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Toxicity of representative organophosphate, organochlorine, phenylurea, dinitroaniline, carbamate, and viologen pesticides to the growth and survival of *H. vulgaris, L. minor*, and *C. elegans*

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Abstract

Pesticides are commonly found in the environment and pose a risk to target and non-target species; therefore, employing a set of bioassays to rapidly assess the toxicity of these chemicals to diverse species is crucial. The toxicity of 9 individual pesticides from organophosphate, organochlorine, phenylurea, dinitroaniline, carbamate, and viologen chemical classes and a mixture of all the compounds were tested in 3 bioassays (*Hydra vulgaris*, *Lemna minor*, and *Caenorhabditis elegans*) that represent plant, aquatic, and soil-dwelling species, respectively. Multiple endpoints related to growth and survival were measured for each model, and EC_{10} and EC_{50} values were derived for each endpoint to identify sensitivity patterns according to chemical classes and target organisms. *L. minor* had the lowest EC_{10} and EC_{50} values for 7 and 5 of the individual pesticides, respectively. *L. minor* was also 1–2 orders of magnitude more sensitive to the mixture compared to *H. vulgaris* and *C. elegans,* where EC_{50} values were calculated to be 0.00042, 0.0014, and

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Author Contributions

Kelly Rivenbark Study design, execution of *in vitro* experiments, preparation of original draft. Hasan Nikkhah: Software, visualization, writing- review and editing. Meichen Wang Study design, execution of *in vitro* experiments, writing- review and editing. Burcu Beykal: Software, visualization, funding acquisition, writing- review and editing. Timothy Phillips: Funding acquisition, writing- review and editing

Statements and Declarations

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Competing Interests

The authors declare that they have no competing interests.

Supplementary Materials

Supplemental materials include 3 additional figures and are linked with this article.

0.038 mM, respectively. *H. vulgaris* was the most sensitive species to the remaining individual pesticides, and *C. elegans* consistently ranked the least sensitive to all tested compounds. When comparing the EC_{50} values across all pesticides, the endpoints of *L. minor* were correlated with each other while the endpoints measured in *H. vulgaris* and *C. elegans* were clustered together. While there was no apparent relationship between chemical class of pesticide and toxicity, the compounds were more closely clustered based on target organisms (herbicide vs insecticide). The results of this study demonstrate that the combination of these plant, soil, and aquatic specie can serve as representative indicators of pesticide pollution in environmental samples.

Keywords

Ecotoxicology; battery of bioassays; herbicides; insecticides; EC_{50}; EC_{10}

1. Introduction

Pesticides are diverse classes of chemicals that are intentionally released into the environment to control unwanted plants, insects, fungi, and other organisms (Fujita et al., 2010; Hernández et al., 2013; Warne and Reichelt-Brushett, 2023). However, only 1% of the applied chemicals has been estimated to effectively control the target organisms, and the remaining residues enter the environment where they can interact with non-target organisms (Grégoire et al., 2022; Hernández et al., 2013; Tudi et al., 2021; Warne and Reichelt-Brushett, 2023). Considering the leaching, run-off from agricultural lands, and spray drifting that can occur after applications, humans, plants, and other organisms living in the surrounding environment are at risk of exposure and possible toxicity (Cunha et al., 2012; Leu et al., 2004; Sharma et al., 2019). For example, aquatic species, bees, and earthworms living near citrus (Cunha et al., 2012) and plum orchards (Reinecke and Reinecke, 2007) have been shown to have elevated risks of toxicity due to spray drifting after pesticide applications. Membrane devices deployed into streams that drain from two golf courses also showed significant toxicity to a fish species, which correlated with the application schedule of chlorinated pesticides on the courses (Metcalfe et al., 2008). Previous studies have also demonstrated that exposure to just 1% of the recommended field dose of metsulfuron methyl and chlorsulfuron was linked to severe growth inhibition of aquatic plant species, food crops (Boutin et al., 2000), and fruit trees (Bhatti et al., 1995; Cedergreen and Streibig, 2005). These studies demonstrate that even low-levels of pesticides in the environment pose a significant risk to the ecosystem.

The global application of all pesticides was approximately 4.2 million tons in 2019 (Warne and Reichelt-Brushett, 2023), and over 1000 individual chemicals are currently registered for use worldwide (Johansen, 2003; Schwingl et al., 2021; WHO, 2022). Pesticides account for roughly 20% of the compounds listed on the Agency for Toxic Substances and Disease Registry Substance Priority List (SPL) (Simonsen et al.), where nearly 75% and 17% of the listed pesticides belong to the legacy organochlorine and organophosphate chemical classes, respectively (ATSDR, 2022). Because of the complexity of these existing applications, presence at priority sites, and the development of new agrochemicals over time, there is a critical need for bioassay methods that can quickly assess the toxicity of individual

compounds and complex mixtures to target and non-target species (Fochtman et al., 2000; Ullah and Zorriehzahra, 2015). To assess the toxicity of individual pesticides and mixtures, the use of several bioassays from similar ecological niches (e.g. a set of aquatic organisms) or different groups (e.g. microorganisms, aquatic species, and other invertebrates) have been used (Hernando et al., 2003; Nowell et al., 2014). Utilizing a battery of bioassays in hazard assessment is advantageous as a single model organism cannot accurately nor reliably depict the toxicity of a chemical or represent the whole environment (Mariani et al., 2006; Repetto, 2013).

Hydra vulgaris is a freshwater cnidarian that is used to assess the toxicity of contaminants to aquatic species because: 1) hydra species play and important role in their ecosystem, 2) it has well-described morphological endpoints, 3) and is sensitive to environmental contaminants (Galliot, 2012; Karntanut and Pascoe, 2000). *Lemna minor* is a floating macrophyte that is used to represent aquatic plants in toxicity testing because: 1) macrophytes play an important ecological role as primary producers (de Alkimin et al., 2020), 2) its ease of cultivation, 3) has a high reproduction rate that yields a genetically-homogenous population, and 4) is sensitive to pesticides (Aliferis et al., 2009; Wang, 1990). *Caenorhabditis elegans* is a soil nematode that is useful for understanding the toxicity of pesticides to soil species because: 1) nematodes account for the largest number of soil-dwelling species and are essential for maintaining soil quality (Sochová et al., 2006), 2) its ease of cultivation, and 3) has diverse histological, biochemical, and behavioral endpoints that are available to characterize chemical toxicity (Hunt, 2017). These bioassays were utilized represent important ecological niches in aquatic and soil environments that contain aquatic, plant, and soil invertebrate species that can be directly or indirectly affected by pesticides. These bioassays are advantageous for toxicity testing of environmental chemicals like pesticides because of they combine the whole-organism approach of *in vivo* methods and the scalability and throughput of smaller, cell-based, *in vitro* systems (Hunt, 2017; Ueda and Nagai, 2021).

In the current study, 9 pesticides from important chemical classes, including organochlorines, organophosphates, urea-type, dinitroanilines, carbamates, and viologens (Table 1) where chosen to represent diverse compounds with varying ranges of toxicities and target organisms. As the SPL is not a list of the most toxic compounds, chemicals that are not currently listed were also included in the study to further represent compounds that have environmental and human relevance. We also included a pesticide mixture because organisms are exposed to a complex mixture of chemicals in the environment, and the toxicities of these mixtures are typically different than the individual compounds. For example, interactions between chemicals in the mixtures may act synergistically or antagonistically to alter the sample toxicity (Bart et al., 2022; Hernández et al., 2013; Hernando et al., 2003; Rider and Simmons, 2018; Wang et al., 2021).

The goals of the current study were to assess the toxicity of diverse chemical classes of insecticides and herbicides to a battery of bioassays and use this information to rapidly 1) compare the sublethal responses of the bioassays and 2) determine if specific classes of pesticides are more toxic to the current set of bioassays than others. These results will be used to identify sensitive endpoints for each bioassay that can be used to rapidly assess

and predict the toxicity of new pesticides, complex mixtures, and environmental samples to monitor soil and water quality and verify remediation efforts.

2. Materials and Methods

2.1 Reagents and Materials

9 pesticides that represent different chemical classes, target organisms, and physiochemical properties were included in the present study (Table 1) (PubChem, NIH).These pesticides also have varying LogP values, molecular weights (MW), and water solubilities, which are known to be important factors for chemical toxicity and bioaccumulation factors (Fujita et al., 2010; Silva et al., 2022). Crystalline 2,4,6-trichlorophenol, pentachlorophenol, lindane, diazinon, glyphosate, linuron, aldicarb, and paraquat dichloride were purchased from Sigma Aldrich (St. Louis, MO). Trifluralin was purchased from ChemServ (Minneapolis, MN). Stock concentrations of individual pesticides were created by dissolving each chemical in water (glyphosate, paraquat, aldicarb) or 100% acetonitrile, which was chosen based on the chemical's water solubility (Table 1) (PubChem, NIH) to prevent chemical precipitation during the experiments. The pesticide mixture was created by dissolving 2 mg/mL of each chemical into 100% acetonitrile. Cell culture grade dimethyl sulfoxide (DMSO) and HPLC grade acetonitrile were purchased from Sigma Aldrich.

