NOVIFLUMURON ACTIVITY in HOUSEHOLD and STRUCTURAL INSECT PESTS

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Abstract Hexaflumuron is a chitin synthesis inhibitor registered in the United States solely as a termite bait active ingredient. A benzoylphenyl urea insect growth regulator, with the proposed common name of noviflumuron, is being developed by Dow AgroSciences for use as the active ingredient in a termite baiting system. In other pests, noviflumuron shows faster activity and a broader spectrum of control than hexaflumuron, demonstrating control of cockroaches, fleas, ants, drywood termites and houseflies. Noviflumuron has activity as a bait toxicant, as well as in liquid and dust formulations. This paper presents a summary of the spectrum of activity, speed of control, control longevity and repellency of the compound in both laboratory and field trials on urban insect pests.

Key Words chitin synthesis inhibitor bait insect growth regulator

INTRODUCTION

Dow AgroSciences is developing a new insect growth regulator for use as the active ingredient in the Sentricon (Trademark of Dow AgroSciences LLC) *Termite Colony Elimination System* (STCES). The new compound has shown broad-spectrum activity in a number of household pests, in addition to being highly active in subterranean termites. This paper will describe the molecule and its activity in household and structural insect pests.

Noviflumuron [N{((3,5-dichloro-2-fluoro-4(1,1,2,3,3,3-hexafluoropropoxy) phenyl)amino)carbonyl}-2,6-difluorobenzamide] is the proposed common name for a benzoylphenyl urea chitin synthesis inhibitor (CSI). This family of compounds is thought to act by affecting chitin synthetase, disrupting chitin deposition in the insect cuticle in a way that prevents successful molting. Noviflumuron was first synthesized by Dow AgroSciences chemists in 1995. Noviflumuron is a crystalline, odorless compound with low water solubility, and a high melting point.

Beginning in 1995, Dow AgroSciences undertook an extensive analysis of the laboratory performance of noviflumuron against cockroaches, termites, houseflies, and cat fleas. In those early trials, noviflumuron was shown to have significantly greater potency and broader spectrum than hexaflumuron, the CSI active ingredient in the STCES. Since 1997, numerous Dow AgroSciences and outside cooperator laboratory and field studies have further characterized the activity of noviflumuron for control of red imported fire ants, Argentine ants, carpenter ants, pharaoh ants, cockroaches, fleas, and termites. Results of key research trials conducted by Dow AgroSciences and cooperating scientists are discussed in this paper.

MATERIALS and METHODS

Intrinsic Activity of Noviflumuron in Subterranean Termites

A field assessment program has followed laboratory characterization of noviflumuron as a potential active ingredient in the STCES. Specifically, the performance of noviflumuron has been compared with that of hexaflumuron. To investigate if differences in the rate of uptake, clearance,

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insect to insect transfer (trophallaxis), or metabolism of noviflumuron can explain why it is faster and more active than hexaflumuron we conducted a series of experiments to measure these kinetic parameters in Eastern subterranean termites, *Reticulitermes flavipes* (K). Termites were fed cellulose-based diets containing either [14C] noviflumuron or [14C] hexaflumuron and the above pharmacokinetic parameters were measured.

A laboratory study was conducted investigating the effect of limited exposure to hexaflumuron and noviflumuron on *R. flavipes*. In this study, termites were permitted to feed on toxicant-treated filter paper for only 7 days, at which time the treated filter paper was removed and untreated filter paper was substituted (Table 1).

Feeding Deterrence

In a laboratory arena test to investigate feeding deterrence of hexaflumuron and noviflumuron, termites were offered their choice of untreated and treated halves of a filter paper circle. Filter paper halves were treated with either plain acetone or the active ingredient in acetone at rates that would yield concentrations of 1, 10, 100, 1,000, or 10,000 ppm (w/w). The paper halves were oven-dried, weighed and placed side by side within a feeding chamber. Termites were permitted to forage freely on the treated and untreated papers for seven days, at which time the papers were oven-dried and re-weighed (Table 2).

