Fumigation of Bed Bugs (Hemiptera: Cimicidae): Effective Application Rates for Sulfuryl Fluoride

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ABSTRACT The bed bug, *Cimex lectularius* L. (Hemiptera: Cimicidae), has resurged recently as a domestic pest in North America with very limited options for decisive control. We report efficacy studies with sulfuryl fluoride (SF) toward use as a structural fumigant to control bed bugs. Laboratory studies were conducted in which eggs, adults, and nymphs from a pesticide susceptible laboratory population were fumigated for 24 h using SF at 99.8% purity in airtight, 3.8-liter glass containers under two temperatures, 25°C and 15°C. Bed bugs were placed in separate ventilated glass vials and wrapped in mattress padding before fumigation. The gas concentration within each jar was determined using quantitative gas chromatography–mass spectrometry. Dose–response trials using eggs of known age (48–96 h) were conducted at five or six target concentrations measured as concentration × time accumulated dosages (g-h/m^3) and one untreated control at each temperature. Each target dose was replicated in four different fumigation containers (replicates), with at least 32 eggs per replicate. The number of hatched and unhatched eggs postfumigation, and number of live and dead nymphs that resulted from hatched eggs, were evaluated daily for at least 1 wk after egg hatch. The lethal accumulated dosage (LAD_{99}) for bed bug eggs was 69.1 (95% fiducial limits [FLs] of 62.9–79.5) g-h/m^3 at 25°C and 149.3 (95% FLs of 134.4–177.9) g-h/m^3 at 15°C. Confirmatory trials with dosages of 1.5× the LAD_{99} were conducted at 25°C and 1.5× the threshold mortality dose at 15°C with at least 15 adults, 13 late-instar nymphs and 79 eggs of known age per replicate. At 25°C, a target dosage of 103.7 g-h/m^3 resulted in 100% mortality of adults and late-instar nymphs. Nymphs emerged and survived from two of 439 eggs treated with SF dosages that were 6–7 g-h/m^3 less than the target dosage. No nymphs emerged from eggs fumigated with dosages >97.9 g-h/m^3 in the validation study. Therefore, the threshold dosage for complete egg mortality (97.9 g-h/m^3) was used, rather than the LAD_{99}, to calculate the monitored field dosage rate of 148.2 g-h/m^3 (= 1.5 × 97.9 g-h/m^3) for control of all life stages of bed bugs at 25°C. Based on these results, at 15°C, 1.5× the threshold dosage for complete egg control (189.7 g-h/m^3) was used to calculate a target dosage of 285 g-h/m^3 for the confirmatory trial, which resulted in 100% mortality of adults, late-instar nymphs, and eggs.

KEY WORDS urban pest control, structural pest control, hematophagy, human ectoparasite, probit analysis

Cimex lectularius L. (Hemiptera: Cimicidae), the bed bug, is a transient ectoparasite that feeds on blood from humans and other warm-blooded animals. Bed bugs are difficult to control. They are nocturnal, cryptic, and readily "hitchhike" on belongings to infest new locations (Harlan 2006, Potter 2011). Bed bug eggs can survive water inundation and extreme temperatures (Naylor and Boase 2010) and adults and nymphs can survive at least three months without a bloodmeal (Harlan 2006).

Nonchemical tools and methods such as bug-proofing mattress encasements, steam machines, sanitation, vacuum cleaning, and diligent monitoring can manage bed bug infestations, but often do not eliminate them (Wang et al. 2009). Bed bugs can be controlled by exposure to extreme cold (Olson et al. 2013) and heat (Potter et al. 2008, Pereira et al. 2009, Naylor et al. 2010). Heat treatments are now widely used for bed bug control. One limitation of heat treatment is undetected thermal refugia, such as near floors, inside walls, within fabrics and furnishings, and other insulating materials, which can harbor bed bugs and prevent their exposure to lethal temperatures.

