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Evaluation of chlorfenapyr for control of the bed bug, *Cimex lectularius* L.

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Abstract

BACKGROUND: The presence of bed bug populations resistant to pyrethroids demands the development of new control tactics, including the use of insecticides with new modes of action. Insecticides that disrupt oxidative phosphorylation in insect mitochondria can be an option. Laboratory assays were used to measure the toxicity of chlorfenapyr to susceptible strains and two strains highly resistant to pyrethroids. The effectiveness of two chlorfenapyr-based formulations was compared, and behavioral responses of bed bugs to dry residues of aerosol sprays were evaluated.

RESULTS: Chlorfenapyr was effective against all bed bug strains, killing them at a similar rate, regardless of their susceptibility status to pyrethroids. Dry residues aged for 4 months were as toxic as fresh dry residues. The aerosol formulation had contact activity and caused faster mortality than a water-based formulation. Bed bugs did not avoid resting on surfaces treated with aerosol.

CONCLUSION: Chlorfenapyr is an option for controlling pyrethroid-resistant bed bugs. While it does not cause quick knockdown, its long residual activity and no avoidance behavior of bed bugs to dry residues appear to make this insecticide suitable for bed bug control. A faster insecticidal effect is obtained with the aerosol formulation, suggesting greater bioavailability of the toxicant.

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Keywords: bed bug; insecticide resistance; chlorfenapyr; laboratory evaluations

1 INTRODUCTION

Recently, the bed bug, *Cimex lectularius* L. (Heteroptera: Cimidae), has re-emerged as a serious and growing problem, not only in North America, but globally.^{1–5} This bed bug resurgence has renewed interest in effective control tactics. Chemical options for bed bug management, however, are limited because of precautions and regulatory restriction pertaining to insecticide treatment in areas where human exposure is possible. Furthermore, the near disappearance of bed bugs in many parts of the world had reduced the interest of industry for registering insecticide products against this pest. The insecticides most commonly used today for bed bug control in the United States are pyrethroids.⁶ Recent laboratory studies reported that some bed bug populations have become highly resistant to this group of insecticides.^{5,7–11} Molecular and synergism studies suggest that target-site insensitivity and metabolic detoxification are involved as resistance mechanisms to pyrethroids in some bed bug populations.^{10,12,13} Failure to eliminate resistant bed bugs could be a contributing factor for the spread of this pest.⁸ Therefore, alternative effective insecticides for bed bug control are of great importance.

Chlorfenapyr is an option that is registered for bed bug control and is increasingly being used commercially.^{6,14,15} However, its effect on pyrethroid-resistant bed bugs has not been fully investigated under laboratory conditions. The only report on the efficacy of chlorfenapyr on bed bugs was conducted with a susceptible laboratory strain that had not been exposed to insecticides for more than two decades.¹⁶ A previous study showed bed bugs did not avoid resting on chlorfenapyr-treated

surfaces.¹⁷ Chlorfenapyr is a halogenated pyrrole that disrupts mitochondrial oxidative phosphorylation.¹⁸ It is a pro-insecticide that must be activated by cytochrome P450 monooxygenases to its more active metabolite.¹⁹ Chlorfenapyr has proved to be an effective non-repellent insecticide against other medically important and household insects, e.g. cockroaches, ants, horn flies, subterranean termites and mosquitoes.^{20–24} The objective of this study was to evaluate the effectiveness of chlorfenapyr against both susceptible and pyrethroid-resistant strains of bed bugs. The toxicity of aged residues of chlorfenapyr to bed bugs was also examined, and the effectiveness of two chlorfenapyr-based formulations was compared.

