

## Toxicity of a Synergized Formulation of Sumithrin to Daphnia magna

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West Nile Virus has stimulated the renewed use of insecticides for the control of adult mosquitos in many places in the US (CDC 2003; NYSDOH 2001). Pyrethroid insecticides are becoming one of the favored classes of insecticides for this purpose (NYSDOH 2000; NYSDEC 2000). These insecticides can be very effective against target mosquitos and tend to have short half-lives in the environment (USEPA 2002a, WHO 1990). In addition they have low toxicities to mammals and birds. Pyrethroid insecticides are very toxic to aquatic organisms (Demoute 1989). Sumithrin is a pyrethroid insecticide which is being used in several states for the control of mosquitos which may be carriers of West Nile Virus (CDC 2003; NYSDOH 2001).

All new pesticide products are reviewed by the USEPA for possible effects to non-target aquatic and terrestrial organisms (40 CFR Part 158 2002). This review is based predominately upon required studies conducted by the pesticide manufacturers, but occasionally data from independent researchers are also included. Toxicity data are reviewed along with environmental fate and other data to insure that non-target risks do not outweigh the benefits of the use of a pesticide. A key component to this review is that toxicity data submitted truly represents the toxicity of the pesticide to various groups of non-target species. If the data are not valid, the review will fail to properly recognize potential non-target toxicity from the use of a pesticide.

The Environmental Fate and Effects Division of the Office of Pesticide Programs, USEPA maintains a database which "incorporates ecological toxicity data which have been reviewed and categorized as fully or partially acceptable for fulfillment of pesticide registration and reregistration guideline requirements as explained under FIFRA Subdivision E, Parts 158.145 and 158.150" (40 CFR Part 158 2002). This Environmental Effects Data Base (EEDB) contain only one acute toxicity study for sumithrin using an aquatic invertebrate (*Daphnia magna*). The 48-hr EC50 associated with this study is >300 mg/L. This value seems to be inordinately high, considering that acute studies of sumithrin with fish yield LC50s in the  $\mu$ g/L range (Worthing and Walker 1987).

The purpose of this study is to determine the acute toxicity of a formulated sumithrin insecticide product to a freshwater aquatic invertebrate, specifically *Daphnia magna*.

## **MATERIALS AND METHODS**

The sumithrin formulation used in this study is marketed under the name Anvil® 10+10 ULV and is produced by Clarke Environmental Mosquito Management, Inc. (Roselle, Illinois). Anvil® 10+10 ULV is a synergized formulation of sumithrin which contains 10% w/w sumithrin and 10% w/w piperonyl butoxide (PBO). The PBO is the synergist, and is frequently used with pyrethroid insecticides (Casida 1980; WHO 1990).

The 24-hr old *Daphnia magna* neonates used in these tests were from cultures maintained at the New York State Rome Field Station. Testing procedures followed standard methods for conducting *Daphnia* tests (USEPA 2002b) and test animals were not fed during the tests.

Testing was conducted in 30-mL plastic containers with 20 mL of test solution. Rome spring water was used as the dilution water (pH = 7.61, hardness = 132 mg/L  $CaCO_3$ ). Temperature was held at  $20.2 \pm 0.3$  °C using a thermostatically controlled water bath. Dissolved oxygen was measured at the beginning and end of each test. All dissolved oxygen levels were >8.3 mg/L.

A stock solution was prepared by dissolving the insecticide in acetone to make a  $120,000 \,\mu\text{g/L}$  sumithrin solution. This stock solution was used to apply the pesticide to the water used in tests. Test concentrations were 0.60, 1.7, 4.5, 8.9, 12.5, and 34.2  $\mu\text{g/L}$ . Two types of controls were used. One set of controls contained only dilution water. The second set of controls is a solvent control which was mixed with a volume of acetone equal to that of the highest sumithrin concentration (acetone =  $0.22 \, \text{mg/L}$ ).

All of the tests were conducted with 1 test animal in each test chamber and 10 replica of each concentration. The tests were repeated five times, with fresh stock solution and test waters mixed for each test.

The tests conducted were 48-hr acute static toxicity tests. Counts were made at 5 hr, 24 hr and 48 hr. Death (or more properly immobility) was determined by a failure to respond to gentle probing. The testing yielded highly comparable results between the 5 tests conducted. In addition, 100% survival was maintained in both the control and solvent control during all 5 tests. Some preliminary tests were conducted using various concentrations of acetone in water. These test results indicated that acetone concentrations equal to 0.50 mg/L would not harm the *Daphnia magna* neonates. In the present study, tests of sumithrin were conducted with a maximum acetone concentration of 0.22 mg/L.

Since each of the five tests yielded similar results, all of the test results were pooled for statistical analysis. The EC50 was calculated using nominal concentrations and the probit or trimmed Spearman-Karber methods (Finney 1978; Hamilton et al. 1977).

