LC50 Determination of the Pyrethroid Pesticides Esfenvalerate and Permethrin

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Abstract Pyrethroids are a fairly new class of pesticides that are widely used in agriculture but whose environmental impacts are largely unknown (Drenner *et al*. 1993). For years, the safety of chemical compounds has been determined with a series of water toxicity tests, but pyrethroids are so hydrophobic that the pesticide will mostly be found in the sediment, not the water, making results of the water tests inaccurate for determining pyrethroids' environmental risks (Vlaming *et al*. 2001). This study found the LC50's, which are the concentration that kills fifty percent of a population, for esfenvalerate and permethrin, both pyrethroids, using the midge *Chironomus tentans,* a test species recommended by the EPA, in a series of sediment toxicity tests utilizing two sediments, one from Glen Ellen, CA and one from the San Pablo dam near Orinda, CA, of varying organic carbon content. The values were found following EPA protocol for measuring pesticide toxicity in sediment, which consists of exposing ten-day old midges to known concentrations of pesticides in the sediment and evaluating survival after ten days (EPA 2000). All concentrations were calculated using ToxCalc to interpret the results of the ten-day tests. The LC50 for permethrin was 1274µg/kg using the Glen Ellen sediment and 756µg/kg for the San Pablo sediment. The LC50 for esfenvalerate was 177µg/kg using the Glen Ellen sediment and 248µg/kg for the San Pablo sediment. These values are all much higher than their concentrations that were found in field samples from different locations in the Central Valley, and therefore the decreased survival found in the field samples could not have been caused by either esfenvalerate or permethrin alone (Weston *et al.* 2002). Because permethrin's LC50 is much greater than esfenvalerate's, it may be beneficial to the ecosystem if esfenvalerate use is replaced by permethrin, assuming permethrin and esfenvalerate are used in the exact same quantities and are comparably effective.

Introduction

In 1999 the Environmental Protection Agency, EPA, initiated a complete review of all pesticides. The first class to be evaluated was the organophosphate pesticides because of their threat to human and all mammalian life (EPA 2003a). As a result of the evaluation, the EPA eliminated organophosphate production in the United States and began a program to phase organophosphates out of use (EPA 1999). In the absence of organophosphates, synthetic pyrethroid pesticides, which were derived from the chrysanthemum in the 1970's, are quickly increasing in popularity because of their high efficiency against insects, greater stability in the environment and a greater safety to farm workers and wildlife (Al-Makkawy et al. 1999). Pyrethroids are just as effective as chlorpyrifos, the most commonly used organophosphate, which puts them in a place to be a realistic and competitive replacement to organophosphates (Tang et al. 1996). The conversion from organophosphate to pyrethroid use will be beneficial to farm workers and the health of other mammals, but it may also have dire consequences on other species such as salmon and trout, important in commercial fishing, as well as other unknown environmental impacts (Edwards 1986).

The effects of pyrethroid use are largely unknown because of the newness of the compounds. Synthetic pyrethroid pesticides were derived in the early 1970's but have not been widely used until recently due to the recent reduction in organophosphate use enacted by the EPA (Drenner *et al*. 1993). Although pyrethroids are generally safer for the environment than organophosphates, it is more difficult to determine if they are causing problems in the environment than organophosphates because they are toxic at such small concentrations (Glickman and Lech 1982). It is very difficult to establish pyrethroid regulations for this reason. Pyrethoids have very low water solubility, which means that when the compounds are exposed to the environment they will associate with the sediment, not remain in the water column like the organophosphates (Lee *et al.* 2002). This presents a potential problem because for years EPA pesticide regulation has been based on the results of water quality and water toxicity tests (EPA 2003b). These tests have aided the EPA in determining whether a compound could be used for commercial and/or residential purposes as well as set restrictions on allowable concentrations (EPA 2003c). Unfortunately pyrethroids are so hydrophobic that the pesticide will mostly be found in the sediment, not the water, making results of the water tests inaccurate for determining pyrethroids' environmental risks. An appropriate measure of environmental pyrethroid toxicity

can be determined by conducting soil toxicity tests; unfortunately there is very little information in the literature on sediment concentration that would be potentially toxic, the few reported levels are in the high ppb to low ppm range (70 µg/kg to grass shrimp (McKenney *et al*. 1998), 1,300 µg/kg to nematodes (Chandler *et al*. 1994), 2,100 µg/kg to a chironomid (Conrad *et al*. 1999), 2,800-3,400 to copepods (Chandler *et al*. 1994). The EPA has years of data from water tests and very little data from soil toxicity tests. When registering a pesticide, the producer needs to show that a compound presents no or very little danger to the environment and ecosystem. Unfortunately the EPA had no suggestions or guidelines on which tests accurately assess the safety of a compound until the recently when the reregistration process included suggestions for further studies (EPA 2003).

This study will identify the LC50's, the concentration that kills 50% of a given population, for both permethrin and esfenvalerate, two of the most commonly used pyrethroids. These values will be obtained by conducting several series of sediment toxicity tests with *Chironomus tentans*, a midge, as the test species using two clean test sediments of varying organic carbon content for both permethrin and esfenvalerate.

