THE PERSISTENCE OF THE INSECT GROWTH REGULATORS UC84572 AND DIFLUBENZURON IN WATER USING

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MOSQUITO BIOASSAYS

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B.V. HELSON

Forest Pest Management Institute Canadian Forestry Service Government of Canada P. O. Box 490 Sault Ste. Marie, Ontario. P6A 5M7

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Director Forest Pest Management Institute Canadian Forestry Service P.O. Box 490 Sault Ste. Marie, Ontario P6A 5M?

INTRODUCTION

UC84572 is a new insect growth regulator (IGR) being developed by Union Carbide Agricultural Products Company (now Rhone-Poulenc). The company was interested in the potential of this compound for forest insect control. They also wanted to know how persistent UC84572 was in the field. This study was undertaken to determine the persistence of UC84572 compared to a standard IGR, diflubenzuron, in an aquatic system by measuring its toxicological activity over time with mosquito larvae.

METHODS AND MATERIALS

Mosquitoes

Aedes aegypti mosquito larvae were used to assess toxicological activity of UC84572 and diflubenzuron. Eggs were hatched under a vacuum for 1 hour to obtain larvae of uniform age. The larvae were reared at 27°C in plastic pans containing 600 larvae in 1 L of water. Each day the larvae were provided with measured amounts of ground dog chow. These highly standardized procedures were used to ensure synchronous development. Third-instar larvae, 72 h of age were used in all tests.

The Aquatic System

Two-ring, plastic, wading pools, ca 0.8 m in internal diameter were inflated with air and lined with 6 mL clear plastic sheets. These were placed outdoors in a completely exposed location. In 1986, one half of a standard roll of landscaping sod was placed on the bottom of each pool and it was filled with 50 L of Sault Ste. Marie tap water 1 day before treatment. In 1987, one-third of a piece of sod and 75 L of water were used to reduce the extent of bacteria and algal growth during hot weather. The depth of water in each pool was then measured with a metal ruler at a marked spot.

Treatment Procedures

In 1986, the pools were treated randomly with several concentrations (see Table 3) of either UC84572 (250 g AI/L Flowable) or diflubenzuron (Dimilin 25% WP) on October 3, 1986. Stock, 0.5% suspensions of each compound were prepared in distilled water. Each stock was serially diluted with distilled water so that 1 mL of each dilution gave the required concentration (ppb AI) in a pool based on the initial volume of 50 L. One mL of pool water was removed before application. The suspensions were shaken well and applied to the pools with an Eppendorf pipette. The water in the pools was stirred well after treatment.

In the two 1987 trials, groups of three pools were treated with UC84572 at 1.0 and 3.0 ppb, and diflubenzuron at 0.3 and 1.0 ppb AI based on the initial volume of 75 L. These concentrations were chosen to detect reductions in quantities of up to 95% continuously over time based on the mortality-concentration relationship for each compound.

Sampling Procedures

Immediately after treatment and at periodic intervals thereafter water samples were collected from each pool for bioassays with mosquito larvae. A 2 L glass beaker was inserted on its side into a pool down to the sod so that the entire column of water was sampled. Water from the beaker was then filtered through window screening to remove large pieces of grass into two 500 mL glass Mason jars. Two jars from each pool were returned to the laboratory, and left to acclimate to room temperature if necessary. Ten larvae were then introduced into each jar along with 0.022 g of ground dog chow which was found to be necessary for normal larval development. These were placed in an environmental chamber at 21°C and 16L:8D. The larvae were checked for mortality every 2 days until all had either died or pupated.

Weather conditions including water temperatures in two control pools were monitored continuously with a Campbell Scientific 21X Portable Weather Station throughout the experimental period. On each sampling day, water depths were measured in all pools to calculate remaining volumes. Water samples were also collected from the control pools to measure pH and dissolved 02.

