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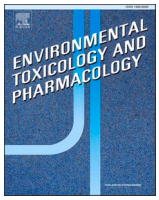

Early life developmental effects induced by dioxins and PCBs in novel bioassays with *C. elegans*

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ABSTRACT

This study assessed the effects of TCDD, two PCB mixtures (Clophen A50 and Aroclor 1254), and field extracts from marine sediments and swimming crab tissues on early-life development in Caenorhabditis elegans. Gravid nematodes were exposed on agar, and isolated eggs and larvae were tested in solution. Larval development was evaluated after 72 hours. Reporter gene assays (DR-CALUX) were also used to quantify dioxin-equivalent toxicity (TEQ). Exposure to 10 pM Clophen A50 and TCDD on agar inhibited L3–L4 transition by 60 % and 50 %, respectively. Liquid exposure to 5 μM Aroclor 1254 or TCDD (10 nM and 10 μM) delayed development by 20–40 %. Field extracts contained TEQ values of 0.67–4.91 ng/kg (0.2–1.47 pM TCDD), reducing L3–L4 development by 40–60 %. Both bioassays effectively assessed the toxicity of persistent organic pollutants in environmental samples. Agar exposure mimics realistic uptake, while liquid assays offer faster, high-throughput screening.

1. Introduction

Dioxins and Polychlorinated biphenyls (PCBs) are organic pollutants that persist in the environment and bioaccumulate through the food web. Dioxins are a family of complex chemicals with similar molecular structures, including polychlorinated dibenzo-p-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) (Srogi, 2008). In vertebrates, the toxicity of dioxins is primarily mediated via binding to the aryl hydrocarbon receptor (AhR), a receptor that preferably binds to dioxins with a planar configuration (Mandal, 2005). PCBs are divided into two groups based on their molecular structures: “dioxin-like” PCBs (DL-PCBs) and “non-dioxin-like” PCBs (NDL-PCBs), each with a different mode of action depending on their interaction with the AhR. NDL-PCBs comprise the larger proportion of PCBs in the environment (Knerr and Schrenk, 2006).

In the present study, we utilized not only a standard dioxin (2,3,7,8-tetrachorodibenzo-p-dioxin (TCDD) but also two comparable technical mixtures of mainly NDL-PCBs: Aroclor 1254 and Clophen A50 and some marine benthic sediment extracts. Aroclor 1254 and Clophen A50 are two of most toxic commercial PCB mixture produced, primarily due to their relatively high concentrations of dioxin-like congeners (Wang et al., 2021). Moreover, these two PCBs have been reported as potential sources of contamination in Hangzhou Bay(Wang et al., 2016), the region where our marine benthic sediment samples were collected. Dioxins and PCBs belong to the chemical class of persistent organic pollutants (POPs). These recalcitrant, persistent, and lipophilic chemicals accumulate in sediments and bioaccumulate in the food chain (Mumbo et al., 2015, Omwoma et al., 2015). POPs can be transferred vertically from the mother to their offspring (González, 2021), both in egg-laying species and mammals (prenatally and via lactation). Dioxins and PCBs are known to induce numerous adverse effects in vertebrates, including retardation of growth and early life development, inducing hepatotoxicity, and interfering with reproduction (Foekema, Parron et al., 2014, Faiad et al., 2022, Aldeli et al., 2023, Faiad et al., 2023, Aldeli et al., 2024). The effects are most severe during the developmental stages of organisms (humans and wildlife) and may persist throughout their lifetime (Binelli and Provini, 2003, Foekema et al., 2012, Doering et al., 2018). In spite of the fact that POPs (including

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PCB's and dioxins) are strictly regulated in the EU by inclusion in Annexes IV and V to Regulation (EU) 2019/1021, these chemicals are omnipresent in the environment. Therefore, it is important to assess the toxic potency of such POPs. We developed *in vitro* bioassays to assess the toxic potency of dioxins and PCBs as chemical standards and extracts of polluted marine sediments, addressing the limitation of chemical methods in assessing the combined toxic effects of complex mixtures.

Bioassays using amphipod crustaceans (Gammarus fossarum) and zebrafish (Danio rerio) embryos are commonly employed to assess development and reproductive toxicities due to their ability to produce large numbers of progeny, the transparency of the embryos and led to the development of high-throughput assays (Geffard et al., 2010, Teixidó et al., 2019). However, these traditional animal tests rely mainly on visual assessment of morphological features and are limited in providing a quantitative benchmark for our study. The DR-CALUX *in vitro* bioassay is an efficient method for quantifying the potency of dioxins and dioxin-like compounds (DLCs) in sediments and assessing their interaction with AhRs (Murk et al., 1996, Murk et al., 1998, Stronkhorst et al., 2002). The relative toxicity of a sample is expressed as the toxic equivalency (TEQ), compared to the level of 2,3,7,8-tetrachorodibenzo-p-dioxin (TCDD), which is the most potent congener. TEQ calculation involves an extensive chemical analysis of all AhR antagonists in a sample, with each concentration being multiplied by its toxic equivalency factor (TEF) (Van den Berg et al., 2006). The DR-CALUX assay directly quantifies the TEQ, with the results often expressed in terms of bioanalytical equivalents (BEQ) (Hoogenboom et al., 2006). Early developmental defects occur after non-lethal exposure of developing amphibian and fish embryos to NDL-PCBs and DL-compounds (Incardona, 2017, Zhao et al., 2017). However, the developmental effects of these compounds on invertebrates have been less extensively studied. Currently, no rapid assay or method exists to assess the adverse developmental effects of NDL-PCBs and DL-PCBs, along with DLCs, in invertebrates.

