

EFFICACY OF THE ZAPPBUG OVEN FOR KILLING SEVERAL LIFE STAGES OF BED BUGS BY HEAT

i2LResearch USA, Inc. 1330 Dillon Heights Avenue Baltimore, MD 21228-1199 USA

> Alicia Kelley October 2012

Certification

This report represents a true and accurate record of all data obtained.

Signed Alucia Allly Date 117/12

Alicia Kelley Study Director

Approved by Date Date

Dr. Robin Todd Executive Director

All raw data and a copy of the final report will be archived at i2LResearch USA, Inc. for a period of five years. At the end of this period all data relating to this report will either be retained by i2LResearch USA, Inc. for a further disclosed period of time, or passed on to the sponsor.

Report circulated to: Cameron Wheeler (1 copy)

i2LResearch USA, Inc. (1 copy)

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Study Information

Efficacy of the ZappBug Oven for Killing Several Life Stages of Bed Bugs by Heat

i. Testing facility:

i2LResearch USA, Inc.

1330 Dillon Heights Ave. Baltimore, MD 21229-1199

USA

ii. Sponsor:

Cameron Wheeler CEO/Co-founder

ZappBug

511 Boren Ave N; Suite 200

Seattle, WA 98109

USA

iii. i2LResearch Study code:

12/283 (545-0030)

iv. Study start date:

10/02/2012

Experimental start date:

10/10/2012

Experimental end date:

10/30/2012

Study end date:

11/7/2012

v. Study Director:

Alicia Kelley

Summary

The ZappBug Oven was tested for its ability to kill bed bug adults, nymphs, and eggs using a heat treatment. The oven was tested for insulated and non-insulated conditions. Insulated bed bugs were placed between three folded towels (terry cloth), while non-insulated bed bugs were placed inside the oven directly on the support net. Control replicates were positioned outside the oven.

There were three replicates of 10 individuals for each life stage in each treatment condition (control, insulated, and non-insulated). A HOBO® data logger was used to record temperature and humidity for each position. An indoor/outdoor thermometer included with the ZappBug Oven kit was also placed in the insulated environment, as directed in the user manual. The oven was switched on and left alone until the temperature in the towels reached 120°F, after which the oven was allowed to remain on for an additional hour. The number of alive, moribund and dead bed bugs was recorded for the adults and nymphs. Additional 24 hour mortality readings were recorded for the nymphs and adults. The eggs were kept until the majority of control bed bug eggs hatched (6 days).

Each run of the ZappBug Oven constituted one true replicate. Three total true replicates were tested as described above. In each replicate, the ZappBug Oven successfully provided complete mortality of all bed bug life stages.

Aim

A laboratory bioassay was required to assess the efficacy of the ZappBug Oven against three bed bug life stages (adults, nymphs, and eggs) using heat treatment as a killing agent.

Methodology

Test systems

Bed bug (Cimex lectularius) adults, nymphs and eggs from the i2LResearch USA field strain bed bugs obtained from DC, DE, MD, ME and NJ from 2006 to 2009 were used in this study. All bed bug adults and nymphs were blood fed within 7 days of testing.

Test treatments and application

One ZappBug Oven was tested, provided by the sponsor. The heat treatment was tested against bed bugs in an insulated condition and in a non-insulated condition. The laboratory area around the oven served as the untreated control environment.

Oven Set-up

The ZappBug Oven was set up according to the user manual provided by the sponsor, using all the accessories and equipment provided (Figure 1). The oven uses support blocks and a support net to suspend items above the oven floor and increase convective heat transfer (shown on page 8 of product manual). On one side of the oven, a stack of three folded towels were placed on top of the support net. On the other side, the bed bugs were placed directly on the support net (Figure 2).



Figure 1. Oven set up, showing proper assembly and positioning of controls.



Figure 2. Oven interior set up, including insulated and non-insulated environments.

Replication

Three replicates of 10 individuals for each life stage were used for each treatment condition: on the support net of the oven (Non-insulated), in between the stack of towels (Insulated), and in the same area outside of the oven (Control). This provided a total of 9 pseudoreplicates for each life stage (adults, nymphs and eggs) during each run of the ZappBug Oven. Each run constituted one true replicate.

Preparation of Treatment Containers

The bottoms of plastic 9-dram vials were removed to make a plastic tube. Over the removed bottom of the vial, a piece of sheer fabric was hot glued. Holes were cut in the lids which accompanied the vials, and another piece of sheer fabric was placed over the top and held secure with the lids. The result is a vial with sheer fabric at both ends to allow ventilation and prevent any bed bugs from escaping.

Preparation and Handling of Bed Bugs

Bed bug adults and nymphs were anesthetized with CO₂ and placed in the treatment containers. Bed bug eggs were obtained by placing several pieces of clean filter paper in a colony of bed bugs. The bed bug colony was allowed to lay eggs on the filter paper pieces for 5 days, after which the papers were removed. The papers were cut into pieces so that only 10 eggs were on a particular piece, or eggs were gently removed from the paper. The eggs were placed in the treatment containers.

Monitor of Internal Temperature

A HOBO® data logger was placed in each treatment environment to record the temperature over time. Additionally, the indoor/outdoor thermometer was placed in the insulated environment and was used to determine when the internal temperature reached 120°F (Figure 3). The time which it took for the oven to reach 120°F was recorded for each environment. The oven was allowed to run for an additional hour after the internal temperature reached 120°F. Then the oven was shut off and the bed bugs removed for examination.



Figure 3. Picture of the insulated environment, including three replicates of each bed bug life stage, a HOBO® data logger, and the indoor/outdoor thermometer.

Observation of Mortality

The duration which the bed bugs were exposed to the heat were recorded. Mortality counts were taken for the bed bug adults and nymphs in each treatment environment. Bed bugs were recorded as Alive, Moribund, or Dead, where moribund bed bugs are those which show movement, but are unable to right themselves when flipped on their backs, or do not respond to stimuli consistent with a normal bed bug.

