PROJECT NO:
434-0032

PROTOCOL NO:
N4340309032A140

STUDY TITLE:
EFFICACY OF *BEST YET*, A DIRECT SPRAY, FOR KNOCKDOWN AND KILL OF ADULT FLEAS AND BED BUGS

IN-LIFE COMPLETION DATE:
March 11, 2009

STUDY COORDINATOR
Niketas C. Spero

PERFORMED BY:
ICR, Inc.
1330 Dillon Heights Avenue
Baltimore, MD 21228

EXECUTIVE SUMMARY

Five replicates of 10 cat fleas (*Ctenocephalides felis*) and 10 bed bugs (*Cimex lectularius*) were placed in plastic payliners and sprayed for 5 seconds (which provided an average of 36.51 grams and 30.71 grams respectively) with CedarCide BEST YET. The insects were thoroughly saturated with this unusually large amount of product. The insects were observed for 15 minutes post-treatment for knockdown and KD_{50}, KD_{90}, and KD_{100} were recorded. The times for KD_{50}, KD_{90} and KD_{100} are reported as the average of the five replications. Mortality was assessed at +24 hours. The results are shown below:

Species	Mean Amount Sprayed (g)	Mean Knockdown Times (sec.)			Mean % 24-Hour Mortality
		KD_{50}	KD_{90}	KD_{100}	
Fleas	36.51	27	34	39	100.0
Bed Bugs	30.71	15	22	28	100.0

BESTYET was completely effective using the large amount of product that was applied in this study against adult fleas and bed bugs. It provided 100 % knockdown of fleas in 39 seconds and 100% knockdown of bed bugs in 28 seconds. Mortality at +24 hours was 100% for both species tested. There was no control mortality.

Niketas C. Spero 3-23-09

Niketas C. Spero Date
Study Coordinator

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OBJECTIVE

To evaluate BEST YET, a direct spray, for knockdown and mortality against adult cat fleas and bed bugs.

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

MATERIALS & METHODS:

The materials and methods used were as described in protocol N4340309032A140 (APPENDIX I).

CedarCide Industries, Inc.supplied the following sample:

1. BEST YET

RESULTS:

Five replicates of 10 cat fleas and 10 bed bugs were placed in plastic payliners and sprayed for 5 seconds (which provided an average of 36.51 grams and 30.71 grams respectively) with CedarCide BEST YET. The insects were thoroughly saturated with this unusually large amount of product. The insects were observed for 15 minutes post-treatment for knockdown and KD_{50}, KD_{90}, and KD_{100} were recorded. The times for KD_{50}, KD_{90} and KD_{100} are reported as the average of the five replications. Mortality was assessed at +24 hours. The results are shown below in Table 1:

Table 1. Knockdown and Mortality produced by BESTYET, a direct spray, applied to three species.

Species	Mean Amount Sprayed (g)	Mean Knockdown Times (sec.)			Mean % 24-Hour Mortality
		KD_{50}	KD_{90}	KD_{100}	
Fleas	36.51	27	34	39	100.0
Bed Bugs	30.71	15	22	28	100.0

BESTYET provided rapid knockdown of both adult fleas and bed bugs. BESTYET worked slightly quicker on bed bugs with 50% being knocked down in 15 seconds. 100% knockdown was achieved in 39 seconds with fleas and 28 seconds with bed bugs. There was no control knockdown (appendix III). Mortality for both species at 24 hours was 100% (there was no control mortality so correction with Abbott's formula was not necessary).

CONCLUSIONS:

BESTYET was completely effective while using the large amount of product that was applied in this study against adult fleas (*Ctenocephalides felis*) and bed bugs (*Cimex lectularius*). It provided 100 % knockdown of fleas in 39 seconds and 100% knockdown of bed bugs in 28 seconds. Mortality at +24 hours was 100% for both species tested.