Toxicity Determinations

The toxicities of UC84572 and diflubenzuron to A. aegypti larvae were also determined in conjunction with these field trials. Extra pools were set up in the same fashion as above to provide a water source for these tests. On each sampling day water was collected from one of these pools with a 2 L beaker and filtered through window screening. Using a graduated cylinder, 400 mL aliquots of water were measured and poured into 500 mL Mason jars. These jars were returned to the laboratory and treated with a series of concentrations (see Table 2) of UC84572 or diflubenzuron. Two jars were treated with each concentration and two were not treated to serve as controls. Appropriate stock suspensions of each IGR were prepared in distilled water and serially diluted so that 1 mL of each dilution provided the desired concentration in the jar. One mL of water was removed from each jar before treatment. The suspensions were shaken well and applied with an Eppendorf pipette. The water in the jars was then stirred and 10 larvae were introduced into each jar along with 0.022 g of dog chow. These larvae were checked as above. Probit analysis has been performed on the combined data from all the tests for each compound in each trial.

RESULTS AND DISCUSSION

Trial 1, October 1986

Water Chemistry and Weather Conditions

These conditions are presented in Table 1. Generally cool, wet conditions prevailed during the experimental period. A total of 9 cm of rain fell which increased the volume of water in the pools to a maximum of 67 L 14 days after treatment. Evaporation and removal of approximately 800 mL of water from each pool each sampling day accounted for the declines in water volumes observed during some periods. Mean water temperatures were substantially higher than mean air temperatures. The temperature probes in the pools were laying on the sod. Heat retention and absorption of radiant energy by the sod may have influenced the temperature readings since both minimum and maximum temperatures were usually higher in the pools. Toxicity of UC84572

Initially it was intended to assess the toxicity of UC84572 after one moult to fourth instars. However preliminary tests revealed that very little mortality occurred after this moult. Most of the mortality occurred after the next moult to pupae. This phenomenon is illustrated in Table 2. With diflubenzuron on the other hand, considerable mortality occurred after one moult and further mortality was evident after the next moult to pupae.

Both UC84572 and diflubenzuron were highly toxic to A. aegypti larvae (Table 2). Diflubenzuron was more toxic with a Relative Potency of 2.3 compared to UC84572. The slopes of the regression lines for the two insect growth regulators were significantly different.

Persistence of UC84572

The mortality of larvae in water samples collected at various times up to 35 days after treatment is presented in Table 3. The persistence of both insect growth regulators as indicated by larval mortality increased with increasing concentration. Mortality consistently occurred longer in the pools treated with UC84572 than with diflubenzuron at equivalent concentrations. Since the toxicity of UC84572 was lower than Dimilin, this means that UC84572 was more persistent than diflubenzuron under these conditions. For instance, when mortalities are converted to equivalent concentrations based on the toxicity equations in Table 2 the concentrations remaining after 3 days in the pools treated with 10 ppb are 2.59 ppb UC84572 and 0.28 ppb Dimilin and after 6 days, 1.12 ppb UC84572 and 0.11 ppb Dimilin. These, and the data from other pools indicate that a 90% decline in initial concentrations of UC84572 occurred in approximately 6 days while a similar decline in initial concentrations of Dimilin occurred in about 1 day.

Trial 2, June 1987

Water Chemistry and Weather Conditions

Hot, sunny, dry conditions prevailed during the experimental period in this trial (Table 4). Since no rain fell, the volume of the pools declined from 75 L to 50 L after 7 days due to evaporation and water sample collections. Mean water temperatures were 4 to 5° warmer than mean air temperatures. Dissolved O_2 and pH did not vary much during the trial.

Toxicity of UC84572

The toxicities of UC84572 and Dimilin based on tests every sampling day are presented in Table 5. The toxicities of both insect growth regulators after the pupal moult were similar to their toxicities in Trial 1. Diflubenzuron was again more toxic than UC84572 with a relative potency of 3.5. The slopes of the regression lines were not significantly different in Trial 2.

Persistence of UC84572 and Dimilin

The mortality of larvae in water samples from the treated pools in Trial 2 is presented in Table 6. Equivalent concentrations for each mortality level have been calculated using the probit equations in Table 5. As in Trial 1, UC84572 was more persistent than Dimilin. Fifty and 90% reductions in concentrations of UC84572 occurred in approximately 12-24 h and 72 h, respectively. Comparable reductions in Dimilin concentrations occurred by 6 h and 12 h. After 24 h, mortality of larvae in Dimilin water samples was insignificant. However, low but stable mortality continued after 96 h in the pools treated with 3.0 ppb UC84572 with little if any further apparent reduction in concentration. Actually all of the larval mortality during this later period was from only one of the three pools. Reductions in UC84572 seemed to occur more quickly in Trial 2 than in Trial 1. Temperatures and amount of sunshine were much higher in Trial 2.