Control and treatment eggs were placed in the same area as the bed bug colonies and kept until the control eggs hatched (6 days). Eggs were recorded as hatched or unhatched, with unhatched being bed bugs which failed to emerge from the egg.

Data analyses

The numbers of alive, moribund, and dead bed bugs were added together for the total mortality. The means and standard deviations per replicate were then calculated. Statistical analyses were not necessary since any live bed bugs or hatched eggs would have constituted a product failure. The oven treatment provided total mortality, and as such, Abbott's formula was not used to correct for mortality in the controls.

Results and Conclusions

Complete data and the results of the data analysis are presented in Appendix III.

The ZappBug Oven was run three times, giving a total of three true replicates. Bed bug adults and nymphs were examined immediately after heat treatment to determine alive, moribund, and dead individuals. They were also checked at 24 hours. Eggs were examined to determine which were hatched or unhatched. Data is summarized below:

Average Number of Live Bed Bugs								
Rep	Reading	Life stage	Control		Non-insulated			
1	6 hr	Adults	10.0	0.0	0.0			
1	24 hr	Adults	10.0	0.0	0.0			
1	6 hr	Nymphs	10.0	0.0	0.0			
1	24 hr	Nymphs	10.0	0.0	0.0			
1	6 day	Eggs	8.0	0.0	0.0			
2	5.3 hr	Adults	9.3	0.0	0.0			
2	24 hr	Adults	8.7	0.0	0.0			
2	5.3 hr	Nymphs	10.0	0.0	0.0			
2	24 hr	Nymphs	10.0	0.0	0.0			
2	6 day	Eggs	7.0	0.0	0.0			
3	5.3 hr	Adults	10.0	0.0	0.0			
3	24 hr	Adults	9.7	0.0	0.0			
3	5.3 hr	Nymphs	10.0	0.0	0.0			
3	24 hr	Nymphs	10.0	0.0	0.0			
3	6 day	Eggs	8.7	0.0	0.0			

Table 1. The average number of live bed bugs recorded in each treatment environment.

In each replicate, all bed bugs which received the heat treatment, whether in the insulated or non-insulated environment, were dead upon examination.



Figure 4. Bed bugs before heat treatment.

Figure 5. Bed Bugs after heat treatment.

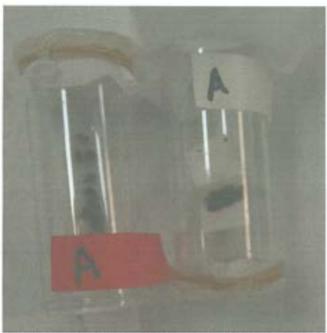


Figure 6. Comparison of heat-treated bed bugs (red label) and control bed bugs (white label).

When used according to the product's manual instructions, the ZappBug Oven was successful in providing complete mortality of all bed bug adults, nymphs, and eggs in each replicate.

Appendix I - Protocol

PROTOCOL NUMBER N5450912030A644

PROJECT NUMBER 545-0030

STUDY TITLE EFFICACY OF THE ZAPPBUG OVEN FOR KILLING SEVERAL LIFE STAGES OF BED BUGS BY HEAT

PROPOSED START DATE

October 2012

PROPOSED COMPLETION DATE

October 2012

STUDY COORDINATOR

Alicia Kelley

SPONSOR

ZappBug 511 Boren Ave N; Suite 200 Seattle, WA 98109

TESTING FACILITY

i2LResearch USA, Inc. 1330 Dillon Heights Avenue Baltimore, MD 21228-1199

OBJECTIVE:

To determine the efficacy of the ZappBug Oven heat treatment product for killing beg bug adults, nymphs and eggs.

This is not a GLP (Good Laboratory Practices) protocol.

USE OF I2LRESEARCH USA'S NAME IN PROMOTIONAL RELEASES

Sponsor agrees not to use i2L's name in any promotional literature, TV, radio, web-based or other media, without the express written permission of i2L management. i2LResearch USA, Inc. reserves the right to grant this permission to the sponsor based on the relation of the promotional text and images to the data generated for the sponsor.

MATERIALS:

FORMULATIONS:

The Sponsor will provide the following test systems:

ZappBug Oven

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.

TEST ARTHROPODS:

Bed bug (Cimex lectularius) adults, nymphs and eggs from the i2LResearch USA Field strain bed bugs obtained from DC, DE, MD, ME and NJ from 2006 to 2009 will be used in this study. All bed bug adults and nymphs will have been blood fed within 7 days of testing.

TREATMENT CHAMBER:

Any area of the laboratory large enough to host the ZappBug Oven. The oven will be tested at an ambient temperature ≥65°F as specified on page 5 of the product manual. The temperature and relative humidity will be recorded for any areas used.

TREATMENT CONTAINERS:

Bed bugs will be placed inside vials with both ends covered with sheer fabric for ventilation

MISCELLANEOUS:

Stop watch, data record forms, forceps, surgical gloves, disposable Tyvek®coveralls, Kestrel® weather meter, HOBO® data logger

METHODS:

Summary

Six replicates of 10 bed bug adults, nymphs and eggs will be placed inside ZappBug Oven ("oven"). Three replicates will be placed on the floor of the "oven" and three replicates will be placed in between six large bath towels. An additional three replicates will be placed outside the "oven" in the same area which will serve as controls. A HOBO® data logger will be placed on the "oven" floor, in the towels and with the controls. The "oven" will be switched on and left alone until the temperature in the towels reaches 120°F after which the "oven" will remain on for an additional hour. After the "oven" is turned off, the number of alive, moribund and dead bed bugs will be recorded for the adults and nymphs. Additional 24 hour mortality readings will be recorded for the nymphs and adults. The eggs will be kept until control bed bug eggs hatch (approximately 2 weeks). The sponsor will be informed of the results and will determine if additional replication is required.

