Lewis and his colleagues (2013) provided insight into the impact of dichlorvos on different stages of *C. elegans* development [104]. They found that exposure to this substance caused several changes in gene expression related to neural growth/repair in L4 worms [104]. Similar data were also obtained while doing research on rodents [105]. The genotoxic effect using human cell culture was also documented by Fiore *et al.* (2013) [106]. They showed that dichlorvos caused disruption of mitotic division, mitotic arrest production, and chromosome aneuploidy/polyploidy in cell population proliferation *in vitro* [106].

Malathion is another organophosphate insecticide widely used in agriculture. This agent is also known for its use during mosquito eradication in public well-being areas. Malathion, as well as its metabolite called malaoxon, was previously published to exert acute neurotoxic effects by inhibiting AChE activity. This situation resulted in accumulation of acetylcholine and subsequent cholinergic overstimulation [107]. These data were also confirmed by experiments performed by Patil and David (2010) in freshwater fish, *Labeo rohita* [108]. The first study, which described the neurotoxic potential of malathion in *C. elegans*, was published by Williams and Dusenbery (1990) [109]. Their results showed a remarkable decrease in the locomotion of exposed worms at concentrations, which did not affect the survival of nematodes [109]. Additional experiments conducted in rats demonstrated that this chemical agent induced significant neurobehavioral deficits and neuronal degeneration in the brain [110]. To prevent or alleviate malathion-induced toxicities, researchers sought alternative biotherapy. For example, the study by Kamaladevi *et al.* (2016) demonstrated the protective mechanism of *Lactobacillus casei* against malathion-induced toxicity in *C. elegans*. In their study, nematodes exposed to malathion alone (300 mM) showed decreased survival, feeding, and locomotion. However, the presence of lactic acid bacteria, specifically *L. casei*, led to protective activity against malathion-induced toxicity and restored all physiological parameters of *C. elegans* [111].

Monocrotophos is an additional organo-phosphate insecticide, which exerted a neurotoxic effect by inhibiting AChE activity [112]. Most countries, including the USA, EU, India, and many others, have banned its use mainly due to its toxicity, fast activity, and non-organism specificity [113]. In early 2001, Olivier *et al.* demonstrated that a high-glucose diet could exacerbate organophosphate toxicity in rats [114]. Further extensive research by Salim and Rajini, (2014) used *C. elegans* to investigate the role of such a diet in

developmental neurotoxicity caused by monocrotophos [115]. They found that exposure of *C. elegans* to a high glucose diet in the egg stage increased the toxic outcome of monocrotophos in terms of physiological, behavioral, and biochemical reactions [115]. Furthermore, the same group showed that the monocrotophos-induced glucose-rich diet aggravated DAergic neuronal dysfunction in *C. elegans* N2, as well as transgenic strains [95]. A study related to the neurotoxicity of monocrotophos indicated that *C. elegans* treated with this agent exhibited changes in locomotion, as well as significant reduction in AChE activity, which are common features of PD [116]. These specific findings related to the decrease in AChE function were later confirmed in a mouse model [117] and also in humans [118].

Glyphosate

Glyphosate is a well-known herbicide with broad-spectrum activity that has been used since 1974 worldwide. Due to its specific chemical structure (the phosphorus atom is attached to the residue of the molecule by a carbon atom, not oxygen), it is classified as an organophosphonate and not as an organophosphate [119]. Glyphosate is structurally similar to other organophosphate pesticides, but is toxicologically distinct and does not inhibit cholinesterase activity [120]. The mode of action of glyphosate is related to its ability to block the shikimic acid pathway, which is crucial for the synthesis of aromatic amino acids, such as tyrosine, phenylalanine and tryptophan, in plants, fungi, and some microorganisms [121]. Although glyphosate alone is essentially non-toxic, some studies performed in *C. elegans* revealed that exposure to glyphosate-containing products was linked to mitochondrial dysfunction. For example, worms treated with a commercial formulation of glyphosate (glyphosate F) exhibited ROS production and an enhanced response to oxidative stress [122]. An additional study by Bailey *et al.* (2018) demonstrated that *C. elegans* treated with the glyphosate-containing herbicide TouchDown (TD) produced higher levels of ROS and hydrogen peroxide [123]. Furthermore, nematodes exposed to the same herbicide also showed neurotoxicity through inhibition of Complex II (succinate dehydrogenase), decreased ATP levels, and increased hydrogen peroxide production [124]. Occupational or chronic exposure to glyphosate (through inhalation and dermal routes) in humans can also cause neurotoxic effects [125]. According to a study by Fuhrimann *et al.* (2022), smallholder farmers exposed to glyphosate in Uganda developed visual memory impairment [126].

Carbamate pesticides

Carbamates are organic compounds synthesized from carbamic acid (NH_2COOH), and more specifically, they are esters of carbamic acid. In terms of structure and mechanism, carbamates are related to organophosphates. Carbamates cause carbamylation of AChE at neuronal synapses and neuromuscular junctions. The difference between carbamates and organophosphates is in their binding to AChE. Whereas organophosphates cause irreversible phosphorylation of AChE, carbamates bind to AChE reversibly. Consequently, carbamates have a toxicological presentation that is comparable to OPs poisoning, with a typical toxicity period of less than 24 hours [127].