Trial 3, October 1987

Weather and Water Chemistry Conditions

This trial was conducted under weather conditions which were hoped would be similar to those during Trial 1 in October 1986. They generally were similar in that cool, wet, cloudy conditions prevailed during most of Trial 3 (Table 7). Mean water temperatures ranged between 6.7 and 12.1°C. Initially, they were similar to mean air temperatures but were 4 to 5°C warmer than the air temperatures by the end of the experiment. Water volumes reached a low of 68 L 2 days after treatment due to evaporation and sampling but increased to 84 L, 4 days post due to heavy rains. Except on treatment day, there was very little bright sunshine. Dissolved O₂ and pH did not vary much during the trial.

Persistence of UC84572 and Dimilin

The mortality of larvae in water samples from the treated pools in Trial 3 is presented in Table 8. For this trial, equivalent concentrations were calculated from probit equations derived from the combined toxicity data from Trials 1 and 2. The equations for UC84572 and Dimilin respectively are Y = 6.02 + 2.95X and Y = 7.67 + 3.35X where Y =probit mortality and $X = \log$ concentration. Limited tests during Trial 3 indicated that the toxicities of UC84572 (LC50 = 0.37 ppb) and Dimilin (LC50 = 0.12 ppb) were again similar to the previous trials.

As in the previous trials, UC84572 was more persistent than Dimilin. There was no apparent decline in UC84572 during the first 24 h after treatment. Concentrations then appeared to fall quite rapidly with a 50% decline occurring between 2 and 3 days post and a 90% decline being reached by about 4 days after treatment. This decline may have been hastened by the 24% increase in pool volumes between days 2 and 4. Low concentrations causing significant mortality did persist thereafter in the pools treated with 3.0 ppb UC84572 for the rest of the experiment without much further apparent reduction. The decline in concentrations of Dimilin in this trial was similar to Trial 2 with a 50% reduction in 6 to 12 h and a 90% reduction by 24 h.

CONCLUSIONS AND RECOMMENDATIONS

- UC84572 is highly toxic to A. aegypti larvae and about 0.3X less potent than diflubenzuron.
- 2. Based on bioassays with A. aegypti larvae, UC84572 is more persistent than diflubenzuron in outdoor sod lined pools.

3. UC84572 appears to decline more quickly under hot and/or sunny conditions than in cool and/or cloudy weather.

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4. In some circumstances, low concentrations of UC84572 amounting to ca 5-10% of initial levels appear to persist at relatively steady levels. The possible occurrence and duration of such a phenomenon with this compound needs to be investigated further.

Date	Days after treatment	Dissolved O ₂ (mg/L)	рН	Mean pool volume (L)	Total rain (mm)	Mean H ₂ 0 ¹ temp (°C)	Mean ^l air temp (°C)	Total light (KW/m ²)	Total ² bright sun (H)
Oct. 2	-1			50.0		<u></u>			•.
3	0	3.0	6.7	51.9					
4	1	3.0	7.7	50.0	3.0	15.4	9.0	32	0
6	3	4.0	8.2	52.6	14.5	11.3	4.9	157	0.5
9	6	3.0	6.9	54.6	27.6	14.0	8.0	507	12.8
13	10	4.0	7.1	64.6	63.2	13.5	8.0	1003	24.6
17	14	No samples		67.0	73.4	12.6	4.6	1276	26.5
20	17	1.5	8.0	64.3	73.4	13.1	5.5	1837	52.6
24	21	2.5	6.9	63.1	73.9	16.0	9.8	2373	77.3
27	24	7.0	7.5	63.7	78.2	13.2	6.9	2793	96.1
31	28	6.5	6.9	60.0	79.9	13.5	6.2	3207	111.2
Nov. 3	31 35	8.5 10.5	6.9 6.9	61.0 61.0	86.4 90.1	11.3 9.3	4.5 1.8	3357 3636	114.5 126.5

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Table 1. Water chemistry and weather conditions during Trial 1, October 1986.

