

**FINAL REPORT:**

Laboratory Efficacy – non-GLP

**DATA REQUIREMENTS:**

40 CFR 158.640 Product Performance – Efficacy

EPA Product Performance Test Guidelines OPPTS 810.3000  
General Considerations for Efficacy of Invertebrate Control Agents

EPA Product Performance Test Guidelines OPPTS 810.3500  
Premises Treatments

**TITLE:**

Efficacy Evaluations of Bed-Bug-Rid Natural Bed-bug Killer Applied as a Direct Application  
Against Bed Bugs and German Cockroaches

**PROJECT NUMBERS:**

SRL Project I.D. # IGR11-1

**SPONSOR:**

Iguana-Rid, LLC  
432 S. Military Trail  
Deerfield Beach, FL 33442

**TESTING FACILITY:**

Sierra Research Laboratories, Inc. (SRL)  
P.O. Box 576886  
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**REPORT AUTHOR:**

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**STUDY COMPLETION DATE:**

29 July 2011

**Study Title:** Efficacy Evaluations of Bed-Bug-Rid Natural Bed-bug Killer Applied as a Direct Application Against Bed Bugs and German Cockroaches

**Study Objectives:**

To determine the knockdown efficacy and mortality of Bed-Bug-Rid Natural Bed-bug Killer against bed bugs and German cockroaches when applied as a direct application treatment.

**SRL Study Personnel:**

William A. Donahue, Jr., Ph.D. – Study Director/Principal Investigator  
Sumiko De La Vega, B.A. – SRL Scientist I, Data Collection  
Bret Vinson, B.S. – SRL Scientist I, Data Collection  
Michael W. Donahue, B.S. – SRL Scientist I, Data Collection  
Wendy Olmstead, B.A. – SRL Scientist I, Data Collection  
Griselda Juarez – SRL Laboratory Technician, Data Collection

**Study Initiation Date:** 20 June 2011

**Experimental Initiation Date:** 20 June 2011

**Experimental Completion Date:** 12 July 2011

**Study Completion Date:** 29 July 2011

**Introduction – Justification for Selection of Test System:**

*In vitro* systems are very efficient in the evaluation of the intrinsic activity of insecticide formulations against selected arthropod pest species. The ability to evaluate several pest arthropods against candidate formulations under controlled laboratory conditions is critical in determining the efficacy of a product to support product registration through various regulatory agencies. These evaluations are designed to determine the knockdown activity and mortality of Bed-Bug-Rid Natural Bed-bug Killer against bed bugs and German cockroaches when applied as a direct application treatment. Data will be used to support marketing claims and regulatory agencies as desired by the sponsor. This study will not be conducted under GLP Guidelines (40 CFR Part 160).

**Definitions:**

**Knockdown** is defined as the inability of the arthropod to right itself or move in an upright deliberate manner. **Moribund** is a condition exhibited by an organism just prior to death or recovery, especially when poisoned, and manifests abnormal reactions to stimuli. **Mortality** is assessed at 24 hours and beyond by “probing” to determine death. **Recovery** is the process leading to partial or complete restoration of a cell, tissue, organ or organism following its damage from exposure to a harmful substance or agent.



### Test Substances:

2 pump spray bottles of Bed-Bug-Rid Natural Bed-bug Killer, Lot No. 8521800061, Net Contents 32 fl. oz. (946 ml) each, Active Ingredients: Cinnamon oil 2.0%, Diatomaceous earth 0.1%, inert ingredients 97.9%. The test substance was received and logged in on 16 June 2011 via Fed Ex Express (Trk. # 7948 6374 5037).

**Chemical Storage Conditions and Environmental Monitoring** - The test substance was stored in the original sealed containers in the SRL Chemical Storage Area under ambient conditions of temperature, relative humidity and light conditions. Laboratory chemical storage area environmental monitoring was conducted and recorded with SRL temperature (°F) and humidity (% RH) recording devices (min/max); data are included in the Appendix.

### Test Species:

1. Bed bug, *Cimex lectularius*

- a. "Harlan" strain (SRL lab susceptible strain), adults and nymphs
- b. "Earl" strain (SRL colony field collected susceptible strain), adults and nymphs
- c. "James" strain (SRL colony field collected susceptible strain), adults, nymphs, and eggs

