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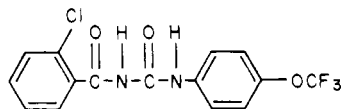
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Factors Affecting the Stability of SIR-8514 (2-Chloro-*N*-[[[4-(trifluoromethoxy)phenyl]amino]carbonyl]benzamide) under Laboratory and Field Conditions

Charles H. Schaefer* and Emil F. Dupras, Jr.

SIR-8514 (2-chloro-*N*-[[[4-(trifluoromethoxy)phenyl]amino]carbonyl]benzamide) is a potent inhibitor of mosquito larval development. Its stability in water is greatly reduced as temperature and pH both become relatively high. Its persistence is not greatly affected by sunlight. In water having a high organic matter content, its persistence appears to be affected by adsorption and by microbial degradation. SIR-8514 is stable on vegetation under field conditions; residues on vegetation can be minimized by application of sand granule formulations. The biological and chemical properties of SIR-8514 are similar to those of diflubenzuron (2,6-difluoro-*N*-[[[4-chlorophenyl]amino]carbonyl]benzamide).

SIR-8514 (2-chloro-*N*-[[[4-(trifluoromethoxy)phenyl]amino]carbonyl]benzamide) is a highly effective inhibitor of mosquito larval development in the laboratory and field (Schaefer et al., 1978). SIR-8514, like diflubenzuron



(2,6-difluoro-*N*-[[[4-chlorophenyl]amino]carbonyl]benzamide) is a potent inhibitor of the terminal polymerization step in chitin formation (Hajjar and Casida, 1978).

Because extensive field testing of SIR-8514 is anticipated, investigations of methods for quantitative analyses and of factors which affects its persistence were conducted in laboratory and small-scale field trials during 1977 and 1978.

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MATERIALS AND METHODS

High-Performance Liquid Chromatograph (LC). A Varian Model 8500 LC having a μ V photometer (254 nm) was utilized for all quantitative analyses. A Micro Pak-CH column (octadecylsilane bonded on 10- μ m particles) 8 mm \times 25 cm, provided reverse-phase separations using 70% acetonitrile-30% water as the mobile phase at a rate of 70 mL/h. At an ambient temperature of 27 $^{\circ}$ C, SIR-8514 has a retention time of 12 min.

Extraction of SIR-8514 from Water. Duplicate 600-mL samples of water were partitioned against 3 \times 200-mL aliquots of dichloromethane (technical grade, redistilled in all glass); these were dried over anhydrous Na_2SO_4 and the combined aliquots were reduced to dryness in a rotary vacuum evaporator. The residues were dissolved in 1 mL of the mobile phase, and 30- μ L samples were subjected to LC. Tap water and field waters collected from mosquito breeding habitats (pasture, pond, and sewage) were fortified with ethanolic solutions of SIR-8514 to give 0.02 (the maximum water solubility as determined

by the manufacturer), 0.01 and 0.005 ppm; these were then extracted and analyzed as above.

Stabilization of SIR-8514 in Water Samples. Because it is frequently not practical to immediately extract water samples, e.g., at field locations, the capability of stabilizing samples at the time of collection is important. Duplicate 600-mL aliquots of tap water, fortified with 0.02 ppm SIR-8514 were mixed with 200 mL of dichloromethane each, and then held for 0, 4, 24, 48, and 72 h at room temperature (ca. 23 °C). The recoveries of SIR-8514 were then determined as above.

Combined Effect of Temperature and pH on SIR-8514 in Tap Water. Tap water was buffered at pH 6.5 (KH₂PO₄ + NaOH), 7.7 [tris(hydroxymethyl)amino-methane + HCl] and 10.0 (NaHCO₃ + NaOH). Thirty samples of 600 mL each, at each pH mentioned, were fortified with 0.02 ppm SIR-8514. These were then held in the dark in capped 1-L bottles in temperature-controlled incubators at 10, 24, and 38 °C. After exposures of 1, 2, 3, 6, and 9 days, duplicate samples from each temperature-pH combination were removed and analyzed. The range of temperature and pH included covered those anticipated where SIR-8514 might be applied in mosquito breeding habitats.