Field Studies

During 1998 through 2000, field studies were conducted with three objectives: 1) obtain performance comparisons for noviflumuron and hexaflumuron with the key termite species found in the United States; 2) obtain comparative data for noviflumuron and hexaflumuron under a wide variety of geographical and environmental conditions; and 3) compare the matrix consumption of both compounds. In these studies, Sentricon stations were applied per commercial label instructions at infested sites. Stations were inspected monthly and auxiliary stations were installed around infested stations. Both in-ground and above-ground baiting were included in the 1998 field research at a total of 34 trial sites; in 1999 and 2000, only in-ground baiting was conducted at a total of 118 trial sites. Most trials involved some characterization of the colony to allow for colony-specific information to be gathered; in some cases, however, characterization was not completed before active stations at a given site were baited.

Table 1. Effect of limited (7 day) exposure of treated filter paper to groups of termites (*Reticulitermes flavipes*) at 2, 4, 6, and 8 weeks post-exposure (wpe)

		Mean number of dead termites (n=50) at wpe:*			
Compound	Concentration (ppm)	2	4	6	8
Hexaflumuron	100	5.5a	17.7b	29.5b	46.5ab
	1000	6.0a	17.5b	32.7b	40.2bc
	10000	8.2a	17.0b	33.0b	41.0bc
Noviflumuron	100	9.5a	17.0b	30.7b	41.2bc
	1000	8.2a	16.7b	28.2b	45.2ab
	10000	10.7a	28.2a	50.0a	50.0a
Control	0	5.2a	6.5c	12.2c	10.7d

*Means within a column sharing the same letter do not differ significantly at the 0.05% level (Student-Newman-Keuls test).

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Table 2. Amounts of feeding by *Reticulitermes flavipes* on treated and untreated filter paper during 1 week in a side-by-side one-way choice test

Treatment	Concentration (ppm)	Mean amount (mg) of treated paper consumed (95% confidence intervals)	Mean amount (mg) of untreated paper consumed (95% confidence intervals)
Noviflumuron	1	9.9 (4.1-15.7)	5.8 (0.1-11.6)
	10	7.9 (-2.0-17.8)	9.4 (-0.5-19.4)
	100	6.3 (-2.5-15.2)	7.4 (-1.4-16.3)
	1000	7.5 (-4.4-19.5)	10.3 (-1.7-22.3)
	10,000	9.1 (-1.8-20.0)	4.1 (-6.8-15.0)
Hexaflumuron	1	5.4 (-5.6-16.4)	11.1 (0.1-22.1)
	10	8.5 (-9.9-27.0)	8.5 (-9.9-27.0)
	100	8.6 (-0.1-17.3)	8.9 (0.2-17.6)
	1000	8.6 (-6.7-24.0)	11.0 (-4.4-26.4)
	10,000	7.9 (-0.3-16.0)	7.1 (-1.1-15.2)
Control	0	9.4 (-1.6-20.5)	8.3 (-2.7-19.4)

Within each choice test, there was no significant difference in consumption between treated and untreated paper (T-test, $p>0.10$).

Colonies were characterized using one of two methods: a single mark-recapture technique described by Grace et al. (1995), or the installation of a Baitube (Trademark of Dow AgroSciences LLC) device containing dyed matrix (0.1% Nile Blue A or 0.5% Neutral Red) (Atkinson, 2000). Both these techniques allow the investigator to determine that termites infesting stations belong to the same colony if dyed individuals are present. Colonies were thus characterized in order to leave one or more "connected" station(s) baited with untreated matrix during the trial and so be able to determine whether feeding deterrence was the cause of a lack of feeding activity on the treated matrix. Termites involved in these trials were *Coptotermes formosanus* (S.), *Heterotermes* spp. and a variety of *Reticulitermes* spp.

Once two or more stations were connected via dye marking of the termites, baiting was conducted by replacing infested monitoring devices with Baitube devices containing matrix treated with either hexaflumuron (0.5%) or noviflumuron (0.5%). If a site comprised two separate colonies, one colony was randomly assigned either hexaflumuron or noviflumuron and the second colony was baited with the other active ingredient. If only one colony was present on a site, it was randomly assigned a treatment of either hexaflumuron or noviflumuron. Baitube devices containing untreated matrix were installed in at least one termite-infested station per colony, as previously described.

In accordance with label directions for the Sentricon System, the baiting process consisted of dislodging termites from the monitoring devices, estimating their number, and placing them into the water-moistened Baitube device. Data recorded included: date baited, date monitored, termite species, estimated number of termites present in baited stations, estimated percent monitoring device or matrix consumed, presence of mold or fungus, presence of non-target arthropods, observed effects on termites (change in coloration, behavior, etc.), and caste proportion changes.

