PROJECT NUMBER:

486-0030

STUDY TITLE:

THE EVALUATION OF **002-BG** AND **002-BG+CA5%**
AS A DIRECT SPRAY AGAINST BED BUGS

PROTOCOL NUMBER:
N4861010030A141

IN-LIFE COMPLETION DATE:
October 27, 2010

STUDY COORDINATOR:
Niketas Spero

PERFORMED FOR:

ACP Worldwide

138 West Drive
Lodi, OH 44254

TESTING FACILITY:

ICR, Inc.
1330 Dillon Heights Avenue
Baltimore, MD 21228

EXECUTIVE SUMMARY

Five replicates of 10 bed bugs were placed in paper buckets and sprayed manually with one of two test formulations. The study consisted of two treatments, **002-BG** and **002-BG+CA5%** and an untreated control. Immediately following application, the bed bugs were observed for knockdown at 0.5, 1, 2, 3, 4 and 5 minutes. The bed bugs were then transferred to clean containers and a final knockdown reading was taken at 15 minutes. Mortality was recorded at 24 hours. The results are shown below:

Treatment	Avg. Wt. Applied (g)	Mean % knockdown							Mean % 24-hr Mortality
		0.5 min	1 min	2 min	3 min	4 min	5 min	15 min	
Control	NA	0	0	0	0	0	0	0	0
002-BG		86	98	100	100	100	100	100	100
002-BG+CA5%		96	100	100	100	100	100	100	100

Both 002-BG and 002-BG+CA5% were effective against the bed bug, *Cimex lectularius*. Spraying bed bugs with either of these test substances knocked down high numbers of bed bugs (>85%) within 30 seconds of spraying. All bed bugs were knocked down at 1minute with 002-BG+CA5% and at 2 minutes with 002-BG. Both 002-BG and 002-BG+CA5% were also successful killing agents, providing 100% mortality of bed bugs 24 hours after treatment.

For Nick Spero

Niketas Spero

Study Coordinator

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OBJECTIVE

To evaluate the efficacy of two 25(B) direct spray formulations **002-BG** and **002-BG+CA5%** for the knockdown and mortality of the bed bug, *Cimex lectularius*.

This is not a GLP (Good Laboratory Practices) study or protocol, and the final report is not intended to be submitted to any regulatory agency as part of a GLP study or to support product registration.

MATERIALS AND METHODS

The materials and methods were as described in Protocol N4861010030A141 (Appendix I).

ACP Worldwide supplied the following for test:

1. **002-BG**
2. **002-BG+CA5%**

Five replicates of 10 bed bugs were placed in paper buckets and sprayed manually with one of two test formulations. The study consisted of two treatments, **002-BG** and **002-BG+CA5%** and an untreated control. Immediately following application, the bed bugs were observed for knockdown at 0.5, 1, 2, 3, 4 and 5 minutes. The bed bugs were then transferred to clean containers and a final knockdown reading was taken at 15 minutes. Mortality was recorded at 24 hours.

RESULTS

The complete knockdown and mortality values for all five replications of bed bugs are shown in Appendix III. The average knockdown and mortality values are listed below in Table 1 and knockdown is also displayed graphically in Figure 1.

Both **002-BG** and **002-BG+5%** provided fast knockdown of bed bugs knocking down >85 % in the first 30 seconds post treatment. Complete knockdown was achieved within the first minute with **002-BG+CA5%** and in the first two minutes with **002-BG**. Both **002-BG** and **002-BG+CA5%** were also successful in killing bed bugs providing 100% mortality within 24 hours. There was no control knockdown or mortality.

Table 1 Knockdown and mortality of untreated bed bugs and bed bugs sprayed with 002-BG or 002-BG+CA5%.

