Hockley International Ltd

Mostyn Duo CS: Efficacy Studies for the Treatment of the Common Bed Bug (*Cimex lectularius*)

Study 1: Thermal and ULV Fogging Application*

Aims of the Study

The study was undertaken in order to establish the knockdown efficacy of Mostyn Duo CS (ZW formulation containing 100 g/l Cyphenothrin CS and 10 g/l Prallethrin EW) when applied by thermal or ULV fogging for the treatment of common bed bugs (*Cimex lectularius*). The study design aimed to emulate field use by conducting the trial in test chambers in which bed bugs were exposed to Mostyn Duo CS for up to 48 hours.

Materials and Methods

Bed bugs were sourced from a colony raised in a specialist laboratory. Batches of 25 adult bed bugs and 25 nymphs were prepared 4 hours prior to the insecticidal treatment. Batches were placed into glass jars with harbourages containing food/water sources. Glass jars containing the bed bug batches were then placed in a test chamber in a vertical, diagonal pattern with two jars being placed at ground level, two batches being suspended at 50cm height and with each batch assuming a separate corner one metre away from the walls. An illustration of the batches' relative location is presented in Figure 1 below. Each replicate conducted in the experimental chamber contained 100 adult bed bugs and 100 nymphs.



Figure 1. Approximate location of the sample batches within the experimental chamber.

The test chambers themselves measured 500m³ in volume (100m² floor area, 5m in height). Temperatures within the chamber were kept consistent between replicates and within the range normally expected in a domestic setting. Cardboard boxes and polystyrene blocks were added to the chamber to represent the obstacles expected in field settings. The cardboard and polystyrene objects, along with the walls, floor and ceiling, were covered with plastic sheets which were replaced between replicates. In addition, the chamber was well ventilated between each treatment.

Application

Mostyn Duo CS was applied in two ways depending on the fogging method. For both treatment types, the nebulizers used were those ordinarily used by professional pest controllers. The equipment used was the Hurricane Dynafog and IGEBA TF 34 for the cold and thermal fogging applications, respectively. Pre-treatment test applications were conducted to ensure the correct flow rate and dosages for each machine (20mg of active substance per cubic metre). To further ensure consistent dose rates, each machine was rigorously rinsed between treatments and the suction tubes were replaced.

Prior to the application, the Hurricane Dynafog or the IGEBA TF 34 - depending on which trial was being performed - was placed in the corner of the chamber with the application nozzle facing the centre. The chamber received no ventilation during treatment or for one hour afterwards. The application equipment remained in the chamber until the full dose had been achieved and was removed by a technician using an 'airlock' doorway.

Results

 Table 1. Percentage rates of knockdown and mortality in Common bed bugs (*Cimex lectularius*)

 after application of Mostyn Duo CS by cold or thermal fogging.

Cold Fogging Application					
Target Organism	Knockdown-1 Hour/ %	Knockdown-4 Hours/ %	Mortality 24 Hours/ %	Mortality 48 Hours/ %	
Cimex lectularius (Adult)	100	100	100	100	
Cimex lectularius (Nymph)	100	100	100	100	

Mostyn Duo CS applied with a Hurricane Dynafog Fogging Machine at a dose rate of 100ml per 500m³, equalling 20mg of active substance content per m³.

Hot Fogging Application

Target Organism	Knockdown-1 Hour/ %	Knockdown-4 Hours/ %	Mortality 24 Hours/ %	Mortality 48 Hours/ %
Cimex lectularius (Adult)	100	100	100	100
Cimex lectularius (Nymph)	100	100	100	100

Mostyn Duo CS applied with an Igeba TF 34 thermal fogging machine at a dose rate of 100ml per 500m³, equalling 20mg of active substance content per m³.

The knockdown effect of the formulation, determined through observations of excitation, uncoordinated movements and paralysis, was recorded after the first hour of exposure. After this, the ventilation was set back to pre-treatment rates and the knockdown rate was once again recorded after 4 hours. The mortality of the insects was observed after 24 and 48 hours separately to record instances of recovery from an initial knockdown effect. The criteria for death/complete mortality would be complete paralysis after 24 and/or 48 hours.