Time of Year, Amount of Bait Consumed

The effect of time of year that baiting was initiated on time to elimination was analyzed as well, for *Reticulitermes* spp. The amount of bait matrix consumed was compared for the two active ingredients.

Cockroaches

Laboratory assays were conducted with German cockroaches, *Blatella germanica* (L.), to determine noviflumuron activity against this pest by different routes of exposure. In these assays, cockroach nymphs were exposed to noviflumuron, hexaflumuron, and hydramethylnon in low-dose, continuous ingestion or contact exposure regimes. To measure ingestion activity, corn meal was treated with the test compounds and the treated corn meal was offered to groups of second and third-instar nymphs for 42 days. Mortality of exposed insects was monitored weekly, and LC_{50} values were calculated from the resultant mortality data.

Two additional aspects of ingestion toxicity of noviflumuron to German cockroaches were also investigated: reproductive effects and horizontal transfer. To examine reproductive effects, pairs of young cockroach adults were exposed to 0.5% noviflumuron in a gel bait formulation for intervals of 1, 3, 7, 14 days or continuously through 28 days. Egg case abortion and production of viable young were the factors recorded.

The residual efficacy of noviflumuron sprayable formulations was determined by formulation and surface substrates to German cockroaches. The suspension concentrate (SC) and wettable powder (WP) formulations (0.05% and 0.1% a.i.) applied to clean and dirty surfaces, and aged through 120 days indoors, were toxic to second and third stage German cockroach nymphs.

House Flies

In a probe laboratory study, noviflumuron and hexaflumuron were applied to fly larval media at concentrations of 0.1, 1.0, 10 and 100 ppm. House fly, *Musca domestica* (L.), eggs were infested onto the treated media. After 20 days, presence of adult flies was recorded.

Fleas

Control of cat flea, *Ctenocephalides felis* (B.), larvae by ingestion of treated media was tested in the lab. In this test, noviflumuron and hexaflumuron were formulated in acetone at concentrations of 1, 10, 100, and 1000 ppm and applied to flea larval medium in glass jars. Second and third instar flea larvae were placed in the media and held for 3-5 weeks, at which time number of emerged fleas was recorded.

Carpenter Ants

Early-stage field trials conducted within Dow AgroSciences in 2001 have indicated that 0.5% noviflumuron in sugar-milk bait could cause an unexpectedly rapid reduction in foraging activity of the black carpenter ant, *Camponotus pennsylvanicus* (De Geer). It was unclear in those field studies whether or not the reduction in foraging activity caused by noviflumuron was due to reduced foraging behavior or direct mortality of worker ants. A laboratory test was conducted to determine the type of adverse effects caused by a continuous oral ingestion of noviflumuron against carpenter ant workers.

Carpenter ant workers, primarily the large or major caste, were placed in round, plastic test arenas (round, 17.8 cm by 7.6 cm) which contained cardboard harborage and a water vial. Four total treatment scenarios were tested, consisting of 2 continuous-exposure feeding regimes: 20% sucrose agar bait alone, or 20% sucrose agar bait and 20% sugar milk bait. In these feeding regimes, ants were exposed either to bait treated with 0.5% noviflumuron or untreated.

Red Imported Fire Ants

Field trials were established during 1999, 2000, and 2001 in Georgia and Texas to evaluate several concepts for control of red imported fire ant, *Solenopsis invicta* (B.), on rangeland and pasture sites. Both broadcast applications and individual mound treatments (IMT) were evaluated as part of this project. All broadcast field trials were established on plots of 0.5 to 2.0 acres.

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Smaller broadcast plots, generally 0.5 to 1.0 acre, were generally replicated 3 times at each location; larger 2.0 acre plots were replicated by location. Individual mound treatment (IMT) trials were typically 10 replicated trials with each mound representing one replicate. All trials were designed to evaluate the potential for fire ant baits using several toxicants, including noviflumuron. The bait used was a corn grit/soybean oil granule with noviflumuron dissolved in the oil. The data is based upon broadcast application rates of 1.5 lbs of bait product per acre or 4 tablespoons of bait product per mound in IMT trials, with percent active mound reduction the reported result.

