Acute Exposure of Deltamethrin and Chlorpyrifos on the Locomotion of Caenorhabditis elegans

Tania Thakur & Melinda Pomeroy-Black

Faculty Sponsor: Dr. Melinda Pomeroy-Black

Department: Biology

Abstract

Deltamethrin and chlorpyrifos are pesticides that have adverse effects on the environment. They are used by a wide variety of farmers for agriculture. *Caenorhabditis elegans* (*C. elegans*) are soil nematodes that serve as a good model organism for locomotion tests because their nervous system is similar to humans' nervous systems. The hypothesis stated that increasing concentrations of chlorpyrifos and deltamethrin will cause a decrease in the locomotion of *C. elegans*. The *C. elegans* were exposed to 0.1 µM, 1.0 µM, or 2.5 µM chlorpyrifos and 0.1 µM, 1.0 µM, or 10 µM deltamethrin. The front body bends of adult *C. elegans* were counted immediately after the treatment (0 hour) and at time points of 24, and 48 hours post-treatment. There was a significant decrease between the control group and the worms treated with either chlorpyrifos or deltamethrin ($p<0.001$). In addition, there was a significant difference between each time point ($p<0.001$). Significant changes in locomotion were somewhat dependent on the compound, as well. Moreover, there was also a significant interaction between time and concentration of each compound ($p<0.001$). The data suggest that more adverse effects were seen at higher concentration with an increased time of exposure. If chlorpyrifos and deltamethrin adversely affect the locomotion of a validated animal model (*C. elegans*), they could potentially have adverse effects on the human nervous system as well.

Introduction

Caenorhabditis elegans (*C. elegans*) are simple non-parasitic nematode that live in soil. They are approximately 1mm in length (Rajini et al., 2018). *C. elegans* have a centralized nervous system. Studies have suggested that *C. elegans* are the first multicellular organism that possesses a complete genome sequence (Cole et al., 2003). The *C. elegans* is as an excellent model for an in vivo experiment because they have a short life cycle and life span, and they are easy to maintain in the laboratory (Rajini et al., 2018).

Deltamethrin and chlorpyrifos are two widely used insecticidal compounds. Deltamethrin is a synthetic pyrethroid that is widely used in agriculture. However, it is hazardous for human health and the environment, decreasing biodiversity (Ncir et al., 2016). Several studies have examined the effect of deltamethrin on *C. elegans* development and reproductive toxicity. The results from the studies illustrate decreases in serum testosterone level, change in reproductive behavior, along with decreases in sperm count, motility rate and the quality of sperm on adult rabbits, rats, and mice (Saillenfait et al., 2016). The major effect of the pyrethroid impairs voltage-gated sodium channels in insects by prolonging the opening of sodium channels, resulting in membrane hyper-depolarization and thereby blocking the nervous system signals (Gutiérrez et al.,2016).

Chlorpyrifos is a widely used organophosphate pesticide in agriculture and in households. It also has highly toxic effects on the environment. Organophosphates (OP) insecticides inhibit acetylcholinesterase enzyme (AChE), which lead to an accumulation of acetylcholine, a major neurotransmitter, in the synaptic cleft. A significant reduction in AChE activity correlates with effects on behavior that may impair survival of the exposed organism (Rajini et al., 2018). Some studies have indicated that chlorpyrifos is also carcinogenic to humans. (Sun et al., 2015).

The purpose of this study was to determine the effect on locomotion of *C. elegans* at different concentrations of deltamethrin and chlorpyrifos. The hypothesis stated that an increase in the concentration of each compound would cause a decrease in locomotion of *C. elegans*. The study will determine the lowest concentration at which each compound will cause a locomotive effect (ED50). The data may also inform scientists which of the two compounds has the lowest lethal effect of 50% of the worms (LD50).

Materials and Methods

Wild type N2 *C. elegans* were maintained on NGM agar with OP50 *E. coli* bacteria. After 72 hours the starved *C. elegans* were kept at 16°C. The *C. elegans* were exposed to chlorpyrifos (0.1µM, 1.0µM, and 2.5µM in K-medium) or deltamethrin (0.1µM, 1.0µM, and 10µM in K-medium) or to 0.1% ethanol in K-medium (control) in 6-well plates. The plate wash method was used to treat and transfer the *C. elegans* from a petri dish to one well of a 6 well plates. The *C. elegans* were washed with 1 ml of a respective treatment into the well. The plate wash repeated once more for each treatment. After two plate washes 1ml of the respective treatment was added directly to the wells, for a total volume of 3 ml in each well. The number of front body bend was counted for 20 seconds immediately after treating (0 hours), at 24 hours and at 48 hours. Ten *C. elegans* were counted for each time point of each treatment. The data were analyzed using 2-way ANOVA (Jamovi) with a Tukey post-hoc test.