Complete knockdown of adults and nymphs was observed in all replicates after 1 hour of exposure to Mostyn Duo CS regardless of the application method. Furthermore, no instances of recovery was witnessed with all individual, juvenile and adult, maintaining paralysis through 24 and 48 hours after exposure.

Separate bed bug samples were placed within the same test chambers and were not exposed to any treatment. In these conditions bed bug mortality did not exceed 5% in all replicates.

Conclusion

In the conditions of this trial, Mostyn Duo CS applied via hot or cold fogging, at a rate of 100ml per 500m³, provided complete efficacy as a curative treatment against Common bed bugs (*Cimex lectularius*) sourced from laboratory-raised strains.

*Simulated-Use Trial of the Insecticide Efficacy of a Product Applied as a Space Treatment (Hot/Cold Fogging) and Intended To Control Various Pests., **Report No. 1937-BF-4b+5b+6b+9b/0515R**

Study 2: Direct Application and No-Choice Residual Application via Surface Spray*

Aims of the Study

Two complementary experiments were undertaken to determine the knockdown and residual efficacy of Mostyn Duo CS (ZW formulation containing 100 g/l Cyphenothrin CS and 10 g/l Prallethrin EW) when treating the Common bed bug (*Cimex* lectularius) on various surfaces. This was achieved through the use of direct spray application and no-choice residue exposure on porous and non-porous surfaces. The time taken to achieve 100% knockdown was recorded in the direct application replicates whilst the rate of mortality after differing time periods was observed throughout the no-choice replicates.

Materials and Methods

Bed bugs were sourced from a colony raised in a specialist laboratory. Batches of 25 adult bed bugs and 25 nymphs were prepared. For the no-choice residue experiment, batches of bed bugs were placed on surfaces pre-treated with Mostyn Duo CS and contained in place using petri dish lids. The surfaces used varied in order to emulate the variety of 'typical' porous and non-porous surfaces expected in the field. Specifically, the treatment surfaces consisted of raw pine wood, bare concrete blocks, ceramic tiles, and steel.

Separate sample batches were exposed to the pre-treated surfaces at Day 0, Day 28, Day 56, and Day 84. Replicates were performed in quadruple. Pre-treated surfaces were made up of 15 x 15cm 'tiles'. Between experiments, the tiles were stored in enclosed conditions without any form of contact between one another. The tiles were arranged randomly and placed flat under fluorescent tubes (2.5m from the ground). Light exposure followed a photoperiod of 16 hours of light trailed by 8 hours of darkness. Other environmental conditions kept constant included the temperature (26°C \pm 2°C); humidity (70% \pm 5%); and, ventilation (1m³/ hour).

The direct application replicates utilised bed bugs from the same source as the no-choice test.

Application

For both experiments, Mostyn Duo CS was diluted at a rate of 100ml of product with 5 litres of water with that mixture allowing for the treatment of 100m². Negative control replicates (water) and positive control replicates (Permost CS: formulation containing 250g/l Permethrin CS) were also utilised in both experiments in an effort to better establish an efficacy comparison. Permost CS was applied at a rate of 25ml per 5 litre of water to allow for treatment of 100m².

No-Choice Residual Application

The tiles to be used in the no-choice experiment were treated in the absence of the test species in a 60m³ room. Application was performed using a professional hand-held, pressurised sprayer, Gloria 8 Litre Sprayer. To ensure the correct dosage, the equipment was calibrated and the amount of insecticide applied was measured by weight. All tiles of each control condition (experimental, positive control, negative control) were treated at once after being randomly placed on the floor.

For each of the conditions, a 'single-use' sprayer was used to perform the respective applications. For each application, an anti-drop nozzle was utilised and the diluted mixture was applied at a pressure that ensured the droplet sizes would wet each surface without causing run-off or excessive vaporisation. Tiles were not touched for at least an hour after treatment. Bed bug batches were placed on the tiles at several set points after treatment. This began with introduction at day 0 after the tiles had dried two hours after treatment. Once placed on the tiles, bed bug adults and nymphs were not able to avoid treated areas and instead forced to stay in direct contact with the treated surfaces. During the one hour of exposure, knockdown and mortality was recorded at regular intervals and after which the bed bugs were carefully removed and placed within an untreated container in which food/water were available and conditions favoured development. Mortality was then recorded at 24 and 48 hours.