RESULTS

Intrinsic Activity of Noviflumuron in Subterranean Termites

Noviflumuron was found to be more toxic and faster acting than hexaflumuron in all the experiments performed in this study. Internal uptake of noviflumuron is generally less than hexaflumuron, especially at the higher diet concentrations. Hexaflumuron is cleared from termites in a first order process with a half life of 8-9 days, whereas the half life of noviflumuron in termites is much longer, 29-91 days depending on the initial concentration fed to the insects. Both compounds are efficiently transferred from treated to untreated termites by trophallaxis, with similar kinetics. The dose of noviflumuron required internally in termites to result in toxicity is at least 5 to 6 times less than that required of hexaflumuron. Thus, noviflumuron is either more active at the target site, and/or has much greater availability to reach the target site than hexaflumuron. It is clear that the faster activity of noviflumuron over hexaflumuron comes about due, at least in part, to a combination of increased toxicity of noviflumuron at a given internal dose, and to its slower clearance from the body of termites.

In the laboratory study, 10,000 ppm noviflumuron-treated paper induced more rapid mortality than hexaflumuron- treated paper indicating that noviflumuron is intrinsically more potent than hexaflumuron (Table 3).

Feeding Deterrence

In this study, no significant feeding deterrence was detected for either noviflumuron or hexaflumuron (Table 4).

Table 3. Comparative days to elimination of subterranean termite colonies for noviflumuron and hexaflumuron trials conducted from 1998 through 2000

	Hexaflumuron	Noviflumuron
Number of colonies baited	53	74
Number of colonies eliminated	53	74
Mean days to elimination	205	107
Median days to elimination	208	90

Table 4. Impact of early vs. late initiation of baiting with noviflumuron and hexaflumuron upon the mean time to elimination of *Reticulitermes* spp.

	Mean days to elimination	
Baiting period	Hexaflumuron	Noviflumuron
Overall	204	109
Baited April – June	162	95
Baited July – October	221	117

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Field Studies

Results of these studies were summarized to compare the time to elimination (an elimination date was recorded once active termites were no longer found in any Baitube, or bait was no longer being consumed for two consecutive monitoring periods). Table 5 shows that noviflumuron resulted in colony elimination in approximately half the time required for hexaflumuron.

Time of Year, Amount of Bait Consumed

The data confirm for hexaflumuron that baiting in the later part of the year, from July–October, results in a longer time to elimination than baiting in the first part of the year, from April –June. For noviflumuron, however, the difference in time to elimination is less for early vs. late season baiting than it is for hexaflumuron. Results indicated that the number of Baitube devices required for elimination of termite colonies is lower for noviflumuron than for hexaflumuron.

Cockroaches

In this series of tests, ingestion activity of noviflumuron was found to be outstanding and superior to that of hexaflumuron (Table 6). Noviflumuron had a slightly slower onset of ingestion activity than hydramethylnon, but its potency was far greater. In related choice tests, noviflumuron was shown not to be repellent or a feeding deterrent to second and third-stage cockroach nymphs, even at a concentration of 10,000 ppm in corn meal.

In all exposures, noviflumuron had a negative impact on cockroach reproduction that would be expected to contribute toward long-term population suppression in the field. To investigate horizontal transfer, frass from toxicant-exposed adult male cockroaches was made available to first-stage nymphs in choice and no-choice trials. In these trials, noviflumuron and hydramethylnon were both effectively transferred to cockroach nymphs via frass. (Table 7)

Twenty-minute exposure to treated clean masonite and stainless steel resulted in 100% mortality of the test insects by 28 days after treatment. Noviflumuron provided less control on dirty surfaces and painted plywood. In a demonstration that cockroach activity of noviflumuron is broader than a single species, it was shown to be an effective toxicant for killing American cockroach, *Periplaneta americana* (L.), nymphs as well.

Table 5. Comparison of Baitube device installation and consumption

	Hexaflumuron	Noviflumuron
Mean number of Baitube devices installed	10.9	7.7
Mean number of Baitube devices consumed	5.8	3.7
Mean active ingredient consumed	1.02 g	0.65 g

Table 6. Effect of low dose, continuous exposure to noviflumuron-, hexaflumuron-, and hydramethylnon-treated cornmeal to 2nd-3rd stage German cockroaches

	Spearman-Karber Calculated LC50 (ppm)*				
Compound	7 days	21 days	28 days	35 days	42 days
Noviflumuron	>200	<<0.19	<<0.19	<<0.19	<<0.19
Hexaflumuron	>200	>200.	>200.	>200.	>200.
Hydramethylnon	123	55.3	29.3	5.9	5.9

*Hamilton et al., 1977.