Test sample

The test system will be stored in a secure area at ambient temperature and humidity until used in the study.

Replication

A total of 9 replicates of 10 bed bug adults, nymphs and eggs will be used for one true replicate. After the first true replicate the sponsor will be informed of the results and will then decide whether to do an additional two true replicates.

Personal Protection Measures

All person(s) involved in treatment may wear disposable Tyvek coveralls and gloves

Preparation of Treatment Containers

A plastic vial will have the bottom removed to make a plastic tube. Over the removed bottom of the vial a piece of sheer fabric will be hot glued. A screw top lid with the top removed will be screwed onto the top of the vial with another piece of sheer fabric. The results will be a vial with sheer fabric at both ends to provide ventilation and prevent any bed bugs from escaping.

Preparation and Handling of Bed Bugs

Ten bed bug adults, nymphs, and eggs will be used per replicate. Each replicate of bed bug adults and nymphs will be anesthetized with CO₂ and placed in the treatment containers. Bed bug eggs will be obtained by placing several pieces of clean filter paper in a colony of bed bugs. The bed bug colony will be allowed to lay eggs on the filter paper pieces for 5 days after which the papers will be removed. Each paper will be cut into pieces so that only 10 eggs are on a particular piece. The egg papers will then be placed inside the treatment containers.

Preparation of "Oven"

The "oven" will be setup according to the user manual instructions. The "oven" uses support blocks and a support net to suspend items above the oven floor and increase convective heat transfer (shown on page 8 of product manual). On one side of the oven, a stack of six large folded towels will be place on top of the support net. On the other side, the bed bugs will be placed directly on the bottom of the oven.

Treatment of Bed Bugs

Three replicates of each bed bug life stage (adult, nymph, egg) will be placed on the bottom of the oven and in between the stack of towels. Control bed bugs will be placed in the same area outside of the "oven" and will not receive any heat treatment.

Monitor of Internal Temperature

A HOBO® data logger will be placed with the control, on the oven bottom and in the stack of towels to record the temperature over time. The time which it took for the "oven" to reach 120°F will be recorded on the data sheet.

Observation of Mortality

After the oven has run its recommended time, the unit will be switched off. The duration which the bed bugs were exposed to the heat will be recorded and mortality counts will be taken on the bed bug adults and nymphs for the oven floor, in between the towels and the controls. Bed bugs will be recorded as Alive, Moribund, or Dead, where moribund bed bugs are those which are unable to right themselves when flipped on their backs, or show any movement not consistent with a normal bed bug.

Control and treatment eggs will be placed in the same area as the bed bug colonies and kept until the control eggs hatch (approximately 2 weeks). Eggs will be recorded as hatched or unhatched, with unhatched being bed bugs which failed to emerge completely from the egg.

DATA ANALYSIS

The number of dead bed bugs per replicate will be added together for a total mortality count.

Abbott's Formula will be used to correct for any mortality among the controls.



ZappBug Heat Treatment Against Bed Bugs Protocol #: N5450912030A644 ICR Project #: 545-0030

DATA ANALYSIS

The number of dead bed bugs per replicate will be added together for a total mortality count. Abbott's Formula will be used to correct for any mortality among the controls.

SCHEDULE OF EVENTS

Date

Procedure

Time 0

Test conducted

At End of Test

Telephone/Fax report

After Test is Conducted

Written report

Following Final Report

Samples returned

STATEMENT OF DEVIATION OR AMENDMENT:

Any amendments to this protocol must be discussed with and approved by the Sponsor. Any amendments to, and/or deviations from, this protocol will be documented in the final report.

Alicia Kelley

Study Coordinator

ICR, Inc.

te Cameron Wheeler

Co-Founder/ CEO

ZappBug

RAW DATA SHEET KNOCKDOWN AND MORTALITY

		KNO	CKDOWN AN	D MORTA	LITY		
Spor	nsor: ZappBug				Date:		-0.0
Tim	e Start:		Tin	ne Stop:			
Trea	tment: ZappBu	g Oven	Control cle one)	Replica	te Number:_		
Tem	perature:	Relative Hu	amidity:				
			Adul	ts			
Donlingto	Time to reach	+	Hour Morta	lity	2	4 Hour Mortali	ty
Replicate	120°F	Alive	Moribund	Dead	Alive	Moribund	Dead
1							
2							
3							
Total							
Mean							
			Nymp	hs			
n 11	Time to reach	+	Hour Morta		2	4 Hour Mortalit	v
Replicate	120°F	Alive	Moribund	Dead	Alive	Moribund	Dead
1							
2							
3							
Total							
Mean							
		Eggs					
D 11	Time to	-00					
Replicate	reach 120°F	Hatched	Unhatched	Date			

Eggs									
Replicate	Time to reach 120°F	Hatched	Unhatched	Date					
1									
2									
3									
Total				454					
Mean									

Notes:

Recording Technician Initials/Date:

Study Coordinator Signature/Date:

Appendix II - Protocol Amendment

i2LResearch USA, Inc. 1330 Dillon Heights Avenue Baltimore, MD, 21228 Telephone: (410) 747-4500 Fax: (410) 747-4928

PROTOCOL AMENDMENT

Project Number:

545-0030

Protocol Number:

N5450912030A644

Sponsor:

ZappBug

Tests Article(s):

ZappBug Oven

Amendment:

In the "Methods" section of the protocol (pages 3 and 4), it states three replicates will be placed between SIX (6) large bath towels inside the ZappBug oven. Per sponsor request, only THREE (3) bath towels will be used.

Impact on the study:

The sponsor determined it would take approximately 9 hours for the temperature inside six bath towels to reach 120°F. Changing the number of towels to three serves only to reduce the amount of time it takes to reach this temperature. It will not impact the integrity of the study.