2 MATERIALS AND METHODS

2.1 Bed bug strains

Insects were obtained from colonies maintained at 26 °C and 65 ± 5% RH under a 12 : 12 h light : dark photoperiod. Two strains used were highly resistant to deltamethrin:^{8,9,12} CIN-1 (collected in 2005 in Cincinnati, OH) and WOR-1 (collected in 2007 in Worcester, MA). Two strains were susceptible:^{8,9} LA-1 (collected in 2006 in Los Angeles, CA) and Fort Dix, which had not been exposed to

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insecticides for more than 30 years.²⁵ Insects were fed in the laboratory through a parafilm-membrane feeder with citrated rabbit blood that was heated to 39 °C with a circulating water bath.²⁶ Evaluations began 7–12 days after adult emergence, and insects had not been fed as adults. The authors had previously found more consistent results of responses to insecticides with such bed bugs than with those that had more recently emerged or fed.

2.2 Evaluations with technical-grade chlorfenapyr

Adults (1:1 sex ratio; three replicates of 20 insects) were continuously exposed to dry deposits of technical-grade chlorfenapyr (99.3% purity; Chem Service, West Chester, PA) that corresponded to the label rate of the commercial formulations. A 5 g L⁻¹ acetone solution of the insecticide (50 µL) was applied to each filter paper disc [Whatman No. 2, cut to 2.27 cm² (1.7 cm diameter)] and allowed to dry completely before being placed in the bottom of individual cells of 24-well cell culture plates. A bed bug was placed in each cell, and the culture plate was then covered. Control discs received 50 µL of acetone only. Continuous exposure to the upper surface of the filter paper was ensured by the tight fit of the paper and by a Fluon AD-1 (polytetrafluoroethylene; Northern Products, Woonsocket, RI) coating on the walls of each cell that prevented individuals from climbing off the treated surface. Temperature was maintained at 26 °C after initiation of the exposure. Mortality was assessed daily for at least 1 week by gently touching each individual with entomological forceps and categorizing bed bugs as alive (coordinated avoidance movement) or dead (moribund, e.g. no response, usually on backs, with no movement of any body parts).

2.3 Evaluations with aged residues of Phantom SC

Adults (1:1 sex ratio; three replicates of 20 insects) were continuously exposed to filter papers that were treated with a water-based formulation, chlorfenapyr 5 g L⁻¹ SC (Phantom SC termiteicide-insecticide; BASF, Research Triangle Park, NC). Treated discs were allowed to dry in room conditions (24 ± 2 °C; 50 ± 10% RH) for 3 h (referred to as fresh dry residue hereafter) or for 1, 2 or 4 months. Control insects were exposed to filter paper discs treated with distilled water only (50 µL). Insect mortality was recorded daily for at least 7 days.

2.4 Comparison of efficacy of two chlorfenapyr formulations

In direct contact assays, a group of ten male (or female) bed bugs from the CIN-1 and WOR-1 strains were directly sprayed with Phantom SC or a chlorfenapyr 5 g L⁻¹ aerosol formulation (Phantom pressurized insecticide; BASF, Research Triangle Park, NC). Groups treated with Phantom SC received two pumps (0.28 mL of insecticide formulation, ca 0.0014 g AI) with a fine mist sprayer (4 oz fine mist spray bottle, SQB-4FMS; ProChemical and Dye, Somerset, MA), which was adequate to wet each individual. Other groups of bed bugs received two brief discharges (about 0.5 s) with the aerosol formulation (0.1 mL, 0.0005 g AI). Controls consisted of sprays of distilled water or aerosol without active ingredient (blank formulation). The distance between spray nozzle and treated bugs was approximately 20 cm. Treated individuals were immediately transferred into individual wells of a 24-well cell culture plate lined with filter paper. Insect mortality was recorded 4 h post-spray and then daily for at least 7 days.

In residual assays, discs of filter paper were treated with 50 µL of Phantom SC or two 0.5 s discharges from Phantom aerosol or

aerosol blank. Each of these applications left the filter paper visibly wet, but there was no run-off. These discs were allowed to dry for 3 h before they were inserted into the 24-well cell culture plate. At this time, untreated individual bed bugs were added onto these filter paper discs. Observations of mortality were the same as in Section 2.2.