Thiocarbamates and dithiocarbamates represent a group of pesticides, which is often used in the USA, EU, and many other countries around the world [128]. As suggested by its name, thiocarbamates are sulfur analogues of carbamates. Compared to monothiol-carbamates, which contain one sulfur atom, dithiocarbamates are composed of two sulfur atoms in combination with zinc salts, ferric salts, and manganese salts giving them additional biological properties [129]. These compounds have greater efficacy, better stability, and less phytotoxicity than elemental sulfur. However, its frequent use has already been associated with the development of various diseases and health problems, including neuropathologies, such as PD, endocrine and reproductive disruption, and the development of cancers [128]. To date, extensive research has been done on the toxicity of these agents has been performed in *C. elegans*. For example, Caito *et al.* (2013) investigated that two thiocarbamate insecticides, as well as a reactive intermediate of their metabolism, promoted neurodegeneration and specific DAergic cell dysfunction in *C. elegans*, and may be an environmental risk factor for the development of PD [130]. However, other neurotransmitter systems, such as cholinergic, glutamatergic, and GABAergic, were not affected [130]. Two dithiocarbamates, mancozeb and maneb, are widely used manganese-containing ethylene-bis-dithiocarbamate (EBDC) fungicides, which helped producers manage several economically important plant diseases [131]. Possible developmental neurotoxicity of the glyphosate-containing herbicide TD and mancozeb was also investigated using *C. elegans* as a model organism. In the

first study, worms (stage L2) were exposed to either product or both products acutely or chronically [132]. The results of this study demonstrated dose-dependent neurotoxicity, with exposure to mancozeb causing the highest level of neurodegeneration [132]. A follow-up study using the same design and chemical agents found that exposure to herbicides containing glyphosate and mancozeb is associated with neurodegeneration of GABAergic and DAergic neurons, showing that these drugs had a more specific impact on the nervous system [133]. Exposure to mancozeb through pesticide applicators was strongly associated with an increased incidence of thyroid cancer in humans [134]. Some existing systematic reviews have already discussed the issue related to the toxic effect in the liver, reproductive system, kidney, and central nervous system caused by mancozeb [127,131,135,136]. From February 2021, the European Commission banned the use of mancozeb mainly due to its toxic properties on the human organism [127].

Conclusions

In daily life, humans are continuously and simultaneously exposed to a variety of chemical compounds primarily through the environment, consumer products, and food. Therefore, it is reasonable to expect that hundreds of chemicals contained in the human body will interact with each other, resulting in additional effects that may be harmful to human health. In this review, we summarize the most recent findings on the neurotoxic effects of heavy metals, heavy metal mixtures, and pesticides, which are often used in agriculture, on *C. elegans*, a host model organism. Nematodes are a favorite and widely used experimental model in biomedical and environmental toxicology. According to existing studies conducted in *C. elegans*, heavy metals caused changes in lethality, reproduction, life span, and protein expression. Similar findings were observed in a case of pesticides, which had a strong impact on reproduction, longevity, gene expression, and mitochondrial function of *C. elegans*. Due to these reasons, heavy metals and pesticides must be strictly controlled in terms of persistence, bioaccumulation, and toxicity in agroecosystems. Furthermore, the toxic activity of heavy metal mixtures should not be neglected when assessing the hazard and environmental health risk because the activity of the mixtures could differ significantly from exposure to a single metal. In conclusion, the toxic consequences of heavy metals and their mixtures, as well as pesticides in the human body, their damage to ecosystems with respect to both short- and long-term exposure should be intensively reported.

Conflict of Interest

There is no conflict of interest.

Acknowledgements

Financial support for this work was provided by a grant from Trnava University in Trnava n. 10/TU/2021 awarded to KM and by grant from Trnava University in Trnava n. 8/TU/2021 awarded to ZB.

References

1. Brenner S. The genetics of *Caenorhabditis elegans*. Genetics. 1974;77:71-94. https://doi.org/10.1093/genetics/77.1.71
2. Consortium TC elegans S. Genome sequence of the nematode C. elegans: a platform for investigating biology. Science (80). 1998;282. https://doi.org/10.1126/science.282.5396.2012
3. Boyd WA, Smith MV, Freedman JH. *Caenorhabditis elegans* as a model in developmental toxicology. Methods Mol Biol. 2012;889:15-24. https://doi.org/10.1007/978-1-61779-867-2_3
4. Tralau T, Riebeling C, Pirow R, Oelgeschläger M, Seiler A, Liebsch M, *et al.* Wind of change challenges toxicological regulators. Environ Health Perspect. 2012;120:1489-94. https://doi.org/10.1289/ehp.1104782
5. Hartung T. Toxicology for the twenty-first century. Nature. 2009;460:208-212. https://doi.org/10.1038/460208a
6. Knight AW, Little S, Houck K, Dix D, Judson R, Richard A, *et al.* Evaluation of high-throughput genotoxicity assays used in profiling the US EPA ToxCastTM chemicals. Regul Toxicol Pharmacol. 2009;55:188-199. https://doi.org/10.1016/j.yrtph.2009.07.004
7. Hunt PR. The C. elegans model in toxicity testing. J Appl Toxicol. 2017;37:50-59. https://doi.org/10.1002/jat.3357