Submitted by:

Study Coordinator

Data

Acknowledged:

Sponsor's Representative

Date

Appendix III – Data Analysis

CONTROL								
Sponsor	Project#	Test start date:	Time start	Time stop	Time to reach 120°F	Replicate		
ZappBug	545-0030 (12/283)	10/10/2012	11:00 AM	4:50 PM	N/A	1		

			ADULTS			
Donlings		6 Hr Mortalit	y	0	24 Hr Mortality	8
Replicate	Alive	Moribund	Dead	Alive	Moribund	Dead
1	10	0	0	10	0	0
2	10	0	0	10	0	0
3	10	0	0	10	0	0
Total	30	0	0	30	0	0
Mean	10.0	0.0	0.0	10.0	0.0	0.0
St. Dev.	0.000	0.000	0.000	0.000	0.000	0.000
			NYMPHS		THE	
Daville Land		6 Hr Mortality	/	24 Hr Mortality		
Replicate	Alive	Moribund	Dead	Alive	Moribund	Dead
1	10	0	0	10	0	0
2	10	0	0	10	0	0
3	10	0	0	10	0	0
Total	30	0	0	30	0	0
Mean	10.0	0.0	0.0	10.0	0.0	0.0
St. Dev.	0.000	0.000	0.000	0.000	0.000	0.000
		EGGS				
Replicate	Hatched	Unhatched	Date of reading			
1	7	3	10/16/2012			
2	7	3	10/16/2012			
3	10	0	10/16/2012			
Total	24	6	8/11/19			
Mean	8.0	2.0	BUCALRI			
St. Dev.	1.732	1.732	100 00 00			

		ZAPPBUG	OVEN: NON-	NSULATED		
Sponsor	Project#	Test start date:	Time start	Time stop	Time to reach 120°F	Replicate
ZappBug	545-0030 (12/283)	10/10/2012	11:00 AM	4:50 PM	15 minutes	1

			ADULTS				
Replicate		6 Hr Mortalit	y .	24 Hr Mortality			
Replicate	Alive	Moribund	Dead	Alive	Moribund	Dead	
1	0	0	10	0	0	10	
2	0	0	10	0	0	10	
3	0	0	10	0	0	10	
Total	0	0	30	0	0	30	
Mean	0.0	0.0	10.0	0.0	0.0	10.0	
St. Dev.	0.000	0.000	0.000	0.000	0.000	0.000	
			NYMPHS				
		6 Hr Mortality	y	24 Hr Mortality			
Replicate	Alive	Moribund	Dead	Alive	Moribund	Dead	
1	0	0	10	0	0	10	
2	0	0	10	0	0	10	
3	0	0	10	0	0	10	
Total	0	0	30	0	0	30	
Mean	0.0	0.0	10.0	0.0	0.0	10.0	
St. Dev.	0.000	0.000	0.000	0.000	0.000	0.000	
		EGGS					
Replicate	Hatched	Unhatched	Date of reading				
1	0	10	10/16/2012				
2	0	10	10/16/2012				
3	0	10	10/16/2012				
Total	0	30	100000				
Mean	0.0	10.0					
St. Dev.	0.000	0.000	ALC: U				

		TAPPE	UG OVEN: INS	ULATED		
Sponsor	Project #	Test start date:	Time start	Time stop	Time to reach 120°F	Replicate
ZappBug	545-0030 (12/283)	10/10/2012	11:00 AM	4:50 PM	4.8 hours	1

			ADULTS				
Replicate		6 Hr Mortalit	y	24 Hr Mortality			
Replicate	Alive	Moribund	Dead	Alive	Moribund	Dead	
1	0	0	10	0	0	10	
2	0	0	10	0	0	10	
3	0	0	10	0	0	10	
Total	0	0	* 30	0	0	-30	
Mean	0.0	0.0	10.0	0.0	0.0	10.0	
St. Dev.	0.000	0.000	0.000	0.000	0.000	0.000	
			NYMPHS		-	4-11	
0		6 Hr Mortality	/		24 Hr Mortality		
Replicate	Alive	Moribund	Dead	Alive	Moribund	Dead	
1	0	0	10	0	0	10	
2	0	0	10	0	0	10	
3	0	0	10	0	0	10	
Total	0	0	30	0	0	30	
Mean	0.0	0.0	10.0	0.0	0.0	10.0	
St. Dev.	0.000	0.000	0.000	0.000	0.000	0.000	
		EGGS			-		
Replicate	Hatched	Unhatched	Date of reading				
1	0	10	10/16/2012				
2	0	10	10/16/2012				
3	0	10	10/16/2012				
Total	0	30					
Mean	0.0	10.0	133 3000				
St. Dev.	0.000	0.000	THE PARTY				

			CONTROL			
Sponsor	Project#	Test start date:	Time start	Time stop	Time to reach 120°F	Replicate
ZappBug	545-0030 (12/283)	10/17/2012	9:00 AM	1:21 PM	N/A	2

			ADULTS			
Replicate		5.3 Hr Mortali	ty		24 Hr Mortality	3
Replicate	Alive	Moribund	Dead	Alive	Moribund	Dead
1*	9	0	0	8	0	1
2*	9	0	0	8	0	1
3	10	0	0	10	0	0
Total	28	0	0	26	0	2
Mean	9.3	0.0	0.0	8.7	0.0	0.7
St. Dev.	0.577	0.000	0.000	1.155	0.000	0.577
			NYMPHS			
		5.3 Hr Mortalit	ty	24 Hr Mortality		
Replicate	Alive	Moribund	Dead	Alive	Moribund	Dead
1	10	0	0	10	0	0
2	10	0	0	10	0	0
3	10	0	0	10	0	0
Total	30	0	0	30	0	0
Mean	10.0	0.0	0.0	10.0	0.0	0.0
St. Dev.	0.000	0.000	0.000	0.000	0.000	0.000
	1	GGS				
Replicate	Hatched	Unhatched	Date of reading			
1	8	2	10/23/2012			
2	7	3	10/23/2012			
3	6	4	10/23/2012			
Total	21	9				
Mean	7.0	3.0	1111/28188			
St. Dev.	1.000	1.000				