Therefore, insecticides continue to be an integral part of bed bug management programs (U.S. Environmental Protection Agency [EPA] 2012). Nonetheless, many insecticide products are currently ineffective in controlling bed bug infestations either because of an inherent lack of toxicity of the active ingredient (Moore and Miller 2006, Wang et al. 2009, Jones and

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Bryant 2012, Singh et al. 2013), or because of widespread bed bug resistance to the most commonly used active ingredients such as various pyrethroids (Romero et al. 2007, Zhu et al. 2010). In addition, pest management professionals often require more than two visits and application of multiple residual insecticides to manage bed bug infestations (Potter et al. 2010).

The history of bed bug control follows the history of many major insecticide groups. Fumigation with hydrogen cyanide was the most effective and efficient treatment to eliminate serious bed bug infestations during the first half of the 20th century (Potter 2011). Dichlorodiphenyltrichloroethane (DDT) became widely available by the mid-20th century and replaced hydrogen cyanide as an inexpensive, relatively safe, and effective treatment that also provided long residual control of bed bugs in structures. Sulfuryl fluoride (SF), SO_2F_2, was first marketed as a structural fumigant in 1961 under the trade name of Vikane (Dow AgroSciences, Indianapolis, IN). SF is currently labeled for bed bug control in the United States and has multiple benefits for this use pattern. SF creates no persistent residues of toxicological concern in fumigated items, there is no known resistance of any insects to SF and one application can eliminate the existing infestation (Thoms and Phillips 2004). SF can be used to safely fumigate numerous household items, including mattresses, bedding, clothing, toys, many furniture items, and electronics (Bell et al. 2003, Mueller 2012), items for which other common bed bug treatments, such as heat or residual insecticides, may cause damage or may not be approved for application. A recent study showed carbon dioxide fumigation in plastic bags can be an affordable method to kill 100% of the bed bugs hiding in household items (Wang et al. 2012); however, carbon dioxide is not registered for controlling bed bugs in the United States. Household clutter, which can hinder the efficacy of other chemical and nonchemical treatments (e.g., Wang et al. 2009, Potter et al. 2010), does not affect SF treatments because this gas readily penetrates into piles of clothing, papers, and other debris to kill bed bugs.

The efficacy of SF to control bed bugs has resulted in the practical use of this fumigant to eliminate bed bug infestations in many types of buildings, including single-family homes, multiunit dwellings, and hotels (Thoms 2010), and in shipping containers filled with household contents (Walker et al. 2008). One deterrent to the use of SF for bed bug control is the cost of fumigation (Miller and Fisher 2008). Before this study, SF was applied at three times the dosage for drywood termites (Isoptera: Kalotermitidae) to control the egg stage (Dow AgroSciences 2011); however, a screening laboratory trial indicated bed bugs eggs could be controlled at a lower dosage (Thoms and Scheffrahn 1994). EPA requires specific testing protocols be conducted to change the pesticide label application rates for control of public health pests, including bed bugs. Therefore, the objective of this study was to conduct laboratory testing to verify the dosages of SF required to control bed bug eggs, adults, and nymphs at two temperatures using a protocol approved by the EPA.

Materials and Methods

Bed Bug Culture. The study used an insecticide-susceptible laboratory strain (Ft. Dix) that was maintained on a human volunteer since 1973 and then on rabbits or an artificial feeding system using defibrinated rabbit blood since 2007–2008. The bed bugs used for the 25°C trials were from the Department of Entomology, Rutgers University, New Brunswick, NJ. These bed bugs were fed weekly with defibrinated rabbit blood (Hemostat Laboratories, Dixon, CA) using the Hemotek membrane-feeding system (Discovery Workshops, Accrington, the United Kingdom). The bed bugs for the 15°C trials were also the Ft. Dix strain provided by Sierra Research Laboratories Inc., Modesto, CA. These bed bugs were fed on rabbits weekly. All bed bug adults and nymphs were fed 2–4 d before overnight delivery from their source colony to Kansas State University. Mated gravid adult female bed bugs were held in the Kansas State University laboratory on pieces of colored construction paper in 5-ml ventilated glass shell vials for 24 h to obtain eggs. Females were then transferred to new vials with new paper each day for up to 6 d at 28°C, ≈70% relative humidity (RH), and a photoperiod of 16:8 (L:D) h (Fig. 1A and B). Females oviposited directly onto the colored paper from which eggs were easily seen and counted. Eggs of known age within 0–24 h were then held for a fumigation experiment. Each replicate in this study contained between 32 and 121 eggs that were 48–96 h old when fumigated for dose–response trials at two temperatures (Fig. 1.C).

