Journal of Environmental Management 237 (2019) 519–525

Contents lists available at ScienceDirect

Journal of Environmental Management

journal homepage: www.elsevier.com/locate/jenvman

Review

A review of toxicity induced by persistent organic pollutants (POPs) and endocrine-disrupting chemicals (EDCs) in the nematode *Caenorhabditis elegans*

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ARTICLE INFO

Keywords:
POPs
EDCs
C. elegans
Different exposures
Toxicity assessments
Toxicological mechanisms

ABSTRACT

Persistent organic pollutants (POPs) and endocrine disrupting compounds (EDCs) are almost ubiquitous in synthetic and natural sources; however these contaminants adversely impact ecosystems and humans. Owing to their potential toxicity, concerns have been raised about the effects of POPs and EDCs on ecological and human health. Therefore, toxicity evaluation and mechanisms actions of these contaminants are of great interest. The nematode *Caenorhabditis elegans* (*C. elegans*), an excellent model animal for environmental toxicology research, has been used widely for toxicity studies of POPs or EDCs from the whole-animal level to the single-cell level. In this review, we have discussed the toxicity of specific POPs or EDCs after acute, chronic, and multigenerational exposure in *C. elegans*. We have also introduced a discussion of the toxicological mechanisms of these compounds in *C. elegans*, with respect to oxidative stress, cell apoptosis, and the insulin/IGF-1 signaling pathway. Finally, we raised considered the perspectives and challenges of the toxicity assessments, multigenerational toxicity, and toxicological mechanisms.

1. Introduction

The rapid pace of industrialization has increased the severity of the global problem of environmental pollution. Among the various types of pollutants, persistent organic pollutants (POPs) and endocrine disrupting compounds (EDCs) are almost ubiquitous in synthetic or natural compounds. POPs are organic compounds characterized by long-range transport, persistence, bioaccumulation and high toxicity, whereas EDCs are a diverse group of organic substances that influenced the processes and functions of the endocrine system (Esplugas et al., 2007; Lohmann et al., 2007). As a result of their widespread use and their physical and chemical properties, many species are exposed to POPs and EDCs at various concentration in the environment (El-Shahawi et al., 2010; Palioura and Diamanti-Kandarakis, 2015). It is suggested that POPs and EDCs adversely impact ecosystems and humans, and that their toxicities may transfer from the parental generation to the subsequent generations (El-Shahawi et al., 2010; Fuhrman et al., 2015). In the future, large amounts of POPs and EDCs will be released to the environment; as such, the risk of POPs and EDCs to human health must be thoroughly considered. However, potential environmental and health risks associated with the POPs and EDCs still require investigation, particularly a consideration of the possible chronic and multigenerational toxicities, and their associated mechanisms, at environmental relevant concentrations.

The nematode *C. elegans*, a free-living nematode, is abundant in soil ecosystems (Brenner, 1974). Owing to its translucent body, genetic manipulability, ease of cultivation, short life cycle, well-characterized genome and sensitivity to toxicants, *C. elegans* has been established as an excellent model animal for environmental toxicology research (Leung et al., 2008). Furthermore, *C. elegans* has been used successfully in environmental and toxicological studies from the whole-animal level to the single-cell level (Das and Kumar, 2016). The assessment

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https://doi.org/10.1016/j.jenvman.2019.02.102
Received 27 September 2018; Received in revised form 9 February 2019; Accepted 21 February 2019

endpoints for *C. elegans* used to evaluate the toxicity of environmental toxicants include development, locomotion behaviors, reproduction, reactive oxygen species (ROS) production, degree of cell apoptosis, and stress response (Zhang et al., 2011; Zhou et al., 2016c). Recently, *C. elegans* has also been used for toxicity assessments of POPs and EDCs, such as hexabromocyclododecane (HBCD), endosulfan, perfluorooctane sulfonate (PFOS), bisphenol A (BPA), phthalates, triclosan (TCS), triclocarban (TCC), and 4-nonylphenol (NP).

In this review, we aim to summarize studies that have evaluated the toxicity and mechanisms of these contaminants using *C. elegans* as the model animal. As *C. elegans* is used as non-mammalian alternative model, the toxicological studies in this species will provide important contributions to the understanding of the toxicity evaluation and toxicological mechanisms of POPs and EDCs in mammals and humans. The related studies will allow further comprehension of the potential risks and mechanism of POPs and EDCs to ecological and human health.

2. Toxicity evaluation of POPs and EDCs

C. elegans has been used as a model system to assess the toxicity of POPs and EDCs such as HBCD, endosulfan, PFOS, BPA, phthalates, and NP. These toxicity studies have investigated various exposure durations, including acute, subacute, chronic, and multigenerational exposure. Different exposure durations resulted in different toxic effects of POPs and EDCs in nematodes. The toxicity evaluation of POPs and EDCs in *C. elegans* after different exposure durations are reported in Table 1.