2.5 Behavioral responses to surfaces treated with chlorfenapyr aerosol

Responses of bed bugs were carried out in 500 mL glass beakers whose bottom-inside surfaces were covered with white filter paper (70 mm diameter, Fisherbrand quality P4), henceforth referred to as arenas. Paper was fixed to the glass with double-sided tape to prevent bed bugs from crawling under the paper. After each assay, papers were removed and beakers were rinsed with acetone. Individual bed bugs were offered two tents made of filter paper (15 × 12 mm, Whatman No. 2) folded in the middle to offer a tent-like shelter of 15 mm length × 5 mm height with two open ends. One tent was treated with a 0.5 s discharge of Phantom aerosol, while the other remained untreated and served as a blank. The treated tent was allowed to dry for 3 h before being placed in an arena. Forty-eight individuals (1:1 sex ratio) from CIN-1 or WOR-1 were evaluated. Behavioral assays lasted about 16 h (from about 4:30 p.m. to 8:30 a.m. the next day) with the following light–dark regimen: lights off at 6 p.m. and lights on at 6 a.m. (the same light cycle used during rearing). During the photophase, each block of 16 arenas was illuminated with a 19 W fluorescent light, which was placed 60 cm above the arena surface (light intensity was approximately 300 lux at arena level). Room temperature remained at 24 ± 2 °C. Insects were acclimated to the arena for 15 min by restricting them in a shell vial (21 mm diameter × 70 mm height), which was placed inverted in the center of the arena. Insects were released by lifting up the shell vial. At the end of each assay it was noted whether the test individual was resting on a treated or untreated tent or wandering in the arena. The number of bed bugs resting on each tent was compared by a chi-square analysis.²⁷ Bed bugs that were wandering were not included in this analysis.

2.6 Data analysis

Successive observations of mortality over time were fitted to the probit, complementary log–log (CLL) or log–probit regression model^{28,29} in order to estimate the time required to kill 50% (LT₅₀) of the exposed insects (Throne JE, <http://ars.usda.gov/Services/docs.htm?docid=11281>). These regression models were chosen from six possible transformations on the basis of chi-square values (lower values better describe the data) and examination of the fitted regression lines compared with the transformed observations.²⁹ The LT₅₀ values of any two treatments with formulated insecticides were compared using lethal time ratios, as described by Robertson *et al.*²⁸ This method was chosen because it is a more powerful method for comparison of lethal times than examination for overlap of 95% confidence intervals.³⁰ According to Robertson *et al.*,²⁸ two LT₅₀ values are not significantly different if the 95% confidence interval (95% CI) of the LT₅₀ ratio includes 1.

3 RESULTS

3.1 Evaluations with technical-grade chlorfenapyr

Time–mortality data from bioassays with dry residues of technical-grade chlorfenapyr showed good regression fit to the probit model

Table 1. Lethal times for four strains of bed bugs exposed to aged deposits of Phantom SC (5 g AI L⁻¹)