8. Johnson TE. Advantages and disadvantages of *Caenorhabditis elegans* for aging research. Exp Gerontol. 2003;38:1329-1332. https://doi.org/10.1016/j.exger.2003.10.020
9. Marsh EK, May RC. *Caenorhabditis elegans*, A model organism for investigating immunity. Appl Environ Microbiol. 2012;78:2075-81. https://doi.org/10.1128/AEM.07486-11
10. Ha NM, Tran SH, Shim YH, Kang K. *Caenorhabditis elegans* as a powerful tool in natural product bioactivity research. Appl Biol Chem. 2022;65. https://doi.org/10.1186/s13765-022-00685-y
11. Nichols ALA, Eichler T, Latham R, Zimmer M. A global brain state underlies C. Elegans sleep behavior. Science (80). 2017;356:1247-1256. https://doi.org/10.1126/science.aam6851
12. Gjorgjieva J, Biron D, Haspel G. Neurobiology of *Caenorhabditis elegans* locomotion: Where do we stand? Bioscience. 2014;64:476-486. https://doi.org/10.1093/biosci/biu058
13. Byrd DT, Jin Y. Wired for insight- recent advance in *C. elegans* Neural Circuits. Curr Opin Neurobiol. 2022;69:159-169. https://doi.org/10.1016/j.conb.2021.02.009
14. Hobert O. Neurogenesis in the nematode *Caenorhabditis elegans*. WormBook. 2010;1-24. https://doi.org/10.1895/wormbook.1.12.2
15. Iliff AJ, Xu XZS. C. elegans: a sensible model for sensory biology. J Neurogenet. 2020;34:347-350. https://doi.org/10.1080/01677063.2020.1823386
16. Emmons SW. The beginning of connectomics: A commentary on White *et al.* (1986) 'The structure of the nervous system of the nematode *Caenorhabditis elegans*.' Philos Trans R Soc B Biol Sci. 2015;370. https://doi.org/10.1098/rstb.2014.0309
17. Tolstenkov O, Van der Auwera P, Costa WS, Bazhanova O, Gemeinhardt TM, Bergs ACF, *et al.* Functionally asymmetric motor neurons contribute to coordinating locomotion of *Caenorhabditis elegans*. Elife. 2018;7:1-28. https://doi.org/10.7554/eLife.34997
18. Zhang C, Zhao N, Chen Y, Zhang D, Yan J, Zou W, *et al.* The signaling pathway of *Caenorhabditis elegans* mediates chemotaxis response to the attractant 2-heptanone in a Trojan Horse-like pathogenesis. J Biol Chem. 2016;291:23618-23627. https://doi.org/10.1074/jbc.M116.741132
19. Ruszkiewicz JA, Pinkas A, Miah MR, Weitz RL, Lawes MJA, Akinyemi AJ, *et al.* C. elegans as a model in developmental neurotoxicology. Toxicol Appl Pharmacol. 2018;354:126-135. https://doi.org/10.1016/j.taap.2018.03.016
20. Tanner CM, Kame F, Ross GW, Hoppin JA, Goldman SM, Korell M, *et al.* Rotenone, paraquat, and Parkinson's disease. Environ Health Perspect. 2011;119:866-872. https://doi.org/10.1289/ehp.1002839
21. Raj K, Kaur P, Gupta GD, Singh S. Metals associated neurodegeneration in Parkinson's disease: Insight to physiological, pathological mechanisms and management. Neurosci Lett. 2021;753:135873. https://doi.org/10.1016/j.neulet.2021.135873
22. Leung MCK, Williams PL, Benedetto A, Au C, Helmcke KJ, Aschner M, *et al. Caenorhabditis elegans*: An emerging model in biomedical and environmental toxicology. Toxicol Sci. 2008;106:5-28. https://doi.org/10.1093/toxsci/kfn121
23. Williams PL, Dusenbery DB. Aquatic toxicity testing using the nematode, *Caenorhabditis elegans*. Environ Toxicol Chem. 1990;9:1285-1290. https://doi.org/10.1002/etc.5620091007, https://doi.org/10.1897/1552-8618(1990)9[1285:ATTUTN]2.0.CO;2
24. Ijomone OM, Miah MR, Akingbade GT, Bucinca H, Aschner M. Nickel-induced developmental neurotoxicity in C. elegans includes cholinergic, dopaminergic and GABAergic degeneration, altered behaviour, and increased SKN-1 activity. Neurotox Res. 2020;37:1018-1028. https://doi.org/10.1007/s12640-020-00175-3
25. Tang B, Williams PL, Xue KS, Wang JS, Tang L. Detoxification mechanisms of nickel sulfate in nematode *Caenorhabditis elegans*. Chemosphere. 2020;260:127627. https://doi.org/10.1016/j.chemosphere.2020.127627
26. Kong L, Gao X, Zhu J, Zhang T, Xue Y, Tang M. Reproductive toxicity induced by nickel nanoparticles in *Caenorhabditis elegans*. Environ Toxicol. 2017;32:1530-1538. https://doi.org/10.1002/tox.22373
27. Chen CY, Wang YF, Lin YH, Yen SF. Nickel-induced oxidative stress and effect of antioxidants in human lymphocytes. Arch Toxicol. 2003;77:123-130. https://doi.org/10.1007/s00204-002-0427-6