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Table 7. Comparative impact of horizontal transfer of Noviflumuron (in a gel bait) and Hydramethylnon (in Maxforce gel bait) in German cockroach nymphs

		Mean # dead (of 20/rep) at X days of exposure to frass*			
Frass from Adult Male Treatment	Nymphs Exposed To:	7 days	14 days	21 days	28 days
0.5% noviflumuron	Frass + dog food	6.00a	13.62b	19.75a	20a
2.15% hydramethylnon		8.25a	16.87a	19.25a	20a
Dog food control		1.75b	1.75c	2.12b	2.25b
0.5% noviflumuron	Frass only	6.50b	13.25b	16.25b	18.37ab
2.15% hydramethylnon		16.25a	20.00a	20.00a	20.00a
Dog food control		3.00c	6.50c	14.62b	18.12b

*Treatments sharing a letter within a column, within a first-instar exposure class do not differ at the 0.05% level of significance (Bonferroni comparisons).

House Flies

In this trial, both compounds were effective at preventing the development of the larvae into adult houseflies at 10 and 100 ppm (Table 8).

Fleas

Results indicate that noviflumuron was very effective at preventing adult cat flea emergence (Table 9).

Carpenter Ants

Under a forced-exposure experimental system, carpenter ant workers readily fed on agar or sugar-milk with 0.5% noviflumuron. Worker ants fed on noviflumuron began to appear sluggish approximately 7-10 days post-treatment. Both treatment regimens of noviflumuron caused toxic effects to workers; however, those receiving sugar-milk treatments became sluggish and died much faster when compared to ants receiving agar treatment only (Table10). These results indi-

Table 8. Impact of noviflumuron- and hexaflumuron-treated fly larval medium on house fly development

Treatment	Rate (ppm w/w dry media)	Number of Replications (of 10) Yielding Adult Flies
Noviflumuron	0.1	5
	1.0	8
	10.0	0
	100.0	0
Hexaflumuron	0.1	8
	1.0	9
	10.0	0
	100.0	0
Control	0	7

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Table 9. Activity of noviflumuron and hexaflumuron treated rearing media against cat flea larvae, as quantified by measuring adult emergence

Compound	Rate (ppm)	% Control
Noviflumuron	1	52
	10	80
	100	100
	1000	100
Hexaflumuron	1	52
	10	70
	100	82
	1000	100

Table 10. Effects of 0.5% Noviflumuron on carpenter ant workers by oral ingestion

		% Dead Worker Ants, Days Post-Exposure						
Treatment Scenario	N	3	7	10	14	17	21	24
20% Sucrose Agar (untreated)	52	3.9	9.6	9.6	11.5	13.5	13.5	19.2
20% Sucrose Agar with 0.5% noviflumuron	55	9.1	12.7	14.6	29.1	30.9	72.7	80.0
20% Sucrose Agar & 20% Sucrose Milk (both untreated)	35	8.6	11.4	11.4	11.4	11.4		
20% Sucrose Agar & 20% Sucrose Milk (both treated with 0.5% noviflumuron)	33	12.1	36.4	63.6	90.9	100.0		

cate that uptake of noviflumuron is faster in worker ants via the sugar-milk. These findings demonstrate that noviflumuron does have the potential to cause slow-acting toxic effects on carpenter ant workers.

Red Imported Fire Ants

Results should be considered preliminary, since these trials were designed to act as initial probe studies for this molecule. Results from these trials consistently demonstrated, however, that noviflumuron provided excellent RIFA control when applied as either a broadcast or an individual mound treatment. Although noviflumuron was initially slower acting than commercial standards, at approximately 2 months post application, noviflumuron provided control equal to or better than commercial standards evaluated. On average, noviflumuron treatments in 2001 broadcast trials provided 73% mound control at 20 DAT, 90% control at 40 DAT and 96% control at 70 DAT. Some limited long-term control results (up to about 1 year after treatment) from the earlier 1999 studies suggest that noviflumuron bait treatments are potentially superior to all of the commercial standards that were evaluated in these trials. Also, in these trials, varying the noviflumuron concentrations in the bait (0.05%, 0.11%, and 0.22%) did not significantly affect long term RIFA control.

SUMMARY and CONCLUSIONS

In summary, noviflumuron is a promising compound for control of multiple household and structural insect pests. Dow AgroSciences is continuing its research and development efforts with a goal to develop and market noviflumuron for professional pest management opportunities.

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