For confirmatory trials, we collected eggs of known age as described above. Adults (7–14 d of age) and third- to fourth-instar nymphs were also accumulated and all life stages were placed in separate vials (see Preparation of Bed Bugs for Fumigation below) for each replicate immediately before fumigation. Eggs for confirmatory tests consisted of two age groups, 48–72 or 72–96 h, which were represented in each replicate. Nymphs were not in their final instar, so they did not molt into adults before or during the fumigation. For the 25°C confirmatory experiments, we evaluated 20 male and female adults, and 20 late-instar nymphs, per replicate. For the 15°C trials, we used 15 female adults and 13–15 late-instar nymphs per replicate.

Preparation of Bed Bugs for Fumigation. A known number of eggs, adults, or nymphs were placed in 3-dram glass shell vials (≈11 ml; 19 by 65 mm; Fisherbrand, Fisher Scientific, Pittsburgh, PA) that were closed with a fine netted cloth to allow fumigant entry, while preventing escape of bugs. The vials were then wrapped in mattress pad bedding material to simulate a habitat that might be subject to SF fumigation for bed bug control (Fig. 2).

Fumigation Containers. The wrapped bioassay vials were placed inside 3.8-liter airtight glass jars (Fig. 2) at ambient relative humidity (35–45%) in controlled

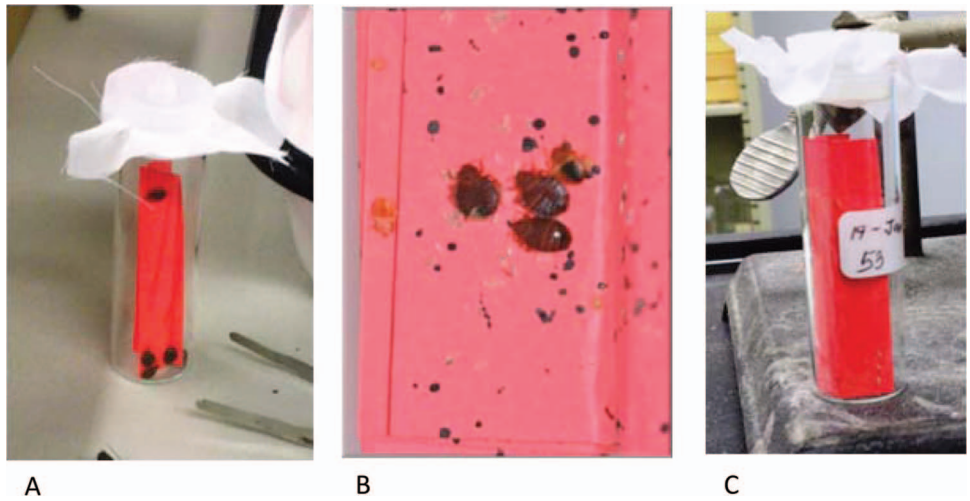

Fig. 1. (A) Blood-fed, mated bed bug females in ventilated, glass vial with colored paper for oviposition. (B) Bed bug females with eggs on paper. (C) Bed bug eggs on paper in vial ready for use in fumigation experiment. (Online figure in color.)