28. M'Bemba-Meka P, Lemieux N, Chakrabarti SK. Role of oxidative stress and intracellular calcium in nickel carbonate hydroxide-induced sister-chromatid exchange, and alterations in replication index and mitotic index in cultured human peripheral blood lymphocytes. Arch Toxicol. 2007;81:89-99. https://doi.org/10.1007/s00204-006-0128-7
29. M'Bemba-Meka P, Lemieux N, Chakrabarti SK. Role of oxidative stress, mitochondrial membrane potential, and calcium homeostasis in nickel subsulfide-induced human lymphocyte death *in vitro*. Sci Total Environ. 2006;369:21-34. https://doi.org/10.1016/j.scitotenv.2006.04.007
30. Chen P, Chakraborty S, Mukhopadhyay S, Lee E, Paoliello MMB, Bowman AB, *et al.* Manganese homeostasis in the nervous system. J Neurochem. 2015;134:601-610. https://doi.org/10.1111/jnc.13170
31. Settivari R, VanDuyn N, LeVora J, Nass R. The Nrf2/SKN-1-dependent glutathione S-transferase π homologue GST-1 inhibits dopamine neuron degeneration in a *Caenorhabditis elegans* model of manganism. Neurotoxicology. 2013;38:51-60. https://doi.org/10.1016/j.neuro.2013.05.014
32. Avila DS, Benedetto A, Au C, Bornhorst J, Aschner M. Involvement of heat shock proteins on Mn-induced toxicity in *Caenorhabditis elegans*. BMC Pharmacol Toxicol. BMC Pharmacol Toxicol. 2016;17:1-9. https://doi.org/10.1186/s40360-016-0097-2
33. Ijomone OM, Miah MR, Peres T V., Nwoha PU, Aschner M. Null allele mutants of trt-1, the catalytic subunit of telomerase in *Caenorhabditis elegans*, are less sensitive to Mn-induced toxicity and DAergic degeneration. Neurotoxicology. 2016;57:54-60. https://doi.org/10.1016/j.neuro.2016.08.016
34. Lawes M, Pinkas A, Frohlich BA, Iroegbu JD, Ijomone OM, Aschner M. Metal-induced neurotoxicity in a RAGE-expressing C. elegans model. Neurotoxicology. 2020;80:71-75. https://doi.org/10.1016/j.neuro.2020.06.013
35. Raj V, Nair A, Thekkuveettil A. Manganese exposure during early larval stages of C. elegans causes learning disability in the adult stage. Biochem Biophys Res Commun. 2021;568:89-94. https://doi.org/10.1016/j.bbrc.2021.06.073
36. Neumann C, Baesler J, Steffen G, Nicolai MM, Zubel T, Aschner M, *et al.* The role of poly(ADP-ribose) polymerases in manganese exposed *Caenorhabditis elegans*. J Trace Elem Med Biol. 2020;57:21-27. https://doi.org/10.1016/j.jtemb.2019.09.001
37. Guilarte TR. Manganese neurotoxicity: New perspectives from behavioral, neuroimaging, and neuropathological studies in humans and non-human primates. Front Aging Neurosci. 2013;5:1-10. https://doi.org/10.3389/fnagi.2013.00023
38. Racette BA, Tabbal SD, Jennings D, Good L, Perlmutter JS, Evanoff B. Prevalence of parkinsonism and relationship to exposure in a large sample of Alabama welders. Neurology. 2005;64:230-235. https://doi.org/10.1212/01.WNL.0000149511.19487.44
39. Gonzalez-Cuyar LF, Nelson G, Criswell SR, Ho P, Lonzanida JA, Checkoway H, *et al.* Quantitative neuropathology associated with chronic manganese exposure in South African mine workers. Neurotoxicology. 2014;45:260-266. https://doi.org/10.1016/j.neuro.2013.12.008
40. Caito S, Aschner M. Neurotoxicity of metals. 1st ed. Handb. Clin. Neurol. Elsevier B.V. 2015. https://doi.org/10.1016/B978-0-12-801238-3.00209-9
41. Crespo-López ME, Macêdo GL, Pereira SID, Arrifano GPF, Picanço-Diniz DLW, Nascimento JLM d., *et al.* Mercury and human genotoxicity: Critical considerations and possible molecular mechanisms. Pharmacol Res. 2009;60:212-220. https://doi.org/10.1016/j.phrs.2009.02.011
42. Martins AC, Ke T, Bowman AB, Aschner M. New insights on mechanisms underlying methylmercury-induced and manganese-induced neurotoxicity. Curr Opin Toxicol. 2021;25:30-35. https://doi.org/10.1016/j.cotox.2021.03.002
43. Amadi CN, Igweze ZN, Orisakwe OE. Heavy metals in miscarriages and stillbirths in developing nations. Middle East Fertil Soc J. 2017;22:91-100. https://doi.org/10.1016/j.mefs.2017.03.003
44. Antonelli MC, Pallarés ME, Ceccatelli S, Spulber S. Long-term consequences of prenatal stress and neurotoxicants exposure on neurodevelopment. Prog Neurobiol. 2017;155:21-35. https://doi.org/10.1016/j.pneurobio.2016.05.005

45. Xing XJ, Rui Q, Du M, Wang DY. Exposure to lead and mercury in young larvae induces more severe deficits in neuronal survival and synaptic function than in adult nematodes. Arch Environ Contam Toxicol. 2009;56: 732-741. https://doi.org/10.1007/s00244-009-9307-x
46. McElwee MK, Freedman JH. Comparative toxicology of mercurials in *Caenorhabditis elegans*. Environ Toxicol Chem. 2011;30:2135-2141. https://doi.org/10.1002/etc.603
47. Ke T, Santamaria A, Rocha JBT, Tinkov A, Bornhorst J, Bowman AB, *et al.* Cephalic neuronal vesicle formation is developmentally dependent and modified by methylmercury and sti-1 in *Caenorhabditis elegans*. Neurochem Res. 2020;45:2939-2948. https://doi.org/10.1007/s11064-020-03142-8
48. Wang Y, Zhao F, Liao Y, Jin Y, Sun G. Effects of arsenite in astrocytes on neuronal signaling transduction. Toxicology. 2013;303:43-53. https://doi.org/10.1016/j.tox.2012.10.024
49. Sinha D, Prasad P. Health effects inflicted by chronic low-level arsenic contamination in groundwater: A global public health challenge. J Appl Toxicol. 2020;40:87-131. https://doi.org/10.1002/jat.3823
50. Yu CW, Liao VHC. Arsenite induces neurotoxic effects on AFD neurons via oxidative stress in *Caenorhabditis elegans*. Metallomics. 2014;6:1824-1831. https://doi.org/10.1039/C4MT00160E
51. Zhang X, Zhong HQ, Chu ZW, Zuo X, Wang L, Ren XL, *et al.* Arsenic induces transgenerational behavior disorders in *Caenorhabditis elegans* and its underlying mechanisms. Chemosphere. 2020;252:126510. https://doi.org/10.1016/j.chemosphere.2020.126510
52. Luz AL, Godebo TR, Smith LL, Leuthner TC, Maurer LL, Meyer JN. Deficiencies in mitochondrial dynamics sensitize *Caenorhabditis elegans* to arsenite and other mitochondrial toxicants by reducing mitochondrial adaptability. Toxicology. 2017;387:81-94. https://doi.org/10.1016/j.tox.2017.05.018
53. Müller L, Soares GC, Josende ME, Monserrat JM, Ventura-Lima J. Comparison of the toxic effects of organic and inorganic arsenic in *Caenorhabditis elegans* using a multigenerational approach. Toxicol Res (Camb). 2022;11:402-16. https://doi.org/10.1093/toxres/tfac010
54. Calderón J, Navarro ME, Jimenez-Capdeville ME, Santos-Diaz MA, Golden A, Rodriguez-Leyva I, *et al.* Exposure to arsenic and lead and neuropsychological development in Mexican children. Environ Res. 2001;85: 69-76. https://doi.org/10.1006/enrs.2000.4106
55. Kapaj S, Peterson H, Liber K, Bhattacharya P. Human health effects from chronic arsenic poisoning-a review. J Environ Sci Heal Part A. 2006;41:2399-2428. https://doi.org/10.1080/10934520600873571
56. Tolins M, Ruchirawat M, Landrigan P. The developmental neurotoxicity of arsenic: Cognitive and behavioral consequences of early life exposure. Ann Glob Heal. 2014;80:303-314. https://doi.org/10.1016/j.aogh.2014.09.005
57. Bornhorst J, Ebert F, Meyer S, Ziemann V, Xiong C, Guttenberger N, *et al.* Toxicity of three types of arsenolipids: Species-specific effects in: *Caenorhabditis elegans*. Metallomics. 2020;12:794-798. https://doi.org/10.1039/d0mt00039f
58. González De Chávez Capilla T. The metabolism of arsenic in humans: Bioaccessibility in the gastrointestinal tract, diffusion across lipid membranes and biotransformations in liver cells. University of Canberra, Australia; 2018. https://researchsystem.canberra.edu.au/ws/portalfiles/portal/33678845/file
59. Müller SM, Finke H, Ebert F, Kopp JF, Schumacher F, Kleuser B, *et al.* Arsenic-containing hydrocarbons: Effects on gene expression, epigenetics, and biotransformation in HepG2 cells. Arch Toxicol. 2018;92:1751-1765. https://doi.org/10.1007/s00204-018-2194-z
60. Meyer S, Matissek M, Müller SM, Taleshi MS, Ebert F, Francesconi KA, *et al.* In vitro toxicological characterisation of three arsenic-containing hydrocarbons. Metallomics. 2014;6:1023-1033. https://doi.org/10.1039/C4MT00061G
61. Mason LH, Harp JP, Han DY. Pb neurotoxicity: Neuropsychological effects of lead toxicity. Biomed Res. 2014;2014. https://doi.org/10.1155/2014/840547
62. Sanders T, Liu Y, Buchner V, Tchounwou PB. Neurotoxic effects and biomarkers of lead exposure: A review. Rev Environ Health. 2009;24:15-45. https://doi.org/10.1515/REVEH.2009.24.1.15
63. Ijomone OM, Gubert P, Okoh COA, Varão AM, Amaral L de O, Aluko OM, *et al.* Application of Fluorescence Microscopy and Behavioral Assays to Demonstrating Neuronal Connectomes and Neurotransmitter Systems in C. elegans. Neuromethods. 2021;172:399-426. https://doi.org/10.1007/978-1-0716-1637-6_18