2.2. *Hydra vulgaris* assay

A population of *H. vulgaris* polyps was obtained from Environment Canada (Montreal, Qc) and was maintained in hydra medium according to established methods (Wang et al., 2021). For toxicity analysis, non-budding polyps were exposed to increasing concentrations of each pesticide and the pesticide mixture (Supplemental Fig. 1) prepared in 4 mL of hydra medium. Each pesticide was tested in at least 4 concentrations. Over 92 hours, the morphology of the polyps was scored according to the Wilby Scale, where a score of 0 represented a dead, disintegrated polyp and 10 represented a normal, healthy polyp (Wang et al., 2021). To prevent the precipitation of the chemicals during the experiment, up to 1% DMSO was included in the test solution to overcome the low water solubility of several of these compounds. Preliminary studies indicated that exposure to 1% DMSO or acetonitrile did not significantly affect the morphology of the polyps. For diazinon and linuron, a metabolic activation package (MAP) (2.4 μg/mL mice hepatic microsomal cytochrome P450, 225 μM NADPH, and 25 μM $MgCl_2$) was included in the test solution. MAP was added to the test solutions because: 1) *H. vulgaris* is metabolically inactive (Galliot, 2012) and 2) these compounds require metabolic activation to exert toxic effects (Ellison et al., 2012; Uren Webster et al., 2015). For each experiment, triplicate analyses of each chemical exposure and vehicle and blank solution controls were included. Relative morphology scores were derived by expressing raw values as percent control, which was set to 1.

2.3. *Lemna minor* assay

A small community of plants was obtained from AquaHabit (Chatham, England) and was maintained in accordance with established methods and standard guidelines (Drost et al., 2007; OECD, 2006) in Steinburg Medium under white, fluorescent lights with a 400 ft-c intensity set to a 16:8 hours light:dark cycle at 25°C (Rivenbark et al., 2022).

Dosimetry experiments were conducted in a sterile, transparent, 24-well plate with a fitted lid (VWR, Radnor, PA). In each well, 2 plants (7–8 fronds total) were exposed to increasing concentrations of each pesticide or the mixture prepared (Supplemental Fig. 1) in 2 mL Steinburg Medium for 7 days under the growth light. Each day, plants were observed for changes in frond number and surface area, which was measured using ImageJ (NIH, Bethesda, MD). At the end of the exposure period, all types of chlorophyll were extracted from the surviving plants by homogenizing (Homogenizer 150, Fisher Scientific) the plants in 1.5 mL of 80% acetonitrile. After a 48-hour incubation in the dark at 4°C, the total chlorophyll content was quantified by measuring the absorbance of the solution using UV/Visible spectroscopy (Shimadzu UV-1800, Kyoto, Japan) set to 663 nm (Drost et al., 2007). Each pesticide was tested in at least 4 concentrations. For each experiment, triplicate analyses of each chemical exposure and vehicle and blank solution controls were included. Preliminary studies demonstrated that the inclusion of 1% acetonitrile in the exposure medium did not adversely impact the growth of plants. Relative surface area, frond number, and chlorophyll content values were derived by expressing raw values as percent control, which was set to 1.

2.4. *Caenorhabditis elegans* assay

Wildtype nematodes (Bristol N2) and *Escherichia coli* (*E. coli*) (strains OP50-1, NA22) were purchased from the Caenorhabditis Genetics Center (University of Minnesota) and maintained according to established protocols on 8P agar seeded with *E.coli* NA22 (Rivenbark et al., 2022). Large populations of larval stage 1 (L1) nematodes were obtained by a bleaching, washing, and incubation process. For dosimetry studies, groups of 2000 nematodes were transferred to a microcentrifuge tube containing 15 μL *E. coli* OP50-1, increasing concentrations of the test chemical (Supplemental Fig. 1), and sufficient K-media complete needed to achieve a final solution volume of 1 mL (Boyd et al., 2012). The tubes were incubated for up to 48 hours on a rocking platform. After 24 and 48 hours of exposure, nematodes were assessed for survival and body length. Survival was quantified by counting the number of alive nematodes in 10 μL of supernatant from each tube using a microscope (Olympus SZ61 zoom stereomicroscope, Olympus, Waltham, MA). Then, the nematodes in the tubes were washed 3 times with M9 solution, transferred to nematode growth media plates containing a lawn of *E. coli* OP50-1, and incubated for 48 hours at 20°C. After 48 hours, the nose touch response was measured following the established procedures (Chatzigeorgiou and Schafer, 2011) to assess the impact of pesticide exposure on neuronal health. Then the nematodes were paralyzed with 25 mM sodium azide and body length was measured using the CellSens Entry (version 3) software that was connected to the microscope. Each pesticide was tested in at least 4 concentrations. All experiments were conducted in triplicate, and relevant vehicle and blank solution controls were included; the inclusion of 1% acetonitrile in the exposure medium did not adversely impact the endpoints of the nematodes. Relative body lengths, survival, and nose-touch responses were calculated by expressing raw values as percent control, which was set to 1.

2.5 Statistical Analysis

An ANOVA with post-hoc Tukey HSD test (GraphPad Software) was used to determine statistical significance in the experimental results, with significance achieved at $p \leq 0.05$.

Each experiment was conducted in triplicate, and the resulting values were employed to compute averages, standard deviations, and produce graphical representations. The dose-response modeling of the experimental results was conducted in R (4.3.0) using the four-parameter log-logistic functional form in the drm function within the drc library (Ritz et al., 2015). The 10% effective concentration (EC_{10}), 50% effective concentration (EC_{50}), and their 95% confidence intervals were estimated using the ED function within the drc library. The delta method was used for confidence interval calculations. A lack-of-fit test was also performed to compare the four-parameter log-logistic model to the one-way ANOVA model where $p \leq 0.05$ was considered statistically significant. The fitted dose-response curves were visualized using the ggplot2 and ggprism libraries in R. In addition, the correlation and the grouping among the 3 model organism endpoints were explored with hierarchical clustering using Spearman correlation as the similarity/dissimilarity index and Ward's minimum variance method with squared distances as the linkage method (Aghayev et al., 2023; Onel et al., 2019). Prior to the clustering analysis, the dataset was checked for missing values, and any missing values were imputed using bagged regression trees via the preprocess function of the caret library. Finally, the clustering results were visualized using the pheatmap library in R.

3. Results

3.1 Toxicity Endpoints and Dosimetry Curves for 2,4,6-trichlorophenol

After exposure to 0–0.051 mM of 2,4,6-trichlorophenol for 92 hours, *H. vulgaris* polyps had a dose- and time-dependent decline in morphological scores (Fig. 1A). Exposure to ≥ 0.032 mM 2,4,6-trichlorophenol caused complete disintegration all polyps after 42 hours (score = 0), while lower doses (0.013 and 0.025 mM) had moderate toxicity to morphology and resulted in scores of 6–8. Based on the 92 hour toxicity data, the EC_{10} and EC_{50} for 2,4,6-trichlorpohneol were calculated to be 0.0090 and 0.022 mM, respectively (Fig. 1B, Table 2). Results of lack of fit testing (p = 1.2E-9) indicated the dose-response curve displayed a better fit to the ANOVA model compared to the four-parameter log-logistic function (Table 2), which may be due to the lack of model fitting to the curve in regions x ≥ 0.032 mM. Dose-response curves for *H. vulgaris* morphological scores corresponding to pentachlorophenol, lindane, diazinon, glyphosate, linuron, trifluralin, aldicarb, paraquat, and the mixture are depicted in the supplemental material (Supplemental Fig. 1). All pesticides showed a similar dose- and time- dependent effect on *H. vulgaris* morphology over the exposure period, and 92 hour EC_{10} and EC_{50} values were derived from these experiments (Table 2).

