Laboratory bioassay to assess the efficacy of the Cryonite system against bed bug adult, nymph and egg stages

Insect Investigations Ltd
Capital Business Park
Wentloog
Cardiff CF3 2PX
UK

Helena Dawe
March 2006

Certification

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

Signed…….. Date……..
Helena Dawe
Study Director

Approved by…… Date……..
Dr Peter M^{c}Ewen
Managing Director

All raw data and a copy of the final report will be archived at I_2L for a period of five years. At the end of this period all data relating to this report will either be retained by I_2L for a further disclosed period of time, or passed on to the sponsor.

Report circulated to: Insect-o-cutor (1 copy)
Insect Investigations Ltd (1 copy)

Contents

Study Information

Laboratory bioassay to assess the efficacy of the Cryonite system against bed bug adult, nymph and egg stages

i. Submitted by: Insect Investigations Ltd
Capital Business Park
Wentloog
Cardiff CF3 2PX

ii. Sponsor: Insect-o-cutor
Oakhurst Drive
Cheadle Heath
Stockport
Cheshire SK3 0XT

iii. I_2L Study code: 06/16

iv. Study start date: 08.03.06
Study completion date: DRAFT COPY

v. Study Director: Helena Dawe

vi. Principal personnel: -

vii. Certification: ORETO Number 175

viii. Test chemicals:

Test Product	I_2L code number
Cryonite system	06031601

Summary

A laboratory bioassay was conducted to assess the efficacy of the Cryonite system against bed bug adults, nymphs and egg stages. The Cryonite system was applied as four bursts into plastic tanks containing bed bug adults, nymphs and eggs. At the time of treatment the bed bugs were on scrunched cardboard to simulate crack/crevices. The number of dead adults and nymphs was assessed at 24 hours and the number of first instar nymphs that hatched was assessed at 7 days post treatment.

The Cryonite system was very effective against bed bugs; almost all adults and all nymphs were dead at 24 hours post application. No nymphs hatched out of the eggs treated with the Cryonite system.

Aim

A laboratory bioassay was conducted to assess the efficacy of the Cryonite system against bed bug adult, nymph and egg stages, in terms of mortality and egg hatching.

Methodology

Test insects

Bed bugs (*Cimex lectularius*) were obtained from a laboratory culture maintained at Sheffield University (Sheffield, South Yorkshire). Mixed sex, mixed age adults and 3-4th instar nymphs were used in the experiments. The egg batches used in the trial were laid by newly moulted female bed bugs over a period of one week.

Treatments and application

The Cryonite system was applied as four bursts for each replicate. An untreated control treatment was also included for comparative purposes, giving a total of two treatments.

Experimental design

Ten adult bed bugs, ten nymph bed bugs and one batch of between 10 and 50 eggs were placed into one plastic tank (33 cm long x 21 cm wide x 21 cm high). The sides of the tanks were coated in Fluon (liquid PTFE) to prevent the insects from escaping. A piece of scrunched cardboard was placed in the tank to simulate crack/crevices. The Cryonite system was then applied to the tank. After application a container with water was placed in the tank to raise the humidity. The number of dead insects was assessed at 24 hours post treatment with the Cryonite. The number of first instar nymphs that hatched was assessed 7 days post treatment application.

Three replicates were performed for each of the treatments, each replicate consisting of one tank.

Temperature ranged from 26 – 30 ºC and relative humidity was 40%.

Results and Conclusions

Treatment with the Cryonite system resulted in almost 100% bed bug mortality (table 1). All the bed bug nymphs and almost all the adults were dead at 24 hours post application. No eggs hatched 7 days post treatment, compared to the untreated control. It can be concluded that the Cryonite system is effective in controlling bed bugs.

Table 1. Effect of the Cryonite system on mortality of *C. lectularius* 24 hours post treatment and subsequent hatching of eggs at 7 days post treatment

Treated				Control			
Replicate	No dead adults	No dead nymphs	No 1st instar nymphs hatched	Replicate	No dead adults	No dead nymphs	No 1st instar nymphs hatched
1	10	10	0	1	0	0	19
2	9	10	0	2	0	0	14
3	10	10	0	3	0	1	42
Mean	**9.7**	**10**	**0**	**Mean**	**0**	**0.3**	**25**
SE	0.3	0	0	SE	0	0.3	8.6

Appendix I Study Protocol

Laboratory bioassay to assess the efficacy of the Cryonite system against bed bug adult, nymph and egg stages.

Proposal submitted by Insect Investigations Ltd to Insect-o-cutor

Submitted by: Helena Dawe, Study Director (Efficacy Group)
7th March 2006

Protocol

Test insects. Bed bugs, *Cimex lectularius*, will be obtained from a laboratory culture maintained at Sheffield University. Mixed sex adults, nymphs and newly laid eggs will be used in the experiment.

Treatments. The Cryonite system will be tested on bed bug adults, nymphs and eggs. A control will also be set up for each bed bug stage, giving a total of two treatments.

Experimental design. Ten adult bed bugs, ten nymph bed bugs and one batch of eggs (size to be determined) will be placed into one plastic tank, measuring approximately 33 cm long x 21 cm wide x 20 cm high. The tank will be coated in Fluon (liquid PTFE) to prevent the insects from escaping. Suitable harbourages, e.g. cardboard rolls, will be placed in the tank to simulate crack/crevices. The Cryonite system will then be applied to the tank according to the sponsor's instructions. The number of live and dead bed bug adults/nymphs will be assessed at 24 hours post treatment application. The number of 1st instar nymphs that hatch from the treated eggs will be assessed at an interval determined by the hatching period of the control eggs. If no treated eggs hatch following a period of one week after the final control eggs have hatched, it will be assumed that the eggs are dead.

Three replicates will be performed for each treatment, each replicate consisting of one tank.

Temperature and humidity will be monitored throughout the experimental period and detailed in the final report.

Appendix II **ORETO certification**

Certificate of

Official Recognition of Efficacy Testing Facilities or Organisations in the United Kingdom

This certifies that

Insect Investigations Limited

complies with the minimum standards laid down in Commission Directive 93/71/EEC for efficacy testing.

The above Facility/Organisation has been officially recognised as being competent to carry out efficacy trials/tests in the United Kingdom in the following categories:

Agriculture/Horticulture

Date of issue	6 January 2004
Effective date	1 January 2004
Expiry date	31 December 2008

Signature _____

Authorised signatory

Certification Number

ORETO 175

Department of Agriculture and Rural Development

PSD
SAFETY FOR PEOPLE AND THE ENVIRONMENT
an Executive Agency of DEFRA

ORET 3 (Rev. 4/01)