Fig. 2. A group of vials containing bed bug eggs that were wrapped in bedding material before fumigation to simulate a habitat for bed bugs that might be subject to fumigation with SF. Screw-on lid with injection port not shown. (Online figure in color.)

environmental chambers set to 25 or 15°C. Each jar, as in previous fumigation studies (e.g., Sekhon et al. 2010), was equipped with a port in the center of the metal screw-on cap and with a rubber gasket between the lid and the top of the jar for a gas-tight fit. The center port of the lid was fitted with a rubber injection septum used for fumigant introduction and headspace gas sampling via gas-tight syringes. The bed bugs equilibrated in environmental chambers for at least 4 h before fumigant introduction.

Fumigant Introduction and Measurement. A commercial supply of SF (Vikane at 99.8% purity; Dow AgroSciences, Indianapolis, IN) from a metal gas cylinder was used for the fumigations, which were conducted at normal atmospheric pressure for Manhattan, KS. SF gas was drawn from the cylinder and held in a Tedlar gas bag (CEL Scientific Corp., Santa Fe Springs, CA) that had been evacuated before use. From this stock gas of 99.8% purity, we used precisely calibrated gas-tight syringes to draw measured aliquots of known volume of SF and dilute it with a known volume of air in additional Tedlar bags to obtain SF of a desired concentration. After removing an equal volume of air from the fumigation jar using a gas-tight syringe, the same volume of pure or diluted SF was injected into a fumigation jar to obtain the desired target concentration. The exposure temperatures of either 25 or 15°C were maintained for jars in the environmental chamber during the 24-h fumigation period. SF was measured in mg/liter and calculated to deliver the desired concentration × time dosages (g-h/m^3) over the 24-h exposure period.

The gas concentration within each jar was measured 30 min after SF introduction and again before the termination of the 24-h exposure period using quantitative gas chromatography–mass spectrometry (GC-MS) using the external standard curve method. The MS was set in the selected ion mode to detect a fragment ion characteristic of SF. The external standard curve method (Sekhon et al. 2010) can calculate a precise concentration at a given sample time. For the external curve method of quantification, a precisely measured known concentration of SF was injected at a volume of 25 μl, and then smaller successive injections of 20, 15, 10, and 5 μl followed. Each volume was injected three times and the area under the peak in mV counts was recorded along with the amount of SF injected. SF amounts were regressed against measured peak areas to generate a straight-line regression equation that had an r^2 value between 0.96 and 0.99 in all cases. When 25 μl samples of gas from the experimental fumigation jars were analyzed by GC-MS, the peak areas were subjected to the regression equation generated from the standard curve obtained just before the sampling, and the concentration of SF in the jar was thus quantified. Three 25 μl samples were drawn and quantified from each jar at a given time to give an average estimated concentration for that specific jar. The average SF concentration for each jar was calculated as the mean of the concentration measured 30 min after starting the fumigation and that measured at 24 h, 3 min before the end of the SF exposure with the opening and ventilation of the jar. The percentage decrease from starting to the ending concentration of SF in our jars over 24 h was 5–20%. Calculation of an accumulated dosage, expressed in g-h/m^3 for a given jar, was the product of the average concentration in a jar and the number of exposure hours (24 h or a value close to it, to the tenth of an hour). Following the fumigation period, the jar lid was removed and the air space in each jar was ventilated in a fume hood for 1–2 h, after which the vials containing bed bugs were removed for assessing mortality.

Dosages Tested and Exposure Times. A dose–response study was conducted to determine the lethal accumulated dosage to kill 99% of a given group, the LAD_{99}, evaluated from either five or seven concentration × time dosages and one untreated control at 25 and 15°C, respectively. Four different fumigation jars, with each jar considered a true replicate, were set up at each target concentration, including the untreated controls. For the 25°C confirmatory tests at 1.5× the LAD_{99} dosage for eggs (see Postexposure Evaluations) for each of five replicates, untreated control bed bugs were maintained in the same vials and environmental conditions, but exposed only to air. Confirmatory testing at 15°C was conducted at 1.5× the threshold dosage for complete egg mortality for each of six replicates. Untreated control bed bugs were maintained in the same vials and environmental conditions, but exposed only to air.