In this study, we evaluated the suitability of *C. elegans*, a free-living nematode and a well-characterized model organism, for use in *in vivo* assays screening for toxicity of PCB/dioxin contaminants. The nematode was chosen for its short life cycle, prolific reproduction, and ease of cultivation in a laboratory environment. *C. elegans* has been validated as a model organism for developmental studies (Boyd et al., 2012). For instance, *C. elegans* has been utilized in toxicity studies to assess the adverse effects of heavy metals (Donkin and Williams, 1995). With its fully sequenced genome, *C. elegans* enables detailed gene expression studies. Recent studies have demonstrated that the effects of genotoxic compounds on *C. elegans* are concentration-dependent (Karengera, Bao et al., 2021, Karengera et al., 2022). Specifically, two PCB mixtures, benzo(a)pyrene (BaP) and Aroclor 1254, have been shown to induce the expression of toxicity-related genes in *C. elegans* genes (Karengera et al., 2022).

In the present study, we describe two robust and inexpensive methods for analysing the effects of direct and indirect exposure to dioxins and PCBs on the early development of *C. elegans* larvae. In the first method, gravid nematodes were exposed to the compounds via the agar with *E. coli* bacteria on which they feed, while in the second assay, nematode eggs were isolated and then exposed to the chemicals via the liquid in which also the subsequently developing larvae lived. The maternal exposure method mimics the natural exposure of invertebrates to these compounds in soil. This method already was developed and used 26 years ago, but has not been previously published. This test was repeated using standard compounds to compare its effectiveness with that of the egg-exposure method. The direct egg-exposure method, being both rapid and straightforward, is suitable for testing numerous samples. Our analysis focused on the effects of dioxin (TCDD), a mixture of two PCBs (Aroclor 1254 and Clophen A50), and extracts from contaminated marine field samples including sediments and edible crab, on the early life development of *C. elegans*. Both the chemical standards as well as the field extracts consistently disrupted the early life development of *C. elegans* in a dose-dependent manner. Although this study was initiated a long time ago, we have not seen recent applications of C. elegans to detect developmental effects induced by dioxins and PCBs. More importantly, our recent studies found gene expression profiling in *C. elegans* has been demonstrated to be a potential bioanalytical tool to detect the complex toxic potency of environmental contaminants (Karengera et al., 2023).

2. Material and methods

2.1. Chemicals

All chemical reagents used in this study were of analytical grade (purity > 99 %). Nitric acid (65 %) and hydrogen peroxide (70 %) were purchased from Changmu company LTD (Hangzhou, China). Dimethyl sulfoxide (DMSO) was purchased from Merck (Darmstadt, Germany). Aroclor 1254 (PCB1254) (analytical standards grade) was purchased from Sigma-Aldrich (St. Louis, MO, USA). Clophen A50 was purchased from Promochem (Wesel, Germany), studies have identified the presence of congeners 2,2′,4,4′,5,5′-hexachlorobiphenyl (PCB 153), 2,2′,3,3′,4,4′,5-heptachlorobiphenyl (PCB 170), and 2,2′,3,4′,5,5′,6-heptachlorobiphenyl (PCB 187) within Clophen A50 (Larsson and Bergman, 2001). 2, 3, 7, 8-tetrachlorodibenzo-p-dioxin (TCDD) was purchased from Schmidt B.V. (Amsterdam, The Netherlands).

2.2. Nematode strain and cultivation

C. elegans wild-type strain (*Bristol N2*) was obtained from the Nematology group at Wageningen University and maintained on Nematode Growth Medium (NGM) agar plates at 16 °C. The media were changed monthly, using fresh NGM agar seeded with *E. coli* bacteria. The *E coli*-NGM was prepared as previously described (Stiernagle, 2006, Karengera et al., 2021).

2.3. Experimental set-up

To assess the impact of dioxin and PCBs on *C. elegans*' early development, we utilized two methods: 1) indirect exposure via the mother and without further exposure of the laid eggs and 2) direct exposure of the isolated eggs. Both methods were performed at 20 °C.

For the maternal exposure method (Fig. 1), dioxin, PCBs, or field sample extracts were dissolved in DMSO before dilution in pre-heated NGM. Briefly, five gravid *C. elegans* hermaphrodites from a clean culture plate were transferred onto NGM agar supplemented with the test compounds or DMSO only as control. After 24 hours, the gravid *C. elegans* were removed, only leaving behind the eggs. After 72 hours, newly developed and exposed gravid hermaphrodites were transferred to 48-well tissue culture plates (one nematode per well). Each well was filled with 130 μl of K-medium. After laying eggs, the exposed gravid C. elegans were removed from the wells. The number of offspring produced in 48-well tissue culture plates was counted, and for all larvae the L1 - L4 (Ambros, 2000) developmental stage was determined microscopically in 72 hours and observed the malformations or behavioural changes during all the developmental stages (Racz et al., 2017).