For *L. minor,* significant toxicity was observed to surface area and frond number content after exposure to ≥ 0.0025 mM 2,4,6-trichlorophenol (Fig. 2A and 2B) ($p \leq 0.05$). Among the measured endpoints, chlorophyll content showed a slightly higher sensitivity to 2,4,6-trichlorophenol exposure as a 15 ± 3% decrease in chlorophyll content was observed after exposure to 0.0013 mM for 7 days (Fig. 2C) ($p \leq 0.05$), but this concentration only inhibited the surface area and frond number by 5.8 ± 0.9% and −3 ± 7%, respectively ($p \geq 0.05$). Lack of fit testing also indicated the ANOVA model fit the dose-response curves the best ($p \leq 0.05$, Table 3). Based on the dosimetry curve (Fig. 2D), the 168 hour EC_{50} values for surface

area, frond number, and chlorophyll content were calculated to be 0.0016, 0.0019, and 0.0011 mM, respectively (Table 3). Dose-response curves for pentachlorophenol, lindane, diazinon, glyphosate, linuron, trifluralin, aldicarb, paraquat, and the mixture with *L. minor* are depicted in the supplemental material (Supplemental Fig. 2). Toxicity studies with all of the pesticides demonstrated that the toxicity was dose- and time-dependent and yielded similar 168 hour EC_{10} and EC_{50} values for surface area and frond number. However, chlorophyll content had a slightly lower EC_{10} value, compared to frond number and surface area ($p = 0.07$ and 0.23, respectively).

After 48 hours of exposure to 0.10 mM 2,4,6-trichlorophenol, nematodes had a 5.9 ± 4% and 16 ± 3% impairment to body length and behavior, respectively (Fig. 3A and 3B) ($p \leq 0.05$). However, this concentration only caused a 2.6 ± 4% decrease in nematode survival (Fig. 3C) ($p \geq 0.05$), and significant lethality was only observed in concentrations ≥ 0.25 mM. Similar to the other bioassays, toxicity was significantly higher after longer exposure durations. The 48 hour EC_{50} values for body length, behavior, and survival were calculated to be 0.18, 0.25, and 0.23 mM, respectively for 2,4,6-trichlorophenol (Table 4). Dose-response curves for body length and survival displayed good fit to the four-parameter log-logistic and ANOVA models ($p \geq 0.05$) while the nose touch data displayed a slightly better fit to the ANOVA model ($p = 0.019$) (Table 4). Dose-response curves for pentachlorophenol, lindane, diazinon, glyphosate, linuron, trifluralin, aldicarb, paraquat, and the mixture with *C. elegans* are depicted in the supplemental material (Supplemental Fig. 3).

Based on the calculated EC_{10} values, *L. minor* was the most sensitive bioassay to 2,4,6-trichlorophenol exposure, followed by *H. vulgaris* then *C. elegans.* When comparing the most sensitive endpoints for each bioassay, the chlorophyll content of *L. minor* had the lowest EC_{10} value compared to the morphology and body length of *H. vulgaris* and *C. elegans*, respectively ($p \leq 0.05$). For EC_{50}, *H. vulgaris* had the lowest values compared to the surface area and body length of *L. minor* and *C. elegans*, respectively ($p \leq 0.05$).

3.2 EC_{10} and EC_{50} Values for the Remaining Pesticides

For pentachlorophenol, the EC_{10} of the chlorophyll content of *L. minor* (0.00073 mM) was 1–2 orders of magnitude lower than the values for frond number and surface area (0.0053–0.0086 mM) and the endpoints in *H. vulgaris* and *C. elegans* (0.0018– 0.21 mM) ($p \leq 0.05$). The morphology of *H. vulgaris* had the lowest EC_{50} value (0.0019 mM), which was similar to the values obtained by the chlorophyll content of *L. minor* (0.0023 mM) ($p = 0.67$) but was 2 orders of magnitude lower than the values for the survival of *C. elegans* (0.11 mM) ($p \leq 0.05$). For lindane, the chlorophyll content of *L. minor* had the lowest EC_{10} and EC_{50} values (0.0039 and 0.0061 mM, respectively), which were 1–2 orders of magnitude lower than the values calculated for *H. vulgaris* and *C. elegans*. The morphology of *H. vulgaris* and the survival of *C. elegans* had similar EC_{10} and EC_{50} values $p = 0.82$ and 0.73, respectively).

The chlorophyll content of *L. minor* had the lowest EC_{10} value for diazinon (0.0016 mM), but it was not significantly lower than the values for the surface area or frond number of *L. minor* or the morphology of *H. vulgaris* ($p \geq 0.05$). However, the morphology of *H. vulgaris* had the lowest EC_{50} value compared to all other measured endpoints (0.055 mM). For glyphosate, *H. vulgaris* was the most sensitive bioassay with an EC_{10} value (0.00036

mM) 2–3 orders of magnitude smaller than values derived from *L. minor* and *C. elegans* experiments. However, *L. minor* had lower EC_{50} values for glyphosate compared to the other bioassays (0.034–0.054 mM vs 0.15–0.22 mM).

For linuron, the chlorophyll content of *L. minor* had the lowest EC_{10} and EC_{50} values (0.00010 and 0.00015 mM, respectively), which was 2–3 orders of magnitude lower than the values derived from *H. vulgaris* and *C. elegans* dosimetry curves. *C. elegans* was the least sensitive to linuron and had EC_{50} values for its endpoints between 0.15–0.65 mM. Similarly, *L. minor* had the lowest EC_{10} and EC_{50} values that were several orders of magnitude smaller than the other bioassays after exposure to trifluralin. Specifically, the chlorophyll content of *L. minor* had an EC_{10} of 0.000042 mM, compared to 0.0057 mM and 0.19 mM for the morphology of *H. vulgaris* and nose touch response of *C. elegans*, respectively ($p \leq 0.05$).

The morphology of *H. vulgaris* had the lowest EC_{10} and EC_{50} after aldicarb exposure (0.0043 and 0.0046 mM, respectively) compared to the other bioassays. Interestingly, the surface area of *L. minor* and nose touch of *C. elegans* are 4 orders of magnitude less sensitive to aldicarb exposure with EC_{50} values of 11 and 9.6 mM, respectively. For the mixture, the chlorophyll content of *L. minor* had the lowest EC_{10} value (0.00013 mM), compared to 0.0014 and 0.037 mM for the morphology of *H. vulgaris* and nose touch of *C. elegans*, respectively.

3.3 Correlations Between Bioassays

The distribution of EC_{10} and EC_{50} values of individual pesticides and the mixture for *L. minor* (Fig. 4A and 4C) and *C. elegans* (Fig. 4B and 4D). *L. minor* was more sensitive to the tested compounds and mixture, indicated by the lower EC_{10} and EC_{50} values. *L. minor* had a greater range of EC_{10} (10^{-4}–10^{0} mM) and EC_{50} (10^{-4}–10^{0} mM) values overall. Effective concentration values for each pesticide are distributed throughout the concentration range and do not overlap significantly. However, *C. elegans* displayed high variability in EC_{10} and EC_{50} values for each individual pesticide, and the range of EC_{10} and EC_{50} values were between 10^{-2}–10^{0} mM and 10^{-1}–10^{1} mM, respectively.

When comparing EC_{10} values, *L. minor* was the most sensitive species to 7 individual pesticides and the mixture, and *H. vulgaris* was the most sensitive species to the remaining 2 pesticides (aldicarb and glyphosate). For EC_{50}, *L. minor* had the lowest values for 5 individual pesticides and the mixture, and *H. vulgaris* had the lowest for the remaining 4 compounds (2,4,6-trichlorophenol, pentachlorophenol, diazinon, and aldicarb). *C. elegans* consistently had the highest EC_{10} and EC_{50} values for all the tested compounds.

Importantly, the toxicity results indicated that some endpoints within a single bioassay were more sensitive to chemical exposure than the others; for example, the chlorophyll content of *L. minor* had the lowest EC_{10} and EC_{50} values for 8 and 5 chemicals, respectively. For *C. elegans*, survival rate had the lowest EC_{10} and EC_{50} values for 4 and 5 pesticides, respectively. The distribution of EC_{10} and EC_{50} values for all pesticides across the most sensitive endpoint for *L. minor* and *C. elegans* is shown in Fig. 4A and 4B. These results demonstrated that *L. minor* has a greater range of EC_{10} and EC_{50} values for the tested pesticides and mixture compared to *H. vulgaris* and *C. elegans.* When comparing the most

sensitive endpoints, *L. minor* generally had the lowest EC_{10} and EC_{50} values for the tested chemicals, followed by *H. vulgaris*, and *C. elegans.*

The relationship between the EC_{10} and EC_{50} values for each bioassay's endpoints after chemical exposures was explored using clustering analysis coupled with dissimilarity matrices (Fig. 6A and 6B) based on the Spearman correlation coefficient. For EC_{10} values, the endpoints were closely clustered into 3 distinct groups that correspond to each bioassay. The decreased distance between pairs of features (< 1), such as frond number-chlorophyll content (0.15) reflects the positive correlation between the features. Increased distance between features (> 1), such as body length morphology (1.73), indicates the strong negative association between the endpoints. The endpoints measured in *L. minor* were the most tightly clustered, with distances of 0–0.15. While the 3 endpoints measured in *C. elegans* were also clustered together, they had a higher pair-wise distance (0.47–0.74). Values derived from *H. vulgaris* are negatively correlated with the endpoints from the other bioassays (distance > 1), except for the weak positive correlation with the survival of *C. elegans* (0.81). For EC_{50} values, the endpoints of each bioassay were clustered into 2 main clades, where *H. vulgaris* and *C. elegans* were clustered in the same clade and *L. minor* was clustered alone (Fig. 6B). Additionally, the overall similarity of endpoints in the sample bioassay decreased. For example, the distances between the endpoints of *L. minor* and *C. elegans* increased to 0.01–0.19 and 0.59–0.90, respectively. Interestingly, the distance between the morphology of *H. vulgaris* and the other measured endpoints decreased slightly and was negatively correlated with the surface area of *L. minor*.