Treatment	Avg. Wt. Applied (g)	Mean % knockdown							Mean % 24-hr Mortality
		0.5 min	1 min	2 min	3 min	4 min	5 min	15 min	
Control	NA	0	0	0	0	0	0	0	0
002-BG		86	98	100	100	100	100	100	100
002-BG+CA5%		96	100	100	100	100	100	100	100

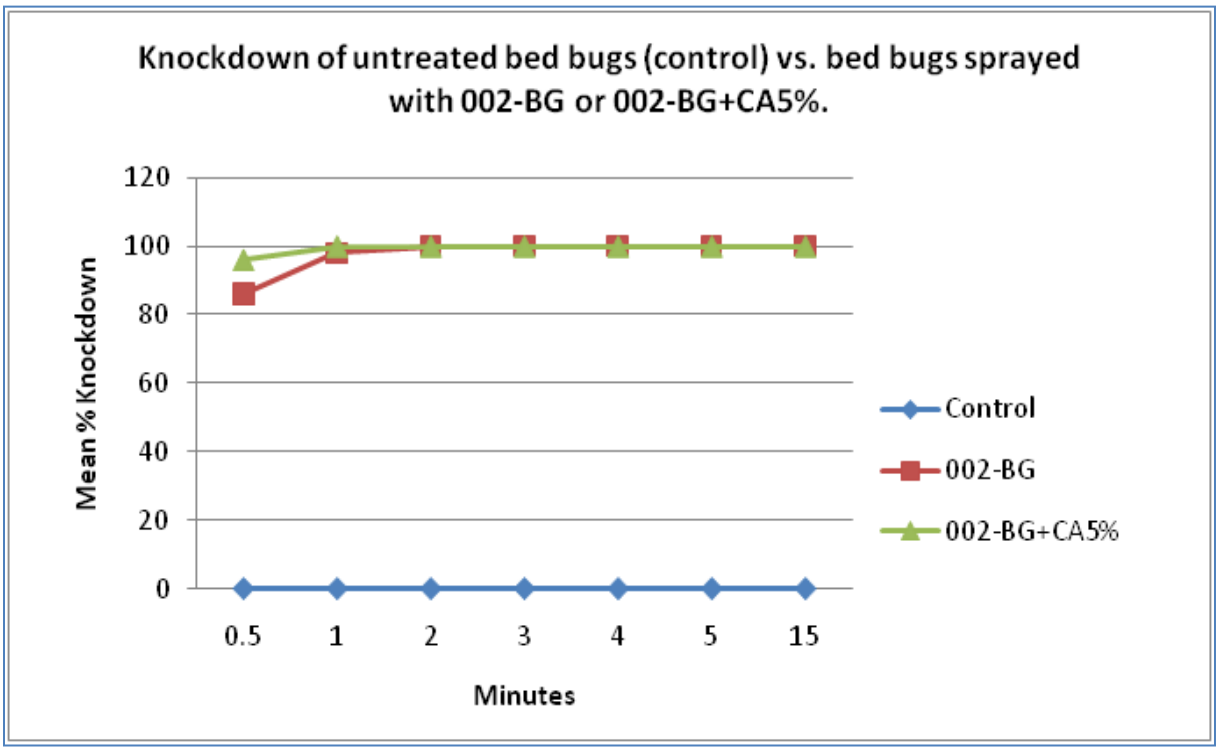

Figure 1 Average knockdown of bed bugs at 0.5, 1, 2, 3, 4, 5 and 15 minutes after treatment with 002-BG or 002-BG+CA5%. Both products were quick to knock down bedbugs, knocking down >85% in the first 30 seconds post treatment. There was no control knockdown.

CONCLUSIONS

Both **002-BG** and **002-BG+CA5%** were effective against the bed bug, *Cimex lectularius*. Spraying bed bugs with either of these test substances knocked down high numbers of bed bugs (>85%) within 30 seconds of spraying. All bed bugs were knocked down at 1minute with **002-BG+CA5%** and at 2 minutes with **002-BG**. Both **002-BG** and **002-BG+CA5%** were also successful killing agents, providing 100% mortality of bed bugs 24 hours after treatment. There was no control knockdown or mortality.