For the direct egg-exposure method (Fig. 2), adult nematodes were transferred from the agar- culture to liquid culture medium and 72 hours later concentrated in a 2 ml tube through centrifugation at 13,000 rpm. Sodium hypochlorite (1 ml; 5 %) was then added to the pellet for bleaching treatment (Karengera et al., 2021) to eliminate nematodes in all developmental stages except the eggs. The pellets, containing only eggs, were resuspended in 0.5 ml of K-medium with a mixture of deionized distilled H_2O and salts (2 mM NaCl and 13 mM KCl). Thereafter, 5 μL of eggs in K-medium was diluted with fresh 2 ml K-medium and aliquoted in 48-well tissue culture plates, ensuring that each well contained at least ten eggs. The number of eggs was confirmed using a binocular microscope. Dioxin, PCBs, or field sample extracts were

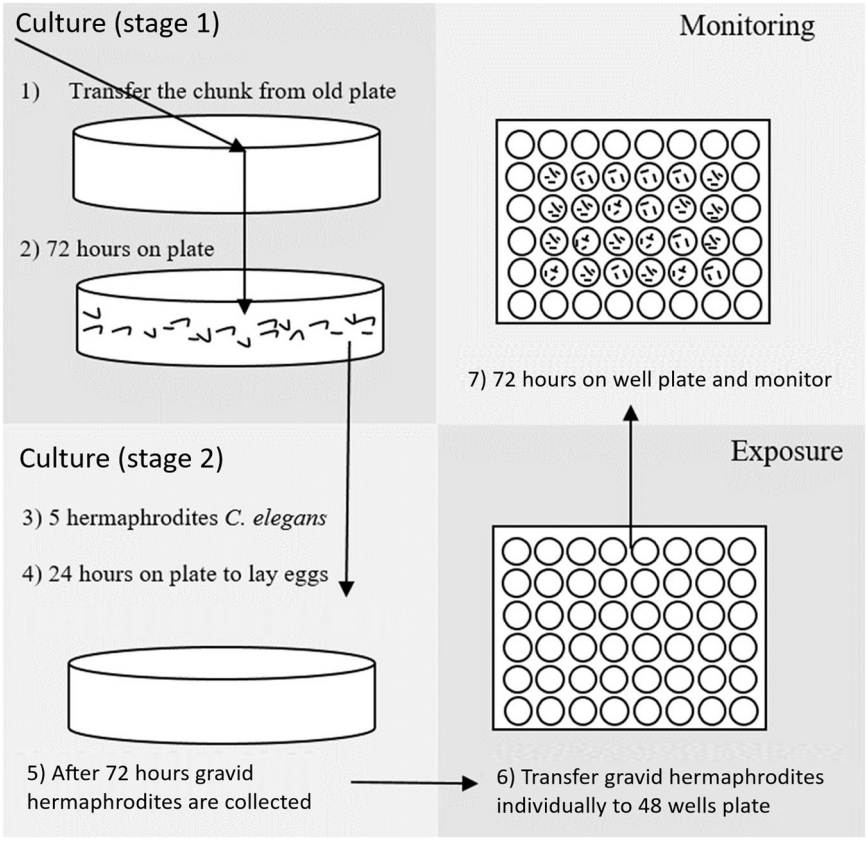


Fig. 1. Schematic overview of the maternal-exposure bioassay method.

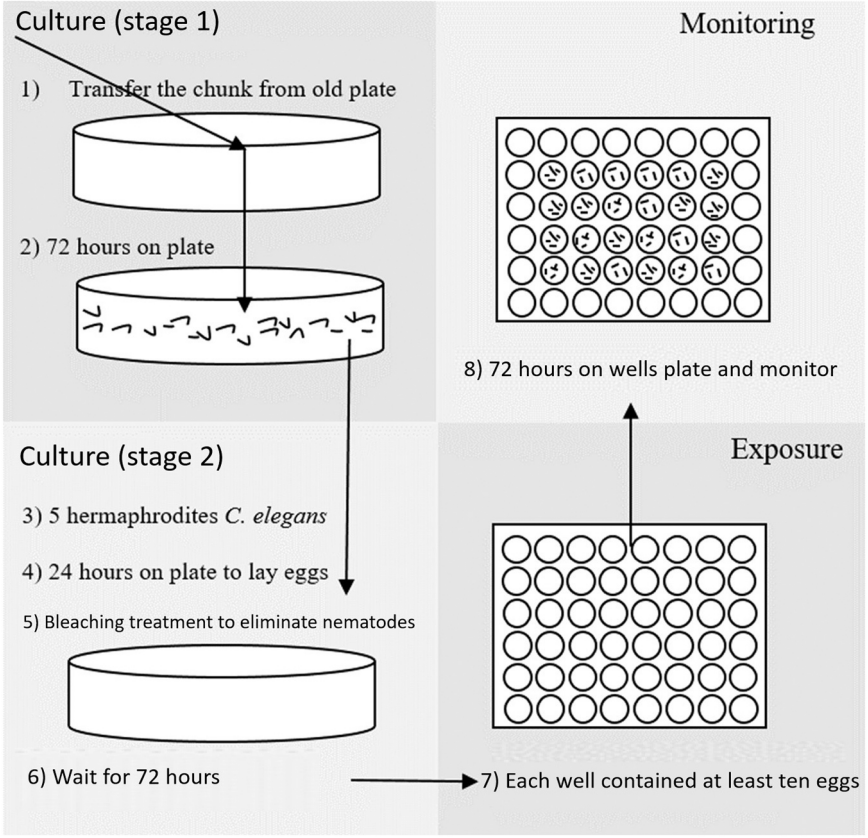


Fig. 2. Schematic overview of the direct egg exposure bioassay method.

