

Editor's note: The research reported here was funded by Applied Science Labs. For additional information about the firm, visit www.vaxinatewith88.com.

There are many choices in the market today to battle bed bugs. In addition to applying traditional insecticides, extreme temperature treatments have grown in popularity. In hospitals, outpatient centers, nursing homes, dormitories, schools and hotel rooms where sensitive health and chemical exposure are concerns, temperature treatments are a common choice. They are effective, however, the main drawback is they leave no residual and these types of sites are areas where incoming pressure is a constant and more of an issue than infestation. Another important reason to use a residual product is because experts believe as much as 60 percent of an infestation consists of the bed bug eggs that will hatch later.

Is it possible to develop a non-toxic chemical that is lethal to bed bugs, yet leaves a residual that is harmless

to humans and other mammals?

The American Academy of Entomological Sciences was approached by a private laboratory to test a product for its effectiveness in killing bed bugs, *Cimex lectularius*, in a field situation with residual potential. Generally, we do not test any product without knowing the active and inert components and the mammalian toxicity.

Dr. Jeff Brown signed a confidential disclosure and received the list of ingredients. When Brown saw that the active was an EPA-exempt 25(b) component, "soybean oil," he contacted the laboratory and explained to management that, in the team's professional opinion, this "active" was not likely to be effective on insects and we were concerned that they would be wasting their money. They insisted and wanted to explain the manufacturing process and what they do with soybean oil to achieve this unique response. Having had

the benefit of their explanation and learning of the background for the product's development, the team agreed to do a small lab test with minimal cost.

The product we tested is commercially available from Applied Science at www.vaxinatewith88.com and as Cimi-Shield Knock Out and Cimi-Shield Protect from www.pestbarrier.com and its distributors. It is available in two strengths: VA88 (high concentrate) and Glow Guard (low-concentration residual).

At first we did not believe that the product would be effective in killing bed bugs. However, once the tests were completed and more was learned about mode of action, the results spoke for themselves. The active is a proprietary protein complex initially engineered from soybean oil involving thousands of proteins and their specific ratios. This active chemical is known as Residulin and is found commercially in VA88, Glow Guard and Cimi-Shield.



After the **TREATMENT**

A year-long efficacy study on bed bugs with a new 25(b) product shows both immediate and residual kill, researchers say.

By Dr. Jeffrey Brown, Kate McGovern and Jeff McGovern



In these initial steps we had proof it worked to kill by direct contact, but the question of residual efficacy remained. The residual efficacy testing project required treating a site with current bed bug activity with a direct spray formulation (prepared in accordance with label directions) and careful periodic monthly evaluations for 12 months after the initial treatment.

SITE DESCRIPTION. The site was a commercial hospitality facility offering overnight and extended stay, single-room accommodations, meeting facilities and other guest services to the public. It is professionally managed with on-site and regional support supervision. The structure is four stories of single rooms, each about 300 square feet. Each room is carpeted, has one to two queen-sized beds with box springs and mattresses, affixed headboards and a chest of drawers. Bearing walls are placed one on top of another without concrete separation. All but end rooms share at least two common walls, one on each side, ceiling and floor joists, except for bottom and top floors which have but one adjacent room above or below, respectively. End rooms share one common wall. Electrical lines are placed in the wall voids and run from room to room.

Rooms are serviced daily by housekeeping with standard hospitality-style wheeled service carts that are pushed from room to room. Carpets are vacuumed with a common machine and were not shampooed during this test period. Soiled bed clothing, towels and wash clothes are gathered daily from each room, placed in common service carts and moved from room to room until filled. They are then moved to an on-site facility and laundered.

INITIAL TREATMENT. Packets of super concentrated material in two formulations (knock down and preventive only) were prepared according to label directions in clean 1-gallon hand pump spray canisters. It should also be noted that the sprayer canisters, hoses and nozzles must be completely free of any surfactant (e.g., soap), other chemicals or residues. This product cannot be combined with other

substances, like IGRs. Each sprayer was equipped with adjustable spray nozzle that permitted both stream and fan spray and all filters were removed. Both formulations of the product contain a UV marker for later detection with a UV light.

In December 2010, 20 rooms (11 infested and 9 uninfested) were selected and treated. The knock-down formulation was applied to infested rooms and the preventive-only version was applied to uninfested rooms sharing a common wall with infested rooms, ceiling or floor joist in accordance with the “instructions for use” and as provided by label directions.

A single pass without overlap, from a hand held wand of a 1-gallon hand held spray canister was made over fabrics and textiles at a distance of approximately 24 to 30 inches at a rate of approximately



An example of a testing site, with a queen-sized bed, as utilized by the authors.

3 to 4 feet per second. This provided for an application rate of about 1 gallon of finished product per 1,200 square feet of surface area and included application to all fabrics and textiles. Treatment included the spraying of all carpeting, upholstered furniture, all sides of box springs, all sides of the mattresses and window coverings, and bed frames including plastic snaps. Headboard and headboard support was removed and these areas were sprayed with a single pass at a distance of approximately 24 to 30 inches moving at about 3 to 4 feet per second. No furnishings were removed. A single pass of all cracks, crevices, junction points and baseboards where carpeting met walls were treated with a spray at a distance of about 24 inches at a rate of approximately 3 to 4 feet per second.