Strain	Aging time, dry residues	Regression model	Slope (\pm SE) ^b	LT ₅₀ ^c (days) (CI 95%) ^d	LT ₉₀ ^c (days) (CI 95%)	χ^2 (df) ^e
FORT DIX	Fresh ^a	Log-CLL	6.51 (\pm 0.70)	3.7 (3.2–4.1) a	5.6 (5.1–6.2) a	8.3 (5)
	1 month	Log-CLL	6.86 (\pm 0.73)	3.7 (3.3–4.1) a	5.5 (5.0–6.1) a	2.9 (5)
	2 months	Log-CLL	7.25 (\pm 0.77)	3.9 (3.4–4.2) a	5.6 (5.2–6.2) a	1.7 (5)
	4 months	Log-CLL	7.28 (\pm 0.80)	3.7 (3.3–4.0) a	5.4 (4.9–5.9) a	3.1 (4)
WOR-1	Fresh	Log-probit	4.74 (\pm 0.49)	4.0 (3.5–4.5) a	7.4 (6.3–9.2) a	10.6 (6)
	1 month	Log-probit	4.10 (\pm 0.41)	3.8 (3.3–4.4) a	7.8 (6.5–9.9) a	12.3 (8)
	2 months	Log-probit	4.20 (\pm 0.43)	3.7 (2.7–5.0) a	7.5 (5.5–13.1) a	16.4* (6)
	4 months	Log-probit	3.90 (\pm 0.40)	3.5 (3.0–4.1) a	7.4 (6.2–9.7) a	3.5 (6)
LA-1	Fresh	Log-logit	8.04 (\pm 0.93)	4.0 (3.5–4.6) a	7.5 (6.4–9.5) a	10.6 (6)
	1 month	Log-logit	9.38 (\pm 1.07)	3.9 (3.2–4.7) a	6.7 (5.5–9.5) a	13.1* (7)
	2 months	Log-logit	7.37 (\pm 0.86)	3.6 (3.1–4.2) a	7.2 (6.0–9.3) a	7.6 (6)
	4 months	Log-logit	5.82 (\pm 0.70)	3.7 (2.6–5.1) a	8.8 (6.1–17.1) a	13.2* (6)
CIN-1	Fresh	Log-logit	8.26 (\pm 0.94)	4.8 (4.2–5.5) a	8.9 (7.6–11.1) a	8.6 (8)
	1 month	Log-logit	8.64 (\pm 0.97)	4.8 (4.2–5.4) a	8.6 (7.4–10.6) a	5.8 (9)
	2 months	Log-logit	9.24 (\pm 1.05)	4.9 (4.4–5.5) a	8.5 (7.4–10.4) a	8.7 (8)
	4 months	Log-logit	8.0 (\pm 0.92)	4.6 (4.1–5.3) a	8.8 (7.4–11.1) a	9.6 (7)

^a Residues were allowed to dry for 3 h before insect exposure.^b SE = standard error.^c LT_{50,90} = time necessary to kill 50% or 90% of individuals.^d CI 95% = 95% confidence interval.^e df = degree of freedom.^{*} Significant ($P < 0.05$) (goodness-of-fit test).The LT₅₀ or LT₉₀ values within strains are not significantly different from one another ($P > 0.05$) following the method of lethal time ratios used by Robertson *et al.*²⁸

for the strains Fort Dix and WOR-1 (χ^2 not significant; $P > 0.05$), but poorer fit for LA-1 and CIN-1 ($P < 0.05$). The rate of mortality of bed bugs exposed continuously to chlorfenapyr, measured by LT₅₀ values, was slower in the susceptible laboratory strain Fort Dix [LT₅₀ = 6.6 days (5.9–7.3); $n = 60$; slope = 0.41 \pm 0.048; $\chi^2 = 5.0$; df = 6] than the field-derived strains WOR-1 [LT₅₀ = 5.3 days (4.9–5.8); $n = 60$; slope = 0.56 \pm 0.056; $\chi^2 = 8.2$; df = 5], LA-1 [LT₅₀ = 5.8 days (2.4–10.1); $n = 60$; slope = 0.30 \pm 0.039; $\chi^2 = 28.5$; df = 5] and CIN-1 [LT₅₀ = 5.4 days (3.9–7.2); $n = 60$; slope = 0.30 \pm 0.037; $\chi^2 = 13.3$; df = 6]. However, no significant differences in LT₅₀ values were detected among Fort Dix and the three field strains.

3.2 Evaluations with aged residues of Phantom SC

The χ^2 values were not significant ($P > 0.05$) for 13 of the 16 time–mortality regressions, indicating good fit of data to the regression models (Table 1). Within each strain, LT₅₀ and LT₉₀ values estimated with mortality from assays with residues aged for different periods of time were not significantly different from one another (Table 1). Overall, mortalities of >50% or >90% were recorded after 3 days or 7 days of continuous exposure to dry residues of chlorfenapyr respectively (Table 1).