Identifying these values is a very important step towards understanding how dangerous pyrethroids are in both the short and long term. They will help policy makers establish the proper regulations on the two compounds as well as help determine the need for future research (EPA 2003c).

Methods

Determining the LC50's for permethrin and esfenvalerate will allow for a comparison with the concentrations of permethrin and esfenvalerate found in field soil samples so that it can be determined whether the field toxicity found by Weston *et al*. (2003) may have been caused by the pyrethroids.

Sediment Preparation Two sediment samples of varying organic carbon content were collected from sources believed to be free of pesticides and other contaminants. One came from the San Pablo Reservoir and the second came from Fern Lake, a reservoir in Glen Ellen, California at Jack London State Park. Each of the sediments was initially sieved with a one-millimeter sieve and the left for the water to settle out. A few days later the overlying water was poured off and this process was repeated until all the excess water was removed. Then the

sediment was homogenized with an electric drill accessory, packaged into labeled one-gallon Ziploc bags and stored in the freezer.

Subsamples were taken for total organic carbon, grain size, total solids, and chemical analysis. The integrity of the sediments samples was confirmed by chemical analysis using gas chromatography. Each sample was analyzed for the presence of the following contaminants: alpha-, beta-, delta-, and gamma-BHC, aldrin, endosulfan I and II, dieldrin, endrin, endrin aldehyde, endrin ketone, heptachlor, methoxychlor, heptachlor epoxide, 4,4'-DDD, 4,4'-DDE, 4,4'-DDT, alpha and gamma isomers of chlordane, chlorpyrifos, cis and trans –permethrin, bifenthrin, and esfenvalerate. If the pesticide was present in the sample, its concentration was reported. Dr. Lyde of Southern Illinios University conducted the analysis using a Hewlett Packard 6890 Series Gas Chromatograph System (HP6890GC) equipped with an electron capture detector (ECD) following modified protocols from USEPA Method #8080A and found both samples to be free of all the listed contaminants. The total solids of each sediment was determined by placing a known weight of each sediment into appropriately labeled tin dished and placed in a 100ºC oven overnight and then reweighed. The ratio of the wet weight to dry weight is the total solids amount. For the San Pablo sediment it was 70% and for the Glen Ellen sediment it was 28.7%.

The LC50's were found for each of the sediment and pesticide combinations. For each series, 350 g of sediment was placed into five glass jars labeled with the sediment origin, and the type and concentration of pyrethroid each jar was spiked with. The appropriate amount of pesticide was added accounting for the total solids of each of the sediments. Once the jars were spiked they were homogenized for a few minutes using a small drill accessory. They were then placed in a 4ºC refrigerator for a week to allow the pesticides to associate with the sediment.

LC50 Determination The day before the sediment test was started, each of the jars of sediment was re-homogenized and then 25-50ml of sediment was added to the appropriately labeled 300ml beaker. 200ml of water was then carefully added to each beaker to minimized sediment disturbance. There were three replicates for each treatment and five concentration steps for each series as well as a control for each sediment type. On the first day of the test ten third instar midges, approximately ten days old were added to each beaker. The midges were then cared for following EPA protocol, which included feeding each beaker 100µl of Tetrafin

slurry every day and changing the water every other day (EPA 2000). The test lasted ten days and survival was evaluated at the end.

For the LC50's to be calculated, the concentration range needed to include almost complete survival and almost complete mortality so that Toxcalc, the computer program used to determine the LC50's, could evaluate the whole survival curve. Toxicity tests were conducted until each series could be accurately calculated.

Analysis Once all the tests were finished, the data was input into Toxcalc, and log and probit transformed. The LC50's for each treatment were then calculated by plotting the survival against the concentration of pyrethroid. The margin of error was reported for each value.

Results

The LC50 for permethrin using the Glen Ellen sediment was 1274µg/kg and 756µg/kg for the San Pablo sediment. The LC50 for esfenvalerate using the Glen Ellen sediment was 177µg/kg while for the San Pablo sediment it was 248µg/kg.