Postexposure Evaluations. Treated and control vials were placed in an incubator at 28°C, ≈70% RH, and a photoperiod of 16:8 (L:D) h, the same conditions as they were maintained for oviposition before testing. For the dose–response trials, the numbers of hatched and unhatched eggs, and the number of live and dead emerged nymphs, were evaluated daily beginning at 24 or 48 h posttreatment, for at least 1 wk postfumigation, which allowed ample time for all viable untreated control eggs to hatch. All eggs that failed to hatch or that produced a nymph that died within the observation period, were considered dead, while all eggs that hatched and had a nymph survive through the observation period were considered alive. The number of treated and dead eggs directly associated with the accumulated dosage (g-h/m^3) quantified for each fumigation jar were analyzed using the PROC PROBIT procedure of SAS (SAS Institute, Cay, NC) to determine the LAD_{99} for each temperature.

In confirmatory dose trials at 25°C, we assessed the number of hatched and unhatched eggs, and the number of live and dead adults or nymphs, 1 wk postfumigation. For the confirmatory dose trials at 15°C, the numbers of hatched and unhatched eggs, and number of live and dead adults or nymphs, were evaluated daily for 1 wk beginning at 72 h postfumigation. Nymphs or adults were designated as dead if they did not show any signs of movement during this 1-wk period. Because confirmatory tests at both temperatures found all fumigated adults and nymphs were dead at 72 h, only eggs and neonate nymphs continued to be evaluated for 9 d postfumigation.

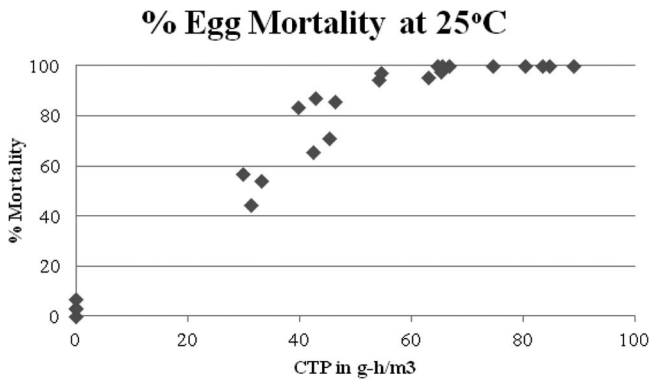

Fig. 3. Mortality of bedbug eggs after 7 d following a 24-h exposure to concentrations of SF, expressed as concentration × time dosage (g-h/m^3) at 25°C.

Results

25°C Trials. The target accumulated dosages at 25°C were 50, 60, 80, 100, and 120 g-h/m^3 for the dose–response trials. The actual accumulated dosages obtained ranged from 31.4 to 89.1 g-h/m^3 across 20 fumigation jars (Fig. 3). Mean mortality of untreated bed bug eggs was 2.6% and values of control mortality were not used to correct treatment values. Bed bugs began to emerge from the untreated eggs at 48 h after the 24-h fumigation period and from fumigated eggs at 72 h after fumigation. Emergence of nymphs from untreated eggs stopped by 96 h postfumigation and emergence ended from SF-fumigated eggs after 144 h. No bed bug nymphs emerged from eggs at accumulated dosages >65.3 g-h/m^3 at 25°C (Fig. 3).