Mean temperature during the period since the last sampling date.
From Sault Ste. Marie Weather Office Records

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			% mortality		
	UC84752			Diflubenzuron	
Conc. (ppb)	3rd moult ¹	4th moult ²	Conc. (ppb)	3rd moult ¹	4th moult ²
Untreated	0	0.6	Untreated	0	0.5
0.01	0	1.4	0.01	0.6	0.6
0.03	1.3	2.0	0.03	0	1.5
0.1	0.5	12.0	0.1	1.8	20.4
0.25	0	20.6	0.2	20.7	56.4
0.375	0	47.1	0.3	37.4	74.0
0.5	0.5	59.4	0.6	62.4	98.9
1.0	0.5	86.3	1.0	82.2	99.5
3.0	7.0	100	3.0	98.0	100
LC50 CL LC95 CL Equation		0.4 ppb 0.26, 0.61 1.79 ppb 1.01, 7.68 Y = 6.01 + 2.53X	LC50 C1 LC95 CL Equation		0.18 ppb 0.17, 0.19 0.54 ppb 0.49, 0.61 Y = 7.56 + 3.44x

Table 2. The toxicity of UC84572 and Diflubenzuron to Aedes aegypti larvae, Trial 1, October 1986.

1 Mortality after all survivors moulted to fourth instars 2 Mortality after all survivors moulted to pupae Y = probit mortality, X = log concentration

Days after treatment				Conc	entrati	on (ppb) in po	ols			
·	Control	0	.3	1	.0	3	•0	10	•0	3	0.0
		UC	Dim	UC	Dim	UC	Dim	UC	Dim	UC	Dim
0	3	100	95	80	100	100	100	100	100	100	100
1	0	82	0	85	0	100	34	100	100	100	100
3	0	10	0	10	0	98	0	98	74	100	100
6	0	0	0	5	0	6	2	87	22	100	100
10	3	0	0	0	0	0	3	3	4	93	85
14	3	0	0	0	0	0	0	10	2	93	78
17	3					0	0	12	2	100	73
21	0							3	2	58	3
24	8							3		21	4
28	0									8	2
31	8									0	2
35	0									0	0
Number of pools	2	1	1	1	1	2	2	3	3	3	3

Table 3. The mean % mortality of *A. aegypti* larvae in water samples collected at different times after treatment from pools treated with UC84572 or Dimilin at the indicated concentrations, Trial 1, October 1986

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Table 4.	Water cl	Table 4. Water chemistry and weather conditions during Trial 2, June 1987.	ather co	onditions du	ring Tri	al 2, June l	987.	
Date	Days post	Dissolved st 02 (mg/L)	Hd	Mean pool volume (L)	Total rain (mm)	Mean H ₂ 0 temp (°C)	Mean air temp (°C)	Total ^l bright sun (H)
June 18	0	3	6.8	75				
June 19	1	1.5	6.8	11	0	26.4	22.2	9.2
20	2	£	7.2	66	0	28.0	23.3	22.5
21	ſ	3.5	7.2	63	0	26.6	20.6	34
22	4	3.5	7.2	61	0	24.7	20.9	34
23	`υ	4.0	7.4	54	0	25.6	20.8	43
24	Q	5.5	7.5	53	0	26.8	23.2	54.4
25	7	3.5	7.5	50	0	27.8	23.9	61.9
1 From Sa	ult Ste.	From Sault Ste. Marie Weather Office Records (No light readings available because pyranometer	Office	Records (No	light re	adings avail	able because	pyranometer

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UC8	4572	Diflubenzuron						
Conc. (ppb)	% mortality ¹	Conc. (ppb)	% mortality ¹					
Untreated	3.3	Untreated	2.9					
0.03	2.2	0.03	0.0					
0.1	0.7	0.1	31.0					
0.25	19.3	0.15	58.8					
0.375			65.4					
0.5 53.2		0.3	85.0					
1.0 82.0		0.6	97.8					
3.0 99.3		1.0	100					
LC50	0.52 ppb	LC50	0.15 рръ					
CL	0.48, 0.56	CL	0.13, 0.16					
LC95 1.62 ppb		LC95	0.44 ppb					
CL	1.38, 2.00	CL	0.37, 0.55					
Equation	Y = 5.95 + 3.31X	Equation	Y = 7.88 + 3.45X					

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Table 5. The toxicity of UC84572 and Diflubenzuron to Aedes aegypti larvae, Trial 2, June 1987.