APPENDIX I: PROTOCOL

PROTOCOL NUMBER:
N4340309032A140

PROJECT NUMBER:
434-0032

PROTOCOL TITLE:
EFFICACY OF *BEST YET*, A DIRECT SPRAY, FOR KNOCKDOWN AND KILL OF ADULT FLEAS AND BED BUGS

PROTOCOL VERSION DATE:
March 2, 2009

PROPOSED START DATE:
March 10, 2009

PROPOSED COMPLETION DATE:
March 11, 2009

STUDY COORDINATOR
Niketas C. Spero

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

OBJECTIVE

To evaluate BEST YET, a direct spray, for knockdown and mortality against adult cat fleas and bed bugs.

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

MATERIALS

TEST FORMULATION: The sponsor will provide the test sample:

BEST YET

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 the complete test sample, control sample, and any reference sample characterizations, and for retaining this doc samples are currently available for consumer use and/or purchased in the marketplace, the sponsor should still conduct the same sample characterizations.

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.

The stability of test, control, and/or reference samples stored under the test site conditions should be determined by the sponsor 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 ORGANISMS: Adult cat fleas, *Ctenocephalides felis,* from EL LABS Inc. of El Soquel, CA. and adult bed bugs *(Cimex lectularius)* from the ICR colony will be used.

TEST SUBSTRATE: Paper towel taped to the bottom of the treatment container.

TREATMENT CHAMBER & CONTAINERS: Treatments will be in a ventilated Peet Grady Chamber (actual size 5.8 m^3). Five gallon plastic payliners (top and bottom outer diameters, 31.8 cm and 26.7 cm, respectively; height 34.3 cm) will be used to contain adult fleas and bed bugs during treatment with spray formulation.

SPRAY EQUIPMENT: A Gilmore Professional Sprayer will be supplied by the sponsor.

MISCELLANEOUS: Aspirators, fine-tipped forceps, 9 dram vials, cotton balls, Tyvek® overalls and boots, latex gloves, respirator, humidity-temperature recording device, measuring tape, Kraft® paper, stop watch, nylon mesh, rubber bands, Fluon.

METHODS

Summary

Five replicates of 10 cat fleas and 10 bed bugs will be placed in plastic payliners and sprayed for 5 seconds with CedarCide BEST YET. The insects will be observed for 15 minutes post-treatment for knockdown and KD_{50}, KD_{90}, and KD_{100} will be recorded. Mortality will be assessed at +24 hours.

Samples

Test samples will be stored in a locked cabinet at ambient temperature and humidity until the study date.

Personnel Safety
Person(s) involved may wear disposable Tyvek® coveralls and booties, a respirator and will follow general containment procedures.

Preparation and Handling of Test Species
Fleas will be obtained from EL LABS of El Soquel, CA. Adult fleas a few days to 2 weeks old will be tested. Fleas will be collected into groups of 10. There will be five replicates for treatment and five for controls.

The bed bugs will have received a blood meal within 7 days of their introduction into the containers. The inside walls of the payliners will be treated with Fluon to prevent the bed bugs from escaping. The bugs will be anesthetized with CO_2 and then 10 will be placed in each treatment container. There will be five replicates for treatment and five for controls.

Direct Spray Treatment and Observation
Insects will be placed on the paper towel taped to the bottom of a treatment payliner. They will be sprayed for 5 seconds using Gilmore Professional Sprayer. The fleas will then be observed for knockdown. Moribund fleas are those that are unable to hop when the observer exhales into the payliner and/or probes the flea. Moribund bed bugs are defined as moving, but with uncontrollable movement, erratic response to external stimuli, or the inability to right themselves if placed on their backs. The times will be recorded when KD_{50}, KD_{90}, and KD_{100} occur. Observations will continue for 15 minutes. If any of the KD's are not reached within 15 minutes, the number knocked down and the knockdown time will be recorded as greater than 15.

After KD observations are finished, payliners will be brought into the laboratory and kept at ambient temperature and humidity for 24 hours. The control payliners which received no spray will be placed in the same location. After twenty-four hours, mortality will be evaluated. Fleas will be evaluated by gently blowing into the liner or probing quiescent fleas and counting the number of fleas jumping. Number of insects alive, moribund and dead will be recorded. For statistical purposes, moribund insects will be counted as dead.