64. Sun L, Wu Q, Liao K, Yu P, Cui Q, Rui Q, *et al.* Contribution of heavy metals to toxicity of coal combustion related fine particulate matter (PM2.5) in *Caenorhabditis elegans* with wild-type or susceptible genetic background. Chemosphere. 2016;144:2392-2400. https://doi.org/10.1016/j.chemosphere.2015.11.028
65. Akinyemi AJ, Miah MR, Ijomone OM, Tsatsakis A, Soares FAA, Tinkov AA, *et al.* Lead (Pb) exposure induces dopaminergic neurotoxicity in *Caenorhabditis elegans*: Involvement of the dopamine transporter. Toxicol Reports. 2019;6:833-840. https://doi.org/10.1016/j.toxrep.2019.08.001
66. Tiwari SS, Tambo F, Agarwal R. Assessment of lead toxicity on locomotion and growth in a nematode *Caenorhabditis elegans*. J Appl Nat Sci. 2020;12:36-41. https://doi.org/10.31018/jans.v12i1.2227
67. Bishak YK, Payahoo L, Osatdrahimi A, Nourazarian A. Mechanisms of cadmium carcinogenicity in the gastrointestinal tract. Asian Pacific J Cancer Prev. 2015;16:9-21. https://doi.org/10.7314/APJCP.2015.16.1.9
68. Schoeters G, Den Hond E, Zuurbier M, Naginiene R, Van Den Hazel P, Stilianakis N, *et al.* Cadmium and children: Exposure and health effects. Acta Paediatr Int J Paediatr. 2006;95:50-54. https://doi.org/10.1080/08035320600886232
69. Wang B, Du Y. Cadmium and its neurotoxic effects. Oxid Med Cell Longev. 2013;2013. https://doi.org/10.1155/2013/898034
70. Chen P, Martinez-Finley EJ, Bornhorst J, Chakraborty S, Aschner M. Metal-induced neurodegeneration in C. elegans. Front Aging Neurosci. 2013;5:1-11. https://doi.org/10.3389/fnagi.2013.00018
71. Zhu JQ, Liu Y, Zhang JH, Liu YF, Cao JQ, Huang ZT, *et al.* Cadmium exposure of female mice impairs the meiotic maturation of oocytes and subsequent embryonic development. Toxicol Sci. 2018;164:289-299. https://doi.org/10.1093/toxsci/kfy089
72. Wang S, Chu Z, Zhang K, Miao G. Cadmium-induced serotonergic neuron and reproduction damages conferred lethality in the nematode *Caenorhabditis elegans*. Chemosphere. 2018;213:11-18. https://doi.org/10.1016/j.chemosphere.2018.09.016
73. Qu Z, Guo P, Zheng S, Yu Z, Liu L, Zheng F, *et al.* Cadmium is detrimental to *Caenorhabditis elegans* via a network involving circRNA , lncRNA and phosphorylated. bioRxiv. 2022. https://doi.org/10.1101/2022.04.16.486470
74. Höss S, Schlottmann K, Traunspurger W. Toxicity of ingested cadmium to the nematode *Caenorhabditis elegans*. Environ Sci Technol. 2011;45:10219-10225. https://doi.org/10.1021/es2027136
75. Bovio F, Sciandrone B, Urani C, Fusi P, Forcella M, Regonesi ME. Superoxide dismutase 1 (SOD1) and cadmium: A three models approach to the comprehension of its neurotoxic effects. Neurotoxicology. 2021;84:125-135. https://doi.org/10.1016/j.neuro.2021.03.007
76. González-Hunt CP, Leung MCK, Bodhicharla RK, McKeever MG, Arrant AE, Margillo KM, *et al.* Exposure to mitochondrial genotoxins and dopaminergic neurodegeneration in *Caenorhabditis elegans*. PLoS One. 2014;9: 1-23. https://doi.org/10.1371/journal.pone.0114459
77. Cedergreen N. Quantifying synergy: A systematic review of mixture toxicity studies within environmental toxicology. PLoS One. 2014;9. https://doi.org/10.1371/journal.pone.0096580
78. Ajsuvakova OP, Tinkov AA, Willkommen D, Skalnaya AA, Danilov AB, Pilipovich AA, *et al.* Assessment of copper, iron, zinc and manganese status and speciation in patients with Parkinson's disease: A pilot study. J Trace Elem Med Biol. 2020;59:126423. https://doi.org/10.1016/j.jtemb.2019.126423
79. Schetinger MRC, Peres T V., Arantes LP, Carvalho F, Dressler V, Heidrich G, *et al.* Combined exposure to methylmercury and manganese during L1 larval stage causes motor dysfunction, cholinergic and monoaminergic up-regulation and oxidative stress in L4 *Caenorhabditis elegans*. Toxicology. 2019;411:154-162. https://doi.org/10.1016/j.tox.2018.10.006
80. Lu C, Svoboda KR, Lenz KA, Pattison C, Ma H. Toxicity interactions between manganese (Mn) and lead (Pb) or cadmium (Cd) in a model organism the nematode C. elegans. Environ Sci Pollut Res. 2018;25:15378-15389. https://doi.org/10.1007/s11356-018-1752-5
81. Wah Chu K, Chow KL. Synergistic toxicity of multiple heavy metals is revealed by a biological assay using a nematode and its transgenic derivative. Aquat Toxicol. 2002;61:53-64. https://doi.org/10.1016/S0166-445X(02)00017-6