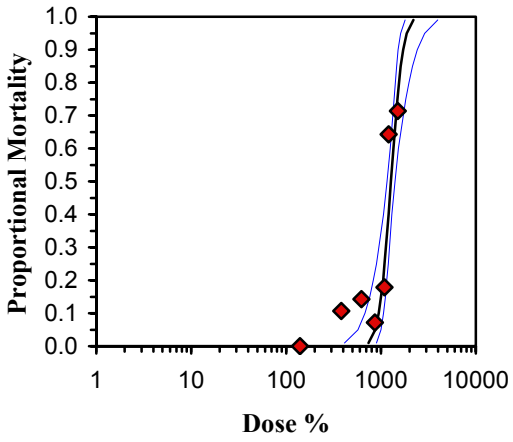

Figure 1. Permethrin Toxicity Curve using Glen Ellen sediment

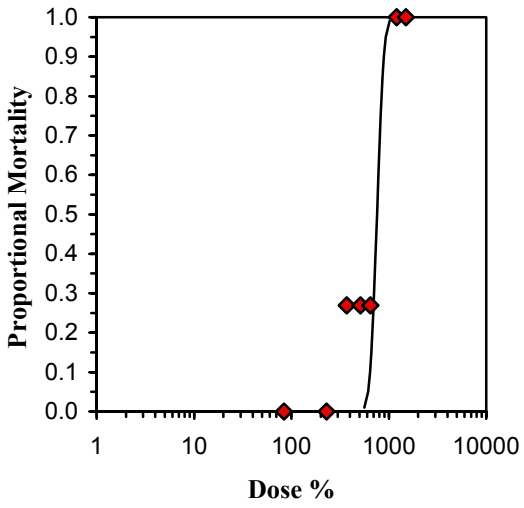

Figure 2. Permethrin Toxicity Curve using San Pablo sediment

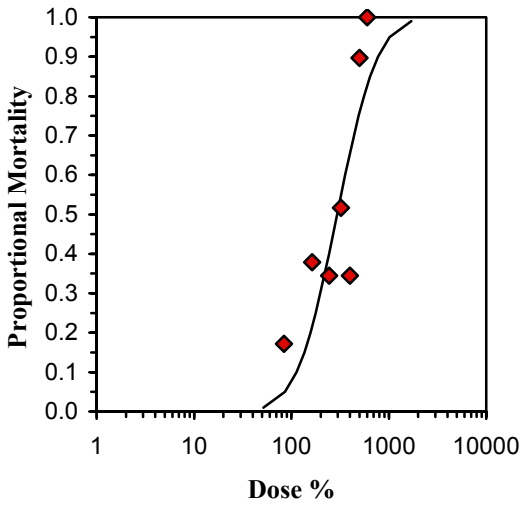

Figure 3. Esfenvalerate Toxicity Curve using Glen Ellen sediment

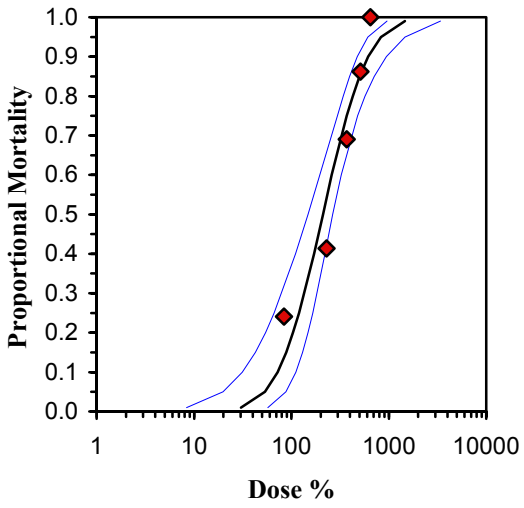

Figure 4. Esfenvalerate Toxicity Curve using San Pablo sediment

Discussion

The LC50's would not be an ideal concentration to establish a maximum allowable concentration of the contaminants in sediments across the country because a concentration that has less of an effect on a population would be more desirable. Unfortunately there are no such values so these LC50's would be the most appropriate values that are available. The values are appropriate because the midge is fairly sensitive to pyrethroids. There are species that are more sensitive to pyrethroid toxicity but these are the first values of their kind and it would be beneficial to have pesticide regulation that was based on scientific research. As additional values are conducted for more sensitive species the regulation will need to be adjusted, but they are a good starting point.

All of the LC50's found by this study were much higher than the concentrations found in Central Valley sediments by Weston *et al*. (2002). For esfenvalerate, the highest reported concentration was 18µg/kg and 458µg/kg for permethrin. This suggests that the toxicity found by Weston *et al*. was not caused by either permethrin or esfenvalerate alone. Assuming permethrin and esfenvalerate are used in the same quantity and for the same purposes, these results do suggest that it may benefit the environment if esfenvalerate users switched to permethrin because permethrin's LC50 is so much higher because the higher LC50 might help more sensitive species survive. There are many unknown factors that could be influenced by such a change that should

be investigated before such changes are made. First of all, the two compounds could have synergistic effects that may or may not intensify if the ratio of permethrin to esfenvalerate use was altered. Also, both are sub lethal so a general reduction in pyrethroid use would be more beneficial.

This study failed to identify the cause of the toxicity found by Weston *et al.* (2002). There are several unknown factors that may be causing the toxicity. Interaction effects between the various contaminants found in the samples may be one cause for the toxicity. The chemical analysis tested for 28 contaminants, all of which were present in one or more samples, and some samples contained the vast majority of the contaminants. The combination of some of the contaminants may have caused the toxicity when their detectable levels were lower that the LC50. Although the chemical analysis checked for 28 contaminants, there are many others that were not examined such as PCB's and hydrocarbons that may have been present in the samples. Such compounds may be responsible for the toxicity while there presence was unknown. Also, as stated earlier, there are very few published sediment LC50's so it is impossible to know if any of the detected compounds could be causing the toxicity or not. More investigation is needed to accurately identify the cause or causes of the toxicity but from this study it can be concluded with certainty that neither permethrin nor esfenvalerate are causing the toxicity in the field samples alone.

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