3.3 Comparison of efficacy of two chlorfenapyr formulations

A good fit to the regression models was observed in all assays, with the exceptions of the mortality regression of direct sprays of CIN-1 with Phantom SC and dry residue exposures of WOR-1 to the same formulation (χ^2 significant; $P < 0.05$) (Table 2). Aerosol treatments with chlorfenapyr, both as direct sprays and as dry deposits, consistently killed CIN-1 and WOR-1 faster

than the liquid formulation (Phantom SC) (at a ratio of LT₅₀ values of 1:2 approximately) (Fig. 1, Table 2). Direct sprays with aerosol had significantly shorter LT₅₀ values than dry residues of the same formulation in evaluations in CIN-1 (1.5 versus 2.5 days) and in WOR-1 (0.9 versus 2.0 days) (Fig. 1). Similar rates of mortality were observed when individuals from CIN-1 were sprayed directly with Phantom SC (LT₅₀ = 5.6 days) or when they were exposed continuously to dry residues of the same formulation (LT₅₀ = 4.6 days). In assays with WOR-1 with Phantom SC, no significant differences were observed between LT₅₀ values of direct sprays (LT₅₀ = 3.1 days) and exposure to dry residues (LT₅₀ = 3.3 days).

3.4 Behavioral responses to surfaces treated with chlorfenapyr aerosol

Bed bugs from CIN-1 did not avoid resting on tents treated with chlorfenapyr aerosol ($\chi^2 = 0.08$; $P = 0.773$) (Fig. 2). No avoidance of chlorfenapyr-treated tents was observed in the strain WOR-1. Individuals of this strain assembled significantly more in tents with dry deposits of chlorfenapyr aerosol than in control tents ($\chi^2 = 8.52$; $P = 0.004$) (Fig. 2). At the end of the test period, 20% of the individuals from the WOR-1 strain were moving sluggishly in the arena, a typical symptom of chlorfenapyr intoxication.

4 DISCUSSION

Management of pyrethroid-resistant bed bugs requires selection of appropriate synergists, insecticides with new modes of action, improved formulations of existing insecticides or incorporation of non-chemical control tactics.^{8,12} The addition of the synergist piperonyl butoxide to pyrethroids has been

Table 2. Time–mortality regression estimates for bed bugs exposed to different formulations of chlorfenapyr

Formulation	Strain							
	CIN-1				WOR-1			
	Type of exposure	Regression model	Slope (\pm SE)	LT ₅₀ (days) (CI 95%)	χ^2 (df) ^a	Regression model	Slope (\pm SE)	LT ₅₀ (days) (CI 95%)
Phantom SC	Direct spray	Log–CLL	6.70 (\pm 0.723)	5.6 (1.8–7.9)	75.6* (7)	Log–probit	3.16 (\pm 0.34)	3.1 (2.5–3.7) (5)
	Dry residue	Log–CLL	4.4 (\pm 0.485)	4.6 (3.8–5.3)	12.9 (8)	Log–probit	4.90 (\pm 0.30)	3.3 (2.6–4.1) (5)
Phantom aerosol	Direct spray	Log–CLL	1.32 (\pm 0.198)	1.5 (0.8–2.5)	1.6 (4)	Log–probit	1.35 (\pm 0.20)	0.9 (0.6–1.6) (1)
	Dry residue	Log–CLL	4.91 (\pm 0.562)	2.5 (2.2–2.9)	5.1 (4)	Log–probit	4.83 (\pm 0.91)	2.0 (1.7–2.3) (2)