82. Chen P, DeWitt MR, Bornhorst J, Soares FA, Mukhopadhyay S, Bowman AB, *et al.* Age- and manganese-dependent modulation of dopaminergic phenotypes in a C. elegans DJ-1 genetic model of Parkinson's disease. Metallomics. 2015;7:289-298. https://doi.org/10.1039/C4MT00292J
83. Goutam Mukherjee A, Ramesh Wanjari U, Eladl MA, El-Sherbiny M, Elsherbini DMA, Sukumar A, *et al.* Mixed contaminants: occurrence, interactions, toxicity, detection, and remediation. Molecules. 2022;27:2577. https://doi.org/10.3390/molecules27082577
84. Moyson S, Town RM, Vissenberg K, Blust R. The effect of metal mixture composition on toxicity to C. Elegans at individual and population levels. PLoS One. 2018;14:1-23. https://doi.org/10.1371/journal.pone.0218929
85. Pei C, Sun L, Zhao Y, Ni S, Nie Y, Wu L, *et al.* Enhanced uptake of arsenic induces increased toxicity with cadmium at non-toxic concentrations on *Caenorhabditis elegans*. Toxics. 2022;10. https://doi.org/10.3390/toxics10030133
86. Meyer D, Williams PL. Toxicity testing of neurotoxic pesticides in *Caenorhabditis elegans*. J Toxicol Environ Heal - Part B Crit Rev. 2014;17:284-306. https://doi.org/10.1080/10937404.2014.933722
87. Rand JB, Nonet ML. C. elegans II. Chapter 22 Synaptic transmission. 2nd ed. Riddle DL, Blumenthal T, Meyer BJ *et al.* (Eds.). New York: Cold Spring Harbor (NY): Cold Spring Harbor Laboratory Press; 1997.
88. Clark DG, McElligott TF, Hurst EW. The Toxicity of Paraquat. Occup Environ Med. 1966;23:126-32. https://doi.org/10.1136/oem.23.2.126
89. Dinis-Oliveira RJ, Duarte JA, Sánchez-Navarro A, Remião F, Bastos ML, Carvalho F. Paraquat poisonings: Mechanisms of lung toxicity, clinical features, and treatment. Crit Rev Toxicol. 2008;38:13-71. https://doi.org/10.1080/10408440701669959
90. Barbeau A, Dallaire L, Buu NT, Poirier J, Rucinska E. Comparative behavioral, biochemical and pigmentary effects of MPTP, MPP+ and paraquat in rana pipiens. Life Sci. 1985;37:1529-1538. https://doi.org/10.1016/0024-3205(85)90185-7
91. Heinz S, Freyberger A, Lawrenz B, Schladt L, Schmuck G, Ellinger-Ziegelbauer H. Mechanistic investigations of the mitochondrial complex I inhibitor Rotenone in the context of pharmacological and safety evaluation. Sci Rep. 2017;7:1-13. https://doi.org/10.1038/srep45465
92. Chia SJ, Tan EK, Chao YX. Historical perspective: Models of Parkinson's disease. Int J Mol Sci. 2020;21:1-14. https://doi.org/10.3390/ijms21072464
93. Henchcliffe C, Beal MF. Mitochondrial biology and oxidative stress in Parkinson disease pathogenesis. Nat Clin Pract Neurol. 2008;4:600-609. https://doi.org/10.1038/ncpneuro0924
94. Ritz BR, Manthripragada AD, Costello S, Lincoln SJ, Farrer MJ, Cockburn M, *et al.* Dopamine transporter genetic variants and pesticides in Parkinson's disease. Environ Health Perspect. 2009;117:964-969. https://doi.org/10.1289/ehp.0800277
95. Wu S, Lei L, Song Y, Liu M, Lu S, Lou D, *et al.* Mutation of hop-1 and pink-1 attenuates vulnerability of neurotoxicity in *C. elegans*: the role of mitochondria-associated membrane proteins in Parkinsonism. Exp Neurol. 2018;309:67-78. https://doi.org/10.1016/j.expneurol.2018.07.018
96. Zhou S, Wang Z, Klaunig JE. *Caenorhabditis elegans* neuron degeneration and mitochondrial suppression caused by selected environmental chemicals. Int J Biochem Mol Biol. 2013;4:191-200.
97. Mello DF, Bergemann CM, Fisher K, Chitrakar R, Bijwadia SR, Wang Y, *et al.* Rotenone modulates *Caenorhabditis elegans* immunometabolism and pathogen susceptibility. Front Immunol. 2022;13:1-14. https://doi.org/10.3389/fimmu.2022.840272
98. Bora S, Vardhan GSH, Deka N, Khataniar L, Gogoi D, Baruah A. Paraquat exposure over generation affects lifespan and reproduction through mitochondrial disruption in C. elegans. Toxicology. 2021;447:152632. https://doi.org/10.1016/j.tox.2020.152632
99. Lu M, Mishra A, Boschetti C, Lin J, Liu Y, Huang H, *et al.* Sea cucumber-derived peptides alleviate oxidative stress in neuroblastoma cells and improve survival in c. elegans exposed to neurotoxic paraquat. Oxid Med Cell Longev. 2021;2021. https://doi.org/10.1155/2021/8842926
100. Ji P, Li H, Jin Y, Peng Y, Zhao L, Wang X. C. Elegans as an in vivo model system for the phenotypic drug discovery for treating paraquat poisoning. PeerJ. 2022;10:1-16. https://doi.org/10.7717/peerj.12866