3.4 Correlation Between Pesticides

Fig. 7A and 7B depict the relationship between the EC_{10} and EC_{50} values for all the pesticides according to the Spearman correlation method. For EC_{10} values, 2 main clades were created from the associated dendrogram (Fig. 6A). In the first clade, glyphosate, paraquat, pentachlorophenol, trifluralin, and the mixture were clustered together. In clade 2, diazinon and aldicarb had the highest dissimilarity from the other pesticides (distance 0.5–1.04 and 0.43–0.86, respectively). When categorized by chemical class, there was no direct relationship with the overall cluster structure. A slight relationship between target organism and cluster structure was observed as 3 herbicides and 1 insecticide where clustered in clade 1, and 3 insecticides and 2 herbicides were clustered in clade 2. The mixture had high similarity between most of the compounds (distance = 0.04–0.32), except for diazinon and aldicarb, where the distance was 0.82 and 0.64, respectively.

For EC_{50} values, the clustering structure also yielded 2 primary clades, where diazinon and aldicarb were strongly clustered away from the other pesticides (Fig. 6B). Diazinon had the highest dissimilarity from the other pesticides (distances = 0.96–1.68). Similar to the EC_{10} results, no clear relationship between the clustering and chemical class was observed. However, several of the chemicals with the same target organism were tightly clustered together and had low distances. For example, insecticides diazinon and aldicarb were consistently clustered closely and had a distance of 0.32. Pairs of herbicides, such as 2,4,6-trichlorohenol-glyphosate and trifluralin-paraquat were closely clustered together and

had pair-wise distances of 0.04. The mixture also displayed high similarity to most of the compounds, except for diazinon and aldicarb (distance 1.68 and 1.07, respectively).

4. Discussion

4.1 Toxicity of Individual Pesticides

The main mode of action for 2,4,6-trichlorophenol toxicity is through acetylcholinesterase inhibition (Matsumura et al., 1997), which causes a variety of effects in plants and animals. In plants, acetylcholinesterase plays a vital role in water retention and photosynthesis (Wessler et al., 2001); in animals, it is essential for neuronal communication (Devi et al., 2023; Matsumura et al., 1997). This mode of action explains the increased sensitivity of *L. minor* to 2,4,6-trichlorophenol exposure for all measured endpoints (Fig. 2 A–D) as well as the rapid deterioration of the nose touch response observed in *C. elegans* at high concentrations (Fig. 3B). The EC_{10} and EC_{50} values derived for *H. vulgaris* and *L. minor* in the current study are in accordance with values for other aquatic organisms, like *Scenedesmus obliquus* (a green microalgae) and *Daphnia magna* (a crustacean) (Xing et al., 2012). Pentachlorophenol inhibits ATP-ases and can produce reactive oxygen species (Maheshwari et al., 2023). Plants have previously been shown to be more sensitive to pentachlorophenol than other organisms due to the inhibition of photosynthesis caused by exposure (Huber et al., 1982; Repetto et al., 2001). The EC_{50} values for the endpoints in *L. minor* and *C. elegans* calculated in the current study are in accordance with published values for these bioassays (Huber et al., 1982; Kammenga et al., 1994; Repetto et al., 2001) and other hydra species (Silva et al., 2001). Because the number of chlorinated substitutions contributes to the toxicity of the compound, EC_{10} and EC_{50} estimates for all the bioassays were lower for pentachlorophenol than 2,4,6-trichlorophenol (Freitag et al., 1994).

Lindane is a legacy organochlorine insecticide that was banned under the Stockholm Convention due to concerns over its toxicity and environmental persistence (Nolan et al., 2012). While there is no main mode of action for insects, the neurotoxicity of lindane is among the most concerning effects after exposures (Nolan et al., 2012), which explains the lower values of EC_{10} and EC_{50} for the nose touch response of *C. elegans* compared to the body length (Yu et al., 2022). *L. minor* was 2 orders of magnitude more sensitive to lindane compared to the other models, likely because of the impact of lindane on plant development and photosynthesis (Pereira et al., 2010). Diazinon is an organophosphate insecticide that has been previously shown to cause toxicity to aquatic species after the contamination of waters (Bailey et al., 2000). The main mode of action is the inhibition of acetylcholinesterase (Velki et al., 2017). Previous studies have also demonstrated that significant toxicity to aquatic species was observed at levels at and below the recommended application doses (Natal-da-Luz et al., 2012), highlighting the importance of investigating the toxicity of low doses to more species. Interestingly, *L. minor*, *H. vulgaris*, and *C. elegans* were similarly susceptible to diazinon toxicity when comparing EC_{10} estimates.

Glyphosate is one of the most commonly used broad-spectrum organophosphate herbicides worldwide that inhibits the biosynthesis of essential amino acids. Specifically, it targets 5-enolpyruvylshikimate-3-phosphate synthase and causes a decrease in plant growth and survival (Gill et al., 2018; Singh et al., 2020). *H. vulgaris* and *L. minor* were sensitive

to glyphosate exposure, likely because aromatic amino acids also play a key role in the health of these species. While *C. elegans* can obtain amino acids from their bacterial food source (Zečić et al., 2019), *L. minor* and *H. vulgaris* mainly rely on the biosynthesis of these molecules to survive, increasing their sensitivity to glyphosate exposure (Trovato et al., 2021). The increased sensitivity of aquatic species and non-target plant species to this compound highlight the importance of using diverse species to understand the environmental impact of chemicals classified as herbicides. Linuron is a phenylurea herbicide that directly decreases the photosynthetic ability of plants by inhibiting the function of the electron transport chain (Maharaj et al., 2020; Santos et al., 2014), which explains why *L. minor* was several orders of magnitude more sensitive to exposure compared to the other bioassays. Importantly. The EC_{50} values for *L. minor* (Gatidou et al., 2015) and *C. elegans* (Zöngür and Sari, 2023) are in accordance with previously published values. Importantly, the EC_{10} values derived for *L. minor* are an order of magnitude lower than levels applied to fields (Durand and Barcelo, 1992), demonstrating the possibility of non-target plant toxicity after applications.

Trifluralin is a dinitroaniline herbicide that inhibits microtubule formation during mitosis in plants (Coleman et al., 2020), but is known to impact aquatic species through the same mode of action (Fernandes et al., 2013). *L. minor* was the most sensitive species to exposure, followed by *H. vulgaris*, and *C. elegans*. The EC_{50} value for *H. vulgaris* was similar to those derived for an aquatic larval frog species, *Lithobates clamitans* (Weir et al., 2012). Perhaps the inclusion of endpoints that are related to mitotic processes in nematodes, like the quantification of offspring (Sant'anna et al., 2016), would have demonstrated a higher sensitivity than the measured endpoints in *C. elegans*. However, the EC_{10} values for all the bioassays were higher than levels reported in soils in cotton fields (3.2–6.0 ppb or 8.9–18 nM) (Li et al., 2021).

Aldicarb is a broad-acting carbamate insecticide that inhibits acetylcholinesterase (Blacker et al., 2010), like 2,4,6-trichlorophenol. However, it has been suggested that these effects are not observed at human or environmentally-relevant exposure levels and are rapidly reversible (Blacker et al., 2010). The low toxicity observed to *L minor* and *C. elegans* in general may be due to the high water solubility and low logP of aldicarb that decreases the baseline toxicity (Mayer and Reichenberg, 2006). Additionally, aldicarb is rapidly metabolized in the environment and in animals to less toxic intermediates (Blacker et al., 2010); since *H. vulgaris* is metabolically inactive, this detoxification process could be inhibited, leading to the increased toxicity that was observed.