2.1. Acute or subacute toxicity of POPs and EDCs in nematodes

2.1.1. Effects of exposure to POPs and EDCs on physiology

Many different assay systems with different exposure durations have been used for the acute or subacute toxicity evaluation of POPs or EDCs in nematodes. The durations of exposure were commonly 24 h or 72 h; L1-larvae, L4-larvae or young adults were chosen for these assays (Guo et al., 2016; Lenz et al., 2017; Roh et al., 2007; Tan et al., 2015; Tseng et al., 2013; Wang et al., 2017; Watanabe et al., 2005; Zhou et al., 2016c). For example, two systems (acute exposure of L4 larvae for 24 h and subacute exposure of L1 larvae for 72 h) were performed to evaluate the adverse effects in nematodes exposed to low concentrations of BPA (Zhou et al., 2016c). Recently, the exposure of young adults for 24 h was explored to assess the toxicological effects of TCS and TCC (Lenz et al., 2017). In addition, short exposure durations (4 h, 6 h, and 8 h) were also used for toxicity assessments in *C. elegans*; with assay systems for the assessment of acute toxicity often using the endpoints of reproduction and gene expression. For example, shorter exposures were used to assess the toxicity of endosulfan and NP with reproduction as the endpoint (Du et al., 2015b; Hoss et al., 2002), and the toxicity of endosulfan was also evaluated using gene expression in *C. elegans* (Wang et al., 2017).

For acute or subacute exposure, the common endpoints were lethality, development, reproduction and locomotion behaviours, and these endpoints were used to evaluate the toxicity of specific contaminants. For example, acute exposure to DEHP at high concentrations induced lethality, and the 24-h LC_{50} of DEHP in *C. elegans* was 22.55 mg/L (Roh et al., 2007). BPA exposure significantly decreased body length, fecundity, and population size in nematodes (Tan et al., 2015). In another study, acute exposure to DEHP, DBP, and DIBP distinctly altered body bending, head thrashing, and reversal frequency in *C. elegans* (Tseng et al., 2013). The endpoints of locomotion behaviours were usually more sensitive than other endpoints. For example, after exposure to 0.01 μM BPA, the locomotion behaviours were significantly decreased, whereas a significant influence on the growth, reproduction, and lifespan were not observed in nematodes (Zhou et al., 2016c).

2.1.2. Effects of exposure to POPs and EDCs on gene expression

The transcription of genes examined by quantitative real-time polymerase chain reaction (qRT-PCR) is not only sensitive to toxic effects but has also provided insight into the underlying mechanisms of the effects. It has been reported that the expression of 16 potential genes related to the stress response were altered in nematodes exposed to low concentrations of BPA, including heat shock proteins (*hsp-16.1*, *hsp-16.2*, and *hsp-16.48*), vitellogenin (*vit-2* and *vit-6*), xenobiotic metabolism enzyme (*cyp35a2*), superoxide dismutases (*sod-1* and *sod-3*), catalase 2 (*ctl-2*), abnormal dauer formation proteins (*daf-12* and *daf-21*), aging alteration protein (*age-1*), tumor suppressor and apoptosis proteins (*cep-1* and *ape-1*), as well as metallothioneins (*mtl-1* and *mtl-2*), which represent oxidative stress or stress responses induced by BPA exposure (Zhou et al., 2016c). It has been indicated that acute exposure to BPA also significantly decreased the gene expression of *gcs-1* (which encodes heavy chain of gamma-glutamylcysteine synthetase) and *gst-4* (which encodes glutathione S-transferase), but significantly increased the gene expression of *sod-3* in nematodes. These data suggested that BPA exposure induced cytosolic oxidative stress and reduced antioxidant ability in *C. elegans* (Tan et al., 2015). Consistently, the gene expression of *cyp-35a2* and *gst-4* were also increased in response to di(2-ethylhexyl)phthalate (DEHP) (Roh et al., 2007). In addition, acute exposure to 2 ppm DEHP caused a significant decrease in the expression of *TTX-1*, *TAX-2*, *TAX-4*, and *CEH-14* required for the differentiation and function of AFD neurons, which indicated that DEHP might induce adverse effects through an influence on the expression of these genes in nematodes (Tseng et al., 2013).

Table 1
The toxicity evaluation of POPs and EDCs in *C. elegans* after different exposure durations.