APPENDIX I: PROTOCOL

PROTOCOL:
N4861010030A141

PROJECT NUMBER:
486-0030

STUDY TITLE
THE EVALUATION OF **002-BG** AND **002-BG+CA5%**
AS A DIRECT SPRAY AGAINST BED BUGS

PROTOCOL VERSION DATE:
October 21, 2010

PROPOSED EXPERIMENTAL START DATE
October 2010

PROPOSED EXPERIMENTAL TERMINATION DATE
October 2010

STUDY COORDINATOR
Niketas Spero

SPONSOR
ACP Worldwide

138 West Drive
Lodi, OH 44254

TESTING FACILITY
ICR, Inc.
1330 Dillon Heights Avenue
Baltimore, MD 21228-1199

OBJECTIVE

To evaluate the efficacy of two 25(B) direct spray formulations **002-BG** and **002-BG+CA5%** for the knockdown and mortality of the bed bug, *Cimex lectularius*.

This is not a GLP (Good Laboratory Practices) study or protocol, and the final report is not intended to be submitted to any regulatory agency as part of a GLP study or to support product registration.

USE OF ICR'S NAME IN PROMOTIONAL RELEASES

Sponsor agrees not to use ICR's name in any promotional literature, TV, radio, web-based or other media, without the express written permission of ICR management. ICR, Inc. reserves the right to grant this permission based on the relation of the promotional text and images to the data generated for the sponsor.

TEST ARTICLE (PRODUCT), INFORMATION AND DISPOSITION

A Material Safety Data Sheet (MSDS) shall be provided for each test, control, and/or reference sample, which will include any hazardous information of the samples. The percentage of all active ingredients and any hazardous constituents must be included in all MSDS.

The sponsor is solely responsible for conducting any test, control, and reference sample characterizations, and for retaining this documentation. If any of the test samples are currently available for consumer use and/or purchased in the marketplace, the sponsor should still conduct the same sample characterizations.

Any determination of the stability of the test, control, and/or reference samples should be determined by the sponsor prior to the experimental start date. When relevant to the conduct of this study, the solubility of each test, control, and/or reference sample should be determined prior to the experimental start date.

If the stability of test, control, and/or reference samples stored under the test site conditions is determined by the sponsor, it should done prior to any studies.

All unused test samples will be returned to the sponsor within 30 days after the final report is sent to the sponsor. The sponsor will be responsible for all costs for the return of the samples, including any costs associated with hazardous materials shipping.

SAMPLE STORAGE

The two test articles **002-BG** and **002-BG+CA5%** will be supplied by the sponsor. The samples will be logged in upon receipt at ICR and stored in a locked cabinet until needed.

TEST ORGANISMS:

The bed bug, *Cimex lectularius*, henceforth 'bed bugs' will be used. Five replicates of 10 bed bugs will be used for the test formulations and the untreated control.

TREATMENT CHAMBER

All formulations will be sprayed in the Peet Grady chamber. Bed bugs will be placed inside treatment containers (pint size paper containers with fluon barrier applied to sides to contain bed bugs). The top and bottom diameters of these containers are ca. 3.75 and 2.87 inches respectively with a height of ca. 3.5 inches.

MISCELLANEOUS EQUIPMENT & SUPPLIES:

Stop watch, data record forms, carbon dioxide source, forceps, brown paper, surgical gloves, respirator, disposable Tyvek® coveralls and booties, hygrothermograph, and cotton balls; rubber bands, Mettler® top-loading balance, forceps and data sheets.

PERSONNEL PREPARATION AND PROTECTION

Applications will take place in the Peet Grady chamber. The applicator involved in treatment will wear disposable Tyvek® coveralls, booties and a respirator.

TESTING METHODS

Test Set-up Design

The formulation will be applied ("push-down" pump spray bottle) directly to each replicate. Immediately following application, the bed bugs will be observed and knockdown recorded at 0.5, 1, 2, 3, 4, and 5 minutes. The bed bugs will then be transferred to clean containers and a final knockdown reading will be taken at 15 minutes. Mortality counts will be taken at 24 hours and if mortality is less than 90%, an additional reading will be taken at 48 hours.

The study design consists of a two treatments 1) **002-BG**, 2) **002-BG+CA5%** and an untreated control.