Paraquat is a viologen and bipyridinium herbicide that inhibits photosynthesis and promotes the formation of reactive oxygen species in plants and animals (Blanco-Ayala et al., 2014). This mode of action explains the increased sensitivity of *L. minor* to exposure compared to the other bioassays. Previous studies have also demonstrated that among other tested pesticides, paraquat was among the most toxic compound to *L. minor* (Tagun and Boxall, 2018). *H. vulgaris* was more sensitive to exposure than the average *C. elegans* response, likely because quaternary ammonium compounds are known to have high toxicity to aquatic species compared to soil organisms (Blanco-Ayala et al., 2014; Zhang et al., 2015).

Importantly, levels of paraquat detected in surface waters (Thi Hue et al., 2018) were above estimated EC_{10} and EC_{50} values derived for *H. vulgaris* and *L. minor*.

Chemical mixtures have previously been shown to have toxicities different from the constituent parts due to additive, synergistic, or antagonistic activity between the individual chemicals (Bart et al., 2022; Hernández et al., 2013; Hernando et al., 2003; Rider and Simmons, 2018; Wang et al., 2021). For *H. vulgaris* and *L. minor*, the pesticide mixture had one of the lowest EC_{10} and EC_{50} values across all the tested chemicals. However, it was not the most toxic substance evaluated according to EC_{50} values, either because of dilution effects or antagonism between the individual pesticides (Hernando et al., 2003; Wang et al., 2021; Zhang et al., 2018). However, the toxicity testing of chemical mixtures is important because the mixture toxicity is difficult to predict (Deneer, 2000).

4.2. Correlation Between Bioassays

Using a set of bioassays that covers diverse ecological niches, levels, and endpoints is advantageous for understanding the toxicity of individual compounds and mixtures (Hernando et al., 2003; Repetto, 2013). The current set of bioassays combined organisms that have standardized toxicity testing guidelines (EPA, 2009; Pollino and Holdway, 1999; Queirós et al., 2019) and have been individually employed previously for the assessment of other pesticides, wastewaters, sediments, polycyclic aromatic hydrocarbons, and complex chemical mixtures (Gatidou et al., 2015; Graves et al., 2005; Höss et al., 2009; Taraldsen and Norberg-King, 1990).

Fairchild et. al utilized *Lemna minor* and *Selenastrum capricornutum* (a green algae) to characterize the toxicity of several herbicides and found that both species were equally sensitive to exposure (Fairchild et al., 1997). The inclusion of addition of *Myriophyllum aquaticum* (a rooted macrophyte) in the bioassay battery was suggested to expand the toxicity assessment to more sensitive aquatic plant species. When exposed to atrazine in aqueous medium, *L. minor* was shown to be more sensitive than the aquatic species *D. magnia* (Klementová et al., 2019) but had comparable results to *M. aquaticum* (Teodorović et al., 2012). *M. aquaticum* was more sensitive to atrazine than *L. minor* when both species were exposed to contaminated sediments, likely because of the roots present on *M. aquaticum* that promote direct contact with the sediment. Because the herbicides in the present study were exposed to the bioassays by spiked aqueous mediums, *L. minor* was likely more sensitive than *M. aquaticum* may have been (Park et al., 2021).

H. vulgaris has been shown to be a more sensitive species to the pesticide 4-chlorophenol than *Hydra viridissima*, but was less sensitive than the fish species *Pimephales promelas* and *Leepomis macrochirus* (Pollino and Holdway, 1999). Consistent with previous studies (Lui and Wrischer, 2002), *H. vulgaris* was the most sensitive species to insecticides and rapid deterioration of the tentacles and morphological scores were observed after exposure, which is consistent with the sensitivity of this species to aldicarb, diazinon, and pentachlorophenol observed in the current study. The inclusion of *H. vulgaris* in toxicity testing is important as these species play an important role in their ecosystem and may be used as an early indicator of pollution in the environment (Beach and Pascoe, 1998; Pollino and Holdway, 1999).

Pesticides are among the most frequently used chemicals for toxicity testing with *C. elegans* (Queirós et al., 2019), and has been shown to produce LD_{50} values that are correlated with oral LD_{50} in rats, mice, and rabbits (Boyd et al., 2012; Hunt, 2017). *C. elegans* also displayed high correlation with zebrafish in 59–79% of tested chemicals in previous studies (Boyd et al., 2012). While less commonly reported to be used in a biological battery, *C. elegans* play an important role in the current study to describe ecological and mammalian toxicity caused by pesticides (Cole et al., 2004; Hunt, 2017).

Interestingly, each bioassay had an endpoint that was more sensitive to pesticide exposure. For *L. minor,* chlorophyll content generally was more sensitive to pesticide exposure compared to surface area and frond number. This may be due to the role of chlorophyll degradation in the plant senescence cycle where chlorophylls are broken down to release nutrients and promote plant viability (Lisiewska et al., 2006). Therefore, the loss of chlorophyll may be an earlier and more sensitive sign of plant toxicity compared to overall surface area. This finding is in accordance with previous studies that demonstrate chlorophyll content is a more sensitive endpoint than frond number for pesticide exposure (Fekete-Kertész et al., 2015). The close clustering of the *L. minor* endpoints was likely because these endpoints are more related to each other than the group of endpoints measured in *C. elegans*. For example, plants with high surface area are more likely to have a higher abundance of fronds and chlorophylls as these endpoints contribute to the overall growth of the plants.

For *C. elegans*, survival rate had the lowest EC_{50} values for most of the pesticides compared to body length and nose touch response. The variation in the dose-response curves for *C. elegans* is larger than those observed in *L. minor* and *H. vulgaris,* possibly due to the endpoints of *C. elegans* corresponding to different mechanisms of toxicity, i.e. survival, growth, and neurological system (Anderson et al., 2001). Nematodes that show sensitivity to nose touch assay may not have growth stunting as neurological endpoints are not shown to be related to growth outcomes directly (Anderson et al., 2001). Therefore, varying endpoints reflecting different mechanisms of action are needed as broad-range indicators for chemical mixtures and environmental samples. Interestingly, lethality endpoints have been shown to be correlative with behavioral and locomotive endpoints (Anderson et al., 2001). This also reflects the wide array of endpoints available in the *C. elegans* model and implemented in the current study to detect toxicity to a variety of different endpoints and biological systems. The varying degrees of clustering and correlation between the endpoints of all organisms demonstrate the need to include different types of model organisms in the toxicity analysis of chemicals and design mixtures to better describe the hazards.

C. elegans consistently ranked the least sensitive to all chemicals among the 3 bioassays. The addition of nematicides in future studies may reveal an increased sensitivity of the nematode model compared to the plant and aquatic models in a set of pesticides. Additionally, it has been previously shown that nematode species may be more sensitive to chemical exposures conducted in solid-phase mediums (soils and sediments) compared to the aqueous phase (Kim et al., 2020). However, conducting the testing in an aqueous medium is advantageous as the chemical is dissolved and freely available, whereas it may be sequestered by organic matter in soil matrices (Sarkar et al., 2021).

5. Conclusions

Because pesticides can ubiquitously occur in soil and aquatic environments and pose a risk to target and non-target organisms in the environment, using a set of bioassays that incorporates environmentally-relevant species to characterize toxicity is important. In the current study, we used a novel set of living organisms that are representative of important environmental niches containing plant, aquatic, and soil species to compare the toxicities of 9 individual pesticides and a mixture. Each bioassay displayed varying sensitivity to the individual pesticides and the mixture *L. minor* had the lowest EC_{10} and EC_{50} values for most of the individual pesticides tested, but these values may not be representative of non-target or more resistant species' responses to these compounds. More importantly, the most sensitive endpoint of each bioassay displayed distinct but slightly overlapping EC_{10} and EC_{50} ranges for all of the chemicals tested. These representative bioassays can be used in combination to characterize the toxicity of individual pesticide exposure to living organisms and predict the toxicity of real-life environmental samples and the prioritization of contaminated sites for further chemical analysis and remediation.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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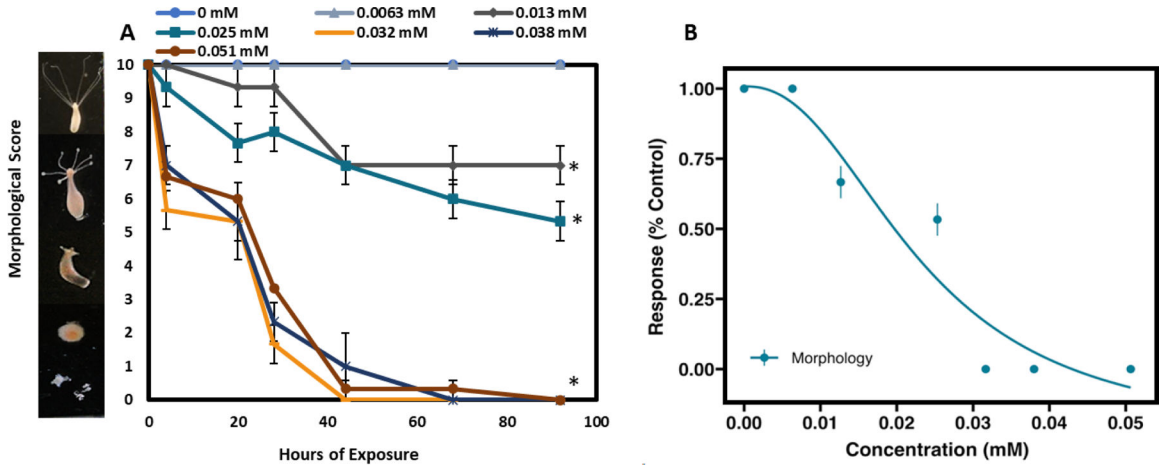

Figure 1:

Toxicity of 2,4,6-trichlorophenol as shown by the morphological scores of H. vulgaris (A) and the corresponding dose-response curve at 92 hours of exposure (B). Data represent the average value from triplicate analysis ± the standard deviation. * Indicates a significant difference ($p \leq 0.05$) from the vehicle control group after 92 hours of exposure.