Treatment of Control Insects
Each control replicate will be subjected to the same procedures outlined above with the exception that they will not be treated. The controls will be housed in the same area as those treated for the duration of the prescribed observation periods.

DATA ANALYSIS

The KD_{50}, KD_{90} and KD_{100} times will be reported as the average of the five replications.

Twenty-four hour mortality will be calculated after adjusting for control mortality with Abbott's formula. Average percent mortality will be calculated.

SCHEDULE OF EVENTS

DATE	PROCEDURE
Time Zero	Test Conducted
At End of Test	Telephone Report
Within 30 Days of Test Completion	Written Report
Within 30 Days of Final Report	Samples Returned

STATEMENT OF DEVIATION OR CHANGE

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

Niketas C. Spero 3-5-09 David Glassel 3-4-09

Niketas C. Spero — Date
Study Coordinator,
Insect Control & Research, Inc.

David Glassel — Date
CedarCide Industries

RAW DATA SHEET
KNOCKDOWN AND +24 HOUR MORTALITY

Company: **CedarCide Ind.** Project No.: 434-0032 Date:

Formulation: **BEST YET** Species:

	Pre-treatment	+ 24 Hr
Temp (° F) :	_____	_____
RH: (%):	_____	_____

Rep	Knockdown Times				24 Hr Mortality		
	KD_{50}	**KD_{90}**	**KD_{100}**	**KD15 min**	Alive	Moribund	Dead
1							
2							
3							
4							
5							
Tot							
Ave							

Technician recording:

Study Coordinator:

APPENDIX II: STATISTICAL CALCULATIONS

Data Reduction

	Sponsor:	CedarCide	Project #	434-0032	Date:	03/19/09

FLEAS

Control

Rep	Wt Sprayed	Knockdown				24 Hour Mortality			% Mortality
		KD_{50}	KD_{90}	KD_{100}	$KD_{15\ min}$	Alive	Moribund	Dead	
1	NA				0	10	0	0	0
2	NA				0	10	0	0	0
3	NA				0	10	0	0	0
4	NA				0	10	0	0	0
5	NA				0	10	0	0	0
Average	NA				0.0				0.0

BESTYET

Rep	Wt Sprayed	Knockdown				24 Hour Mortality			% Mortality
		KD_{50}	KD_{90}	KD_{100}	$KD_{15\ min}$	Alive	Moribund	Dead	
1	40.72	26	32	41		0	0	10	100
2	36.88	27	36	40		0	0	10	100
3	31.02	27	34	40		0	0	10	100
4	38.27	26	32	36		0	0	10	100
5	35.64	29	36	39		0	0	10	100
Average	36.51	27.0	34.0	39.2					100.0

BED BUGS

Control

Rep	Wt Sprayed	Knockdown				24 Hour Mortality			% Mortality
		KD_{50}	KD_{90}	KD_{100}	$KD_{15\ min}$	Alive	Moribund	Dead	
1	NA				0	10	0	0	0
2	NA				0	10	0	0	0
3	NA				0	10	0	0	0
4	NA				0	10	0	0	0
5	NA				0	10	0	0	0
Average	NA				0.0				0.0

BESTYET

Rep	Wt Sprayed	Knockdown				24 Hour Mortality			% Mortality
		KD_{50}	KD_{90}	KD_{100}	$KD_{15\ min}$	Alive	Moribund	Dead	
1	36.10	15	24	34		0	0	10	100
2	30.46	12	23	29		0	0	10	100
3	27.90	13	16	21		0	0	10	100
4	25.06	14	21	25		0	0	10	100
5	34.01	20	25	32		0	0	10	100
Average	30.71	14.8	21.8	28.2					100.0