Comparison of Stabilities to Sunlight of SIR-8514 and Diflubenzuron. Eight 140-mm Petri dishes were treated with 1 µg in 1 mL of ethanol/dish of SIR-8514 and another 8 µg in the same manner with diflubenzuron. The ethanol was evaporated under dry nitrogen and the dishes were placed outside in direct sunlight and exposed for 0 (immediate extraction with no exposure), 3, 6, and 9 h. Each dish was washed with 100 mL of dichloromethane, which was reduced to dryness; the residue was dissolved in 1 mL of the mobile phase and 30-µL samples were analyzed by LC. Diflubenzuron was analyzed as previously reported (Schaefer and Dupras, 1977).

Six 1-gal jars containing 3 L of tap water each were treated to give 0.02 ppm SIR-8514; a second set was treated similarly with diflubenzuron. Jars were held outside where they received direct sunlight during the daytime (August 1978). After 0 (immediate extraction with no exposure), 8, 24, 48, 72, and 96 h, one jar of each treatment was brought into the laboratory and duplicate 600-mL aliquots from each were extracted and analyzed. Jars remaining outside during the exposure were refilled daily to the 3-L level, with tap water.

Negligible loss due to volatilization was expected, based on vapor pressure data supplied by the manufacturers.

Stability of SIR-8514 to Microorganisms. Sewage lagoon waters were utilized as in earlier tests with diflubenzuron (Schaefer and Dupras, 1976). Autoclaved and untreated aliquots (600 mL each) were fortified with 0.02 ppm SIR-8514 and then extracted and analyzed after 0 (immediate), 6, and 24 h of holding under laboratory conditions (23 °C, 30% RH).

Adsorption of SIR-8514 onto Straw. To determine the potential loss of active ingredient from water onto organic matter, 0–3.0 g amounts of straw were added to 600-mL aliquots of tap water, fortified with 0.02 ppm SIR-8514; one set of samples was held for 24 h and another for 48 h at 21 °C in the dark. Methods for separation of straw and water and for extraction of straw were as previously reported (Schaefer and Dupras, 1976). Residual SIR-8514 in all of the water sample was determined; but for the straw, only those from the 3.0 g/600-mL water samples were extracted and analyzed.

Persistence of SIR-8514 and Diflubenzuron on Pasture Grasses. Applications of aqueous sprays of

Table I. Recovery of SIR-8514 from Different Types of Water

type of water	concn, ppm		
	0.02	0.01	0.005
tap	0.02	0.0098	0.0050
	0.02	0.0099	0.0048
	0.02 ^a	0.0098	0.0049
pasture	0.021	0.010	0.0047
	0.020	0.010	0.0052
	0.021	0.010	0.0049
pond	0.019	0.0095	0.0045
	0.019	0.010	0.0054
	0.019	0.0098	0.0050
sewage	0.016	0.0050	0.0026
	0.011	0.0057	0.0027
	0.014	0.0054	0.0026

^a Third row under each water type are averages.

diflubenzuron result in high amounts of long-lasting residues on vegetation (Schaefer and Dupras, 1977). It was of interest to compare the persistence of SIR-8514 residues on vegetation following aqueous spraying with those of diflubenzuron. A 0.05-acre pasture plot in Kern County, CA, was divided into two halves and one was treated with SIR-8514 and the other with diflubenzuron. Each treatment rate was 0.05 lb of active ingredient (AI)/acre and both were applied as aqueous sprays by handcan. Samples of vegetation (ca. 1 lb each) were collected at random just before and at 1 h and at 3, 7, 14, 22, 28, 35, and 42 days following treatment from each plot. Vegetation samples were held in sealed (double), plastic bags at -20 °C, or below, until analyzed. The extraction and clean-up methods of Diprima et al. (1978) were used for diflubenzuron with final analysis by LC (Schaefer and Dupras, 1977). The same methods were used for SIR-8514 except that dichloromethane was used for extraction of vegetation, and LC as reported herein.

Release of SIR-8514 from Sand Granules. Formulations of SIR-8514 on sand were utilized in order to study the potential for penetration of vegetative canopies without deposit of significant residues. A 1% and 0.5% granule were evaluated for release of active ingredient into water. Large glass bottles (5 gal) containing 18 L of water each were treated with 36 mg of 1% and 72 mg of 0.5% granules to give a potential concentration of 0.02 ppm each (assuming a 100% release rate). After 4 h, water samples were pipetted from the top, middle, and lower portions of each bottle. Each was poured into a 1-L graduate until a 600-mL sample was obtained, which was then extracted and analyzed for SIR-8514.