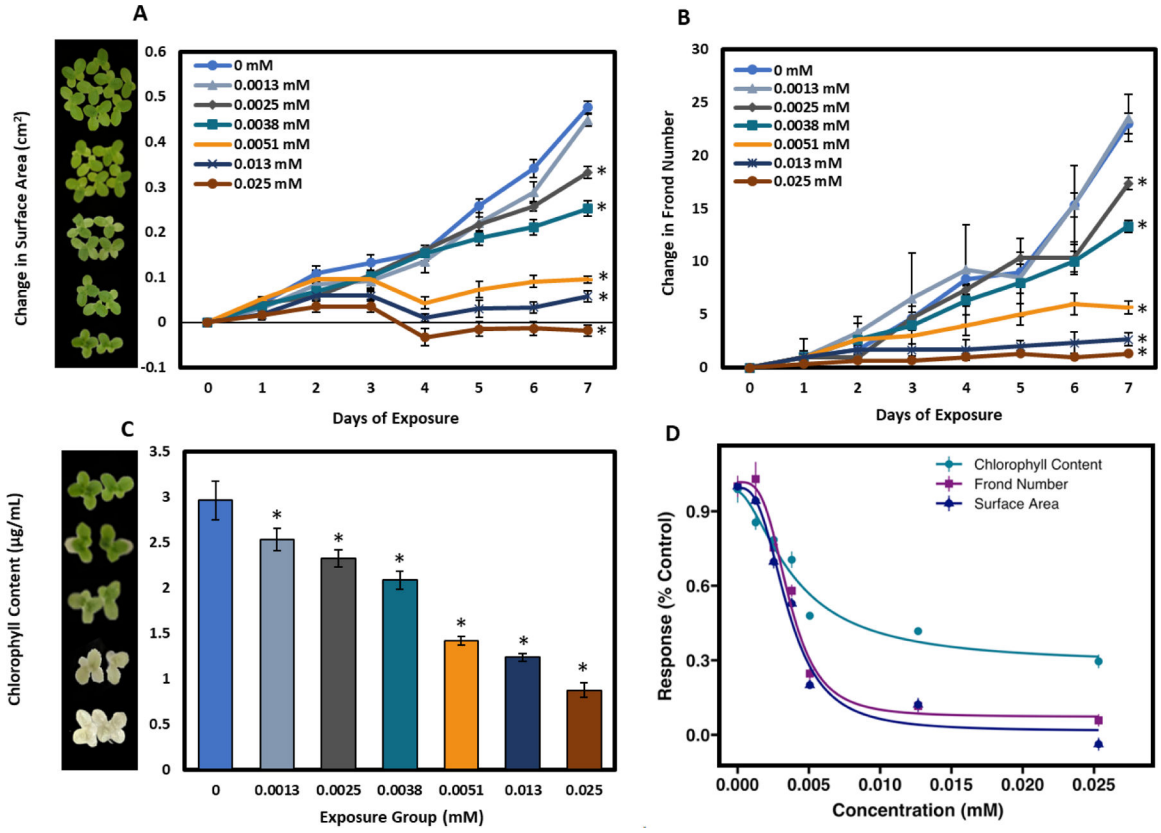

Figure 2:

Toxicity of 2,4,6-trichlorophenol on the surface area (A), frond number (B), and chlorophyll content (C) during a 7 day exposure. Dose-response curves of the 7 day toxicity data for all 3 measured endpoints (D). Data represent the average value from triplicate analysis ± the standard deviation. * Indicates a significant difference ($p \leq 0.05$) on day 7 of exposure compared to the vehicle control group.

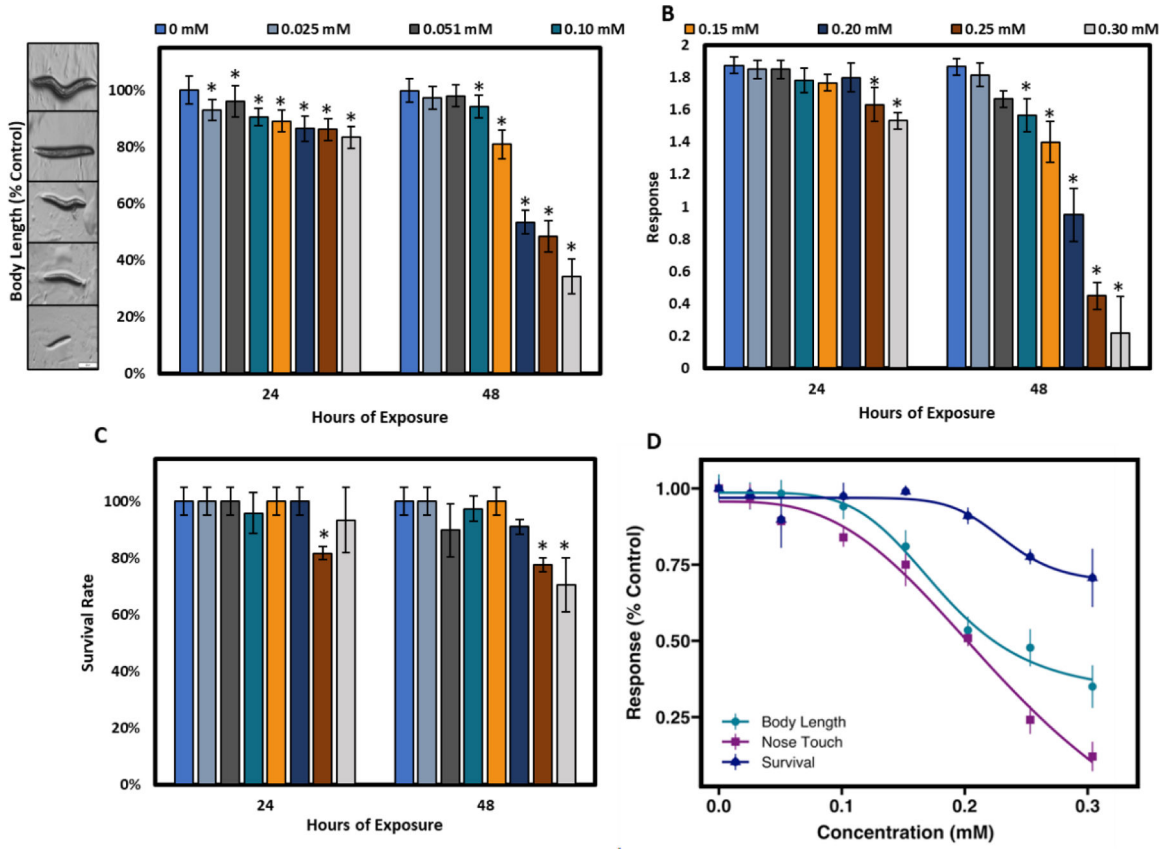

Figure 3:

Toxicity of 2,4,6-trichlorophenol on the body length (A), nose touch response (B), and survival rate (C). The 48 hour toxicity data was used to create the dose-response graphs for the 3 endpoints (D). Data represent the average value from triplicate analysis ± the standard deviation. * Indicates a significant difference ($p \leq 0.05$) after 48 hours of exposure compared to the vehicle control group.