APPENDIX III: RAW DATA SHEETS

RAW DATA SHEET
KNOCKDOWN AND +24 HOUR MORTALITY

Company: **CedarCide Ind.** Project No.: 434-0032 Date: 3-10-09

Formulation: **BEST YET** Species: FLEAS

	Pre-treatment	+ 24 Hr
Temp (° F) :	75	77
RH: (%):	38	40

	Rep	Spray Amt.	Knockdown Times				24 Hr Mortality		
			KD_{50}	KD_{90}	KD_{100}	KD15 min	Alive	Moribund	Dead
10:51	1	40.72g	0'26"	0'32"	0'41"		0	0	10
10:52	2	36.88	0'27"	0'36"	0'40"		0	0	10
10:54	3	31.02	0'27"	0'34"	0'40"		0	0	10
10:55	4	38.27	0'26"	0'32"	0'36"		0	0	10
10:56	5	35.64	0'29"	0'36"	0'39"		0	0	10
	Tot	182.53					0	0	50
	Ave	36.51	0'27"	0'34"	0'39"				

Technician recording: NCpw 3-10-09 (KAB) 3/10/09 CFC 3/10/09

Study Coordinator: NCpw 3-11-09

RAW DATA SHEET
KNOCKDOWN AND +24 HOUR MORTALITY

Company: **CedarCide Ind.** Project No.: 434-0032 Date: 3-10-09 10:40

Formulation: **Control** Species: FLEAS

	Pre-treatment	+ 24 Hr
Temp (° F) :	73.4°	77
RH: (%):	50%	40

Rep	Spray Amt.	Knockdown Times				24 Hr Mortality		
		KD_{50}	KD_{90}	KD_{100}	KD15 min	Alive	Moribund	Dead
1					0	10	0	0
2					0	10	0	0
3					0	10	0	0
4					0	10	0	0
5					0	10	0	0
Tot					0	50	0	0
Ave								

Technician recording: NOpm 3-10-09 CFQ 3/10/09
3-11-09

Study Coordinator: NOpm 3-10-09

RAW DATA SHEET
KNOCKDOWN AND +24 HOUR MORTALITY

Company: **CedarCide Ind.** Project No.: 434-0032 Date: 3-10-09

Formulation: **BEST YET** Species: BEDBUGS

	Pre-treatment	+ 24 Hr
Temp (° F) :	75	77
RH: (%):	36	39

	Rep	Spray Amt.	Knockdown Times				24 Hr Mortality		
			KD_{50}	KD_{90}	KD_{100}	KD15 min	Alive	Moribund	Dead
9:30	1	36.10g	0'15"	0'24"	0'34"		0	0	10
9:30	2	30.46	0'12"	0'23"	0'29"		0	0	10
9:33	3	27.90	0'13"	0'16"	0'21"		0	0	10
9:33	4	25.06	0'14"	0'21"	0'25"		0	0	10
9:33	5	34.01	0'20"	0'25"	0'32"		0	0	10
	Tot	153.53					0	0	50
	Ave	30.71	0'14.8"	0'21.8"	0'28.2"				

Technician recording: (MB) 3/10/09 CFE 3/10/09 N Lpw 3-10-09 3/10/09

Study Coordinator: N Lpw 3-11-09

RAW DATA SHEET
KNOCKDOWN AND +24 HOUR MORTALITY

Company: **CedarCide Ind.** Project No.: 434-0032 Date: 9:00/ 3-10-09

Formulation: **Control** Species: BEDBUGS

	Pre-treatment	+ 24 Hr
Temp (° F) :	76°	77
RH: (%):	52%	39

Rep	Spray Amt.	Knockdown Times				24 Hr Mortality		
		KD_{50}	KD_{90}	KD_{100}	KD15 min	Alive	Moribund	Dead
1					0	10	0	0
2					0	10	0	0
3					0	10	0	0
4					0	10	0	0
5					0	10	0	0
Tot					0	50	0	0
Ave								

Technician recording: NCfw 3-10-09 CFC 3/10/09
3-11-09

Study Coordinator: NCfw