Preparation and Handling of Bed Bugs

Ten bed bugs will be used per replicate: 5 replicates for the test formulations and 5 replicates serving as the untreated control. Each replicate of bed bugs will be placed in separate paper containers.

Application of Test Samples

To maintain consistency in the way the material is sprayed, all treatment will be applied by the same individual. A set quantity of formulation (five pumps of the sprayer dispenses ca. 0.83g of product) will be sprayed upon the container of bed bugs (actual amount sprayed per replicate will be documented). The test material will be applied uniformly on the bottom of the treatment containers. The test material will be applied manually from a vertical distance of 5-6 inches from the bottom of the treatment container.

Five replicate containers will be sprayed with each test formulation.

Untreated Control

The five control replicates will be treated in the same manner as test samples except that they will not be sprayed.

Calculation of application Rate

The weight of the spray delivered per replicate of insects will be calculated from the weights of each sample before and after treatment.

Treatment of Test Bed Bugs

Each replicate container will be sprayed with five pumps of the product spray bottle which typically dispenses ca. 0.167 mL of test material per pump for a total of 0.83 mL.

Treatment of Control Bed Bugs

Each replicate of control bed bugs will be prepared according to the same procedures outlined above with the exception that they will not be sprayed. The control bed bug containers will be housed in the same area as those treated for the duration of the prescribed observation periods.

Observation of Knockdown

After treatment the bed bugs will be observed for up to 15 minutes for knockdown.

Following Initial Observation Interval

Immediately following the 5 minute observation interval, bed bugs will be transferred from the treatment containers to clean paper containers with a nylon screen that will be held in place with a lid (center removed). The containers will be held in the laboratory under ambient temperature and humidity with a 16:8 light:dark cycle for 24 hours.

The controls will be maintained in a similar fashion.

24 and conditional 48 Hour Mortality

Mortality counts will be made at 24 hours and if, at that time, the mortality is less than 90% an additional mortality count will be made at 48 hours provided the total untreated control mortality (dead plus moribund) is $\leq$ 10%.

Bed bugs will be classified as alive if they can crawl normally, and dead if there is no movement (even after probing); those bed bugs that show some movement but lack coordination and are unable to crawl normally; will be classified as moribund. Mortality will be based only upon dead bed bugs.

The treated and control bed bugs will be maintained in the laboratory under ambient temperature and humidity conditions with a 16:8 light dark cycle for 24 hours. Temperatures and humidity will be recorded in the treatment chamber, and in the laboratory where bed bugs are subsequently held for the duration of the test.

DATA ANALYSIS:

Abbott's Formula will be used to correct for any mortality among the controls. Knockdown and mortality data will be analyzed with appropriate statistical tests to discriminate between test sample efficacies. This analysis is usually by Analysis of Variance (ANOVA) followed by Duncan's New Multiple Range Test or an equivalent procedure if data are appropriate.

SCHEDULE OF EVENTS

DATE	PROCEDURE
Time Zero	Test Conducted
At End of Test	Verbal Report
After The Field Test Is Conducted	Written Report
After Final Report Has Been Issued	Samples Returned

STATEMENT OF AMENDMENT OR DEVIATION

All amendments to, and/or deviations from this protocol will be documented in the final report.

Lanny Lingenfelter Date
President
ACP Worldwide, Inc.

Niketas C. Spero Date
Study Coordinator
ICR, Inc.

DATA COLLECTION SHEET

Date:_____ **Start Time:**_____ **AM PM** (Circle one) **Species:** ***Cimex lectularius***

Temp (trt): _____°F **RH (trt):**_____% **Temp (lab):**_____°F **RH (lab):**_____%

TEST ARTICLE ID: _____

Rep	Total	Amt Sprayed	Knockdown (min)							+24 Hour Mortality		
			0.5	1	2	3	4	5	15	Alive	Moribund	Dead
1												
2												
3												
4												
5												
Total												

Notes:

Rep	+48 Hour Mortality		
	Alive	Moribund	Dead
1			
2			
3			
4			
5			
Total			

Recording Technician / Date **Study Coordinator / Date**

APPENDIX II: STATISTICAL ANALYSIS

Data Reduction Table											
PROJECT #: 486-0030			SPONSOR:	ACP Worldwide			**DATE :**	11/1/2010 0:00			
Rep #	Total #	ALIVE	MORI-BUND	DEAD		Control Mortality	Treated Mortality	Abbott's correction	Ave	Asin	Mn Asin
CONTROL - KD +.5 min											
1	10		0			0.0					
2	10		0			0.0					
3	10		0			0.0					
4	10		0			0.0					
5	10		0			0.0			0.0		
002-BG- KD +.5 min											
1	10		9			0.00	90.0	90.0		71.57	
2	10		8			0.00	80.0	80.0		63.43	
3	10		9			0.00	90.0	90.0		71.57	
4	10		8			0.00	80.0	80.0		63.43	
5	10		9			0.00	90.0	90.0	86.0	71.57	68.31
002-BG+CA5%- KD +.5 min											
1	10		10			0.00	100.0	100.0		90.00	
2	10		10			0.00	100.0	100.0		90.00	
3	10		9			0.00	90.0	90.0		71.57	
4	10		10			0.00	100.0	100.0		90.00	
5	10		9			0.00	90.0	90.0	96.0	71.57	82.63
CONTROL - KD +1 min											
1	10		0			0.0					
2	10		0			0.0					
3	10		0			0.0					
4	10		0			0.0					
5	10		0			0.0			0.0		
002-BG- KD +1 min											
1	10		10			0.00	100.0	100.0		90.00	
2	10		9			0.00	90.0	90.0		71.57	
3	10		10			0.00	100.0	100.0		90.00	
4	10		10			0.00	100.0	100.0		90.00	
5	10		10			0.00	100.0	100.0	98.0	90.00	86.31
002-BG+CA5%- KD + 1 min											
1	10		10			0.00	100.0	100.0		90.00	
2	10		10			0.00	100.0	100.0		90.00	
3	10		10			0.00	100.0	100.0		90.00	
4	10		10			0.00	100.0	100.0		90.00	
5	10		10			0.00	100.0	100.0	100.0	90.00	90.00

Data Reduction Table											
PROJECT #: 486-0030				SPONSOR:		ACP Worldwide			**DATE :**	11/1/2010 0:00	
Rep #	Total #	ALIVE	MORI-BUND	DEAD		Control Mortality	Treated Mortality	Abbott's correction	Ave	Asin	Mn Asin
CONTROL - KD +2 min											
1	10		0			0.0					
2	10		0			0.0					
3	10		0			0.0					
4	10		0			0.0					
5	10		0			0.0			0.0		
002-BG- KD +2 min											
1	10		10			0.00	100.0	100.0		90.00	
2	10		10			0.00	100.0	100.0		90.00	
3	10		10			0.00	100.0	100.0		90.00	
4	10		10			0.00	100.0	100.0		90.00	
5	10		10			0.00	100.0	100.0	100.0	90.00	90.00
002-BG+CA5%- KD + 2 min											
1	10		10			0.00	100.0	100.0		90.00	
2	10		10			0.00	100.0	100.0		90.00	
3	10		10			0.00	100.0	100.0		90.00	
4	10		10			0.00	100.0	100.0		90.00	
5	10		10			0.00	100.0	100.0	100.0	90.00	90.00
CONTROL - KD +3 min											
1	10		0			0.0					
2	10		0			0.0					
3	10		0			0.0					
4	10		0			0.0					
5	10		0			0.0			0.0		
002-BG- KD +3 min											
1	10		10			0.00	100.0	100.0		90.00	
2	10		10			0.00	100.0	100.0		90.00	
3	10		10			0.00	100.0	100.0		90.00	
4	10		10			0.00	100.0	100.0		90.00	
5	10		10			0.00	100.0	100.0	100.0	90.00	90.00
002-BG+CA5%- KD + 3 min											
1	10		10			0.00	100.0	100.0		90.00	
2	10		10			0.00	100.0	100.0		90.00	
3	10		10			0.00	100.0	100.0		90.00	
4	10		10			0.00	100.0	100.0		90.00	
5	10		10			0.00	100.0	100.0	100.0	90.00	90.00
CONTROL - KD +4 min											