dissolved in dimethyl sulfoxide (DMSO). The final DMSO concentration in all the plates was 0.5 %, a concentration at which no adverse effects on *C. elegans* were observed *(Wang,* Wang et al., 2010*)*. The eggs and subsequent nematode reproductive stages in the well-plates were monitored every 24 hours for 72 hours using a stereo microscope as previously described based on their size and activities (Hope, 1999, Racz et al., 2017).

2.4. Field samples

Apolar, non-organic extracts from sediment and swimming crab samples were prepared and tested for their effect on the performance of offspring from exposed nematodes using the maternal exposure nematode assay. Field samples, including marine sediment and swimming crab samples containing dioxin and PCB residues, were collected in 1996 from the Netherlands (Murk et al., 1996) and the Hangzhou Bay area in China (crabs in 2019) (Bao, Cai et al., 2021). For the extraction of field samples, 10 g of each sample was dried overnight at 35°C and mixed with 1 g of NaSO4. A hexane/acetone (1:1) liquid-liquid extraction method was used (Murk et al., 1998, Banjoo and Nelson, 2005). De-sulphuration was achieved by adding tetrabutylammonium sulfite (Paré et al., 1998), followed by further cleaning using a multilayer acid-base silica column, as previously described, and dissolving in DMSO (Murk et al., 1996). To mimic natural exposure concentrations, the concentration of sediment extracts in the agar was the same as that in the wet sediment samples.

2.5. DR-CALUX bioassay

The extracts dissolved in DMSO were analysed using the DR-CALUX bioassay as previously described (Bovee et al., 1998). Briefly, 40 µL of the sample, it comes from roughly 1 g environmental samples, in DMSO was dissolved in 2 ml of the cell culture incubation medium. This corresponds to an environmental equivalent concentration of 0.25 g/ml or 0.05 g/well. After 24 h, the medium was aspirated, and the cells were washed and lysed before measuring the luciferase content using a Luminometer. The resulting signal was expressed as bioanalytical equivalents (BEQ) (Hoogenboom et al., 2006), calculated using the TCDD calibration curve (curve fitting of the Sigmoid dose-response variable slope) using GraphPad Prism software v 5.0.

2.6. Statistical analysis

Data were analysed using the GraphPad PRISM 5.0 software. Comparison between groups was performed using the unpaired *t*-test at a two-tailed $P < 0.05$.

3. Results

In both the maternal and egg exposure control groups, 80 % of the larvae reached stage L4 after 72 hours, aligning with reported results from other studies (Helmcke et al., 2009). The effects of exposure to the PCB mixture Clophen A50 and TCDD in the maternal exposure method on larval development were initially recorded every 24 hours. However, due to the time-consuming nature and lack of additional relevant information from these observations, we decided to limit further observations to 72 hours after hatching. Fig. 3 shows the number of larvae at each specific developmental stage (L1 - L4), recorded 72 hours after the removal of the gravid nematode from the observation wells. Exposure to both Clophen A50 and TCDD caused a dose-dependent delay in *C. elegans* development, most notably in the transition from the L3 to L4 stage. Even the lowest concentrations of Clophen A50 or TCDD, both at 10 pM significantly reduced the development into the L4-stage with respectively 60 % and 80 % of the larvae counted at 72 hours. Not only were larvae halted in the L3 stage, these larvae also experienced dose-dependent mortality (Fig. 4), a phenomenon observed in

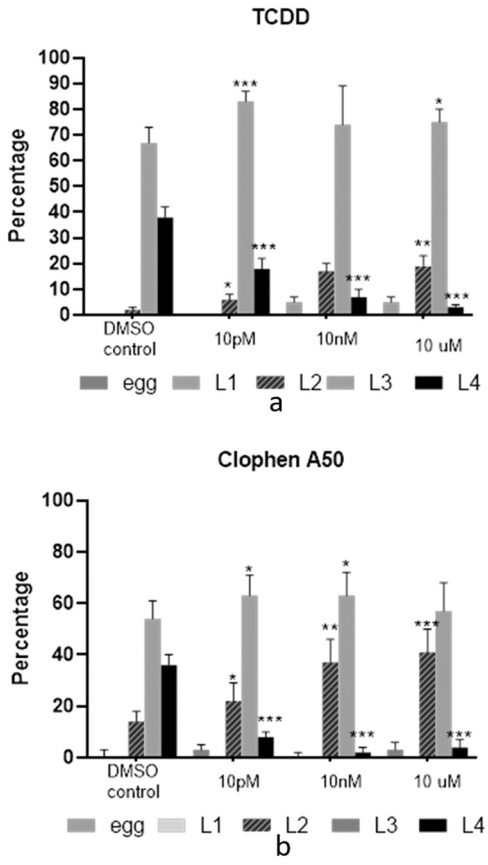


Fig. 3. Proportional distribution in developmental stages of C. elegans larvae hatched from eggs laid by gravid hermaphrodites exposed to (a) dioxin, 2, 3,7,8-tetrachloordibenzo-p-dioxine (TCDD), and (b) a mixture of PCB (Clophen A50). The developmental stage of the larvae was recorded over 72 hours after egg-laying. At least ten nematodes were assessed for a given POP. * represents $P < 0.05$; * * represents $P < 0.001$; * ** represents $P < 0.0001$.