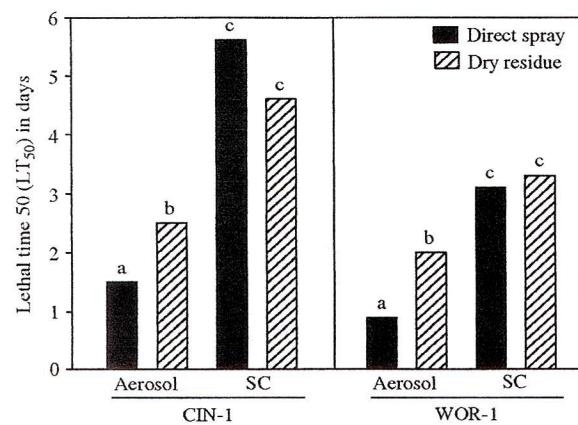
^a Values for goodness of fit of the regression models to the observed mortality data.^b Significant ($P < 0.05$) (goodness-of-fit test).

Figure 1. Toxicity of chlorfenapyr formulations, Phantom aerosol and Phantom SC, applied to adult bed bugs of two pyrethroid-resistant strains as direct contact sprays and as dry residues. The bars within strains with the same letter are not significantly different from one another ($P > 0.05$) following the method of lethal time ratios used by Robertson *et al.*²⁸

attempted to control pyrethroid-resistant bed bugs, but its effectiveness varies among populations.¹² Bed bugs with *kdr*-like insensitivity or any other resistance mechanism that is not inhibited by PBO would limit the effectiveness of this synergist.^{12,13} Insect growth regulators (IGRs), such as hydroprene and methoprene, are potential alternatives to pyrethroids for managing bed bugs. Laboratory results showed that dry residues of IGRs can cause production of infertile adults, morphological malformations, incomplete ecdysis and supernumerary nymphs in individuals treated as nymphs.^{31,32} However, IGRs are slow-acting insecticides on bed bugs and are generally used by the pest control industry in conjunction with other effective fast-acting insecticides.⁶

Chlorfenapyr is one of the few current insecticides with a different mode of action against bed bugs. When formulated as Phantom SC or aerosol it is labeled for indoor use as a 'low-pressure spot or crack and crevice spray that can be applied to places where pests are found or are likely to infest'.³³ Sprays of mattresses, a common location for bed bugs, are restricted to seams, folds and

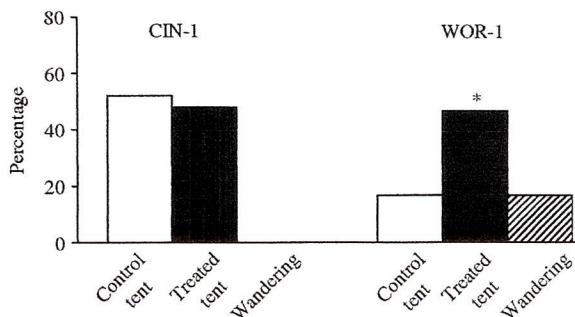


Figure 2. Preference of individual bed bugs from two strains for a paper tent treated with chlorfenapyr Phantom aerosol or a blank control tent. After a 16 h test period, the place where the insect was found was recorded (resting on insecticide, blank tent or wandering in the arena). The asterisk indicates significant differences between the control and insecticide-treated harborage (χ^2 test, $P < 0.05$). Wandering insects were not included in the analysis.

edges. Present laboratory evaluations indicate that chlorfenapyr kills bed bugs as both a contact spray and dry residue. In dry residue assays with the technical-grade material, mortality of >50% was recorded after 3 days of continuous exposure. Moore and Miller¹⁶ reported a much longer exposure time of bed bugs to hardboard panels treated with chlorfenapyr EC to obtain 50% mortality. The slower effect of chlorfenapyr on bed bugs, compared with conventional neurotoxicants such as pyrethroids on susceptible insects, can be explained by its differing mode of action. The active metabolite of chlorfenapyr (AC 303,268) inhibits the ion transport system of the respiratory chain in mitochondria, preventing the production of the energy molecule adenosine triphosphate (ATP), so leading to insect death.¹⁸ Typical symptoms of chlorfenapyr intoxication in bed bugs include sluggish movements, prostration and limited responses upon contact stimulus (Romero A, personal observation). Such symptoms may be less apparent in commercial practice, and users should understand that bed bugs treated with chlorfenapyr succumb more slowly compared with some other insecticides.