Direct Application

Batches of bed bugs of the same origin as above were contained within glass containers on the chamber floor. A paper filter was placed on the floor to sponge any excess liquid and avoid the insects being flooded by the treatment. The direct application used four laboratory sprayers rather than the Gloria 8L pressurised sprayer. This was done to deliver smaller doses accurately. Before the application to the insects, the sprayer was first activated in a sink to remove any deposits that might be present in the nozzle. Once ready to apply, the nozzle was placed at a 30cm height above the container. Then 2ml of the water mixture was applied at the same dilution rate described above.

After application, the knockdown occurrence was recorded at 30 second intervals for up to 30 minutes. After this time, the bed bugs were carefully removed from the test container and placed within an unsoiled petri dish wherein they had access to food/water. Mortality was subsequently recorded after 24 hours.

Results

Table 2. Time taken to achieve complete knockdown (KT100 in intervals of 30 seconds) and the mortality rate (MR in %) of Common bed bugs (*Cimex lectularius*) after direct application of Mostyn Duo CS, a positive control (Permost CS) and a negative control (water).

Direct Application				
Treatment	Knockdown Time/ intervals of 30 seconds	Mortality Rate after 24 Hours/ %		
Mostyn Duo CS	30 Seconds	100		
Permost CS	30 Seconds	100		
Water	n/a	0		

Both Mostyn Duo CS and the positive control, Permost CS, achieved complete knockdown of adult Common bed bugs (*Cimex lectularius*) in a very short period of time after direct application, with all individuals experiencing knockdown symptoms (excitation, uncoordinated movements and/or paralysis) before the beginning of the second measurement interval. Furthermore, there were no instances of recovery amongst adult bed bugs treated with Mostyn Duo CS or Permost CS as all individuals were confirmed dead 24 hours after exposure to the treatment. The treatment with water under the same conditions exhibited no effect in relation to knockdown or mortality.

In the residue exposure section of the study, Mostyn Duo CS and Permost CS produced markedly longer times to produce complete knockdown as to be expected when the products aren't directly applied. A positive correlation existed between the time taken to produce a complete knockdown and the length of time since the tile surfaces had been treated. This being as a result of the insecticide residues decreasing over time.

Performance differences were apparent between the surface materials and between Mostyn Duo CS and the positive control; Permost CS did not offer any improved performance over Mostyn Duo CS at any stage or on any surface type. Conversely, Mostyn Duo CS produced a noticeably improved response on porous surfaces at 4 weeks (Day 28) although the pattern then changed for the remainder of the trial, with Mostyn Duo CS offering no significantly improved performance over the Permost CS on porous surfaces, but at least a 50% reduction in knockdown times on the non-porous surfaces. This may be explained by an improved retention of the Prallethrin-containing EW element of Mostyn Duo CS on non-porous surfaces. Although the increased potency of Cyphenothrin in comparison to the sole active in Permost CS, Permethrin, may also offer some explanation.

Regardless of the surface type and period following the treatment, Mostyn Duo CS and Permost CS produced 100% mortality of adult bed bugs and nymphs in all replicates. Meanwhile, the treatment with water produced knockdown and mortality rates that never exceeded 5% in any of the residual testing replicates.

Residual No-Choice Application						
		Knockdown	Time/ hours	Mortality Rate/ %		
Time (Days) After Treatment	Tile Material	Mostyn Duo CS	Permost CS	Mostyn Duo CS	Permost CS	
	Concrete	1	1	100	100	
Day 0	Wood	1	1	100	100	
Day U	Ceramic	1	1	100	100	
	Steel	1	1	100	100	
	Concrete	1	2	100	100	
Day 29	Wood	1	2	100	100	
Day 20	Ceramic	1	1	100	100	
	Steel	1	1	100	100	
	Concrete	2	2	100	100	
	Wood	2	2	100	100	
Day 50	Ceramic	1	2	100	100	
	Steel	1	2	100	100	
	Concrete	24	24	100	100	
Day 84	Wood	24	24	100	100	
Day 84	Ceramic	8	24	100	100	
	Steel	8	24	100	100	

Table 3. Time taken to achieve complete knockdown (KT100 in hours) and the mortality rate (MR in %) of Common bed bugs (*Cimex lectularius*) after exposure to surfaces pre-treated with Mostyn Duo CS and a positive control (Permost CS).