Classes	Contaminants	Exposure duration	Endpoints assessed	Reference
POPs	Endosulfan	Acute exposure	Life span; Reproduction; Cell apoptosis; Gene expression; Mutations	Du et al. (2015b)
	PFOS	Subacute exposure	Growth; Life span; Reproduction; ROS; DNA damage; Mitotic germ cells; Cell apoptosis; Transgenic strains	(Guo et al., 2016) (Wang et al., 2017)
	PFOS	Chronic exposure	Lifespan; Transgenic strains	Xu et al. (2016)
	HBCD	Chronic exposure	Growth; Locomotion behaviors; ROS; Lipofuscin accumulation; Cell apoptosis; Genes expressions	Wang et al. (2018)
	Lindane	Multigenerational exposure	Fat storages; Enzyme; Level of insulin; Gene expression	Chen et al. (2018)
EDCs	Phthalate	Acute exposure	Mortality; Growth; Reproduction; ROS; AFD neurons; Gene expression;	(Roh et al., 2007) (Tseng et al., 2013)
	TCS; TCC	Acute exposure	Mortality; Reproduction; Life span; Transgenic strains	Lenz et al. (2017)
	BPA	Acute or subacute exposure	Growth; Reproduction; Feeding behavior; Lifespan; Cell apoptosis; Lipofuscin accumulation; Lipid peroxidation; Gene expression; Transgenic strains; Mutations	Kohra et al. (2002); Watanabe et al. (2005); Tan et al. (2015); Zhou et al. (2016c)
	NP	Acute or subacute exposure	Growth; Reproduction; Feeding behavior	(Hoss et al., 2002) (Kohra et al., 2002)
	BPA	Chronic exposure	Growth; Locomotion behaviors; Lifespan; Lipofuscin accumulation; Genes expressions	Zhou et al. (2016a)
	NP	Chronic exposure	Locomotion behaviors; ROS; Transgenic strains; Genes expressions	Cao et al. (2019)
	BPA	Multigenerational exposure	Growth; Reproduction; Locomotion behaviors; Lifespan; Genes expressions	Zhou et al. (2016b)

2.1.3. Effects of exposure to POPs and EDCs on transgenic strains

Transgenic strains with specific transgene expression may also provide more specific information about the toxicity mechanisms from a molecular perspective. The HUS-1:GFP foci, used to investigate whether the DNA damage response machinery, was significantly increased in nematodes exposed to PFOS, which stimulated cellular DNA damage in the reproductive system of nematodes (Guo et al., 2016). Similarly, the accumulation of HUS-1:GFP foci was observed after exposure to endosulfan in nematodes (Du et al., 2015b). In addition, *hsp*, *gcs-1*, and *gst* act to protect cells from oxidative against environmental stimuli, and some transgenic strains (e.g., HSP-16.2:GFP, DAF-16:GFP, GCS-1:GFP, and GST-4:GFP) were occasionally used to assess the stress response in nematodes. Tan et al. reported that the expression of HSP-4:GFP, GCS-1:GFP, and GST-4:GFP significantly decreased by BPA exposure, whereas the expression of HSP-16.2:GFP increased dose-dependently in *C. elegans* (Tan et al., 2015). Similarly, previous studies indicated that the expression of hsp-16.2:GFP, and hsp-16.48:GFP, used to reflect stress responses, was reduced by DEHP exposure (Roh et al., 2007). It has also been reported that the relocalization of DAF-16:GFP from the cytoplasm to the nucleus was observed in nematodes exposed to TCS and TCC, which implied that both compounds induced oxidative stress in *C. elegans*. Moreover, germline toxicity was also observed using a xol-1:GFP transgenic strain, which suggested that TCS and TCC induced systemic toxic effects (Lenz et al., 2017).

2.1.4. Effects of exposure to POPs and EDCs on mutations

The toxicity from POPs and EDCs not only depends on the duration of exposure, but also depends on the genetic composition of *C. elegans*. A number of techniques allow the assignment of mutations to particular genes within the completely sequenced genome, the introduction of altered genes into the organism, and, finally, to monitor the activity of the tagged genes. Mutant strains enable investigators to identify the role of specific molecular targets based on gene expression analysis, and provide a variety of options to manipulate and study *C. elegans* at the molecular level (Menzel et al., 2007). Mutants of *C. elegans* are used to expose to toxicant and are screened for increased sensitivity or resistance. When a sensitivity or resistant mutant is identified, the mutant will be screened using two-point and three-point mapping (Leung et al., 2008). For example, the mutants with *bis-1(nx3)*, *dpy-2(e8)*, *dpy-7(e88)*, and *dpy-10(e128)* were sensitive to BPA, and the *bis-1(nx3)* mutant was hypersensitive to BPA (Watanabe et al., 2005). In addition, the mutants of *pre-1*, *pre-7*, and *pre-33* were resistant to the synergistic effects of oxygen in proportion to their resistance to phosphine, and the mutant of *pre-33* was showed complete resistance to the synergism (Cheng et al., 2003). Similarly, mutants of *bre-2, bre-3, bre-4,* and *bre-5* were resistant to crystal protein intoxication compared to wild-type nematodes (Griffitts et al., 2003).