¹ Mortality after all survivors moulted to pupae Y = probit mortality, X = log concentration

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			UC84	572			Dimi	llin	
Time post (h)	Untreated Mortality (%)	ntreated 1.0 ppb		3.0 p	pb	0.3 p	pb	1.0 p	
(")		Mortality (%)	Conc. (ppb)	Mortality (%)	Conc. (ppb)	Mortality (%)	Conc. (ppb)	Mortality (%)	Conc. (ppb)
0	2.5	71.7	0.76	98.3	2.26	70.0	0.21	100	> 0.60
3	5.0					37.9	0.11	96.6	0.49
6	2.5					16.9	0.07	98.6	0.63
12	5.0	48.3	0.48	79.3	0.89	1.7	< 0.04	31.7	0.10
24	0.0	33.3	0.38	86.7	1.12	1.7	0.04	0.0	< 0.04
48	0.0	11.4	0.22	62.7	0.65	1.7	0.04	1.6	0.04
72	0.0	0.0	< 0.12	20.3	0.29	1.7	0.04	1.7	0.04
96	2.5	3.4	0.10	7.0	0.16				
120	3.3	1.7	< 0.12	13.8	0.22				
144	0.0	3.4	0.15	12.1	0.23				
168	0.0	0.0	< 0.12	8.3	0.20				

Table 6. The mean % mortality of *A. aegypti* larvae and equivalent concentrations¹ in water samples collected from pools treated with UC84572 or Dimilin at indicated concentrations at intervals after treatment, Trial 2, June 1987

 1 Concentrations calculated using % mortalities corrected for control mortality by Abbott's method

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Date	Days post	Dissolved O ₂ (mg/L)	рН	Mean pool volume (L)	Total rain (mm)	Mean H ₂ 0 temp (°C)	Mean air temp (°C)	Total ¹ bright sun (H)
Oct. 14	0	5.5	6.4	75	······		<u></u>	<u> </u>
15	1	5.5	6.7	70	0	12.1	11.9	8.5
16	2	5.5	6.8	68	0.3	9.0	9.1	8.5
17	3	7.0	6.7	74	9.4	11.5	12.5	8.6
18	4	6.5	6.7	84	32.8	9.7	7.6	8.6
19	5 ,	6.0	6.4	83	32.8	8.1	6.9	11.6
20	6	7.0	6.7	81	33.0	7.6	5.0	19.4
21	7	7.0	6.8	81	34.0	8.2	5.2	25.0
22	8				34.3	7.2	2.9	27.9
23	9	9.0	6.7	80	37.6	6.7	1.9	28.1

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Table 7. Water chemistry and weather conditions during Trial 3, October 1987

¹ Hours of bright sunshine from Sault Ste. Marie weather station Pyranometer malfunctional

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			UC84	572	Dimilin					
	Untreated	Untreated 1.0 ppb		3.0 ppb			pb	1.0	opb	
Time post (h)	Mortality (%)	Mortality (%)	Conc. (ppb)	Mortality (%)	Conc. (ppb)	Mortality (%)	Conc. (ppb)	Mortality (%)	Conc. (ppb)	
0	2.5	95.1	1.63	100	> 2.37	96 . 7	0.56	100	> 0.69	
3	3.3	100	> 2.37	100	> 2.37	86.9	0.34	100	> 0.69	
6	0.0	96.6	1.88	100	> 2.37	61.7	0.20	100	> 0.69	
12	17.5	98.2	2.18	100	> 2.37	16.4	< 0.04	56.7	0.15	
24	5.0	100	> 2.37	100	× 2.37	10.7	0.06	16.7	0.07	
48	2.5	81.4	0.89	100	> 2.37	5.2	0.04	6.7	0.05	
72	0.0	28.3	0.29	88.1	1.14	1.6	0.04	0.0	< 0.04	
96	2.5	3.3	0.07	40.0	0.36	1.7	< 0.04	1.7	< 0.04	
120	0.0	0.0	< 0.09	50.0	0.45					
144	0.0	1.7	0.09	13.1	0.19					
168	0.0	0.0	< 0.09	18.3	0.22					
216	0.0	5.1	0.13	13.3	0.19					

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Table 8. The mean % mortality of *A. aegypti* larvae and equivalent concentrations¹ in water samples collected from pools treated with UC84572 or Dimilin at indicated concentrations at intervals after treatment, Trial 3, October 1987

1 see Table 6