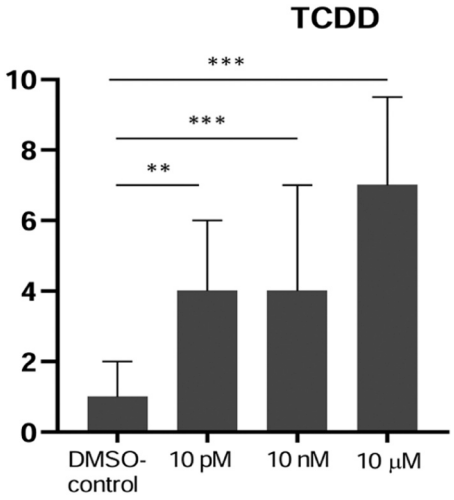


Fig. 4. Average number of dead *C. elegans* offspring out of 10 animals after maternal exposure to TCDD during 72 hours. The assessment was performed 72 hours after removal of the mother nematodes. * * represents $P < 0.001$; * ** represents $P < 0.0001$.

nematodes exposed to both TCDD and Clophen A50. Supplementary figure C1 illustrates the number of offspring C. elegans offspring produced in each exposure group at 72 hrs of development. However,

exposure to dioxins and mixture of PCBs delayed larvae development, shown by few larvae that had reached the L4 stage within 72 hours.

Additionally, animals exposed as eggs showed significant impairment in their development into L4 relative to the control group. The reduction in percentage of nematodes reaching L4 after exposure to 5 μM Aroclor 1254 or 10 nM TCDD was 20 %, and to 10 μM TCDD 40 % (Fig. 5). Similar to what was observed in the maternal exposure method, more nematodes still were in stage L3 at 72 hrs. The animals hatching from eggs exposed to 5 μM Aroclor 1254, 10 nM TCDD, and 10 μM TCDD also experienced significant mortality (Fig. 6).

Using the egg-exposure method, extracts from swimming crabs (fat or meat) and marine sediments significantly impeded development from L3 to L4 by 40–60 % (Fig. 7), corresponding to a TEQ of 0.67–4.91 ng/kg (equivalent to 0.2 – 1.47 pM TCDD). Results from the maternal exposure method using Dutch sediment extracts are shown in Fig. 8. There was a 20–50 % reduction in L4 larvae compared to L1-L3 larvae exposed to TEQs ranging from 0.3 to 15.6 ng/kg, equivalent to 0.09 – 4.66 pM TCDD. Notably, sample 6 shows a high reduction in L4 development despite low TEQ values, suggesting the presence of other organic pollutants. All TCDD-equivalent toxic potencies, expressed as BEQ or TEQ, were analysed using the DR-CALUX bioassay. The results are shown in the tables under Figs. 7 and 8.

4. Discussion and conclusions

Two novel exposure methods demonstrated that TCDD and PCB mixtures significantly disrupt the early life development of *C. elegans*, particularly at and above contamination levels that are environmentally relevant. The field sample extracts of the lipophilic persistent contaminant fraction contained dioxin-like contamination levels are 0.7 – 4.91 TEQ ng/kg in crab tissues and 0.67–156 TEQ ng/kg in sediment samples. Studies found comparable environmental concentrations are 3.8 TEQ ng/kg (Knutzen et al., 2003) for total dioxin-like compounds in the liver and hepatopancreas of crab samples. Similarly, Manning et al. found 4.9 – 22 TEQ ng/kg in marine crab samples from Australia (Manning et al., 2017). Our samples have similar concentrations from 4.09 – 23 TEQ ng/kg significantly lower the L4 stage of *C. elegans* demonstrate that our developed bioassays have potential for detecting developmental toxicity in environmental samples. It cannot be excluded that other contaminants extracted also contributed to the observed effects. Our findings highlight the sensitivity of these small-scale *in vivo* bioassays in detecting developmental effects when exposing nematode early life stages to realistic levels of organic extracts including dioxin-like compounds. The impact of the compounds on larvae was more pronounced in maternally exposed nematodes compared to those directly exposed as egg (Method 2). This difference may stem from the longer exposure period in the maternal exposure method and the possibility that the nematode eggs are less permeable to these chemicals (Hartman et al., 2021). Furthermore, accumulated POPs in the lipids, deposited in the yolk, become directly available to the developing larvae from the onset of development, as they begin utilizing these lipids. It takes time before the levels inside exposed eggs reach their highest level so the developing embryo may be hardly exposed in the first hours of development. Additionally, it is plausible that the hermaphrodite nematodes experienced sublethal effects from the exposure, potentially leading to the production of lower-quality eggs.