Screening of the mutants with specific transgenes may provide more information about toxicity mechanisms in nematodes. For example, a previous study showed that subacute exposure to PFOS at a concentration of 0.25 μM significantly increased the number of germ cell corpses in wild-type nematodes. However, a significant increase in germ cell death was not observed in the *ced-4* (*n1162*) and *ced-3* (*n717*) mutants, which suggested that the core apoptotic pathway was required for germ cell death induced by PFOS (Guo et al., 2016). Similarly, a significant increase in germ cell apoptosis was observed in the *mev-1*(*kn-1*) mutant exposed to endosulfan, whereas the levels of apoptosis were decreased in mutants of *cep-1*(*w40*), *egl-1*(*n487*), and *hus-1*(*op241*) (Du et al., 2015b). Another study also reported that acute exposure to endosulfan significantly increased germ cell apoptosis in *mev-1*(*kn-1*) mutant compared with N2-wild nematodes, whereas levels of apoptosis were decreased in the mutants of *jnk*-1/JNK-*MAPK*, *sek-1/MAP2K*, and *pmk-1/p38-MAPK* (Wang et al., 2017).

2.2. Chronic toxicity of POPs and EDCs in nematodes

2.2.1. Effects of POPs and EDCs exposure on physiology

Chronic exposure to POPs and EDCs causes more severe toxicity than acute or subacute exposure in nematodes, and the toxicological study of toxicants employing chronic exposures invertebrates has been recommended (Gikas et al., 2009). The chronic toxicity assay system exposed the nematodes to xenobiotics, and the exposures were performed for 10 days in an incubator at 20 °C in the presence of a food source. To prevent the production of offspring, 5-fluoro-2′-deoxyuridine (5-FUDR), an inhibitor of DNA synthesis, was added to the test plates at a final concentration of 25 mM (Shen et al., 2009). Multiple endpoints, including lifespan, growth, locomotion behaviors, ROS, and lipofuscin accumulation, were tested after chronic exposure of *C. elegans* to test compounds for 10 days. For example, chronic exposure to PFOS at concentrations of 0.2–200 μM reduced lifespan in nematodes (Xu et al., 2016). Another study also suggested that the exposure to BPA at concentrations of 0.1 μM significantly altered the growth, locomotion behaviors and lifespan, but did not have a significant effect on lipofuscin accumulation in nematodes (Zhou et al., 2016a). Chronic exposure to HBCD at concentrations above 20 nM significantly altered ROS formation, lipofuscin accumulation, and cell apoptosis of nematodes compared with control (Wang et al., 2018). Recently, a significant reduction in locomotion behaviors was observed in nematodes exposed to 10 μg/L NP (Cao et al., 2019).

2.2.2. Effects of POPs and EDCs exposure on gene expressions

Gene expression was also used to test the effect of chronic exposure of *C. elegans* to the test compounds for 10 days, and it is thought that related research will be helpful for our understanding of the toxic effects and mechanisms of these contaminants at a molecular level. Previous studies revealed that chronic exposure to BPA led to increases in the mRNA levels of many genes such as *hsp-16.1*, *hsp-16.2*, *sod-1*, *sod-3*, *ctl-2*, and *cep-1*, which suggested that BPA exposure induced an obvious stress response in nematodes (Zhou et al., 2016a). It has also been consistently reported that chronic exposure to 200 nM HBCD significantly increased the expression of stress-related genes (e.g., *hsp-16.2*, *hsp-16.48*, *sod-1*, *sod-3,* and *cep-1*). Among these genes, the gene expressions of *sod-1, sod-3,* and *cep-1* were significantly correlated with HBCD-induced physiological effects by the Pearson correlation test, indicating that *sod-1, sod-3,* and *cep-1* played important roles in HBCD-induced toxicity in nematodes. (Wang et al., 2018).

2.2.3. Effects of POPs and EDCs exposure on mutations

In a previous report, chronic exposure to PFOS significantly decreased the average lifespan in the *daf-2(e1370)* and *daf-16b(KO)* mutants compared with wild-type nematodes, whereas chronic exposure to PFOS did not decrease the average lifespan in *daf-16(mu86)* and *age-1(hx546)* mutants, which suggested these genes were involved in the longevity and aging process in nematodes (Xu et al., 2016). It has been also reported that the effects of mutations of *sod-1 (tm776), sod-3 (tm760),* and *cep-1 (gk138)* were performed to evaluate HBCD-induced toxicity with chronic exposure. The results demonstrated that the mutations of *sod-3* and *cep-1* induced more severe toxicity compared to wild-type nematodes, which indicated that *sod-3* and *cep*-1 might function to protect nematodes against HBCD-induced toxicity (Wang et al., 2018).