The LAD_{99} of SF calculated from probit analysis of the dose–response data was 69.1 (95% FLs of 62.9–79.5) g-h/m^3 at 25°C (Table 1). We then used this 1.5× the LAD_{99} for killing bed bug eggs at 25°C to derive a target dosage of 103.7 g-h/m^3 to use in our confirmatory trial at 25°C. Of the five replicate confirmatory fumigations we conducted, our target was exceeded only in replicate 3, at 106.6 g-h/m^3, with the lowest confirmatory dose of the other four replicates being 95.9 g-h/m^3 (Table 2). Despite this slight under-dosing in four replicates, we observed only three eggs out of 439 hatched and produced healthy nymphs that survived for 7 d. These surviving eggs were from 97.1 and 97.9 g-h/m^3 dosages (6–7 g-h/m^3 below the target dosage of 103.7 g-h/m^3). All other eggs, nymphs, and adults treated in the 25°C confirmatory trials were killed by SF (Table 2).

Table 1. Log-probit analysis of the dose response data for bed bug eggs exposed for 24 h to SF at 25 and 15°C to derive the LAD_{99}

Temperature	Estimated accumulated SF dosage (g-h/m^3) for LAD_{99}	95% FL	
		Lower	Upper
25°C	69.14	62.94	79.52
15°C	149.82	134.39	177.99

Accumulate dosage is expressed in the concn x time as g-h/m^3.

15°C Trials. The target accumulated dosages applied to eggs for the 15°C dose–response trials were 90, 115, 140, 165, 190, 215, and 250 g-h/m^3. The actual accumulated dosages ranged from 72.8 to 213.6 g-h/m^3 across 20 fumigation jars (Fig. 4). Average mortality of untreated, control bed bug eggs was 10%. Bed bugs began to emerge from fumigated and untreated eggs at 48 h after the fumigant exposure period. Emergence of nymphs from untreated eggs stopped by 96 h post-fumigation and emergence ended from SF-fumigated eggs after 144 h. No bed bugs emerged from eggs at accumulated dosages >189.7 g-h/m^3.

Table 2. Confirmatory tests at 25°C of bed bug eggs, late-instar nymphs, and adults at 1.5× the LAD_{99} of SF determined for bed bugs eggs for a target dosage = 103.7 g-h/m^3.

Repetition	Stage[a]	Dosage (g-h/m^3)	No. treated	Alive[b]	Dead[c]	% mortality[d]
Control[e]	E	0	293	291	2	0.7
	N		87	82	5	5.7
	A		96	88	8	8.3
1	E	95.9	91	0	91	100
	N		20	0	20	100
	A		20	0	20	100
2	E	97.9	84	1	83	98.8
	N		20	0	20	100
	A		20	0	20	100
3	E	106.6	91	0	91	100
	N		20	0	20	100
	A		20	0	20	100
4	E	97.1	79	1	78	98.7
	N		20	0	20	100
	A		20	0	20	100
5	E	97.1	94	1	93	98.9
	N		20	0	20	100
	A		20	0	20	100

[a] Bed bug life stages tested: E, eggs; N, nymphs; A, adults.

[b] Eggs that hatched and had surviving nymphs 7 d after fumigation; nymphs or adults that survived up to 7 d after fumigation.

[c] Eggs that failed to hatch for 7 d after fumigation; nymphs hatching from eggs that died within the 7 d after fumigation; adults that died within the 7 d after fumigation.

[d] Proportion of number dead divided by number treated multiplied by 100.

[e] Individuals held in clean air distributed among three jars at the same conditions as treated insects, and then observed for mortality under same conditions as treated insects.

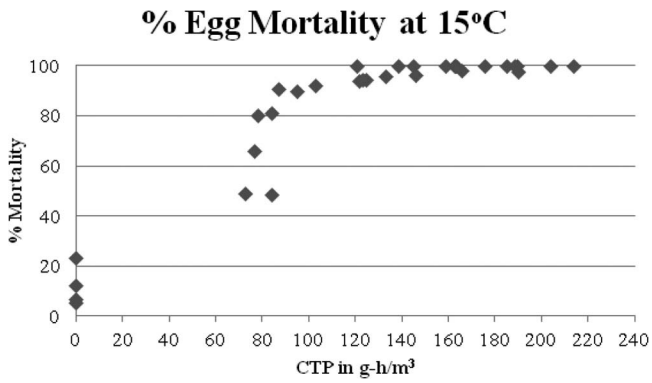

Fig. 4. Mortality of bedbug eggs after 7 d following a 24-h exposure to concentrations of SF, expressed as concentration × time dosage (g-h/m^3) at 15°C.