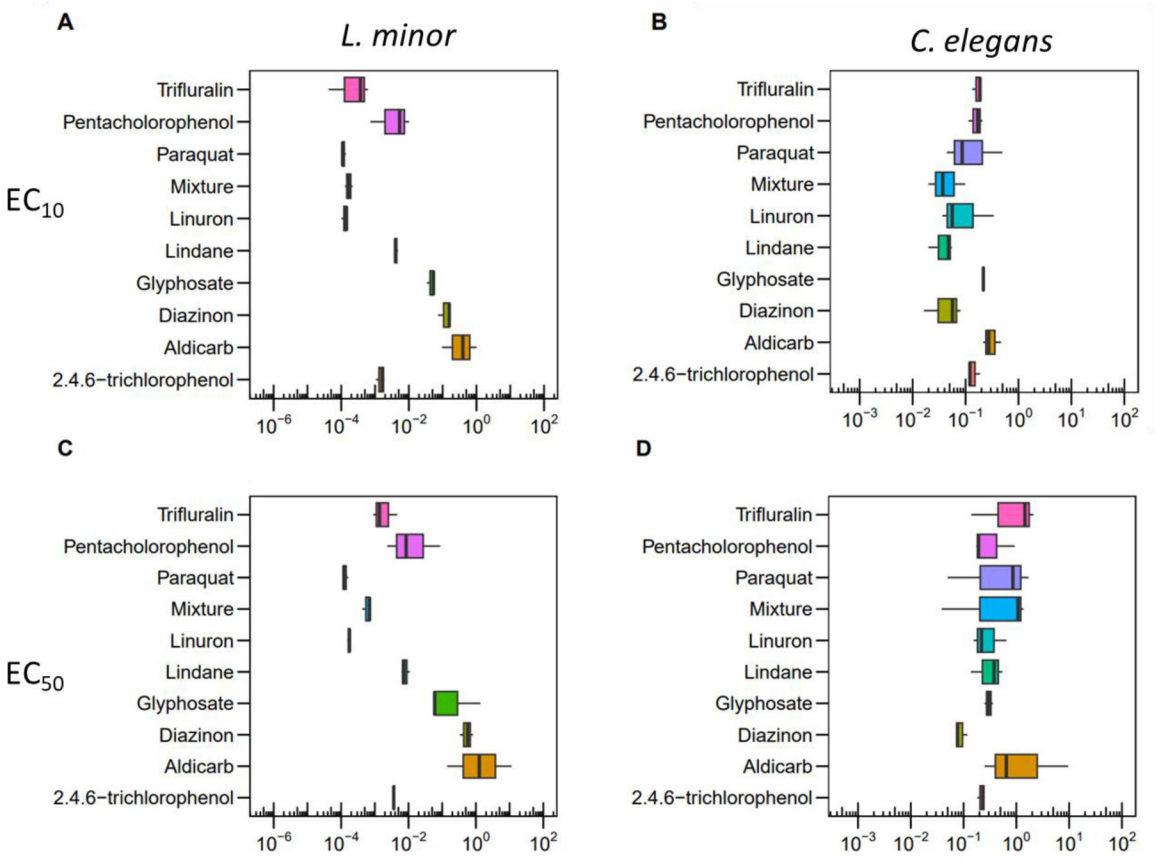

Figure 4:

Distribution of EC_{10} (A-B) and EC_{50} (C-D) values (mM) for each pesticide between the measured endpoints for *L. minor* and *C. elegans.*

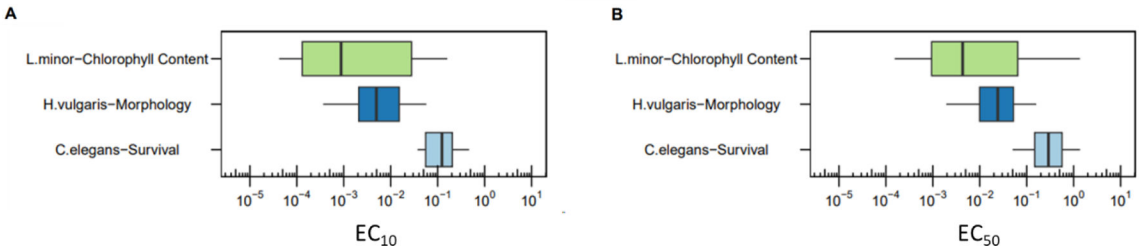

Figure 5:

Distribution of EC_{10} (A) and EC_{50} (B) (mM) values across all tested pesticides for the most sensitive endpoint in each bioassay.

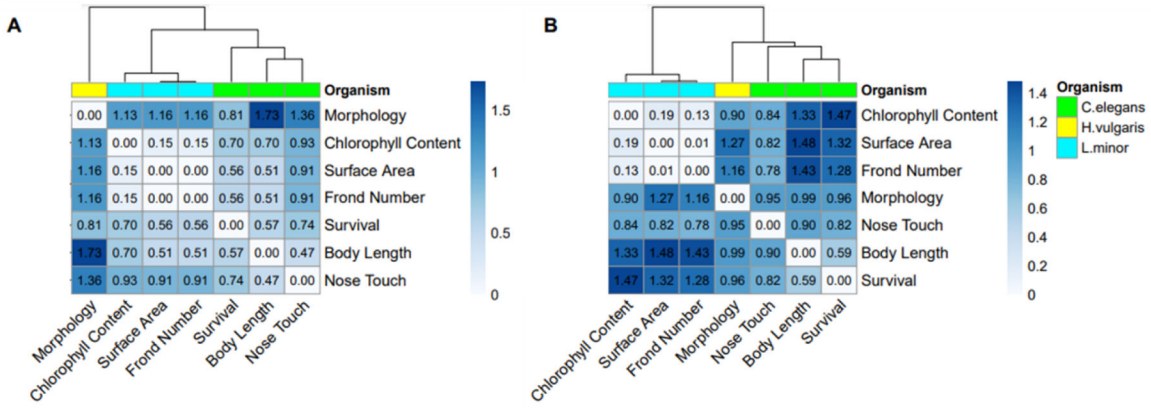

Figure 6:

Clustering heatmap with distance matrix and dendrograms depicting the relationships between the measured endpoints for all model organisms based on their EC_{10} (A) and EC_{50} (B) values for all chemical exposures based on the Spearman correlation coefficient.

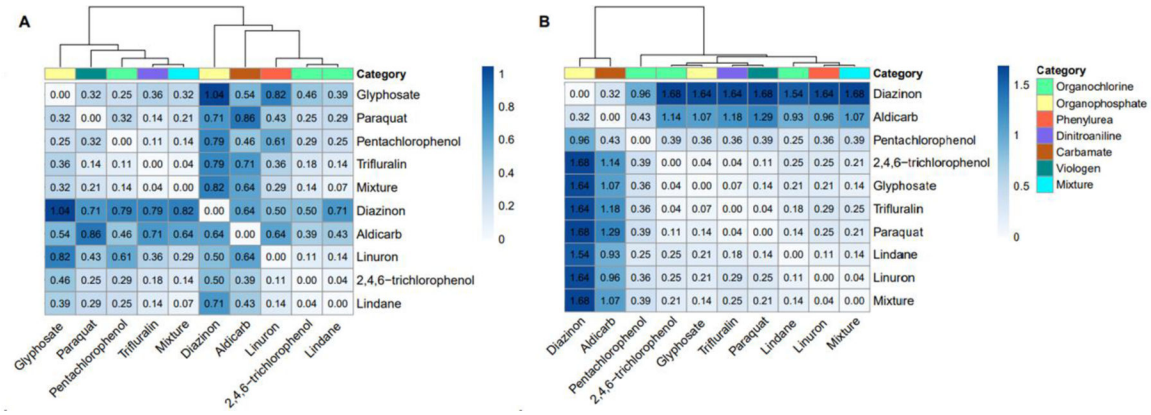

Figure 7:

Clustering heatmap with distance matrix and dendrograms depicting the comparison of EC_{10} (A) and EC_{50} (B) values of all the tested pesticides and mixture according to the Spearman correlation coefficient.

Table 1:

List of the test chemicals with their chemical class, ranking on the SPL, and important physiochemical properties.

Chemical Class	Chemical	CAS #	SPL Rank	LogP	MW	Solubility	Target
Organochlorine	2,4,6-trichlorophenol	88-06-2	87	3.7	197.4	800	Herbicide
	Pentachlorophenol	87-86-5	54	5.1	266.3	14	Insecticide
	Lindane	58-89-9	34	3.8	290.8	2	Insecticide
Organophosphate	Diazinon	333-41-5	40	3.8	304.4	40	Insecticide
	Glyphosate	1071-83-6	N.L.	−4.6	169.1	1050	Herbicide
Phenylurea	Linuron	330-55-2	N.L.	3.2	249.1	75	Herbicide
Dinitroaniline	Trifluralin	1582-09-8	162	5.3	335.3	0.22	Herbicide
Carbamate	Aldicarb	116-06-3	N.L.	1.1	190.3	>4900	Insecticide
Viologen	Paraquat	4685-14-7	N.L.	1.7	186.3	>6.2E5	Herbicide

SPL: substance priority list, N.L. not listed, MW: molecular weight (g/mol), solubility in water (mg/L) (at neutral pH and 20–25°C).