2.3. Multigenerational toxicity of POPs and EDCs in nematodes

Most studies mainly focused on the observed toxic effects of xenobiotics within a generation, whereas the multigenerational toxicity of xenobiotics remains largely unknown. Recently, some previous reports indicated that multigenerational effects induced by other toxicants have been investigated in nematodes owing to the ease of cultivation, short life cycle, and sensitivity to toxicants (Moon et al., 2017; Schultz et al.,

2016; Yang et al., 2016; Yu and Liao, 2016; Yu et al., 2016). Endpoints, including development, locomotion behaviors, reproduction, and stress response, were used for the study of multigenerational exposure. A previous study indicated that after exposure to low-concentration BPA for four generations, a variety of physiological effects varied in magnitude and direction. At the biochemical level, 16 genes potentially related to stress response were significantly changed in both G1 and G4 generations, and gene expression in the G1 generation was more obviously increased than the G4 generation, which indicated that the decreased extent of stress response may have arisen from an evolutionary response or adaptive process (Zhou et al., 2016b). In another study, *C. elegans* was exposed to lindane at a concentration of 1.0 ng/L for four consecutive generations (F0 to F3), indirectly exposed nematodes (T1 and T1′) and un-exposed nematodes (T3 and T3′). The data showed that exposure to lindane stimulated fat storage in all generations, but induced different effects among generations. Lindane influenced insulin levels and the gene expression of *daf-2*, *sgk-1,* and *daf-16* in F0 and F3, and altered insulin and gene expression of *daf-2*, *akt-1,* and *daf-16* in T1 and T3. The differences between *akt-1* (T1 and T3) and *sgk-1* (F0, F3, T1, and T3′) suggested different response strategies in nematodes (Chen et al., 2018).

3. Toxicity mechanisms of POPs and EDCs in nematodes

A series of studies has been performed to elucidate the toxicity mechanisms of various POPs and EDCs using *C. elegans* a model system, and oxidative stress, cell apoptosis, and insulin/IGF-1 signaling pathway usually involved in the toxicity of POPs and EDCs exposure in *C. elegans*. The summary of the toxicity mechanisms of POPs and EDCs in *C. elegans* are reported in Table 2.

3.1. Toxicological mechanisms of oxidative stress

Oxidative stress is well recognized as the imbalance between cellular oxidant species production and antioxidant capability, and ROS plays a critical role in oxidative stress (Mates et al., 2008). ROS production, expressed as relative fluorescent units (RFU) by ImageJ software, is usually examined by the use of a molecular probe (1 μM CM-H_2DCFDA) (Rui et al., 2013). When ROS generation increases, animals are said to be under oxidative stress. As exposure to POPs and EDCs can trigger oxidative stress in nematodes; oxidative stress may directly or indirectly be a potential toxic mechanism that ultimately induces toxicity.

ROS production induced by POPs and EDCs is one of the most common phenomena that leads to oxidative stress in nematodes. For example, exposure to HBCD and BPA significantly increased intracellular ROS production in nematodes, which suggested that oxidative stress played an important role in the induction of adverse effects (Tan et al., 2015; Wang et al., 2018). Another study also reported that the ROS production was significantly higher in nematodes exposed to 0.25–25.0 μM PFOS than in the untreated populations. In addition, PFOS exposure caused the accumulation of HUS-1:GFP, which relegalized and form distinct foci in response to DNA damage. These data indicated that oxidative stress induced by ROS played a critical role in the impairment of gonadal development toxicity (Guo et al., 2016). Recently, ROS production was markedly increased by exposure to NP, and the accumulation of ROS in the head of nematodes was significantly elevated with the increase of NP at concentrations from 10 to 200 μg/L. These results suggested that NP could induce neurotoxicity through oxidative stress in nematodes (Cao et al., 2019), which were consistent with the findings in DEHP-exposed nematodes (Tseng et al., 2013).

Antioxidant treatments offer an important molecular basis for the roles of oxidative stress in the toxicity of environmental toxicants. The antioxidants N-acetyl-L-cysteine (NAC) or ascorbate were used to inhibit the formation of oxidative stress, and nematodes exposed to xenobiotics were sometimes treated with 5 mM NAC or 10 mM ascorbate for 24 h, as described previously (Huang and Lemire, 2009). Treatment with the antioxidant of ascorbic acid significantly suppressed ROS production, ameliorated behavioral defects, and protected AFD neurons from DEHP exposure in nematodes. The results further suggested that oxidative stress played a critical role in DEHP-induced neurotoxicity (Tseng et al., 2013). Similarly, treatment with the antioxidants of ascorbate and NAC also suppressed the adverse physiological effects induced by HBCD (Wang et al., 2018). Recently, the ROS production induced by NP was found to be restored, to some extent, as shown in antioxidant experiments (Cao et al., 2019).

Studies have demonstrated that ROS production influenced the gene expression required for the control of oxidative stress. For example, chronic exposure to HBCD significantly increased ROS production and the expression of stress-related genes (e.g., *hsp-16.2*, *hsp-16.48*, *sod-1,* and *sod-3*) (Wang et al., 2018). Moreover, the expression pattern of *hsp-16.1* and *hsp-16.2* was increased in nematodes exposed to DEHP, representing the stress response induced by BPA exposure (Roh et al., 2007). Similarly, subacute exposure to BPA caused a significant increase in expression of some oxidative stress-related genes, including *hsp-16.1*, *hsp-16.2*, *hsp-16.48*, *cyp35a2*, *sod-1*, *sod-3*, and *ctl-2* (Zhou et al., 2016c). The decrease in GST-4 and GCS-1 expression involved a

Table 2
Toxicity mechanisms of POPs and EDCs in *C. elegans*.