		ZAPPBUG	OVEN: NON-	NSULATED		
Sponsor	Project#	Test start date:	Time start	Time stop	Time to reach 120°F	Replicate
ZappBug	545-0030 (12/283)	10/17/2012	9:00 AM	1:21 PM	30 minutes	2

			ADULTS			
Replicate		5.3 Hr Mortali	ty	24 Hr Mortality		
Replicate	Alive	Moribund	Dead	Alive	Moribund	Dead
1	0	0	10	0	0	10
2	0	0	10	0	0	10
3	0	0	10	0	0	10
Total	0	0	30	0	0	30
Mean	0.0	0.0	10.0	0.0	0.0	10.0
St. Dev.	0.000	0.000	0.000	0.000	0.000	0.000
			NYMPHS			
		5.3 Hr Mortali	ty	24 Hr Mortality		
Replicate	Alive	Moribund	Dead	Alive	Moribund	Dead
1	0	0	10	0	0	10
2	0	0	10	0	0	10
3	0	0	10	0	0	10
Total	0	0	30	0	0	30
Mean	0.0	0.0	10.0	0.0	0.0	10.0
St. Dev.	0.000	0.000	0.000	0.000	0.000	0.000
	1	EGGS				
Replicate	Hatched	Unhatched	Date of reading			
1	0	10	10/23/2012			
2	0	10	10/23/2012			
3	0	10	10/23/2012			
Total	0	30				
Mean	0.0	10.0				
St. Dev.	0.000	0.000	No			

		ZAPPE	UG OVEN: INS	ULATED		
Sponsor	Project #	Test start date:	Time start	Time stop	Time to reach 120°F	Replicate
ZappBug	545-0030 (12/283)	10/17/2012	9:00 AM	1:21 PM	4.3 hours	2

			ADULTS			
Replicate		5.3 Hr Mortali	ty		24 Hr Mortality	2
Replicate	Alive	Moribund	Dead	Alive	Moribund	Dead
1	0	0	10	0	0	10
2	0	0	10	0	0	10
3	0	0	10	0	0	10
Total	0	0	30	0	0	30
Mean	0.0	0.0	10.0	0.0	0.0	10.0
St. Dev.	0.000	0.000	0.000	0.000	0.000	0.000
			NYMPHS			
Danillanta	5.3 Hr Mortali					
Replicate	Alive	Moribund	Dead	Alive	Moribund	Dead
1	0	0	10	0	0	10
2	0	0	10	0	0	10
3	0	0	10	.0	0	10
Total	0	0	30	0	0	30
Mean	0.0	0.0	10.0	0.0	0.0	10.0
St. Dev.	0.000	0.000	0.000	0.000	0.000	0.000
		EGGS				
Replicate	Hatched	Unhatched	Date of reading			
1	0	10	10/23/2012			
2	0	10	10/23/2012			
3	0	10	10/23/2012			
Total	0	30	-			
Mean	0.0	10.0	g Type To			
St. Dev.	0.000	0.000	The same of the			

CONTROL								
Sponsor	Project #	Test start date:	Time start	Time stop	Time to reach 120°F	Replicate		
ZappBug	545-0030 (12/283)	10/24/2012	8:40 AM	2:00 PM	N/A	3		

			ADULTS			
Replicate		5.3 Hr Mortali	ty	24 Hr Mortality		
Replicate	Alive	Moribund	Dead	Alive	Moribund	Dead
1	10	0	0	10	0	0
2	10	0	0	9	0	1
3	10	0	0	10	0	0
Total	30	0	0	29	0	1
Mean	10.0	0.0	0.0	9.7	0.0	0.3
St. Dev.	0.000	0.000	0.000	0.577	0.000	0.577
		•	NYMPHS			
		5.3 Hr Mortali	ty	24 Hr Mortality		
Replicate	Alive	Moribund	Dead	Alive	Moribund	Dead
1	10	0	0	10	0	0
2	10	0	0	10	0	.0
3	10	0	0	10	0	0
Total	30	0	0	30	0	0
Mean	10.0	0.0	0.0	10.0	0.0	0.0
St. Dev.	0.000	0.000	0.000	0.000	0.000	0.000
	E	GGS				
Replicate	Hatched	Unhatched	Date of reading			
1	8	2	10/30/2012			
2	8	2	10/30/2012			
3	10	0	10/30/2012			
Total	26	4				
Mean	8.7	1.3				
St. Dev.	1.155	1.155	0 100			

		ZAPPBUG	OVEN: NON-I	NSULATED		
Sponsor	Project #	Test start date:	Time start	Time stop	Time to reach 120°F	Replicate
ZappBug	545-0030 (12/283)	10/24/2012	8:40 AM	2:00 PM	25 minutes	3

			ADULTS			
Replicate	5	5.3 Hr Mortali	ty		24 Hr Mortality	
Replicate	Alive	Moribund	Dead	Alive	Moribund	Dead
1	0	0	10	0	0	10
2	0	0	10	0	0	10
3	0	0	10	0	0	10
Total	0	0	30	0	0	30
Mean	0.0	0.0	10.0	0.0	0.0	10.0
St. Dev.	0.000	0.000	0.000	0.000	0.000	0.000
			NYMPHS			
B W		5.3 Hr Mortali	ty		24 Hr Mortality	02
Replicate	Alive	Moribund	Dead	Alive	Moribund	Dead
1	0	0	10	0	0	10
2	0	0	10	0	0	10
3	0	0	10	0	0	10
Total	0	0	30	0	0	30
Mean	0.0	0.0	10.0	0.0	0.0	10.0
St. Dev.	0.000	0.000	0.000	0.000	0.000	0.000
	Ε	GGS				
Replicate	Hatched	Unhatched	Date of reading			
1	0	10	10/30/2012			
2	0	10	10/30/2012			
3	0	10	10/30/2012			
Total	0	30	The state of the s			
Mean	0.0	10.0	100000			
St. Dev.	0.000	0.000	the same of the			