Previous research indicates that dioxin-like compounds exert their toxic effects primarily through activation of the Aryl hydrocarbon Receptor (AhR) pathway. Most sensitive are teratogenic effects and this includes early life developmental delay in vertebrates(Faiad et al., 2022, Faiad et al., 2023). In contrast, invertebrate AhR homologs have been shown not to bind dioxins and related chemicals (Hahn et al., 2006) and dioxin-like compounds do not activate the AhR of *C. elegans* (Powell-Coffman et al., 1998, Karengera et al., 2021). This suggests that the effects observed in this study are not mediated through the AhR. The observed developmental effects might be attributed to narcosis (general toxicity), wherein the narcotic effect leads to membrane disruption and deterioration (Sikkema et al., 1994), which induces non-specific disruption of the integrity (Escher et al., 2002, Karengera et al., 2022). Clophen A50 and the dioxin-like PCB77 have been reported to delay the early life development of amphibians, such as *Xenopus laevis* and *Rana temporaria*, by prolonging metamorphosis, potentially through thyroid hormone disruption (Gutleb et al., 2000, Gutleb, Mossink et al., 2007) Those experiments have the disadvantage that it concerns vertebrates, require much larger volumes and take 60 days instead of

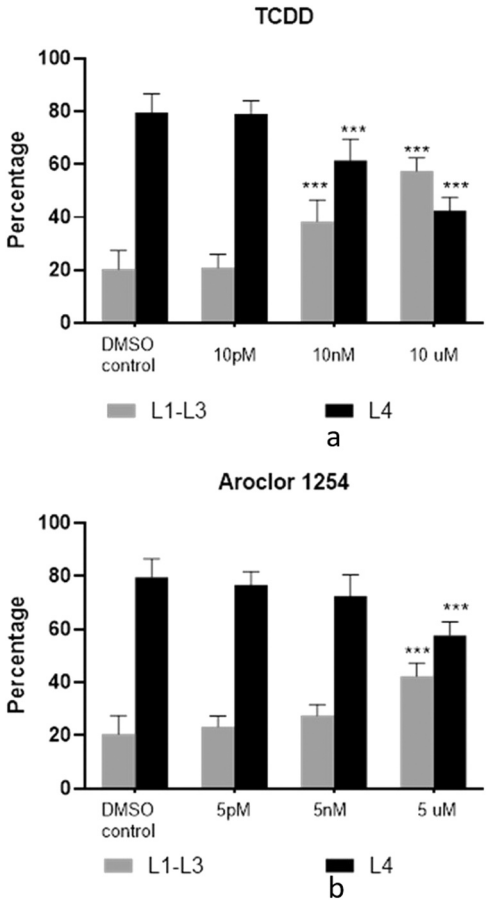


Fig. 5. Proportional distribution in developmental stages of *C. elegans* larvae from eggs directly exposed to (a) the dioxin, 2, 3, 7, 8-tetrachloordibenzo-p-dioxine (TCDD), and (b) the technical PCB mixture Aroclor 1254. The larvae at stage 4 (L4) or stage 1–3 (L1 – L3) were determined after 72 hours of exposure. At least ten nematode eggs were exposed each time. * ** represents P < 0.0001.

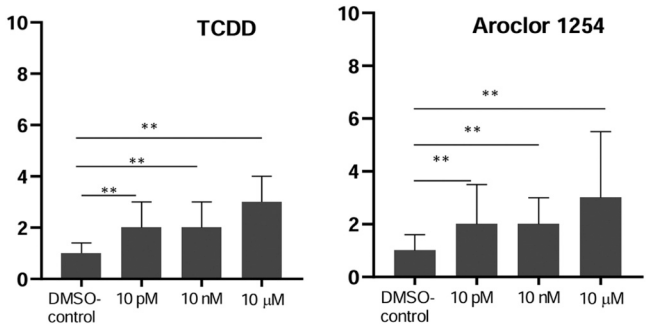
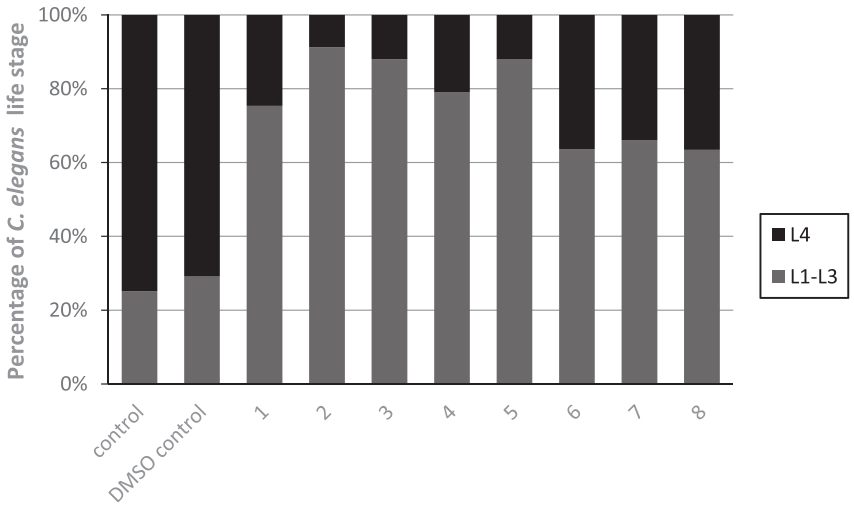


Fig. 6. the average number of dead first-generation *C. elegans* offspring after direct egg exposure to TCDD during 72 hours. The assessment was performed after 72 hours after exposure of the eggs. * * represents P < 0.001.