Conclusion

In the conditions of this trial, Mostyn Duo CS provided rapid and complete knockdown of adult bed bugs when applied directly to the insects. In addition, surfaces treated with Mostyn Duo CS up to 8 weeks (56 days) earlier led to the knockdown and immobilisation of adult bed bugs and nymphs within several hours of contact. Complete mortality was observed in all bed bugs batches exposed to Mostyn Duo CS and Permost CS for the duration of the trial, up to 12 weeks after the tiles were treated. Mortality occurred regardless of the surface material.

*Laboratory Trial of Insecticide Efficacy of a Product Against Various Pests, **Report No.1937-BF** 1a+2a+3a+4a+5a+6a+9a/0515

Study 3: Surface Spray Trial under Simulated Field Conditions*

Aims of the Study

Laboratory studies were performed, utilising a simulated infestation design to evaluate the efficacy and residual persistence of Mostyn Duo CS (ZW formulation containing 100 g/l Cyphenothrin CS and 10 g/l Prallethrin EW) when applied to treat Common bed bug (*Cimex lectularius*) infestations in conditions that emulated 'real life' scenarios.

Materials and Methods

The study was performed in 5 separate test chambers, each measuring $12m^3$ in volume ($6m^2$ floor area, $3m \times 2m \times 2m$). The chambers were used simultaneously for the five replicates of each condition: exposure to insecticide residues at 0 days and 12 weeks using a test product (Mostyn Duo CS), a positive control (Permost CS, Permethrin 250g/I CS), and a negative control (water).

The temperature within the chambers was kept at a constant of $22^{\circ}C \pm 1^{\circ}C$ during the period of testing. Similarly, a ventilation rate of $<1m^{3}/$ hour was activated for the duration of the assays and the relative humidity remained at 60% \pm 5%. A photoperiod of 8 hours per day was provided at 800 lux.

In addition to maintaining these environmental conditions within the chambers - similar to that expected in domestic and commercial field environments – several surface materials were utilised in order to replicate the variation likely to be present in the field. In doing so, the ability for surface type (porous or non-porous) to affect the performance of Mostyn Duo CS was also accounted for. Specifically, the chamber walls were made up of non-sorbent epoxide panels and the floor consisted of ceramic tiles, with each chamber receiving ceramic and cement-based tiles treated with insecticide.

Given the natural behaviour of common bed bugs, the chambers were supplied with several cardboard boxes in order to provide suitable harbourage spaces. Food and water was also provided for the duration of the trial. These resources were present in four locations and did not require the bed bugs to come into contact with surfaces treated with insecticide. The insecticide treatments were instead localised to one wall and the two aforementioned treated panels.



Figure 2. Approximate location of the treated panels and surfaces; food/water sources and harbourages within the chamber.

The test species, common bed bugs, were sourced from a specialist laboratory which raises the insects on rabbit blood. For each replicate, 50 adults and 50 nymphs of varying developmental stages were used. The sample sizes thereby totalled 250 adults and 250 nymphs per experimental condition. During introduction, the samples were placed within the chamber harbourages away from the insecticide-treated tiles. Bed bugs were then free to roam throughout the chamber for up 24 hours, after which the mortality rates were evaluated.

Application

The positive control and test products were diluted with water before application, whereas water was applied at the same volume per m² for the negative control. The respective treatments were applied through the use of a professional Gloria 8 Litre handheld pressurised sprayer with an anti-drop nozzle. To ensure the correct dosages, the amount of insecticide applied was weighed to verify the respective application rates. Treatments were applied according to their label or, in the case of water, at the same volume per m².

Table 4. Application rates adhered to throughout the trial for the respective treatments (experimental product, positive control and negative control).

Treatment	Dose/ ml	Dilution Rate/ ml	Application Area/ m ²
Experimental Product Mostyn Duo CS	100	5000	100
Positive Control Permost CS	25	5000	100
Negative Control Water	-	5000	100

Before introduction of the insects, one wall (6m² total) was treated per chamber along with two panels, one ceramic and one concrete. The two treated panels covered half of the chambers' floor area (3m² total). The insects were then introduced to the chamber following placement of the harbourages, food and water. These resources were all accessible from the untreated half of the floor and, therefore, the bed bugs were not forcibly exposed to the insecticidal treatments. After 24 hours the bed bugs were removed and the mortality rates were recorded.