ZAPPBUG OVER: INSULATED									
Sponsor	Project #	Test start date:	Time start	Time stop	Time to reach 120°F	Replicate			
ZappBug	545-0030 (12/283)	10/24/2012	8:40 AM	2:00 PM	4.3 hours	3			

			ADULTS			
Replicate	-	5.3 Hr Mortalit	ty		24 Hr Mortality	
Replicate	Alive	Moribund	Dead	Alive	Moribund	Dead
1	0	0	10	0	0	10
2	0	0	10	0	0	10
3	0	0	10	0	0	10
Total	0	0	30	0	0	30
Mean	0.0	0.0	10.0	0.0	0.0	10.0
St. Dev.	0.000	0.000	0.000	0.000	0.000	0.000
			NYMPHS			
		5.3 Hr Mortalit	ty		24 Hr Mortality	C.
Replicate	Alive	Moribund	Dead	Alive	Moribund	Dead
1	0	0	10	0	0	10
2	0	0	10	0	0	10
3	.0	0	10	0	0	10
Total	0	0	30	0	0	30
Mean	0.0	0.0	10.0	0.0	0.0	10.0
St. Dev.	0.000	0.000	0.000	0.000	0.000	0.000
DIE T		GGS	1-11-1-1			
Replicate	Hatched	Unhatched	Date of reading			
1	0	10	10/30/2012			
2	0	10	10/30/2012			
3	0	10	10/30/2012			
Total	0	30				
Mean	0.0	10.0				
St. Dev.	0.000	0.000				

Appendix IV - Raw Data Sheets



Heat Treatment Against Bed Bugs Protocol #: N5450912030A644 ICR Project #: 545-0030

RAW DATA SHEET KNOCKDOWN AND MORTALITY

Sponsor: Za	ppBug	22	Date: 10/10/12	
Time Start:	11.00am	Time Stop: _	4:50 pm	Hobo #1
Treatment:	ZappBug Oven		cate Number:	

Temperature: 45° F /Relative Humidity: 58%

	Adults									
Replicate Time to reach 120°F	Time to reach + 5 Hour Mortality				24 Hour Mortality					
	Alive	Moribund	Dead	Alive	Moribund	Dead				
1		10	0	0	10	0	0			
2		10	0	D	10	0	0			
3	-	10	0	0	10	0	0			
Total	V	30	0	0	30	0	0			
Mean	/	10	0	0	10	0	0			

	Nymphs								
Replicate	Time to reach	+ 5 Hour Mortality			24 Hour Mortality				
Replicate	120°F	Alive	Moribund	Dead	Alive	Moribund	Dead		
1	_	10	0	0	10	0	0		
2		10	0	0	10	6	0		
3		10	0	0	- 10	0	0		
Total	V	30	0	0	36	6	0		
Mean		10	0	0	10	0	0		

	Eggs								
Replicate	Time to reach 120°F	Hatched	Unhatched	Date					
1	-	7	30+	10/16/12					
2		7	300	10/16/12					
3	-	10	0	10/16/12					
Total	- V/	24	10						
Mean		8	2	18 18 18 18 18 18 18 18 18 18 18 18 18 1					

AF 10/10/12

Notes:

Recording Technician Initials/Date: Ak 10/10/12 14k 10/11/12

Study Coordinator Signature/Date: Alicia Killy 10/11/12



Heat Treatment Against Bed Bugs Protocol #: N5450912030A644 ICR Project #: 545-0030

RAW DATA SHEET KNOCKDOWN AND MORTALITY



Sponsor: ZappBug

Date: 10/10/12

___ Hobo #3

Time Start: 11:00 am

Time Stop: 4.50 pm

Treatment: (ZappBug Oven

Control

Replicate Number:

Temperature: 45°F /Relative Humidity: 58%

	Adults								
Replicate	Time to reach	+ 5 Hour Mortality		2	4 Hour Mortalit	ty			
	120°F	Alive	Moribund	Dead	Alive	Moribund	Dead		
1	in matt. 8 15 min	0	0	10	0	0	10		
2	15 min	0	0	10	O.	0	10		
3		0	0	10	D	0	10		
Total		0	0	30	0	0	30		
Mean	*	0	0	10	0	0	10		

	Nymphs								
Replicate	Time to reach	+ 5 Hour Mortality			2	4 Hour Mortali	ty		
Replicate	120°F	Alive	Moribund	Dead	Alive	Moribund	Dead		
1	15 min	0	0	10	0	0	10		
2	1	0	0	10	0	0	10		
3		0	0	10	0	0	10		
Total	3 3	0	0	30	0	0	30		
Mean	*	D	0	10	_	0	10		

Eggs								
Replicate	Time to reach 120°F	Hatched	Unhatched	Date				
1	15 min	0	10	10/10/12				
2	1	0	10	10/14/12				
3		O	10	10/16/12				
Total		0	30	- State of				
Mean	9	0	10					

Notes:

Recording Technician Initials/Date: pk 10/10/12 pk 10/11/12

Study Coordinator Signature/Date: Alicia Kelly 19/11/12



Heat Treatment Against Bed Bugs Protocol #: N5450912030A644 ICR Project #: 545-0030

RAW DATA SHEET KNOCKDOWN AND MORTALITY



Sponsor: ZappBug

Date: |0 |0 |2

Time Stop:

Hobo #2

Time Start: 11.00 am

Control

Replicate Number:

Treatment: (ZappBug Oven

(circle one)