Code	types	BEQ ng/kg	Decreased L4
1	Crab Fat	4.91	46%
2	Crab Fat	4.09	62%
3	Crab Fat	4.91	59%
4	Crab Fat	4.58	50%
5	Crab Meat	0.14	59%
6	Crab Meat	0.70	35%
7	Sediment	0.80	37%
8	Sediment	0.67	34%

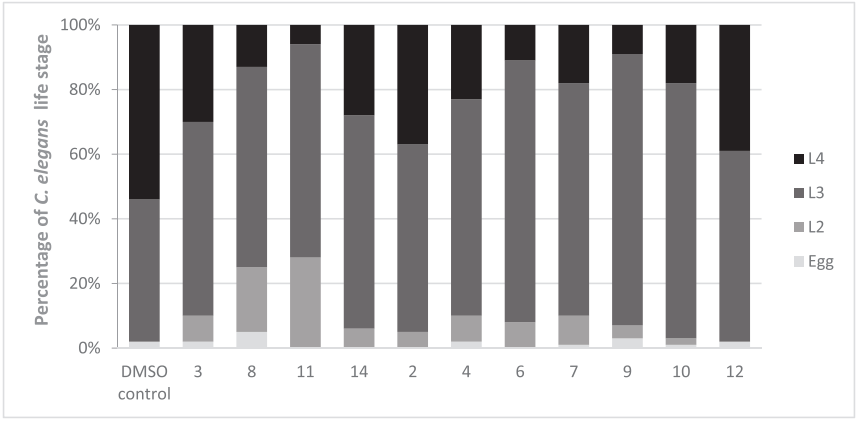
Fig. 7. The toxic potency and effects of crab extracts on the early life stage development of *C. elegans* following egg exposure were exposed the extracted residues. Samples 1–4 were extracted from the fatty parts, and samples 5–6 were extracted from the muscle tissue of swimming crabs. Samples 7–8 were extracted from marine sediments. All the samples come from the Hangzhou bay region in China. BEQs of the sediments and crab samples were determined using an in vitro reporter gene assay (DR-CALUX bioassay).

72 hours. As the amphibians go through more life stages, Gutleb et al., (2007) calculated penalty points integrating developmental delay over more life stages. Interestingly, the sensitivity was I the same order of magnitude as what we found.In *C. elegans*, both PCBs and dioxins have been shown to inhibit vitellogenin metabolism (Won, 2006). Vitellogenin is a glycolipoprotein that belongs to a family of lipid transport proteins(Sparling, 2016) and transports among others sterols (Kimble and Sharrock, 1983) but can also bind lipophilic contaminants. Vitellogenin is actively transferred into the egg in the gravid hermaphrodites, which may explain why the maternal exposure route is more effective. PCBs and dioxins have been shown to inhibit vitellogenin metabolism also in other aquatic vertebrates like zebrafish (King Heiden et al., 2006) or the fish *Abramis brama and Cyprinus carpio* (Schug et al., 2011) so it cannot be excluded that the maternal exposure results in production of lower quality eggs. Interestingly, the observed developmental effects of PCBs in nematodes resemble effects seen in ahr-1 mutant mouse strains and include regulating fatty acid synthesis and homeostasis (Aarnio et al., 2010). Aroclor 1254 dysregulates the expression of lipid metabolism genes in *C. elegans* (Karengera et al., 2022). Aroclor 1254 also downregulates the *C. elegans* genes associated with development, lipogenesis, and membrane fluidity like *daf-22* (Joo et al., 2009), *emb-8* (Li, Li et al., 2017), *fasn-1* (D'Erchia et al., 2006), or *pcyt*-1 (Svensk et al., 2013) (which were upregulated), and *lips-15* (Chan et al., 2018) genes. Therefore, given the unclear effects of dioxin and NDL-PCBs on fatty acid and vitellogenin metabolism in *C. Elegans*, further detailed studies are recommended.

The effects observed in our study following maternal exposure suggest the transmission of Clophen A50 and TCDD to offspring, though the possibility of indirect or epigenetic effects cannot be excluded either. In a side-experiment, exposure has been extended over multiple generations by having the F1 gravid hermaphrodites lay their eggs on new exposure plates and repeating the observations. This did not increase the severity of the effects compared to only exposing the first generation (data not shown). Interestingly, in the F3 generation, unexposed offspring of the original gravid females, were readily picked up during transfer using pig hair. Exposed animals appeared "older" than their control counterparts, exhibiting softer, more wrinkled skin with reduced elasticity. Shortly after being transferred to microscope slides covered with thin glass, exposed animals burst under light pressure, in contrast to control animals, which withstood this pressure unscathed. These observations suggest adverse effects on the metabolism of cholesterol, fatty acids, and/or lipids, which are crucial for maintaining membrane rigidity (Ruiz et al., 2018, Koyiloth and Gummadi, 2022).