Contaminants	Findings	Mechanisms	Reference
DEHP	↓*hsp-16.1* and *hsp-16.2* gene; ↑cyp 35a2 and *gst-4* gene; ↑Phase I and phase II of xenobiotic metabolism enzymes	Oxidative stress	Roh et al. (2007)
DEHP, DBP, and DIBP	↑ROS; ↓ROS pretreatment with antioxidant ascorbic acid	Oxidative stress	Tseng et al. (2013)
BPA	↑ lipofuscin accumulation; ↑lipid peroxide; ↑ROS; ↓expression of HSP-4, HSP-6 HSP-70 GCS-1, and GST-4; ↑ expression of HSP-16.2 and SOD-3	Oxidative stress	Tan et al. (2015)
NP	↑ROS; ↓ROS treatment with antioxidant; ↑*sod-1*, *sod-3*, *ctl-2*, *ctl-3* and *cyp-35a2* genes; ↑ROS in mutation of *sod-3*	Oxidative stress	Cao et al. (2019)
PFOS	↑ROS; ↑Cell apoptosis; ↑Distinct foci of HUS-1:GFP;	Oxidative stress; Cell apoptosis	Guo et al. (2016)
HBCD	↑ROS; ↑Lipofuscin accumulation; ↑Cell apoptosis; ↑*hsp-16.2*, *hsp-16.48*, *sod-1*, *sod-3*, and *cep-1* genes	Oxidative stress; Cell apoptosis	Wang et al. (2018)
BPA	↑Cell apoptosis; ↑Numbers of germ line cell apoptosis; ↑*cep-1* gene	Cell apoptosis	Zhou et al. (2016a); Zhou et al. (2016c)
Endosulfan	↑Germ cell apoptosis; ↑germ cell apoptosis in *mev-1(kn-1)* mutant; ↓Apoptotic in mutants of *cep-1(w40)*, *egl-1(n487)*, and *hus-1(op241)*; ↑HUS-1::GFP foci	Cell apoptosis	Du et al. (2015a); Du et al. (2015b); Wang et al. (2017)
PFOS	↓Average lifespan in *daf-2(e1370)* and *daf-16b(KO)* mutants	Insulin/IGF-1 Signaling pathway	Xu et al. (2016)
Lindane	↓Level of insulin; ↓*daf-2*, *sgk-1*, *akt-1* and *daf-16* genes	Insulin/IGF-1 Signaling pathway	Chen et al. (2018)

reduction antioxidant ability, and the increased expression of *sod*-3 might be caused by an elevation in ROS production caused by BPA exposure (Tan et al., 2015). Similarly, the expression of stress-related genes, including *sod-1*, *sod-3*, *ctl-2*, *ctl-3,* and *cyp-35A2* gene, was significantly increased in nematodes exposed to 200 mg/L NP (Cao et al., 2019).

3.2. Toxicological mechanisms of cell apoptosis

Apoptosis is defined as a distinctive mode of programmed cell death that involve the genetically determined elimination of cells. When cells are unable to repair an injury, apoptosis occurs as a defense mechanism (Kim and Ryu, 2013). Cell apoptosis is an active gene-regulated process in response to environmental changes, and premature apoptosis can induce adverse effects on physiology (Vaux and Korsmeyer, 1999). Cell apoptosis is usually investigated by acridine orange staining and expressed as relative fluorescent units (RFU) by ImageJ software (Wang et al., 2007). Previous studies have revealed that cell apoptosis is considered to be one of the major toxicity mechanisms of POPs and EDCs in nematodes.

It is well established that oxidative stress induced by ROS production can result in cell apoptosis. A previous study have indicated PFOS exposure induce mitotic cell cycle arrest and apoptosis in the germ line, accompanied by an increase in the distinct foci of HUS-1:GFP and in ROS. These results suggested that oxidative stress induced by ROS production might play an important role in transient mitotic cell cycle arrest and apoptosis (Guo et al., 2016). Similarly, ROS-induced oxidative stress also resulted in cell apoptosis in nematodes exposed to HBCD (Wang et al., 2018).

Exposure to POPs and EDCs altered the gene expression required for the control of cell apoptosis in nematodes. A previous study suggested that the gene expression of *cep-1* regulated the multiple stress responses and mediated apoptosis and was obviously associated with adverse effects at the physiological and molecular levels. The result indicated that *cep-1* plays an important role in BPA-induced toxicity in chronically exposed *C. elegans* (Zhou et al., 2016a). Similarly, *cep-1* expression was also significantly correlated with the physiological effects induced by HBCD, and more severe toxicity was induced in *cep-1* mutants than in wild-type nematodes. Therefore, HBCD exposure induced cell apoptosis by oxidative stress, and *cep*-1 has an important role in the protection of nematodes against toxicity (Wang et al., 2018).