The procedure was repeated as described above 12 weeks later. However for this test the original treated tiles were reintroduced and the insecticides were not reapplied. Between the trials, the treated tiles and wall panels were stored under the same conditions as that outlined in the previous section. Mortality was once again recorded after 24 hours in the newly introduced bed bug batches.

Results

Table 5. Mortality rates of Common bed bug (*Cimex lectularius*) adults and nymphs after exposure to insecticide-treated surfaces for 24 hours in a simulated field, choice-test trial.

Mostyn Duo CS	0 Days After Treatment			13	2 Weeks Aft	er Treatme	nt	
Target	Replicate	No. Alive	No. Dead	Mortality Rate/ %	Replicate	No. Alive	No. Dead	Mortality Rate/ %
	1	0	50	100	1	0	50	100
	2	0	50	100	2	0	50	100
Adults	3	0	50	100	3	0	50	100
	4	0	50	100	4	0	50	100
	5	0	50	100	5	0	50	100
	1	0	50	100	1	0	50	100
	2	0	50	100	2	0	50	100
Nymphs	3	0	50	100	3	0	50	100
	4	0	50	100	4	0	50	100
	5	0	50	100	5	0	50	100

CS Permost	0 Days After Treatment			L.	z weeks Aft	er Treatmei	nt	
Target	Replicate	No. Alive	No. Dead	Mortality Rate/ %	Replicate	No. Alive	No. Dead	Mortality Rate/%
	1	0	50	100	1	0	50	100
	2	0	50	100	2	0	50	100
Adults	3	0	50	100	3	0	50	100
	4	0	50	100	4	0	50	100
	5	0	50	100	5	0	50	100
	1	0	50	100	1	0	50	100
	2	0	50	100	2	0	50	100
Nymphs	3	0	50	100	3	0	50	100
	4	0	50	100	4	0	50	100
	5	0	50	100	5	0	50	100

0 Days After Treatment **12 Weeks After Treatment** Water Mortality Replicate Mortality Replicate No. Alive No. Dead No. Alive No. Dead Target Rate/% Rate/% Adults Nymphs

The mortality rates of bed bugs in the negative control did not exceed 2%. This occurred in one replicate only wherein a bed bug nymph died during the trial after exposure to tiles treated with water at day 0. The level of mortality strongly indicates this death was not as a result of the treatment. Therefore, the level of mortality observed in the control group validates the results observed in the test groups (those treated with Mostyn Duo CS) and the positive control (those treated with Permost CS). No significant differences were apparent between the performance of Mostyn Duo CS and Permost CS treatments with both products exhibiting a very high level of efficacy and complete mortality of adult bed bugs and nymphs in all replicates at day 0 and after 12 weeks.

Conclusion

In the conditions of this study, the recorded mortality rates of Common bed bugs (*Cimex* lectularius) after exposure to rooms with surfaces treated with Mostyn Duo CS reinforces the evidence obtained in previous laboratory studies wherein the short term and long term, residual effect of the formulation is established. Furthermore, given the design the researchers adopted for the trial, in which 'real life' environments were emulated and potential variables which may affect the insecticide's performance were accounted for, it can be suggested the complete mortality observed during this trial has more application than the same results observed in the laboratory-based trials.

*Simulated Trial of the Efficacy of an Insecticidal Product Against Various Pests, Report No. 1937-BF 4b+5b+6b+9b/0515

Study 4: Surface Spray Application under Field Conditions

Aim of the study

Following various laboratory-based trials, the formulation of Mostyn Duo CS (Cyphenothrin 100 g/l CS + Prallethrin 10 g/l EW) was used as a primary chemical treatment in several real world infestations to establish the efficacy and residual control when treating wild infestations of Common bed bugs (*Cimex lectularius*) in homes and hotel rooms.