While the maternal exposure route, which is more representative of real-world conditions, was more sensitive and also showed effects on the elasticity of the adult animal, it is notably more time-consuming compared to the egg exposure method. A faster bioassay procedure can provide the scanning tool to identify dioxin and non-dioxin-like compounds in toxicity identification evaluation study. Conversely, the maternal exposure method is preferable for more detailed studies and could be applied later in a tiered approach.

This study demonstrates that environmentally relevant concentrations of dioxins and non-dioxin-like PCBs (NDL-PCBs), present in both polluted sediments and seafood, can disrupt the early development of C. elegans. Two complementary in vivo bioassays—maternal exposure via agar and direct egg exposure in liquid—proved sensitive in detecting developmental delays and increased mortality at picomolar to micromolar levels. Beyond these findings, the study establishes C. elegans as a



Code	locations	types	TEQ ng/kg	Decreased L4
2	Nieuwe waterweg beneluxtunnel	Sediment	16	/
3	Nieuwe waterweg splistingsdam	Sediment	78	27%
4	Loswal Noord Vak 53	Sediment	10	28%
6	Noordwijk 2km off shore	Sediment	3	47%
7	Ijmuiden outer harbour	Sediment	23	38%
8	Ijmuiden inner harbour	Sediment	156	42%
9	NZK10 Buitenhuizen	Sediment	87	48%
10	NZK18 Amerikahaven	Sediment	12	37%
11	NZK29 Oragnjesluis	Sediment	31	52%
12	IJssdmmer Enkhuizen	Sediment	36	/
14	Oosterschelde Oesterput	Sediment	29	24%

Fig. 8. The toxic potencies and effects of sediment extracts on the early-stage development of *C. elegans*. The gravid *C. elegans* adults were fed on agar with food supplemented with sediment extracts in the same concentration as from the sediment it came from. The effect on the early life stage development was analyzed after 72 hours of gravid *C. elegans* exposure to the extract. The compounds were extracted from 11 marine sediments obtained from the Netherlands [23]. TEQs of the sediment extracts were determined using in vitro reporter gene assay (DR-CALUX bioassay).

practical model for screening environmental samples for developmental toxicants. The maternal exposure method offers a more ecologically relevant assessment of early-life stage exposure pathways, while the egg-exposure assay supports higher throughput screening.

These bioassays can fill gaps left by current vertebrate models and cell-based in vitro systems, offering a cost-effective, ethical, and alternative for routine environmental hazard screening. Their application could support toxicity identification evaluations, sediment quality assessments, and seafood safety evaluations. We recommend that future work focuses on mechanistic studies—particularly on lipid and vitellogenin metabolism—as well as integration with gene expression profiling for mode-of-action analysis. Broader application of these bioassays across diverse field samples will strengthen their utility in environmental monitoring and regulatory frameworks addressing persistent organic pollutants.

CRediT authorship contribution statement

Karengera Antoine: Writing – review & editing. **Bao Cong:** Writing – original draft, Visualization, Formal analysis, Data curation. **Wieland Willemien:** Writing – review & editing, Methodology. **Murk Alber-Tinka J.:** Writing – review & editing, Project administration, Methodology, Data curation. **Kammenga Jan:** Writing – review & editing, Methodology. **Dinkla Inez:** Writing – review & editing.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Cong Bao reports was provided by Wageningen University & Research. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper

Appendix

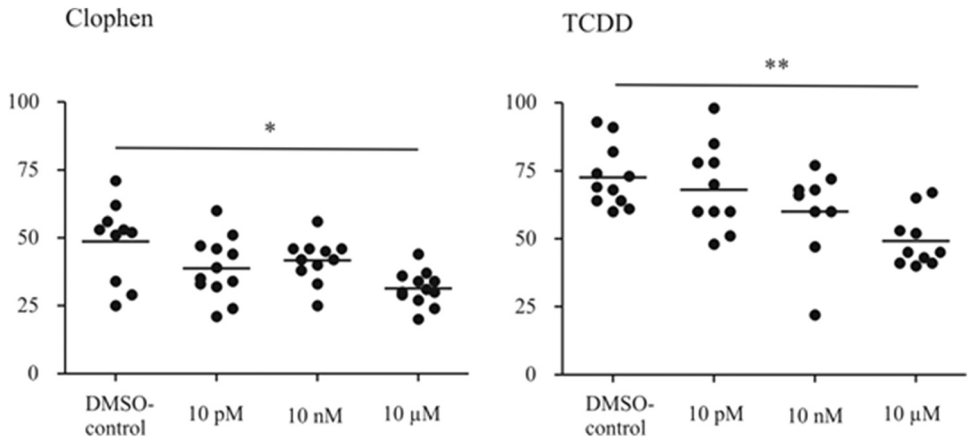


Figure C1. Number of *C. elegans* offspring of the groups maternally exposed to TCDD or Clophen A50. The number of offspring counted was counted after 72 hours of development. *Represents $P < 0.01$; * * represents $P < 0.001$

Data Availability

Data will be made available on request.

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