Temperature: 75 F/Relative Humidity: 58%

	Adults								
Replicate	Time to reach	+ 5 Hour Mortality			2	4 Hour Mortali	ty		
Replicate	120°F	Alive	Moribund	Dead	Alive	Moribund	Dead		
1	4.8 hrs	0	0	10	0	0	10		
2	4.8 hrs	0	0	10	. 0	0	10		
3	4.8 hrs	0	0	10	0	0	10		
Total		0	0	36	0	0	30		
Mean		0	0	10	0	0	10		

	Nymphs								
Replicate	Time to reach	+	Hour Mor	tality	2	4 Hour Mortali	ty		
Replicate	120°F	Alive	Moribund	Dead	Alive	Moribund	Dead		
1	4.8 hrs	0	0	10	0	0	10		
2		0	0	10	0	0	10		
3		0	0	10	- 0	0	10		
Total		0	0	30	0	0	30		
Mean	U.	0	0	10	0	0	10		

	Eggs								
Replicate	Time to reach 120°F	Hatched	Unhatched	Date					
1	4.8 hrs	0	10	10/16/12					
2		0	10	Io/IIo/12					
3		0	10	Jollid12					
Total	2/-	0	30	A SHOW					
Mean	0	0	10	100000					

Notes:

Recording Technician Initials/Date:

10/10/12

Study Coordinator Signature/Date:

Alicia Kelley 10/11/12



Heat Treatment Against Bed Bugs Protocol #: N5450912030A644

ICR Project #: 545-0030

RAW DATA SHEET KNOCKDOWN AND MORTALITY

Sponsor	: Zap	pBug

Hobo #1

Time Start: 9:00 am

Time Stop: 1:21 pm

Treatment:

ZappBug Oven

Control

Replicate Number:

Temperature: 73 Relative Humidity: 52 %

Adults								
Time to reach		+ 4.3 Hour Mortality			24 Hour Mortality			
Replicate	120°F	Alive	Moribund	Dead	Alive	Moribund	Dead	
1	NIA	9+	0	0	8	0	1	
2	1	9+	0	0	8	0	- 1	
3		10	0	0	10	0	0	
Total		28	0	0	26 °	0	2	
Mean		9.3	0	0	8-107	0	0.107	

	Nymphs								
Replicate	Time to reach	+4	3 Hour Mor	tality	24 Hour Mortality				
Replicate	120°F	Alive	Moribund	Dead	Alive	Moribund	Dead		
1	N/A	10	0	0	10	6	0		
2		10	0	0	10	0	0		
3		10	0	0	10	0	0		
Total		30	0.	0	30	0	0		
Mean		O	0	0	10	0	6		

	Eggs								
Replicate	Time to reach 120°F	Hatched	Unhatched	Date					
1	NIA	- 8	2	1923/12					
2	1	7	3	10/23/12					
3		10	4	10/23/12					
Total		2.1	9	A CONTRACTOR					
Mean	V	7	3	11/15/10					

Notes:

Recording Technician Initials/Date: AK 10/17/12

Study Coordinator Signature/Date: Alicia Kelly 1423/12



Heat Treatment Against Bed Bugs Protocol #: N5450912030A644

ICR Project #: 545-0030

RAW DATA SHEET KNOCKDOWN AND MORTALITY

Sponsor: ZappBug

Date: 10/17/12

Hobo #2

Time Start:

9:00 am

Time Stop: 1:21 pm

Treatment: (ZappBug Oven

Topulated

Control

Replicate Number: 2

(circle one)

Temperature: 734 /Relative Humidity: 52

	Adults									
D Linear	Time to reach	+ 4	.3 Hour Mort	tality	24	24 Hour Mortality				
Replicate	120°F	Alive	Moribund	Dead	Alive	Moribund	Dead 10 10 10 30			
1	1 4.3 hrs		0	10	0	0	10			
2		0	0	10	0	0	10			
3		0	0	10	0	0	10			
Total		0	0	30	0	0	30			
Mean	-	0	0	10	0	0	10			

	Nymphs									
Danlicata	Time to reach	+ 4	.3 Hour Mort	24	Hour Mortalit	y				
Replicate	120°F	Alive	Moribund	Dead	Alive	Moribund	Dead			
1	4.3 hrs	0	0	10	6	0	10			
2		0	0	10	O	0	10			
3		0	0	10	0	0	10			
Total		0	0	30	0	0	30			
Mean		0	0	10	0	0	10			

Eggs									
Replicate	Time to reach 120°F	Hatched	Unhatched	Date					
1	4.3 hrs	0	10	10/23/12					
2		0	10	10/23/12					
3		0	10	10/23/12					
Total		0	30						
Mean		0	10	TOTAL TR					

Notes:

Recording Technician Initials/Date: AK 1917/12

Study Coordinator Signature/Date: Alicia Kelley 10/23/12



Heat Treatment Against Bed Bugs Protocol #: N5450912030A644

ICR Project #: 545-0030

RAW DATA SHEET KNOCKDOWN AND MORTALITY



Sponsor: ZappBug

Date: 10/17/12

Hobo #3

Time Stop: 1:21 pm

Time Start: 9:00 am

Control

Replicate Number: 2

Treatment: (ZappBug Oven) (circle one)

Temperature: 737 /Relative Humidity: 52 %

Adults										
Replicate	Time to reach	+4	3 Hour Mort	tality	24	24 Hour Mortality				
Replicate	120°F	Alive	Moribund	Dead	Alive	Moribund	Dead			
1	30 min	0	0	10	0	0	10			
2		0	0	10	0	0	10			
3		0	0	10	0	0	10			
Total		0	0	30	0	0	30			
Mean	-	0	0	10	0	0	10			

			Nymp	hs		100000 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1	
Replicate	Time to reach	+4	3 Hour Mort	tality	24 Hour Mortality		
	120°F	Alive	Moribund	Dead	Alive	Moribund	Dead
1	30 min	0	0	10	0	0	10
2		0	0	10	0	0	10
3	N - T 7	0	0	10	0	0	10
Total		0	0	30	0	0	30
Mean	1	0	0	10	0	0	10