The mutants may provide more information to identify signaling pathways that regulate toxicity in organisms. For example, endosulfan exposure significantly influenced lifespan and germ cell apoptosis, which indicated that endosulfan induced germ cell apoptosis in nematodes. In addition, apoptotic effects were blocked in *cep-1*(*w40*), *egl-1*(*n487*), and *hus-1*(*op241*) mutants, which implied that conserved genotoxic response genes played a key role in the germ cell apoptosis induced by endosulfan (Du et al., 2015b). Endosulfan dramatically influenced the level of germ cell apoptosis and germ cell cycle arrest in nematodes, and hus-1 specifically altered the fecundity, hatchability, and sexual ratio through *hus-1*, *egl-1*, and *cep-1* mutants (Du et al., 2015a). It has also been consistently reported that endosulfan exposure significantly increased germ cell apoptosis in mutants of *mev-1*(*kn-1*), and that cell apoptosis was also blocked in *jnk*-1/JNK-*MAPK*, *sek-1/MAP2K*, and *pmk-1/p38-MAPK* mutants. The results indicated that these genes played an important role in germ cell apoptosis and regulate mitochondrial function, JNK and p38 MAPK cascades (Wang et al., 2017).

3.3. Toxicological mechanisms of insulin/IGF-1 signaling pathway

The insulin/IGF-1 signaling (IIS) pathway is evolutionarily conserved, and connects nutrient levels to metabolism, development, longevity, and behavior in *C. elegans* (Taniguchi et al., 2006). The insulin-like peptide ligands are involved in the regulation of the IIS pathway through binding to the insulin/IGF-1 transmembrane receptor (IGFR) ortholog *daf-2*. Further, the ligands that bind to DAF-2/InR activate several kinases, including AGE-1/PI3K, 3-phosphoinositide-dependent kinase 1 (PDK)-1, AKT-1/2, and serine/threonine-protein kinase (SGK)-1 (Lapierre and Hansen, 2012). Finally, the DAF-16/FOXO transcription factor DAF-16 is inactivated by AKT and SGK-1, which prevent its translocation to the nucleus (Wolff and Dillin, 2006). Therefore, DAF-16/FOXO induces the expression of genes related to the promotion of resistance to various stresses (Lapierre and Hansen, 2012).

It has been reported that lifespan was influenced by the IIS pathway, especially the expressions of *daf-16*, *daf-2,* or *age-1*. Chronic exposure to PFOS significantly decreased the average lifespan in the *daf-2(e1370)* and *daf-16b(KO)* mutants. These data demonstrated that PFOS exposure accelerated the aging process in *C. elegans*, and that the IIS pathway was involved in the PFOS-induced reduced lifespan of *C. elegans* (Xu et al., 2016). Similarly, exposure to 1.0 ng/L lindane inhibited the levels of insulin in different generations, which was consistent with the gene expressions of *daf-2*, *akt-1*, *sgk-1,* and *daf-16* in *C. elegans*. These results suggested that effects of lindane were also involved in an insulin-like signaling pathway (Chen et al., 2018).

However, the numbers of germ cell corpse mutants in the *age-1(hx546)*/PI3K, *daf-2(e1370)*/IGFR, and *daf-16(mu86)*/FOXO were not significantly different from that of N2 nematodes, which indicated that the IIS pathway was not related to the regulation of apoptosis induced by endosulfan in *C. elegans* (Wang et al., 2017). Similarly, exposure to TCS or TCC led to a reduced lifespan, together with the activation of DAF-16/FOXO, which appears to contradict what has been described for the insulin/IGF-1 pathway (Lenz et al., 2017).

4. Perspectives and challenges

It is anticipated that large amounts of POPs and EDCs will be released to the environment in the future, and ecological and human health risk of POPs and EDCs should be paid much more attentions. Based on the studies of toxicity assessments and toxicity mechanisms of POPs and EDCs in nematodes, we identified four challenges for future studies in this field. The challenges and perspectives of POPs and EDCs for future studies are reported in Table 3.

Table 3
The challenges and perspectives of POPs and EDCs for future studies.

NO.	Problem	Challenges and Perspectives
1. Exposure of nematodes to other POPs and EDCs	The currently available toxicity of POPs and EDCs have mainly focused on aquatic and terrestrial organisms.	The toxicity of others POPs and EDCs are assessed using *C. elegans*
2. Toxicity studies of POPs and EDCs in the real-world environment	High doses of POPs and EDCs obscured the effects induced by the low concentrations found in the environment.	The toxicity of POPs and EDCs are performed under real-world circumstances
3. Multigenerational toxicity of POPs and EDCs	The toxicity evaluation of POPs and EDCs has mainly focused on a single generation.	Multigenerational effects of POPs and EDCs are investigated using *C. elegans.*
4. Elucidation of the genomic basis of toxicity of POPs and EDCs	Most results describe the underlying molecular mechanism based on the alteration of gene expression.	Genomic assays will identify more of the underlying mechanisms involved in toxicity.