Data Reduction Table											
PROJECT #: 486-0030			SPONSOR:	ACP Worldwide			**DATE :**	11/1/2010 0:00			
Rep #	Total #	ALIVE	MORI-BUND	DEAD		Control Mortality	Treated Mortality	Abbott's correction	Ave	Asin	Mn Asin
1	10		0			0.0					
2	10		0			0.0					
3	10		0			0.0					
4	10		0			0.0					
5	10		0			0.0			0.0		
002-BG- KD +4 min											
1	10		10			0.00	100.0	100.0		90.00	
2	10		10			0.00	100.0	100.0		90.00	
3	10		10			0.00	100.0	100.0		90.00	
4	10		10			0.00	100.0	100.0		90.00	
5	10		10			0.00	100.0	100.0	100.0	90.00	90.00
002-BG+CA5%- KD + 4 min											
1	10		10			0.00	100.0	100.0		90.00	
2	10		10			0.00	100.0	100.0		90.00	
3	10		10			0.00	100.0	100.0		90.00	
4	10		10			0.00	100.0	100.0		90.00	
5	10		10			0.00	100.0	100.0	100.0	90.00	90.00
CONTROL - KD +5 min											
1	10		0			0.0					
2	10		0			0.0					
3	10		0			0.0					
4	10		0			0.0					
5	10		0			0.0			0.0		
002-BG- KD +5 min											
1	10		10			0.00	100.0	100.0		90.00	
2	10		10			0.00	100.0	100.0		90.00	
3	10		10			0.00	100.0	100.0		90.00	
4	10		10			0.00	100.0	100.0		90.00	
5	10		10			0.00	100.0	100.0	100.0	90.00	90.00
002-BG+CA5%- KD + 5 min											
1	10		10			0.00	100.0	100.0		90.00	
2	10		10			0.00	100.0	100.0		90.00	
3	10		10			0.00	100.0	100.0		90.00	
4	10		10			0.00	100.0	100.0		90.00	
5	10		10			0.00	100.0	100.0	100.0	90.00	90.00
CONTROL - KD +15 min											
1	10		0			0.0					
2	10		0			0.0					

Data Reduction Table											
PROJECT #: 486-0030			SPONSOR:	ACP Worldwide			**DATE :**	11/1/2010 0:00			
Rep #	Total #	ALIVE	MORI-BUND	DEAD		Control Mortality	Treated Mortality	Abbott's correction	Ave	Asin	Mn Asin
3	10		0			0.0					
4	10		0			0.0					
5	10		0			0.0			0.0		
002-BG- KD +15 min											
1	10		10			0.00	100.0	100.0		90.00	
2	10		10			0.00	100.0	100.0		90.00	
3	10		10			0.00	100.0	100.0		90.00	
4	10		10			0.00	100.0	100.0		90.00	
5	10		10			0.00	100.0	100.0	100.0	90.00	90.00
002-BG+CA5%- KD + 15 min											
1	10		10			0.00	100.0	100.0		90.00	
2	10		10			0.00	100.0	100.0		90.00	
3	10		10			0.00	100.0	100.0		90.00	
4	10		10			0.00	100.0	100.0		90.00	
5	10		10			0.00	100.0	100.0	100.0	90.00	90.00
CONTROL - 24-hour mortality											
1	10	10	0	0		0.0					
2	10	10	0	0		0.0					
3	10	10	0	0		0.0					
4	10	10	0	0		0.0					
5	10	10	0	0		0.0			0.0		
002-BG- 24-hour mortality											
1	10	0	0	10		0.0	100.0	100.0		90.00	
2	10	0	0	10		0.0	100.0	100.0		90.00	
3	10	0	0	10		0.0	100.0	100.0		90.00	
4	10	0	0	10		0.0	100.0	100.0		90.00	
5	10	0	0	10		0.0	100.0	100.0	100.0	90.00	90.00
002-BG+CA5% - 24-hour mortality											
1	10	0	0	10		0.0	100.0	100.0		90.00	
2	10	0	0	10		0.0	100.0	100.0		90.00	
3	10	0	0	10		0.0	100.0	100.0		90.00	
4	10	0	0	10		0.0	100.0	100.0		90.00	
5	10	0	0	10		0.0	100.0	100.0	100.0	90.00	90.00