Residues of SIR-8514 in Water and on Vegetation in 1978 Field Tests. The 0.5% sand granules were used in field trials. In one test two 0.05-acre pasture plots having bermuda grass covered with 6–8 in. of water were treated at rates of 0.01 and 0.02 lb of AI/acre. At 2, 24, and 48 h posttreatment, duplicate 600-mL water samples were collected from each plot; to these, 200 mL of dichloromethane was added to each. They were shaken and transported to the laboratory for extraction and analysis. Mortality of mosquito larvae in each plot was monitored.

A 12-acre pasture near Fresno, CA, was treated by aircraft; 0.5% SIR-8514 sand granules were applied to give a treatment rate of 0.05 lb of AI/acre. Immediately before treatment and at 4, 24, and 48 h posttreatment, four 600-mL water samples and a composite vegetation sample (ca. 2 lb, collected over entire field) were taken. These were extracted and analyzed as described earlier. A pre-treatment sample of the vegetation was fortified at 0.01 ppm and carried through the extraction and analysis with

Table II. Effects of Temperature and pH on the Stability of SIR-8514 in Tap Water

pH	held at °C	posttreatment time, days				
		1	2	3	6	9
6.5	10	0.011	0.018	0.016	0.017	0.017
		0.016	0.018	0.016	0.017	0.013
		0.014 ^a	0.018	0.016	0.017	0.015
7.7	10	0.017	0.019	0.017	0.017	0.017
		0.017	0.018	0.017	0.018	0.017
		0.017	0.018	0.017	0.017	0.017
10.0	10	0.016	0.018	0.016	0.016	0.014
		0.017	0.018	0.016	0.016	0.014
		0.016	0.018	0.016	0.016	0.014
6.5	24	0.019	0.018	0.019	0.017	0.017
		0.018	0.019	0.019	0.017	0.015
		0.018	0.019	0.019	0.017	0.016
7.7	24	0.018	0.020	0.019	0.017	0.017
		0.019	0.019	0.019	0.018	0.017
		0.018	0.020	0.019	0.017	0.017
10.0	24	0.015	0.016	0.014	0.010	0.0050
		0.014	0.015	0.013	0.010	0.0057
		0.015	0.016	0.014	0.010	0.0053
6.5	38	0.017	0.019	0.019	0.014	0.014
		0.017	0.019	0.018	0.015	0.0086
		0.017	0.019	0.019	0.015	0.011
7.7	38	0.016	0.019	0.018	0.015	0.012
		0.019	0.017	0.018	0.015	0.0060
		0.018	0.018	0.018	0.015	0.0092
10.0	38	0.012	0.0077	0.0036	0.0012	0.00046
		0.014	0.0077	0.0032	0.00089	0.00029
		0.013	0.0077	0.0034	0.0011	0.00037

^a Third row under each pH-temperature combination are averages.

the field samples. Effects of the treatments on the naturally occurring populations of mosquito larvae present at the times of treatment were determined.

RESULTS AND DISCUSSION

Recovery of SIR-8514 from Water. Recoveries of SIR-8514 from waters of representative mosquito breeding habitats are shown in Table I. For sewage water the recoveries were 70, 54, and 52% for samples fortified at 0.02, 0.01, and 0.005 ppm, respectively. Recoveries from tap, pond, and pasture waters were 98%, or better. Using the 600-mL sample size, it was possible to estimate SIR-8514 concentration to a detection limit of 0.0002 ppm (minimum peak was twice background). The lower recoveries from sewage water are presumably due to losses of adsorption onto organic matter.

Stabilization of SIR-8514 in Water Samples. When 600-mL tap water samples fortified with 0.02 ppm SIR-8514 were stabilized with dichloromethane, average recoveries were 99.7, 98.4, 94.6, 92.1, and 93.1% after holding periods of 0, 4, 24, 48, and 72 h, respectively. Thus, if samples were extracted within 24 h of having added the first aliquot of dichloromethane, the maximum loss is ca. 5%. However, if samples are collected on Friday and not extracted until the following Monday, a loss of 7–8% can be expected.