The LAD_{99} of SF calculated from probit analysis of the dose–response data was 149.8 (95% FLs of 134.4–177.9) g-h/m^3 (Table 1). Based on our observation at 25°C that two eggs survived fumigation at a dose equal to ≈1.5× the LAD_{99} at that temperature, we selected the observed threshold concentration for complete egg mortality found in the dose–response study, 189.7 g-h/m^3, and not the LAD_{99} of 149.8 g-h/m^3, to calculate a target dosage for the confirmation trial. Therefore, 285 g-h/m^3, calculated from 1.5 × 189.7 g-h/m^3, was then evaluated for the confirmatory trial at 15°C. The actual accumulated dosages for the confirmatory trial ranged from 238.1 to 269.7 g-h/m^3 (Table 3). All five dosages fell below the target dosage of 285 g-h/m^3. Complete (100%) control of all bed bug eggs, adults and nymphs was obtained at these dosages.

Discussion

Toxicity experiments with bed bugs conducted here revealed that mortality can be delayed longer than expected. A delay in egg hatch after fumigation with SF has been previously observed in other insects exposed to SF (E. T., unpublished data) and is not uncommon with other fumigants and insect species (reviewed in Phillips et al. 2012). Most of the bed bug nymphs emerging from fumigated eggs in the current study died 1–2 d after their emergence and were therefore scored as "dead." This delayed mortality, also referred to as latent mortality of insects after exposure to SF, is well-documented (Osbrink et al. 1987, Su and Scheffrahn 1990, Thoms and Scheffrahn 1994).

All adult and nymph bed bugs were killed in the confirmatory testing with SF, but three nymphs emerged and survived from 439 fumigated eggs in the 25°C trials. These surviving eggs were from dosages that were 6–7 g-h/m^3 below the target dosage of 103.7 g-h/m^3. Therefore, it was determined that 1.5× the LAD_{99} of SF did not provide a sufficient margin of error for field fumigations to ensure complete mortality of bed bug eggs at 25°C. No nymphs emerged from eggs in the confirmatory trials at 25°C that were fumigated with dosages >97.9 g-h/m^3. Therefore, we propose that the threshold dosage of 97.9 g-h/m^3 SF for complete egg control in our confirmatory test should be used rather than the LAD_{99} of 69.1 g-h/m^3 to calculate the monitored field dosage rate of 148.2 g-h/m^3 (=1.5 × 97.9 g-h/m^3) for mortality of all life stages of bed bugs at 25°C. Similarly, the confirmatory trial at 15°C, with actual accumulated dosages tested that were at least 15 g-h/m^3 lower than the target dosage, demonstrates there is sufficient margin of error incorporated in proposed dosage of 285 g-h/m^3 to

Table 3. Confirmatory tests at 15°C of bed bug eggs, late-instar nymphs, and adults at 1.5× the threshold mortality dosage of 189.7 g-h/m^3 of SF determined for bed bugs eggs in initial dose–response studies, for a target dosage = 285.0 g-h/m^3

Repetition	Stage[a]	Dosage (g-h/m^3)	No. treated	Alive[b]	Dead[c]	% mortality[d]
Control[e]	E	0	585	565	20	3.4
	N		87	86	1	1.2
	A		90	90	0	0
1	E	253.3	134	0	134	100
	N		15	0	15	100
	A		15	0	15	100
2	E	251.8	135	0	135	100
	N		14	0	14	100
	A		15	0	15	100
3	E	238.1	136	0	136	100
	N		13	0	13	100
	A		15	0	15	100
4	E	264.7	110	0	110	100
	N		15	0	15	100
	A		15	0	15	100
5	E	269.7	99	0	99	100
	N		15	0	15	100
	A		15	0	15	100
6	E	255.0	110	0	110	100
	N		15	0	15	100
	A		15	0	15	100

[a] Bed bug life stages tested: E, eggs; N, nymphs; A, adults.