101. Okoroiwu HU, Iwara IA. Dichlorvos toxicity: A public health perspective. Interdiscip Toxicol. 2018;11:129-137. https://doi.org/10.2478/intox-2018-0009
102. Stanley J, Sah K, Jain SK, Bhatt JC, Sushil SN. Evaluation of pesticide toxicity at their field recommended doses to honeybees, Apis cerana and A. mellifera through laboratory, semi-field and field studies. Chemosphere. 2015;119:668-674. https://doi.org/10.1016/j.chemosphere.2014.07.039
103. Du L, Li S, Qi L, Hou Y, Zeng Y, Xu W, *et al.* Metabonomic analysis of the joint toxic action of long-term low-level exposure to a mixture of four organophosphate pesticides in rat plasma. Mol Biosyst. 2014;10:1153-1161. https://doi.org/10.1039/C4MB00044G
104. Lewis JA, Gehman EA, Baer CE, Jackson DA. Alterations in gene expression in *Caenorhabditis elegans* associated with organophosphate pesticide intoxication and recovery. BMC Genomics. 2013;14:1. https://doi.org/10.1186/1471-2164-14-291
105. Wani WY, Gudup S, Sunkaria A, Bal A, Singh PP, Kandimalla RJL, *et al.* Protective efficacy of mitochondrial targeted antioxidant MitoQ against dichlorvos induced oxidative stress and cell death in rat brain. Neuropharmacology. 2011;61:1193-201. https://doi.org/10.1016/j.neuropharm.2011.07.008
106. Fiore M, Mattiuzzo M, Mancuso G, Totta P, Degrassi F. The pesticide dichlorvos disrupts mitotic division by delocalizing the kinesin Kif2a from centrosomes. Environ Mol Mutagen. 2013;54:250-260. https://doi.org/10.1002/em.21769
107. Abou-Donia MB. Pesticides. In: Abou-Donia MB, editor. Neurotoxicology. Boca Raton, FL: CRC Press; 1995. p. 437-478.
108. Patil VK, David M. Behavioral and morphological endpoints: As an early response to sublethal malathion intoxication in the freshwater fish, Labeo rohita. Drug Chem Toxicol. 2010;33:160-165. https://doi.org/10.3109/01480540903196816
109. Williams PL, Dusenbery DB. A Promising Indicator of Neurobehavioral Toxicity Using the Nematode *Caenorhabditis elegans* and Computer Tracking. Toxicol Ind Health. 1990;6:425-440. https://doi.org/10.1177/074823379000600306
110. Abdel-Rahman A, Dechkovskaia AM, Goldstein LB, Bullman SH, Khan W, El-Masry EM, *et al.* Neurological deficits induced by malathion, deet, and permethrin, alone or in combination in adult rats. J Toxicol Environ Heal - Part A. 2004;67:331-356. https://doi.org/10.1080/15287390490273569
111. Kamaladevi A, Ganguli A, Balamurugan K. Lactobacillus casei stimulates phase-II detoxification system and rescues malathion-induced physiological impairments in *Caenorhabditis elegans*. Comp Biochem Physiol Part - C Toxicol Pharmacol. 2016;179:19-28. https://doi.org/10.1016/j.cbpc.2015.08.004
112. Rajini PS, Melstrom P, Williams PL. A comparative study on the relationship between various toxicological endpoints in *Caenorhabditis elegans* exposed to organophosphorus insecticides. J Toxicol Environ Health - Part A Curr Issues. 2008;71:1043-1050. https://doi.org/10.1080/15287390801989002
113. Leelaja BC, Rajini PS. Biochemical and physiological responses in *Caenorhabditis elegans* exposed to sublethal concentrations of the organophosphorus insecticide, monocrotophos. Ecotoxicol Environ Saf. 2013;94:8-13. https://doi.org/10.1016/j.ecoenv.2013.04.015
114. Olivier K, Liu J, Karanth S, Zhang H, Roane DS, Pope CN. Parathion-Induced Neurotoxicity. J Toxicol Environ Health - Part A. 2001;253-271. https://doi.org/10.1080/15287390151143659
115. Salim C, Rajini PS. Glucose feeding during development aggravates the toxicity of the organophosphorus insecticide Monocrotophos in the nematode, *Caenorhabditis elegans*. Physiol Behav. 2014;131:142-148. https://doi.org/10.1016/j.physbeh.2014.04.022
116. Ali SJ, Rajini PS. Elicitation of Dopaminergic Features of Parkinson's Disease in C. elegans by Monocrotophos, an Organophosphorous Insecticide. CNS Neurol Disord - Drug Targets. 2013;11:993-1000. https://doi.org/10.2174/18715273112110800008
117. Ali SJ, Rajini PS. Effect of monocrotophos, an organophosphorus insecticide, on the striatal dopaminergic system in a mouse model of Parkinson's disease. Toxicol Ind Health. 2016;32:1153-1165. https://doi.org/10.1177/0748233714547733
118. Paz-Trejo C, Gómez-Arroyo S. Genotoxic evaluation of common commercial pesticides in human peripheral blood lymphocytes. Toxicol Ind Health. 2017;33:938-945. https://doi.org/10.1177/0748233717736121