In addition, no observed effect concentrations (NOEC) and lowest observed effect concentrations (LOEC) were determined using TOXSTAT<sup>©</sup> software (West, Inc and Gulley 1996).

## RESULTS AND DISCUSSION

Synergized sumithrin was highly toxic to *Daphnia magna*. The EC50s, NOECs, and LOECs are presented in Table 1. Other pyrethroid insecticides have EC50 levels to daphnids in the 0.5-20  $\mu$ g/L range (Mayer and Ellersieck 1986).

The toxicity values found in the present study indicate that sumithrin formulation is far more toxic than the 48-hr static toxicity value contained in the EEDB (EC50 > 300 mg/L, NOEC = 300 mg/L). Permethrin and resmethrin are two other pyrethroids which are commonly used for mosquito control. Sumithrin, permethrin, and resmethrin are all Type I pyrethroids, that do not contain an  $\alpha$ -cyano group. Their toxic action is similar. Formulated products containing PBO synergized permethrin and resmethrin are applied at 7.8 g/ha for mosquito control. Synergized sumithrin is applied at 3.9 g/ha for mosquito control, which suggests that the active ingredient sumithrin is at least as toxic to mosquitoes as permethrin and resmethrin.

Fish toxicity data from the EEDB for these three pyrethroid active ingredients indicates that sumithrin is somewhat less toxic to rainbow trout (*Oncorhynchus mykiss*) by a little less than one order of magnitude. Bluegill sunfish (*Lepomis macrochirus*) toxicity data from the EEDB are also very similar among these three pyrethroids. These data suggest that the toxicity of sumithrin is about the same as that of resmethrin and permethrin.

The EEDB lists the *Daphnia magna* static 48-hr EC50 for permethrin as 0.32-1.26  $\mu$ g/L and for resmethrin as 3.1  $\mu$ g/L. Assuming that the same pattern as is seen in fish holds true, it would be expected that the LC50 of sumithrin to *Daphnia magna* would be less than 13  $\mu$ g/L. The 48-hr LC50 in the present study falls within this expected range.

**Table 1.** Effective concentrations (EC50), no observed effect concentrations (NOEC), and lowest observed effect concentrations (LOEC) of synergized sumthrin to *Daphnia magna*.

	Time		
EndPoints (µg/L)	5-hr	24-hr	48-hr
EC50 (95% Confidence Interval)	27.2 (21.9-37.0)	11.8 (10.4-13.4)	7.1 (6.4-8.0)
NOEC	8.9	4.5	1.7
LOEC	12.5	8.9	4.5

Miyamoto (1976) presents "preliminary data" on the toxicity of fenothrin (AKA sumithrin) to Daphnia pulex . He reported a 3-hr LC50 > 50 mg/L which could be used to support the EEDB acute value, but he also presents a 3-hr No Effect Level as 1  $\mu$ g/L. The difference between this LC50 and No Effect Level (> 5 orders of magnitude) suggests a very flat dose-response curve which would be very atypical for a pyrethroid insecticide. The data presented by Miyamoto do not seem to be in agreement with each other. In addition, our 5-hr LC50 is in strong contrast to this 3-hr LC50, but our 5-hr NOEC would be in closer agreement with the No Effect Level presented by Miyamoto .

The presence of the PBO synergist may explain some of the difference between our data and the EEDB, because data in the EEDB typically reflects the toxicity of the unsynergized, technical grade active ingredient. PBO has been documented to affect the toxicity of pyrethroids to target and non-target organisms. The effect on non-target aquatic animals can be small or great (Paul and Simonin 1995, 1996). It is, however, unlikely that the difference in toxicity between our data and the EEDB is strictly due to the presence of the PBO synergist.

The EEBD also contains a 21-d chronic *Daphnia magna* study with an LOEC = 0.81  $\mu$ g/L. This value seems to be in agreement with the results of our study. It seems therefore that the 48-hr EC50 of >300 mg/L for sumithrin contained in the EEDB is in error.

This error is confirmed by reviewing the Data Evaluation Record (DER) completed by the Ecological Effects Branch of the Office of Pesticide Programs, USEPA for the 48-hr EC50 of >300 mg/L study. The original DER, dated 9 May 1979, accepts the study as valid, and states that it "satisfies core data requirements." (USEPA 1979). The DER includes handwritten notes, apparently added at a later date, stating that the study needed to be repeated. The DER also contains a second page, completed in 1987, which states that the 1979 study "...is not on sumithrin, it is on tetramethrin, and can not be used." The value reported in the EEDB is for another substance and not for sumithrin (potentially tetramethrin, but it is impossible to say for certain). It obviously has no value in assessing the non-target toxicity of sumithrin.

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