Table 2:

Summary of the 92 hour EC_{10} and EC_{50} values (mM), the 95% confidence intervals in brackets, and the results of ANOVA (*p*) fit testing derived for 9 individual compounds and the mixture in *Hydra vulgaris*.

Pesticides		Morphology	*p*
2,4,6-trichlorophenol	EC_{10}	0.0090 (0.0038–0.014)	1.2E-9
	EC_{50}	0.022 (0.015–0.031)	
Pentachlorophenol	EC_{10}	0.0018 (0.00044–0.0032)	1.5E-7
	EC_{50}	0.0019 (0.00040–0.0035)	
Lindane	EC_{10}	0.018 (0.016–0.020)	2.4E-6
	EC_{50}	0.022 (0.016–0.025)	
Diazinon	EC_{10}	0.032 (0.026–0.039)	2.4E-6
	EC_{50}	0.055 (0.044–0.066)	
Glyphosate	EC_{10}	0.00036 (0–0.0043)	0.15
	EC_{50}	0.16 (0–3.5)	
Linuron	EC_{10}	0.057 NA	1E-6
	EC_{50}	0.063 NA	
Trifluralin	EC_{10}	0.0057 (0.0049–0.0065)	0.18
	EC_{50}	0.010 (0.0095–0.011)	
Aldicarb	EC_{10}	0.0043 (0.0042–0.0044)	0.91
	EC_{50}	0.0046 (0.0042–0.0047)	
Paraquat	EC_{10}	0.0031 (0.00090–0.0053)	0.10
	EC_{50}	0.044 (0–0.10)	
Mixture	EC_{10}	0.0014 (0.00058–0.0022)	0.0029
	EC_{50}	0.0099 (0.0039–0.016)	

Table 3:

Summary of the 168 hour (7 day) EC_{10} and EC_{50} values (mM), 95% confidence intervals in brackets, and the results of ANOVA (*p*) fit testing derived for 9 individual compounds and the mixture in *Lemna minor*.

Pesticides		Surface Area	*p*	Frond Number	*p*	Chlorophyll Content	*p*
2,4,6-trichlorophenol	EC_{10}	0.0016 (0.0011–0.0021)	5.8E-8	0.0019 (0.0015–0.0023)	0.0010	0.0011 (0.00030–0.0018)	0.00014
	EC_{50}	0.0035 (0.0032–0.0040)		0.0036 (0.0033–0.0040)		0.0040 (0.0028–0.0052)	
Pentachlorophenol	EC_{10}	0.0086 (0–0.031)	0.00022	0.0053 (0.0037–0.0070)	2.2E-5	0.00073 (0.00027–0.0012)	0.00026
	EC_{50}	0.088 (0–0.35)		0.0084 (0.0033–0.014)		0.0023 (0.0013–0.0034)	
Lindane	EC_{10}	0.0040 (0.0021–0.0059)	0.78	0.0048 (0.0036–0.0060)	0.41	0.0039 (0.0016–0.0061)	0.18
	EC_{50}	0.011 (0.0066–0.015)		0.0073 (0.0067–0.0079)		0.0061 (0.0045–0.0078)	
Diazinon	EC_{10}	0.031 (0–0.12)	0.11	0.030 (0–0.140)	0.87	0.016 (0–0.071)	0.0075
	EC_{50}	0.57 (0–2.6)		0.33 (0–1.9)		0.26 (0–1.2)	
Glyphosate	EC_{10}	0.053 (0.036–0.071)	0.057	0.034 (0.022–0.046)	0.98	0.054 (0–0.30)	2.7E-7
	EC_{50}	0.060 (0.057–0.062)		0.057 (0.048–0.066)		0.094 (0.046–0.143)	
Linuron	EC_{10}	0.00014 (0.00013–0.00016)	0.28	0.00015 (0.00013–0.00016)	0.079	0.00010 (0.000069–0.00013)	2.7E-6
	EC_{50}	0.00018 (0.00017–0.00019)		0.00018 (0.00017–0.00019)		0.00015 (0.00013–0.00018)	
Trifluralin	EC_{10}	0.00062 (0–0.0014)	0.0074	0.00037 (0–0.00075)	0.0025	0.000042 (0–0.00015)	0.00014
	EC_{50}	0.0014 (0.00044–0.0023)		0.0018 (0–0.019)		0.0046 (0–0.039)	
Aldicarb	EC_{10}	1.0 (0–3.9)	0.16	0.40 (0.28–0.52)	0.59	0.096 (0.017–0.17)	0.12
	EC_{50}	11 (0–51)		1.26 (0.62–1.89)		0.11 (0.052–0.17)	
Paraquat	EC_{10}	0.000098 (0.000060–0.000013)	0.064	0.000090 (0.000080–0.000010)	0.050	0.00014 (0.00012–0.00016)	0.00074
	EC_{50}	0.00011 (0.00010–0.00013)		0.00012 (0.00012–0.00013)		0.00016 (0.00013–0.00019)	
Mixture	EC_{10}	0.00022 (0.00013–0.00031)	8.2E-5	0.00017 (0.000062–0.00027)	6.0E-5	0.00013 (0.000065–0.00019)	0.0057
	EC_{50}	0.00070 (0.00046–0.00095)		0.00042 (0.00017–0.00067)		0.00070 (0.00036–0.0010)	

Table 4:

Summary of the 48 hour EC_{10} and EC_{50} values (mM), 95% confidence intervals in brackets, and the results of ANOVA (*p*) fit testing derived for 9 individual compounds and the mixture in *Caenorhabditis elegans*.

Pesticides		Body Length	*p*	Nose Touch	*p*	Survival	*p*
2,4,6-trichlorophenol	EC_{10}	0.12 (0.094–0.14)	0.33	0.12 (0.11–0.15)	0.019	0.19 (0.15–0.23)	0.17
	EC_{50}	0.18 (0.16–0.21)		0.25 (0.12–0.38)		0.23 (0.20–0.27)	
Pentachlorophenol	EC_{10}	0.21 (0–0.98)	4.2E-5	0.17 NA	9.7E-7	0.11 (0.099–0.13)	0.21
	EC_{50}	0.18 (0–0.96)		0.19 NA		0.17 (0.14–0.21)	
Lindane	EC_{10}	0.048 (0–0.21)	0.005	0.020 (0–0.051)	0.082	0.055 (0–0.45)	0.0001
	EC_{50}	0.54 (0–2.7)		0.14 (0–2.3)		0.038 (0-.35)	
Diazinon	EC_{10}	0.016 (0.0083–0.024)	0.011	0.080 (0.068–0.093)	0.0001	0.057 (0.052–0.061)	0.39
	EC_{50}	0.077 (0.0073–0.15)		0.12 (0.054–0.18)		0.071 (0.068–0.075)	
Glyphosate	EC_{10}	0.22 (0.13–0.31)	0.74	0.15 NA	NA	0.21 NA	8.2E-10
	EC_{50}	0.25 (0.12–0.38)		1.0 NA		0.36 NA	
Linuron	EC_{10}	0.036 (0.0030–0.070)	0.040	0.056 (0.029–0.083)	0.005	0.29 (0–1.3)	0.64
	EC_{50}	0.22 (0–64)		0.15 (0.078–0.23)		0.65 (0–3.0)	
Trifluralin	EC_{10}	0.19 (0–1.2)	0.014	0.19 (0–0.59)	0.00015	0.13 (0.13–0.14)	2.2E-5
	EC_{50}	0.18 (0.16–0.21)		0.22 (0.12–0.32)		0.14 (0.11–0.17)	
Aldicarb	EC_{10}	0.22 (0–0.62)	0.33	0.27 (0–1.6)	0.21	0.47 (0.29–0.65)	0.99
	EC_{50}	0.25 (0.14–0.35)		9.6 (0–70)		0.65 (0.50–0.80)	
Paraquat	EC_{10}	0.086 (0–0.63)	0.68	0.50 (0–1.3)	0.00065	0.044 (0–0.21)	0.35
	EC_{50}	1.7 (0–14)		0.86 (0–2.5)		0.050 (0–0.22)	
Mixture	EC_{10}	0.099 (0–0.75)	0.075	0.020 (0.012–0.028)	0.51	0.037 (0–0.23)	0.00014
	EC_{50}	1.07 (0–8.7)		0.038 (0.028–0.049)		1.36 (0–9.2)	