APPENDIX III: RAW DATA SHEETS

DATA COLLECTION SHEET

Date: 10-26-10 **Start Time:** 12:20 AM PM (Circle one) **Species:** *Cimex lectularius*

Temp (trt): 75 °F **RH (trt):** 64 % **Temp (lab):** 75 °F **RH (lab):** 82 %

TEST ARTICLE ID: CONTROL

Rep	Total	Amt Sprayed	Knockdown (min)							+24 Hour Mortality		
			0.5	1	2	3	4	5	15	Alive	Moribund	Dead
1	10	N/A	0	0	0	0	0	0	0	10	0	0
2	10		0	0	0	0	0	0	0	10	0	0
3	10		0	0	0	0	0	0	0	10	0	0
4	10		0	0	0	0	0	0	0	10	0	0
5	10		0	0	0	0	0	0	0	10	0	0
Total	50		0	0	0	0	0	0	0	50	0	0

Notes:

Rep	+48 Hour Mortality		
	Alive	Moribund	Dead
1			
2			
3			
4			
5			
Total			

Recording Technician / Date

NC/pw 10-26-10

NC/pw 10-27-10

Study Coordinator / Date NC/pw 10-26-10

DATA COLLECTION SHEET

Date: 10-26-10 **Start Time:** 1:12 AM PM (Circle one) **Species:** *Cimex lectularius*

Temp (trt): 75 °F **RH (trt):** 65 % **Temp (lab):** 75 °F **RH (lab):** 82 %

TEST ARTICLE ID: 002-BG

	Rep	Total	Amt Sprayed	Knockdown (min)							+24 Hour Mortality		
				0.5	1	2	3	4	5	15	Alive	Moribund	Dead
1:12	1	10	0.92g	9	10					10	0	0	10
1:14	2	10	0.91	8	9	10				10	0	0	10
1:16	3	10	0.90	9	10					10	0	0	10
1:18	4	10	0.92	8	10					10	0	0	10
1:20	5	10	0.92	9	10					10	0	0	10
	Total			43	49	50				50	0	0	50

Notes:

Rep	+48 Hour Mortality		
	Alive	Moribund	Dead
1			
2			
3			
4			
5			
Total			

Recording Technician / Date

NCho 10-26-10
10/26/10
NCho 10-27-10

Study Coordinator / Date NCho 10-26-10

DATA COLLECTION SHEET

Date: 10-26-10 **Start Time:** 1:39 **AM PM** (Circle one) **Species:** *Cimex lectularius*

Temp (trt): 75 °F **RH (trt):** 65 % **Temp (lab):** 75 °F **RH (lab):** 82 %

TEST ARTICLE ID: 082-BG + CA 5%

	Rep	Total	Amt Sprayed	Knockdown (min)							+24 Hour Mortality		
				0.5	1	2	3	4	5	15	Alive	Moribund	Dead
1:39	1	10	0.85g	10						10	0	0	10
1:41	2	10	0.85	10						10	0	0	10
1:43	3	10	0.83	9	10					10	0	0	10
1:45	4	10	0.85	10						10	0	0	10
1:47	5	10	0.87	9	10					10	0	0	10
	Total			48	50					50	0	0	50

Notes:

Rep	+48 Hour Mortality		
	Alive	Moribund	Dead
1			
2			
3			
4			
5			
Total			

Recording Technician / Date

NCfw 10.26.10
10/26/10
NCfw 10-27-10

Study Coordinator / Date

NCfw 10-26-10