Pyrethroid-resistant strains of bed bugs evaluated here were as susceptible as the laboratory strains. It has been suggested

that resistant arthropod populations with enhanced monooxygenase activity might have increased sensitivity to chlorfenapyr (a case of a phenomenon known as negative resistance), because pyrethroid resistance can be caused by detoxification by the same enzymes (P450 monooxygenases) that activate the pro-insecticide chlorfenapyr.³⁴ Negative cross-resistance between pyrethroids and chlorfenapyr has been reported in pyrethroid-resistant housefly and tobacco budworm strains.^{34,35} In the housefly, however, a multiple-pyrethroid-resistant strain did not show negative cross-resistance to chlorfenapyr.³⁴ In the present study, the similar rate of mortality caused by chlorfenapyr to all strains, regardless of their pyrethroid susceptibility status, suggests that the presence of pyrethroid resistance in bed bugs might not limit (or enhance) the effectiveness of chlorfenapyr.

Also of importance to bed bug management are present findings concerning the duration of residual toxicity resulting from chlorfenapyr residues. In this study, dry residues of Phantom SC on filter paper aged indoors for 1, 2 or 4 months remained as toxic as fresh deposits. The ability of chlorfenapyr to remain effective over an extended period of time is encouraging because bed bugs that are not sprayed directly may still succumb after residing on treated surfaces. Most insecticides available today have limited potency as a dry deposit against pyrethroid-resistant bed bugs. Dry residual action of chlorfenapyr might also aid in preventing new infestations if likely areas of infestation are previously treated. Duration of residual toxicity can vary depending on the extent of insecticide migration into the substrate, or degradation on the surface.³⁶ Dry residues of chlorfenapyr, for example, were more toxic to stored-product pests on concrete than on vinyl tile and plywood surfaces.³⁷ In contrast, sprays of chlorfenapyr caused significant mortality to Pharaoh ants on both absorbent and non-absorbent substrates.²² Further study is warranted on the longevity and availability of chlorfenapyr on wood, fabric and similar substrates commonly occupied by bed bugs and the suitability of prophylactic applications in bed bug management programs.

Chlorfenapyr aerosol had residual as well as contact activity, causing mortality about 1.5 and 3 times faster, respectively, than when chlorfenapyr was formulated as a liquid (Phantom SC). The difference in mortality rate between the two formulations could be due to greater bioavailability of the active ingredient or synergism with other ingredients in the aerosol formulation. Direct sprays with the aerosol caused 30% mortality to both pyrethroid-resistant strains within 4 h (data not shown) and about 50% mortality in 24 h. In contrast, direct sprays of the water-based formulation took at least 3 days (WOR-1) or 5 days (CIN-1) to cause 50% mortality. Direct sprays of the blank aerosol (formulation without chlorfenapyr) killed 25% of individuals within 4 h, which indicates that some formulation ingredients also have some contact activity. Insecticides that kill bed bugs upon contact are widely used by pest control companies because they can quickly suppress populations and provide some relief to their customers.³⁸

Bed bugs from strains CIN-1 and WOR-1 showed no avoidance of surfaces treated with the chlorfenapyr aerosol. Similar responses were seen with these strains in previous evaluations with chlorfenapyr and Phantom SC.¹⁷ Continued occupancy of harborage treated with chlorfenapyr enhances exposure to the insecticide and presumably lessens the potential spread of bed bugs to adjoining areas, which may occur with some pyrethroids and insecticides that bed bugs tend to avoid.¹⁷

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