



PROJECT NUMBER:

486-0032

STUDY TITLE:

THE EVALUATION OF **002-BG**
AS A DIRECT SPRAY AGAINST BED BUG EGGS

PROTOCOL NUMBER:

N4861110032A141
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IN-LIFE COMPLETION DATE:

December 21, 2010

STUDY COORDINATOR:

Niketas Spero

PERFORMED FOR:

ACP Worldwide
138 West Drive
Lodi, OH 44254

TESTING FACILITY:

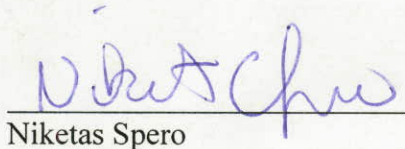
ICR, Inc.
1330 Dillon Heights Avenue
Baltimore, MD 21228

EXECUTIVE SUMMARY

Five replicates of ten eggs were separately sprayed with **002-BG**. The formulation was applied ("push-down" pump spray bottle) directly to each replicate. Immediately following application, the bed bugs eggs were transferred to clean containers. Mortality (% unhatched) counts were taken at three separate times; 10 to 14 days post treatment, 17 to 20 days post treatment, and at 21 days post treatment. The results are shown below:

Treatment	Avg. Wt. Applied (g)	Mean % Mortality		
		10-14 day	17-21 day	+21 day
Control	NA	10	6	0
002-BG	0.85	100	100	100

Spraying bed bug eggs with **002-BG** prevented egg hatch in all treated eggs. All of the untreated bed bugs emerged by 21 days post treatment (90% had emerged by 10 days) while none of the treated eggs hatched.



Niketas Spero

Study Coordinator



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OBJECTIVE

To evaluate the efficacy of a single direct spray formulation **002-BG** for killing bed bug, *Cimex lectularius*, eggs.

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 N4861110032A141 (Appendix I).

ACP Worldwide supplied the following for test:

1. **002-BG**

Five replicates of ten eggs were separately sprayed with 002-BG. The formulation was applied (“push-down” pump spray bottle) directly to each replicate. Immediately following application, the bed bugs eggs were transferred to clean containers. Mortality (% unhatched) counts were taken at three separate times; 10 to 14 days post treatment, 17 to 20 days post treatment, and at 21 days post treatment.

RESULTS

The complete mortality values for all five replications of bed bugs are shown in Appendix III. The average mortality values are listed below in Table 1.

Treatment **002-BG** was successful in killing bed bug eggs providing 100% mortality. Numbers of hatched and unhatched eggs were counted 10, 17 and 21 days after treatment. By 21 days all control bed bugs had emerged (90% had emerged by 10 days) while all eggs treated with **002-BG** failed to hatch.

Table 1 Mortality (% unhatched) of untreated bed bug eggs and bed bug eggs sprayed with 002-BG.

Treatment	Avg. Wt. Applied (g)	Mean % Mortality		
		10-14 day	17-21 day	+21 day
Control	NA	10	6	0
002-BG	0.85	100	100	100

CONCLUSIONS

Spraying bed bug eggs with **002-BG** prevented egg hatch in all treated eggs. All of the untreated bed bugs emerged by 21 days post treatment (90% had emerged by 10 days) while none of the treated eggs hatched.



APPENDIX I: PROTOCOL



PROTOCOL:
N4861110032A141
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PROJECT NUMBER:
486-0032

STUDY TITLE
THE EVALUATION OF **002-BG**
AS A DIRECT SPRAY AGAINST BED BUG EGGS

PROTOCOL VERSION DATE:
November 8, 2010

PROPOSED EXPERIMENTAL START DATE
November 2010

PROPOSED EXPERIMENTAL TERMINATION DATE
November 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 a single direct spray formulation **002-BG** for killing bed bug, *Cimex lectularius*, eggs.

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

FORMULATIONS:

002-BG

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 be 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 test material 002-BG 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:

Adult and large nymph ICR Field strain bed bugs (*Cimex lectularius*) obtained from DC, DE, MD, ME and NJ from 2006 to 2009. All bed bugs will have been blood fed within 7 days of testing. 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

Five replicates of ten eggs will be separately sprayed with 002-BG. An additional five replicates of eggs will not be sprayed and will serve as an untreated control. The formulation will be applied (“push-down” pump spray bottle) directly to each replicate. Immediately following application, the bed bugs eggs will be transferred to clean containers. Mortality counts will be taken at three separate times; 10 to 14 days post treatment, 17 to 20 days post treatment, and at 21 days post treatment.

The study design consists of a single treatment **002-BG** and an untreated control.

Preparation and Handling of Bed Bugs

3-5 days before the planned date of testing, blooded adults will be picked from the ICR field strain colony and allowed to oviposit on 10-15 paper substrates. Remove excess eggs (greater than 10) with forceps or snip off the paper to which they are attached. Ten eggs oviposited on filter paper (in some cases their will be more than a single piece of paper), will be transferred (egg side up) to open petri dishes, which in turn, will be placed into the bottom of pint sized 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 bug eggs (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 Bug Eggs

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.

Observation of Hatching

After treatment the bed bug eggs will be set aside for the normal incubation period (ca. 10 days). Observations of hatching will be taken at three separate times; 10 to 14 days post treatment, 17 to 20 days post treatment, and at 21 days post treatment.

Treatment of Control Bed Bug Eggs

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

Following Treatment

Immediately following the treatment, bed bug eggs 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.



Egg Mortality

Three mortality counts will be made; 10 to 14 days post treatment, 17 to 20 days post treatment, and at 21 days post treatment.