4.1. Exposure of nematodes to other POPs and EDCs

POPs comprise 23 chemicals (e.g., endrin, chlordane, dieldrin, heptachlor, mirex, and toxaphene) that have been included in the Stockholm Convention for consideration of global elimination (www.pops.int). EDCs comprise a long list of products in use, such as PCBs, dioxins, phthalates. The POPs and EDCs are almost ubiquitous in synthetic or natural sources, and these contaminants are known to adversely impact ecosystems and humans. Owing to their potential toxicity, concerns have been raised about the effect of POPs and EDCs on ecological and human health. The currently available toxicity evaluations of POPs and EDCs have mainly focused on aquatic and terrestrial organisms. The evaluation of the toxicity of POPs and EDCs to soil nematodes is limited. Currently, *C. elegans* has only been used for studies on the toxicology of POPs and EDCs such as BPA, HBCD, endosulfan, PFOS, phthalates, TCS, TCC, and NP. Thus, it is necessary to assess the toxicity of others POPs and EDCs using *C. elegans* in the future, because of the unique features of *C. elegans* in toxicology research.

4.2. Toxicity studies of POPs and EDCs in the real-world environment

Experiments performed under conditions that can mimic real conditions, including exposure, dose, and environmentally relevant concentrations, for most POPs and EDCs are urgently required. Recent studies have demonstrated that *C. elegans* could be successfully used for toxicity evaluation and underlying mechanisms of BPA, HBCD, and PFOS at the environmentally relevant concentrations. These studies avoided the use of high concentrations of POPs and EDCs for assessment of the toxicity and mechanisms as they may have obscured the effects induced by the low concentrations found in the environment. In addition, combined exposure to POPs and EDCs at predicted environmentally relevant concentrations should be further investigated in nematodes, because the presence of other chemicals might influence the toxicity evaluation or mechanisms of specific POPs and EDCs under real-world circumstances.

4.3. Multigenerational toxicity of POPs and EDCs

The toxicity evaluation of POPs and EDCs has mainly focused on a single generation; multigenerational studies are largely unavailable because of the low throughput of in vivo experiments. A series of studies has investigated the multigenerational effects of some other toxicants in nematodes. For example, subacute exposure to traffic-related fine particulate matter ($PM_{2.5}$) altered the reproduction and locomotion behaviors of the next generation of nematodes (Zhao et al., 2014). Further, exposure of the F0 generation to CMC-nZVI decreased brood size in the subsequent generation of nematodes (Yang et al., 2016). Recently, maternal exposure (F0) to arsenite caused reproductive toxicity in subsequent generations of *C. elegans* (Yu and Liao, 2016). The impairment of germ cells caused by the maternal exposure to gold nanoparticles (F0 generation) might transfer to subsequent generation and induced multigenerational effects (Kim et al., 2013). In addition, multigenerational exposure of organisms to POPs or EDCs are occasionally performed in natural ecosystems, and multigenerational research could identify acclimation, cumulative damage or adaptive responses. Therefore, it is essential to consider multigenerational testing to highlight the biological impacts over multiple generations.

4.4. Elucidation of the genomic basis of toxicity of POPs and EDCs

Several mechanisms have been proposed to explain the toxicity of POPs and EDCs to *C. elegans,* as discussed above. In particular, the mechanisms of oxidative stress, cell apoptosis, and the IIS pathway may be involved in the regulation of the formation of toxicity. However, as most results describe the underlying molecular mechanism based on the alteration of gene expression, further investigated is required. Genomics methods provide suitable technologies to analyze complex situations and obtain information on gene expression and individual genotypes (Meyer and Gut, 2002). Thus, it is recommended that systematic genomics assays should be performed to investigate the underlying mechanism. Genomic assays will identify more of the underlying mechanisms involved in toxicity. Furthermore, they may become a basis for studies of other POPs and EDCs to identify signaling pathways.

5. Conclusion

This review aimed to summarize the evaluation of the toxicity and mechanisms of action of POPs and EDCs that have used *C. elegans* as the model animal. It is anticipated that, in future, a large amount of POPs and EDCs will be released to the environment, and the potential human exposure to these two types of organic pollutants would be increased in the future. Therefore, there is an urgent need for further evaluation and exploration of the underlying mechanisms of POPs and EDCs in *C. elegans*, as it has been established as an excellent animal model for studies.

Acknowledgements

This work was supported jointly by the National Key Research and Development Plan (2016YFC0206200), the National Natural Science Foundation of China (41877377), the Program of Shanghai Academic/Technology Research Leader (18XD1424100), and the Open Foundation of State Key Laboratory of Environmental Criteria and Risk Assessment Chinese Research Academy of Environmental Sciences (SKLECRA2016OFP19). We also would like to thank the anonymous referees for their helpful comments on this paper.

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