Effects of Temperature and pH on the Stability of SIR-8514 in Water. Table II shows the persistence of SIR-8514 at selected temperature-pH combinations. For unknown reasons, the recoveries of all samples at 10 °C were low at 1 day; these values were all higher at 2 days. There is little, if any, effect of pH at 10 °C; at 24 °C, only the highest pH (10.0) clearly reduced persistence. At 38 °C, there is little difference between the lower pH levels but persistence is markedly reduced at the upper one. These results are quite similar to effects of temperature and pH on the hydrolytic stability of diflubenzuron (Schaefer and Dupras, 1976).

Table III. Stability of SIR-8514 and Diflubenzuron on Glass Surfaces Exposed to Direct Sunlight (in Percent Recovery)

compound	exposure time in direct sunlight, h			
	0	3	6	9
SIR-8514	100	91.1	80.0	71.1
	100	84.4	82.1	68.9
	100 ^a	87.8	81.1	70.0
diflubenzuron	100	98.3	91.7	86.7
	100	96.7	95.0	88.3
	100 ^a	97.5	93.3	87.5

^a Averages.

Table IV. Stability of Aqueous Solutions of BAY-8514 and Diflubenzuron Held in Direct Sunlight, August 1978

exposure period, h	diflubenzuron		BAY-8514	
	concn, ppm	% of initial concn	concn, ppm	% of initial concn
0 ^a	0.020		0.017	
	0.019		0.018	
	0.020 ^b	100	0.017	100
8	0.018		0.016	
	0.018		0.016	
	0.018	94	0.016	93
24	0.019		0.014	
	0.018		0.014	
	0.018	94	0.014	83
48	0.015		0.012	
	0.016		0.012	
	0.015	78	0.012	70
72	0.011		0.014	
	0.012		0.012	
	0.012	59	0.013	76
96	0.0080		0.011	
	0.0091		0.012	
	0.0084	43	0.012	67

^a Extracted immediately; no exposure to sunlight.

^b Third row under each exposure period are averages.

Table V. Recovery of SIR-8514 from Water Containing Straw (in ppm)

quantity straw, g/600 mL of H ₂ O	holding time	
	24 h	48 h
0.0	0.015	0.016
0.5	0.0072	0.0043
1.0	0.0045	0.0032
1.5	0.0042	0.0030
2.0	0.0040	0.0027
3.0	0.0032 ^a	0.0020 ^b

^a Straw contained 2.07 ppm SIR-8514. ^b Straw contained 2.09 ppm SIR-8514.

Stabilities of SIR-8514 and Diflubenzuron to Sunlight. The persistence of films of each compound on glass plates to direct sunlight is shown in Table III. SIR-8514 appears to be slightly less persistent than diflubenzuron. When aqueous solutions of each compound were held in direct sunlight (Table IV), SIR-8514 appears to have slightly greater persistence; this could be due to UV absorption by the water which thereby reduces direct effects on SIR-8514.

Stability of SIR-8514 to Microorganisms. When autoclaved aliquots of sewage water fortified with SIR-8514 were extracted and analyzed after holding times of 0, 6, and 24 h, average recoveries were 88.8, 52.6, and 48.8%, respectively. Nonautoclaved sewage water showed average recoveries of 87.1, 43.3, and 33.0% at the same holding times, respectively. The recoveries from the zero-time samples were similar and even higher than those reported earlier for sewage water (Table I). There was only at 3.8%

Table VI. Residues of SIR-8514 and Diflubenzuron on Pasture Grasses Sprayed with 0.05 lb of AI/acre on July 22, 1977

sample collection time	date (1977)	SIR-8514		diflubenzuron	
		% H ₂ O	ppm ^a	% H ₂ O	ppm ^a
pre	7-22	68.0	ND ^b	68.0	ND ^c
1 h	7-22	66.0	1.03	64.7	3.75
day 3	7-25	61.7	2.74	57.5	3.92
day 7	7-29	66.7	2.66	69.4	2.77
day 14	8-5	64.4	0.74	55.3	1.61
day 22	8-13	62.5	0.31	52.5	1.15
day 28	8-19	63.3	0.26	66.3	1.56
day 35	8-26	58.5	0.32	61.2	0.70
day 42	9-2	65.5	0.17	61.8	0.32

^a Adjusted to dry weight basis. ^b ND = not detected (<0.0059 ppm SIR-8514). ^c ND = not detected (<0.0038 ppm diflubenzuron).