[b] Eggs that hatched and had surviving nymphs 7 d after fumigation; nymphs or adults that survived up to 7 d after fumigation.

[c] Eggs that failed to hatch for 7 d after fumigation; nymphs hatching from eggs that died within the 7 d after fumigation; adults that died within the 7 d after fumigation.

[d] Proportion of number dead divided by number treated multiplied by 100.

[e] Individuals held in clean air distributed among six jars at the same conditions as treated insects, and then observed for mortality under same conditions as treated insects.

ensure all life stages of bed bugs will be controlled at this lower temperature. Increasing laboratory derived lethal dosages by 1.5× to develop field applied rates meets a standard that has now been used for >50 yr in the United States to convert laboratory SF dosages to field dosages applied by fumigators (Thoms and Scheffrahn 1994).

The EPA application labels for SF fumigants intended for controlling multiple species of drywood termites (DWT) in residential structures use the SF treatment recommendations for the western drywood termite, *Incisitermes minor* Hagen (Isoptera: Kalotermitidae), as the basis for dosage calculations. These SF labels assign a dosage factor equal to "1" for DWT. For other target structural pests that are less susceptible to SF than are DWT, the label calls for higher dosages as multiples of the dosage required to kill DWT. The work reported here verifies the bed bug dosages required for a monitored SF fumigation are 148.2 and 285 g-h/m^3 at 25 and 15°C, respectively, which are 1.9× greater than the DWT dosages of 78 and 150 g-h/m^3 required at these same temperatures. This 1.9× the DWT dosage rate for control of all life stages of bed bugs is less than the 3× factor required by labeling for SF to control bed bugs before results reported here. Our revised 1.9× dosages were reviewed and approved by scientists at the EPA and specific state regulatory agencies just before submission of this report for publication. The approach to the research reported here considers important key variables that must be optimized for successful application in the laboratory and in commercial application for pest control with fumigation: target species, most fumigant-tolerant life stage of that species, gas concentration, gas exposure time, and the temperature at which the treatment is applied (Phillips et al. 2012). The common bed bug has resurged as a serious pest in the United States after a hiatus of many decades, so fumigant application details were in need of updating, which this research has done. All of our dose–response studies to determine the effective dosages used eggs as the target life stage because in all tested arthropods, eggs are the life stage most tolerant to SF (Thoms and Scheffrahn1994). Mortality from most fumigants is directly and positively proportional to temperature, such that insects are most difficult to kill when ambient temperatures are low, such as the 15°C we tested, and insects are easier to kill at warmer temperatures, such as the 25°C tested herein. It follows, therefore, that effective dosages at moderate temperatures like 25°C should also be effective at higher ambient temperatures.

In conclusion, this research supports the recent modification to the application label for Vikane to lower the previous 3× DWT dosage factor to 1.9× DWT to eliminate all life stages of bed bugs as a cost-effective structural pest mitigation method. The 1.9× dosage factor has a robust margin of error to account for variability encountered in conducting field fumigations. The research reported here verifies the 1.9× dosage factor to be effective at both standard indoor ambient (25°C) and lower temperatures (15°C). The 1.9× DWT dosage factor is over one-third less SF than specified in the 3× DWT applied dosage and should represent a substantial cost reduction for the fumigant. Bed bugs are very difficult pests to control because of their widespread resistance to pyrethroids, their hiding behavior, and their close association with human resting and sleeping areas where residual pesticide applications are often not permitted (Romero et al. 2007, Zhu et al. 2010, Potter 2011). Proper fumigation with SF to eliminate bed bugs in human habitations offers one of the most effective and definitive controls for this serious pest insect.

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