All treated rooms were fitted with Bug

Dome traps and inspected monthly. There was also an inspection with a trained bed bug dog once each year. In the ensuing 12 months, no live bed bugs were found in the treated rooms. In the 11 rooms that were initially treated for active infestations, all showed dead bugs one month after treatment. Only two rooms had an additional occurrence in the 12-month period where dead bed bugs were found. In the nine rooms treated preventively, only three occurrences were observed during the same period as dead bed bugs were found. The last four months of the observation period showed no evidence of bed bugs in any of the treated rooms.

Although the subsequently found dead bed bugs could be attributed to the product's residual effect, more conclusive data was desired — we still wanted to know

how effective the residual was. After twelve months, fresh bed bugs were brought in from outside the site. In this project we chose one room randomly that had been previously treated and a room (for our control) that had never been treated. The treated room had no observed odor, stains or damage. It was vacuumed and darkened in order to inspect the residual condition with a 350 nm UV black light (the greater the lumens, the easier to observe the “sparkle” or “glow”). This was possible as the formula contains a UV reflective element so as to allow for confirmation of application on inspection.

At 6 to 8 inches, it was apparent through visual inspection where product had been originally applied on textile surfaces.

The bed bugs were placed on the carpeting near the head of the bed under an inverted arena of a new plastic 10-gallon aquarium with the bottom inch of the aquarium treated with petroleum jelly to prevent the bed bugs from escaping.

Hourly checks were made in the evening hours to avoid disturbance by guests or housekeeping and to simulate the nocturnal cycle of the insects. Light was used only at the time of inspections and then with a flashlight. Observations were recorded until all insects were dead or moribund. Instead of introducing CO₂ gas from a cylinder, the observer would slowly breathe into the inverted glass arena using

a flexible tube for five breathes (about 10 seconds) to induce movement of the bed bugs across the carpeting.

In the treated room, 10 adult and 3 nymphs were placed. The nymphs were all dead by hour 4 and the adults were all dead by hour 13. In the control room, an identical setup with 12 adults was placed and observed at the same time. There were no deaths except for three that were presumably injured in transfer from travel container to observation arena. At the end of the observation (hour 13), the control group was anesthetized with CO₂, placed in the upright aquarium, allowed to revive, and sprayed with the “knockdown” formulation. Mortality of all bed bugs was observed within 25 seconds.

This field study clearly indicates that the residual was effective 12 months after the initial application. The project ended January 2012. The “knockdown” formula can be used to obtain control over an infested area. The preventive formulation

can be used in areas where no infestation is wanted. It can also be used as an appropriate “top dressing” following an extreme temperature treatment, either heat or freezing (Cryonite) providing residual protection for at least a year afterward, even with regular vacuuming. It is important to note that regular sanitation and maintenance of this site contributed to the success of the treatment.

SO HOW DOES THIS WORK? We have been asked several times about the mode of action of the proteins, and this is our understanding — the protein complexes uncouple electron transfers and increase transmembrane leak of the protons in the membrane of the waxy layer of the insect. This is similar to standard chemical uncoupling described in organic chemistry. In plain English: the waxy layer is breaking down (in some cases rapidly), creating a hole. This is why it is essential that the bed bugs make adequate contact with the

chemical via applications to textiles rather than hard surfaces. Because Residulin affects the waxy layer, rather than depending on getting into a receptor site, I believe that the insect cannot become resistant to the protein complex active. That’s really big news, and it invites further study into its application to other insect species. **PCT**

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For tables with data on the monthly/periodic on-site monitoring of Bug Dome traps, annual inspection with trained bed bug dogs, physical inspections and customer complaints, visit www.pctonline.com and click on “online extras.”

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TABLE 1 (HOSPITALITY FACILITY)

Monthly periodic on site monitoring of Bug Dome traps and once a year inspection with trained bed bug dogs physical inspection provide the following, plus no complaints from customers were recorded by management.

Room #	Jan	Feb	March	April	May	June	July	August	September	October	November
1	M	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	M	0	0	0	0	0	0
3	M	0	0	M	0	0	0	0	0	0	0
4	M	0	0	0	0	0	0	0	0	0	0
5	M	0	0	0	0	0	0	0	0	0	0
6	M	0	0	0	0	0	0	0	0	0	0
7	0	M	0	0	0	0	0	0	0	0	0
8	0	0	0	0	0	0	0	0	0	0	0
9	0	0	0	0	0	0	0	0	0	0	0
10	0	0	0	0	0	0	0	0	0	0	0
11	0	0	0	0	0	0	0	0	0	0	0
12	M	0	0	0	0	0	0	0	0	0	0
13	M	0	0	0	0	0	0	0	0	0	0
14	M	0	0	0	0	0	M	0	0	0	0
16	M	0	0	0	0	0	0	0	0	0	0
17	0	M	0	0	0	0	0	0	0	0	0
18	0	0	0	0	0	0	0	0	0	0	0
19	0	0	0	0	0	0	0	0	0	0	0
20	M	0	0	0	0	0	0	0	0	0	0

L = living
M = moribund
0 = no evidence

TABLE 2

Mortality

Group 'A' Room 1 Treated												
Hour 1	Hour 2	Hour 3	Hour 4	Hour 5	Hour 6	Hour 7	Hour 8	Hour 9	Hour 10	Hour 11	Hour 12	Hour 13
0	0	0	0	3*	0	0	0	4	3	2	0	1
Group 'B' Room 2 Control												
1**	0	0	0	0	0	0	0	0	0	0	0	0

* three (3) nymphs were dead

**we are assuming that the researcher damaged the bed bug with forceps while removing from the travel container before placing in the arena.