Table VII. Release of SIR-8514 into Water from 0.5 and 1.0% Sand Granules after 4 h (in ppm)

granule	sampling depth in bottle		
	top	middle	bottom
1%	0.011	0.011	0.012
0.5%	0.010	0.011	0.011

reduction in the recovery of SIR-8514 from autoclaved sewage water when the holding time was increased from 6 to 24 h; however, with nonautoclaved sewage water, during the same interval there was a 10.3% reduction in recovery. The latter appears to be due to microbial degradation. These results differ from similar tests with diflubenzuron, where no differences in recoveries between boiled and unboiled sewage waters were observed at the same holding times reported here (Schaefer and Dupras, 1976).

Adsorption of SIR-8514 onto Straw. Table V shows that there is an increased loss of SIR-8514 from water onto straw as the amount of straw and/or the holding time is (are) increased. Analysis of the straw for SIR-8514 substantiates its presence. These data support the conclusion that recovery of SIR-8514 from sewage water (Table I) may be reduced because of adsorption onto organic constituents.

Persistence of SIR-8514 and Diflubenzuron on Pasture Grasses. The residues of SIR-8514 and diflubenzuron on pasture grasses treated with aqueous sprays calculated to give 0.05 lb of AI/acre are shown in Table VI. The generally lower residues for SIR-8514 are apparently due to the partial crystallization of active ingredient when the emulsifiable concentrate formulation was pipetted into water in the spray can; unfortunately, we were unaware of this crystallization problem at the time of this field application (July 22, 1977). It is apparent that residues of SIR-8514 are relatively persistent on vegetation, as is true for diflubenzuron.

Release of SIR-8514 from Sand Granules. When 0.5 or 1.0% SIR-8514 sand granules are applied to containers of tap water, 50–60% of the active ingredient is released within 4 h and by that time the compound is evenly distributed with water depth (Table VII). This release rate is much higher than for previous tests with 1% diflubenzuron sand granules (Schaefer and Dupras, 1977) and indicates good potential for use under field conditions.

Table VIII. Residues of SIR-8514 in Pasture Water Treated in 0.01 and 0.02 lb of AI/acre on June 28, 1978 (in ppm)

rate (lb of AI/acre)	sample collection time (h)			
	0 ^a	2	24	48
0.01	ND ^b	0.0038	0.00078	N.D.
	ND	0.0036	0.00068	N.D.
	ND ^c	0.0037	0.00073	N.D.
0.02	ND	0.012	0.00099	0.00057
	ND	0.012	0.0012	0.00051
	ND ^c	0.012	0.0011	0.00054

^a Pretreatment. ^b ND is not detected (<0.0002 ppm). ^c Averages.

Residues of SIR-8514 in Water and on Vegetation in 1978 Field Tests. When 0.5% SIR-8514 sand granules were applied to pasture plots (water pH 8.6) a complete mortality of mosquito larvae (mixed population of *Aedes nigromaculis* and *Aedes melanimon*) occurred at treatment rates of 0.01 and 0.02 lb of AI/acre. Residues of SIR-8514 in the pasture water (Table VIII) declined steadily and could not be detected at the lower rate by 48 h.

The aerial application of 0.05 lb of AI, using 0.5% sand granules, also resulted in 100% mortality of mosquito larvae (*Aedes nigromaculis*). Water samples (pH 8.3) showed average concentrations of 0.017, 0.0035, and 0.0012 ppm at 4, 24, and 48 h after treatment. Samples of vegetation collected immediately before and at 4, 24, and 48 h after treatment showed no detectable residues (<0.0059 ppm). Pretreatment vegetation samples fortified with 0.01 ppm SIR-8514 gave average recoveries of 96.7%. Thus, the 0.5% SIR-8514 sand granules can be applied through a vegetative cover to achieve mosquito control without depositing persistent residues on vegetation.

The methods described allow for the quantitative determination of SIR-8514 from water and vegetation samples; its concentration in water can be stabilized for several days by the addition of dichloromethane. The persistence of SIR-8514 in water lowers as pH and temperature simultaneously increase and presumably is due to hydrolysis. Loss from water by adsorption onto organic matter increases as organic content does. Degradation of SIR-8514 in polluted water by microorganisms apparently occurs to a limited extent, e.g., 10% in 24 h; this may be an important factor with respect to long-term persistence in foul waters. SIR-8514 residues on vegetative surfaces are persistent but can be avoided by the application of granular formulations. The persistence of SIR-8514 is very similar to that of diflubenzuron, which was previously described (Schaefer and Dupras, 1976).

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