Material and Methods

Five locations in which a pre-existing bed bug infestation were already present were utilised as the locations for this field trial. The location included two apartments and three hotel rooms. A baseline measure of infestation was established before application of Mostyn Duo CS. The population measurements were established through the use of monitoring traps. In each location, ten sticky pad traps were placed overnight in locations bed bugs are likely to inhabit. The traps were then collected the next morning and sent to a laboratory to identify and record the number of bed bug adults and nymphs.

Once a baseline measure of infestation had been established, the Mostyn Duo CS applications commenced. Unused sticky pad monitoring traps were placed overnight in the rooms at several intervals after the insecticide application. This allowed the recording of populations at 1, 2, 4, 8, and 12 weeks after treatment.

In addition to establishing a baseline measure of infestation pre-treatment, each field location was assessed in relation to the general hygiene level. Whilst the determination of hygiene level can potentially be subjective; in this scenario efforts were made to standardise the evaluations by focussing on recordable factors such as the number of occasions the room is cleaned. The hygiene level was assessed in order to identify any experimental variables that may affect the performance of Mostyn Duo CS.

Location No.	Location Type	Hygiene Level
1	Hotel Room	Low
2	Hotel Room	High
3	Apartment	Medium
4	Apartment	Low
5	Hotel Room	High

Table 6. Location type and the corresponding general hygiene level following pre-trial assessment.

Application

As with the laboratory-based surface spray trials, Mostyn Duo CS was applied through the use of a professional, Gloria 8 L pressurised, handheld sprayer with an anti-drop nozzle. The product was diluted at a rate of 50ml of insecticide per 5 litres of water for the treatment of 100m². The amount of insecticide applied did not differ as a result of the pre-assessed hygiene level or level of infestation (number of insects caught).

Once prepared the insecticide mixture was applied as a crack crevice treatment to the bed frame, bedside tables and potential harbourage areas around the bed, such as skirting boards. Particular attention was paid to the bed slats, the corners where the frame meets the legs, under the bed, and the headboard. Application of Mostyn Duo CS occurred once per location.

Location No.	Total Area of the Room/ m ²	Area of the Room Treated/ m ²	Amount of Product Applied/ ml	Amount of Water Applied/ ml
1	25	6	3	300
2	32	12	6	600
3	48	12	6	600
4	53	15	7.5	750
5	41	10	5	500

Table 7. The treatment area and total amount of Mostyn Duo CS applied in each location.

Results & Conclusion

The number of insects caught within the sticky-pad monitoring traps varied significantly between locations (13-88). The number of insects caught observed no correlation between the hygiene level of the room, the size of the room or the type of location (hotel room or apartment). All of which supported the general consensus that bed bugs can establish themselves in any property once introduced.

Table 8. The number of bed bugs caught in overnight monitoring traps before treatment withMostyn Duo CS.

Location No.	Location Type	Pre-trial Infestation Level: No. of Insects Caught
1	Hotel Room	13
2	Hotel Room	31
3	Apartment	14
4	Apartment	88
5	Hotel Room	29

Following the application of Mostyn Duo CS, monitoring indicates the size of the bed bug population initially reduced by 98% across the five locations with a total of four insects being caught within the overnight traps one week after treatment. From the second week and through to week twelve, no bed bugs were observed in overnight monitoring traps.

The study does include data for the weeks immediately following the trial. Therefore, it is not possible to comment as to whether the populations of bed bugs recovered in any form. Nevertheless, the data established throughout the trial by monitoring the bed bug population pre-

trial and throughout the next twelve weeks does indicate a large reduction in the number of bed bugs in each location, if not a complete elimination.



Figure 3. The percentage of Common bed bugs (Cimex lectularius) remaining in five field trial locations after initial monitoring and a subsequent treatment of Mostyn Duo CS.

When also considering the data generated in several laboratory-based studies, it can be concluded that Mostyn Duo CS offers a very high level of efficacy when used to target 'wild' bed bug infestations. The effect generated by Mostyn Duo CS is fast acting upon application with the most significant reductions being observed in immediate period following application (0-1 weeks). Following this large initial population reduction, Mostyn Duo CS remains active on surfaces for twelve weeks, further reducing the adult population whilst preventing re-establishment as the nymphs develop and come into contact with treated surfaces.

T.E.C. Field Trial of the Efficiency of a Residual Insecticide Spray to Control Bed Bugs, **Report No. 2341-BB-FT/0618; 1-12; 2018.**