	TD:448 - 80 II	Eggs		
Replicate	Time to reach 120°F	Hatched	Unhatched	Date
1	30 min	0	10	10/23/12
2		0	10	102312
3		0	10	10/23/12
Total		0	30	
Mean		0	10	= 14VVI

Notes:

Recording Technician Initials/Date: AK 10/17/12

Study Coordinator Signature/Date: Alicia Killy



Heat Treatment Against Bed Bugs Protocol #: N5450912030A644 ICR Project #: 545-0030

RAW DATA SHEET KNOCKDOWN AND MORTALITY

Sponsor: ZappBug

Date: 1924/2

Time Start: 8:40 am

Time Stop: 2:00 pm

Treatment: ZappBug Oven

Control (circle one)

Replicate Number:

BK 1924/12-

Temperature: 75 F/Relative Humidity: 56%

Hobo #

			Adul	ts			
Replicate	Time to reach	+ 5	3 Hour Mort	tality	2	4 Hour Mortali	ty
Replicate	120°F	Alive	Moribund	Dead	Alive	Moribund	Dead
1	45.3 Hrs NA	10	6	0	10	0	0
2	NA	10	0	0	9	0	1
3	NA	10	0	0	10	0	0
Total		30	0	0	29	0	1
Mean	1	10	0	0	9.7	0	0.3

	Nymphs									
Replicate	Time to reach	+5	+ 5.3 Hour Mortality 24 Hour Mor				ality			
Replicate	120°F	Alive	Moribund	Dead	Alive	Moribund	Dead			
1	N/A-	10	0	0	10	6	0			
2	1	10	6	0	10	0	0			
3		10	0	0	10	0	0			
Total	1/-	36	6	0	. 30	0	0			
Mean	8	10	0	0	10	0	0			

		Eggs		1 1000
Replicate	Time to reach 120°F	Hatched	Unhatched	Date
1	N/A	8	2	19/30/12
2	j j	8	2	10/30/2
3		10	0	10/30/12
Total	0	26	4	
Mean	V.	8.67	1.33	THE RE

Notes:

Recording Technician Initials/Date: Ak 10/24/12 AK 10/25/12 AK 10/30/12

Study Coordinator Signature/Date:

Alicia Kelley 10/30/12



ZappBug Heat Treatment Against Bed Bugs Protocol #: N5450912030A644 ICR Project #: 545-0030

RAW DATA SHEET KNOCKDOWN AND MORTALITY

Sponsor:	ZappBug	

Date: 10/24/12

Time Start:

Time Stop: 2:00 pm

Treatment: (ZappBug Oven

Control

Replicate Number: 3

Temperature: 757/Relative Humidity: 56%

(circle one)

Hobo #2

	Adults										
Replicate	Time to reach	+5	3 Hour Mort	tality	2	4 Hour Mortalit	ty				
Replicate	120°F	Alive	Moribund	Dead	Alive	Moribund	Dead 10 10 10 30				
1	4.3 hrs	0	0	10	0	0	10				
2		0	0	10	0	6	10				
3		6	0	10	0	6	10				
Total		0	0	30	0	0					
Mean	1	0	0	10	0	0	10				

	Nymphs									
Replicate	Time to reach	+5	+ 5.3 Hour Mortality 24 Hour Mortal							
Replicate	120°F	Alive	Moribund	Dead	Alive	Moribund	Dead			
1	43 hrs	0	0	10	0	0	10			
2		0	0	10	0	0	10			
3		0	0	10	0	0	10			
Total		0	0	30	0	0	30			
Mean	4	0	0	10	0	0	10			

	00000	Eggs			
Replicate	Time to reach 120°F	Hatched	Unhatched	Date	
1	4.3 hrs	0	10	10/30/12	
2	-	0	10	1930/12	
3		0	10	10/30/12	
Total		0	30	SELENCE.	
Mean	4	0	10		

Notes:

Recording Technician Initials/Date: Ak 10/24/12

AK 10/25/12 AK 10/20/12

Study Coordinator Signature/Date: Alicia Kelley 10/20/12



ZappBug. Heat Treatment Against Bed Bugs Protocol #: N5450912030A644

ICR Project #: 545-0030

RAW DATA SHEET KNOCKDOWN AND MORTALITY

Sponsor: Za	ppBug			Date:/o	24/12	
Time Start:	8:40 am	Time	Stop:	9:00 ;	2:00 pm	
Treatment:	ZappBug Oven	Control	Replic	ate Number:_	3	

Temperature: 75°F /Relative Humidity: 56 %

Hobo #3

Adults							
Replicate	Time to reach	+ 5.3 Hour Mortality		24 Hour Mortality			
Replicate	120°F	Alive	Moribund	Dead	Alive	Moribund	Dead
1	25 min	0	0	10	6	0	10
2		0	0	10	0	0	10
3		0	0	10	0	0	10
Total		0	0	30	0	0	30
Mean	4	0	0	10	0	0	10

Nymphs							
Replicate Time to reach		+ 5.3 Hour Mortality		24 Hour Mortality			
Replicate	120°F	Alive	Moribund	Dead	Alive	Moribund	Dead
1.	25 min	0	0	10	0	6	10
2	1	0	0	10	0	6	10
3		0	0	10	0	0	10
Total		6	0	30	0	0	30
Mean	4	0	0	10	Ŏ	0	10

		Eggs		
Replicate	Time to reach 120°F	Hatched	Unhatched	Date
1	25 min	0	10	193912
2	ſ	0	10	10/30/12-
3		0	10	Id3d12-
Total		0	30	No.
Mean		0	10	Williams

Notes:

Recording Technician Initials/Date: AK 10/24/12 AK 10/30/12

Study Coordinator Signature/Date: Alixin Kelley 10/30/12