119. Stalikas CD, Konidari CN. Analytical methods to determine phosphonic and amino acid group-containing pesticides. J Chromatogr A. 2001;907:1-19. https://doi.org/10.1016/S0021-9673(00)01009-8
120. Schmitt F, Babylon L, Dieter F, Eckert GP. Effects of pesticides on longevity and bioenergetics in invertebrates-The impact of polyphenolic metabolites. Int J Mol Sci. 2021;22. https://doi.org/10.3390/ijms222413478
121. Meftaul IM, Venkateswarlu K, Dharmarajan R, Annamalai P, Asaduzzaman M, Parven A, *et al.* Controversies over human health and ecological impacts of glyphosate: Is it to be banned in modern agriculture? Environ Pollut. 2020;263:114372. https://doi.org/10.1016/j.envpol.2020.114372
122. Kronberg MF, Clavijo A, Moya A, Rossen A, Calvo D, Pagano E, *et al.* Glyphosate-based herbicides modulate oxidative stress response in the nematode *Caenorhabditis elegans*. Comp Biochem Physiol Part - C Toxicol Pharmacol. 2018;214:1-8. https://doi.org/10.1016/j.cbpc.2018.08.002
123. Bailey DC, Todt CE, Burchfield SL, Pressley AS, Denney RD, Snapp IB, *et al.* Chronic exposure to a glyphosate-containing pesticide leads to mitochondrial dysfunction and increased reactive oxygen species production in *Caenorhabditis elegans*. Environ Toxicol Pharmacol. 2018;57:46-52. https://doi.org/10.1016/j.etap.2017.11.005
124. Burchfield SL, Bailey DC, Todt CE, Denney RD, Negga R, Fitsanakis VA. Acute exposure to a glyphosate-containing herbicide formulation inhibits Complex II and increases hydrogen peroxide in the model organism *Caenorhabditis elegans*. Environ Toxicol Pharmacol. 2019;66:36-42. https://doi.org/10.1016/j.etap.2018.12.019
125. Costas-Ferreira C, Durán R, Faro LRF. Toxic effects of glyphosate on the nervous system: a systematic review. Int J Mol Sci. 2022;23. https://doi.org/10.3390/ijms23094605
126. Fuhrimann S, van den Brenk I, Atuhaire A, Mubeezi R, Staudacher P, Huss A, *et al.* Recent pesticide exposure affects sleep: A cross-sectional study among smallholder farmers in Uganda. Environ Int. 2022;158:106878. https://doi.org/10.1016/j.envint.2021.106878
127. Cocco P. Time for Re-Evaluating the Human Carcinogenicity of Ethylenedithiocarbamate Fungicides? A Systematic Review. Int J Environ Res Public Health. 2022;19. https://doi.org/10.3390/ijerph19052632
128. Ajiboye TO, Ajiboye TT, Marzouki R, Onwudiwe DC. The versatility in the applications of dithiocarbamates. Int J Mol Sci. 2022;23:1-36. https://doi.org/10.3390/ijms23031317
129. Hogarth G. Metal-dithiocarbamate complexes: chemistry and biological activity. Mini-Reviews Med Chem. 2012;12:1202-1215. https://doi.org/10.2174/138955712802762095
130. Caito SW, Valentine WM, Aschner M. Dopaminergic neurotoxicity of S -ethyl N,N -dipropylthiocarbamate (EPTC), molinate, and S -methyl- N,N -diethylthiocarbamate (MeDETC) in *Caenorhabditis elegans*. J Neurochem. 2013;127:837-851. https://doi.org/10.1111/jnc.12349
131. Runkle J, Flocks J, Economos J, Dunlop AL. A systematic review of Mancozeb as a reproductive and developmental hazard. Environ Int. 2017;99:29-42. https://doi.org/10.1016/j.envint.2016.11.006
132. Negga R, Rudd DA, Davis NS, Justice AN, Hatfield HE, Valente AL, *et al.* Exposure to Mn/Zn ethylene-bis-dithiocarbamate and glyphosate pesticides leads to neurodegeneration in *Caenorhabditis elegans*. Neurotoxicology. 2012;32:331-341. https://doi.org/10.1016/j.neuro.2011.02.002
133. Negga R, Stuart JA, Machen ML, Salva J, Lizek AJ, Richardson SJ, *et al.* Exposure to glyphosate-and/or Mn/Zn-ethylene-bis-dithiocarbamate-containing pesticides leads to degeneration of c-aminobutyric acid and dopamine neurons in *Caenorhabditis elegans*. Neurotox Res. 2012;21:281-290. https://doi.org/10.1007/s12640-011-9274-7
134. Nordby KC, Andersen A, Irgens LM, Kristensen P. Indicators of mancozeb exposure in relation to thyroid cancer and neural tube defects in farmers' families. Scand J Work Environ Heal. 2005;31:89-96. https://doi.org/10.5271/sjweh.855
135. Bianchi S, Nottola SA, Torge D, Palmerini MG, Necozione S, Macchiarelli G. Association between female reproductive health and mancozeb: Systematic review of experimental models. Int J Environ Res Public Health. 2020;17. https://doi.org/10.3390/ijerph17072580
136. Román GC. Autism: Transient in utero hypothyroxinemia related to maternal flavonoid ingestion during pregnancy and to other environmental antithyroid agents. J Neurol Sci. 2007;262:15-26. https://doi.org/10.1016/j.jns.2007.06.023
137. Salim C, Rajini PS. Glucose-rich diet aggravates monocrotophos-induced dopaminergic neuronal dysfunction in *Caenorhabditis elegans*. J Appl Toxicol. 2017;37:772-80. https://doi.org/10.1002/jat.3426