Bed bug eggs will be classified as hatched if the egg corion shows that a nymph emerged. Mortality will be based only upon unhatched bed bug eggs.

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:

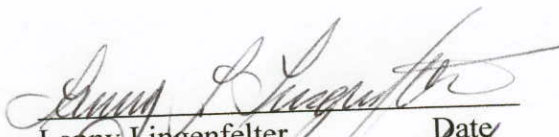
Abbott's Formula will be used to correct for any mortality among the controls. Knockdown and mortality data will be analyzed with appropriate statistical tests to discriminate between test sample efficacies.

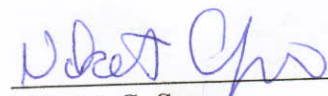
SCHEDULE OF EVENTS

<u>DATE</u>	<u>PROCEDURE</u>
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
 President
 ACP Worldwide, Inc. Date 11/16/10

 11-17-10
 Niketas C. Spero
 Study Coordinator
 ICR, Inc. Date



DATA COLLECTION SHEET

Date: _____ Start Time: _____ AM PM Species: Cimex lectularius

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

TEST ARTICLE ID: 002-BG

Rep	Total	Amt Sprayed	10-14 Day Mortality		17-20 Day Mortality		+21 Day Mortality	
			Hatched	Unhatched	Hatched	Unhatched	Hatched	Unhatched
1								
2								
3								
4								
5								
Total								

Notes:

Recording Technician / Date

Study Coordinator / Date



APPENDIX II: STATISTICAL ANALYSIS



NIT DATA REDUCTION												
Project #:		486-0032		ACP Worldwide					Date:	1/6/2011 0:00		
Rep #	Total #	Hatched	Unhatched		Control Mortality	Treated Mortality	Abbott's correction	Mean Abbott's	Arcsine	Average	UnCorrect ed Mean %	
Control 10-14 day												
1	10	10	0		0.0				0.00			
2	10	10	0		0.0				0.00			
3	10	10	0		0.0				0.00			
4	10	9	1		10.0				18.43			
5	10	6	4		40.0				39.23	11.53	10.0	
002-BG 10-14 day												
1	10	0	10		10.0	100.0	100.000		90.00			
2	10	0	10		10.0	100.0	100.000		90.00			
3	10	0	10		10.0	100.0	100.000		90.00			
4	10	0	10		10.0	100.0	100.000		90.00			
5	10	0	10		10.0	100.0	100.000	100.000	90.00	90.00	100.0	
Control 17-20 day												
1	10	10	0		0.0				0.00			
2	10	10	0		0.0				0.00			
3	10	10	0		0.0				0.00			
4	10	9	1		10.0				18.43			
5	10	8	2		20.0				26.57	9.00	6.0	
002-BG 17-20 day												
1	10	0	10		6.0	100.0	100.000		90.00			
2	10	0	10		6.0	100.0	100.000		90.00			
3	10	0	10		6.0	100.0	100.000		90.00			
4	10	0	10		6.0	100.0	100.000		90.00			
5	10	0	10		6.0	100.0	100.000	100.000	90.00	90.00	100.0	
Control +21 day												
1	10	10	0		0.0				0.00			
2	10	10	0		0.0				0.00			
3	10	10	0		0.0				0.00			
4	10	10	0		0.0				0.00			
5	10	10	0		0.0				0.00	0.00	0.0	
002-BG +21 day												
1	10	0	10		0.0	100.0	100.000		90.00			
2	10	0	10		0.0	100.0	100.000		90.00			
3	10	0	10		0.0	100.0	100.000		90.00			
4	10	0	10		0.0	100.0	100.000		90.00			
5	10	0	10		0.0	100.0	100.000	100.000	90.00	90.00	100.0	



APPENDIX III: RAW DATA SHEETS



DATA COLLECTION SHEET

Date: 11/30/10 Start Time: 12:00 AM PM Circle one Species: Cimex lectularius

Temp (trt): 74 °F RH (trt): 52 % Temp (lab): _____ °F RH (lab): _____ %

TEST ARTICLE ID: 002-BG Untreated control

Rep	Total	Amt Sprayed	10-14 Day Mortality		17-20 Day Mortality		+21 Day Mortality	
			Hatched	Unhatched	Hatched	Unhatched	Hatched	Unhatched
1		0	10	0	10	0	10	0
2		0	10	0	10	0	10	0
3		0	10	0	10	0	10	0
4		0	9	1	9	1	10	0
5		0	6	4	8	2	10	0
Total		0	^{WExp 12-10-10} 345	5	47	3	50	0

Notes:

Recording Technician / Date YC 11/30/10
YC 12-17-10
YC 12-21-10

Study Coordinator / Date ncpo
12-10-10
ncpo 12-17-10
ncpo 12-21-10

DATA COLLECTION SHEET

Date: 11/30/10 Start Time: 12:00 AM PM Circle one Species: Cimex lectularius

Temp (trt): 74 °F RH (trt): 52 % Temp (lab): _____ °F RH (lab): _____ %

TEST ARTICLE ID: 002-BG



Rep	Total	Amt Sprayed	10-14 Day Mortality		17-20 Day Mortality		+21 Day Mortality	
			Hatched	Unhatched	Hatched	Unhatched	Hatched	Unhatched
1		0.87g	0	10	0	10	0	10
2		0.79g	0	10	0	10	0	10
3		0.84g	0	10	0	10	0	10
4		0.82g	0	10	0	10	0	10
5		0.93g	0	10	0	10	0	10
Total			0	50	0	50	0	50

Notes:

Recording Technician / Date 11/30/10

Study Coordinator / Date 12-10-10
12-17-10
12-24-10