German cockroach, *Blattella germanica* (SRL "Jamaican" lab strain), adults

### Type and Frequency of Tests and Measurements (Efficacy Evaluations):

**Knockdown** – Efficacy evaluations were conducted on caged bed bugs and German cockroaches, treated directly with Bed-Bug-Rid Natural Bed-bug Killer in a laboratory spray chamber to avoid cross contamination between other treatments and untreated controls. Treatment containers for bed bugs consisted of 12 oz clear plastic drink cups, lined with coffee filters to absorb excess liquid. German cockroaches were treated in 16 oz squat cups, with a thin layer of petroleum jelly/mineral oil along the rim of the cup to prevent escape. Five replicates were treated for each species with approximately ten bed bugs and ten German cockroaches per replicate. At five minutes post treatment, insects were scored and transferred to clean containers for the remainder of the evaluation. German cockroaches were transferred to 8 oz. squat cups and bed bugs were transferred to 10 cm Petri dishes, lined with 7.5 cm filter papers. Control replicates were set-up, transferred, and scored in the same manner as the treated groups. Treated replicates were assessed for knockdown at 5, 15, 30, 45, 60 min and 2 and 4 hours and for mortality at 24 hours post-treatment. At 4 hours post-treatment, Fluker's® Orange Cube Cricket diet was provided to German cockroaches for moisture and nourishment.

**Ovicidal Treatments** – Bed bug "seed" colonies were set up in 5.5 cm Petri dishes with 5.5 cm filter paper discs in the bottom. After blood feeding, approximately five to seven females and two males were placed in each "seed" colony. The filter paper discs were harvested when eggs numbered approximately ten to twenty. Discs were randomly assigned to either the untreated control or Bed-Bug-Rid Natural Bed-bug Killer test group. Treatment bottle and filter papers

were weighed before and after treatment to determine the amount of test substance dispensed and the actual amount applied on each filter paper. Eggs were scored for hatch daily, up to three days after total hatch in the control group. Eggs were also scored for condition and changes in appearance.

### **Results and Discussion:**

At 5 minutes post-treatment, Bed-Bug-Rid Natural Bed-bug Killer demonstrated  $\geq 90.0\%$  efficacy for knockdown against bed bugs (“James”, “Earl”, and “Harlan” strains) and German cockroaches (Table 1). All three bed bug strains demonstrated slight recovery from the treatment at 4-hours and continued through 96-hours post-treatment. Recovery rates varied with each strain of bed bug with a range of 14-34%. Control mortality for all test insects is reported in Table 2.

Efficacy against German cockroaches was very consistent with 98% knockdown observed at 15 minutes post-treatment and remained at that level through 96-hours with no recovery observed at any data point (Table 1).

Bed bug eggs (“James” strain) exhibited 0% hatch from one to eleven days following treatment with Bed-Bug-Rid Natural Bed-bug Killer compared to 98.8% hatch at Days 10 and 11 in the control replicates (Table 3). Bed-Bug-Rid Natural Bed-bug Killer demonstrated excellent ovicidal activity against bed bugs.

### **Conclusions:**

Bed-Bug-Rid Natural Bed-bug Killer, when applied as a direct application treatment, demonstrated excellent knockdown of bed bugs up to 4-hours (82-98%) depending on strain. Recovery was observed in all three test strains through 96-hours post-treatment.

Bed-Bug-Rid Natural Bed-bug Killer, when applied as a direct application treatment, prevented hatch of bed bug eggs.

Bed-Bug-Rid Natural Bed-bug Killer demonstrated excellent activity (98% mortality) against German cockroaches.



ADAM H. PUTNAM  
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MAY 31, 2011

IGUANA-RID, LLC  
432 S. MILITARY TRAIL  
DEERFIELD BEACH, FL 33442  
UNITED STATES

FAID: 0090158001  
CO ID: 10158001

THIS IS CONFIRMATION THAT THE FOLLOWING 3 PRODUCT BRAND(S) HAVE BEEN REGISTERED BY FLORIDA.

NOTE: ALL PERMITS AND REGISTRATIONS EXPIRE ON DECEMBER 31ST OF THE EVEN REGISTRATION YEAR AND MUST BE RENEWED FOR THE NEXT REGISTRATION PERIOD BY PAYMENT OF THE APPLICABLE FEES AS REQUIRED UNDER CHAPTER 487, FLORIDA STATUTES.

FIFRA SECTION 18 EMERGENCY EXEMPTION PETITIONS ARE NOT SUBJECT TO STATE REGISTRATION FEES, BUT ARE LISTED WITH OTHER PRODUCT BRANDS IN THE SYSTEM FOR AUDITING PURPOSES.

PRODUCT BRAND NAME	FL/EPA REG NO	EFFECTIVE DATE	EXPIRATION DATE
BED-BUG-RID	EXEMPT	05/31/2011	12/31/2012
IGUANA-RID	EXEMPT	05/31/2011	12/31/2012
PEST-RID	EXEMPT	05/31/2011	12/31/2012