Results

There was a dose-dependent decrease in locomotion of *C. elegans* treated with chlorpyrifos and deltamethrin ($p<0.001$ and $p< 0.001$ respectively). Locomotion after the treatment with both of these compounds at each concentration was significantly less than the control ($p<0.001$). However, locomotion of *C. elegans* did not significantly decrease when the lowest concentrations of both were compared ($p=0.459$). *C. elegans* demonstrated more adverse effects in locomotion at the highest concentrations (10.0 µM deltamethrin and 2.5 µM concentration) of both compounds as compared with the lowest concentrations (0.1 µM deltamethrin and 0.1 µM chlorpyrifos). Furthermore, there was a significant difference associated with times of exposure ($p<0.001$). At 24 hours and 48 hours post-treatment with deltamethrin, the locomotion in *C. elegans* significantly decreased compared to the control group ($p <0.001$) (Figure 1).

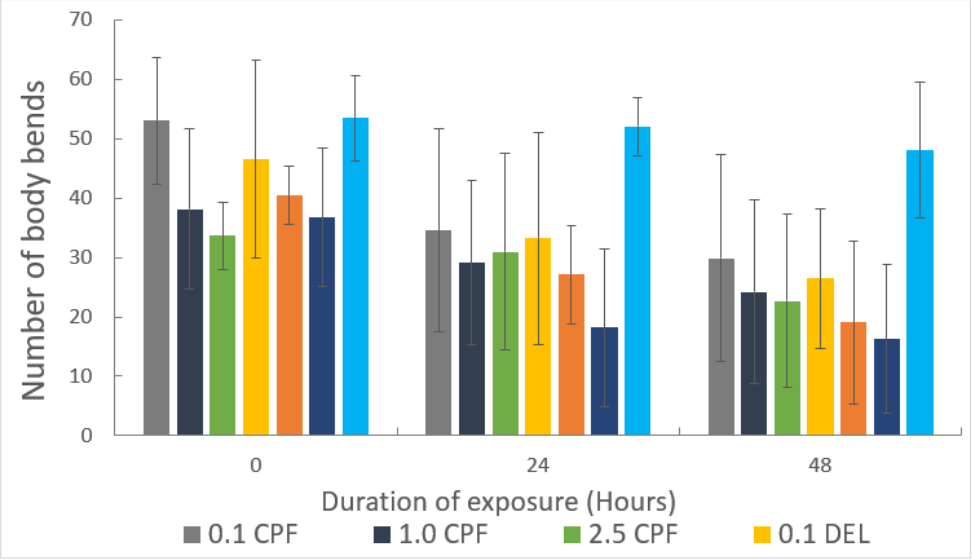

Figure 1. The relationship between number of body bends and duration of exposure (hours) when *C. elegans* were exposed to different concentrations of chlorpyrifos and deltamethrin. The first six bars represent different concentrations of both compounds and the last bar represent the control group.

The locomotion significantly decreased when time and concentration interact with each other ($p<0.001$). The lowest concentrations (0.1 µM chlorpyrifos and deltamethrin) for a shorter time exposed (0 hours) demonstrated less adverse effects on locomotion of *C. elegans*, while the highest concentrations (2.5 µM of chlorpyrifos and 10.0 µM of deltamethrin) with long exposure (48 hours) demonstrated more adverse effects. There was a significant decrease in locomotion of *C. elegans* treated with 0.1 µM CPF or 0.1 µM deltamethrin for 0 hours compared to all higher chlorpyrifos and deltamethrin concentrations across all time points ($p \leq 0.002$ and $p \leq 0.01$, respectively) (Fig. 1). The locomotion of *C. elegans* treated with 0.1 µM CPF or 1.0 µM CPF decreased over time of exposure ($p < 0.001$ and $p = 0.006$, respectively). This was not observed at the highest chlorpyrifos concentration. The highest concentration of each compound appeared to cause paralysis of the *C. elegans*. As the time of exposure increased, paralysis led to the death of *C. elegans*. These two compounds produced approximately 30-60% mortality, depending on the time of exposure. The lowest concentration of each treatment (0.1µM) produced approximately 5-15% lethality depending on the time point. Finally,

the data indicate that there was no trend suggesting that either of these compounds more adversely affected locomotion than the other.

Discussion

The data supported the hypothesis that as the concentration of chlorpyrifos and deltamethrin increased, the locomotion of *C. elegans* would decrease. The most adverse effects were observed at the highest concentration of both compounds. However, this effect was not observed until at least 24 hours after exposure. The effect was most noticeable at 48 hours post-exposure.

Deltamethrin and chlorpyrifos are pesticides that are widely used in agriculture. This study provides evidence that locomotion of *C. elegans* is dependent on the concentration of either compound. The data from this study suggest that the ecosystem of the soil will be adversely affected by high-dose, short-term exposure to either chlorpyrifos and deltamethrin. Since farmers are constantly exposed to deltamethrin and chlorpyrifos, these compounds could have a potential effect on the nervous system of farmers. People who are in contact with these two pesticides should be aware of their side effects.

The pesticides are typically used concurrently. A follow-up study should examine the interaction between chlorpyrifos and deltamethrin to determine if there are additive and synergistic effects.

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