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BioGenius

## Final Report

BioGenius GmbH, Biology  
TechnologiePark, Building 56  
Friedrich-Ebert-Straße  
51429 Bergisch Gladbach, Germany

Study no. : Mo3758  
Report no : BIO017/09  
Date : 2009-03-23  
Study Director : K.-H. Lüpkes

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## Biological Test Report

### Efficacy against Bed bugs

Efficacy after direct spray treatment against Bed bug *Cimex lectularius*.

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**Test Sample:** Chrysamed Universal Insektenspray

**Author** : K.-H. Lüpkes

**Study Completion Date** : 2009-03-23

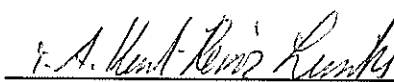
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### Approval of the Biological Test Report

**Signature of the Study Director:**

2009-03-23

Date

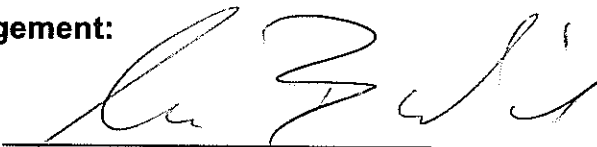


K.-H. Lüpkes

**Signature of the Test Facility Management:**

2009-03-23

Date



Mike Bublitz

The biological results are only valid for the samples received in the laboratory. This report contains the unpublished results of biological work by BioGenius GmbH. These results may not be published, either wholly or in part, or reviewed or quoted in any other publication without the authorisation of the sponsor.



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### 1 General Information

Sponsor: Mr. Aktas  
CHRYSAMED Vertrieb GmbH  
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A-5020 Salzburg

Study Director: K.-H. Lüpkes  
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Study dates: Start of experiments : 2009 week 12  
End of experiments : 2009 week 12

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## 2 Summary

The product Chrysamed Universal Insektenspray (based on 0.06 % Esbiothrin and 0.12 % Permethrin) was tested on efficacy against Bed bugs *Cimex lectularius* (adults) by direct spray treatment.

Bed bugs *Cimex lectularius* (adults) were sprayed directly with Chrysamed Universal Insektenspray. An amount of 1.4 gram (corresponding to one stroke by the products pump spray bottle) was applied onto the bed bugs. Five bed bugs were treated together at the same time out of a distance of 30 cm. The time when the last of the five treated bed bugs of one replicate showed knock down effect (followed by mortality), was determined. Five replicates were done.

Untreated bed bugs were used as untreated control.

The product Chrysamed Universal Insektenspray gave good efficacy against Bed bugs *Cimex lectularius* by direct spray treatment.

100 % of knock down (followed by mortality), was given after ca. 3 minutes, on average by 5 replicates. The range of the 5 replicates was 2 minutes 10 seconds to 3 minutes 50 seconds.

For all data see table 1.

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**3 Sample**

Name: Chrysamed Universal Insektenspray

Charge Nr: UN 001

Prod. Dat.: 02/01/2009

"Ablauf Dat.": 02/01/2011

Content: 0.06 % Esbiothrin  
0.12 % Permethrin  
0.82 % emulsifier Chrysa  
99.00 % water

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### 4 Materials and Methods

#### 4.1 Materials

Test insects: Bed bugs *Cimex lectularius*  
adults  
(breeding method see appendix)  
Equipment: direct spray treatment unit (holder), balance  
Various: aluminium rings, paper tissues, glass rings (Ø 9.5 cm)  
talcum, beakers, pump spray bottle

#### 4.2 Climate Conditions

temperature: 20C  
rel. humidity: 21%  
light regime: artificial light during hours of work, additionally a little of day  
light is given

#### 4.3 Number of Samples

1

#### 4.4 Mode of Application

1 (spraying by pump spray bottle)

#### 4.5 Dosage

1 stroke (= 1,4 gram)

#### 4.6 Bed bugs treated together at the same time

5

#### 4.7 Spray Distance

30 cm

#### 4.8 Replicates

5 (untreated control: 2)

#### 4.9 Test Criteria

% knock down (followed by mortality)

#### 4.10 Knock down time determined

When last of the treated bed bugs of one replicate (five bed bugs treated together at the same time) showed knock down effect (followed by mortality)

#### 4.11 Test Times

knock down efficacy: up to 2 hours continuously  
mortality: 24 and 48 hours after treatment

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### 4.12 Execution of Method BioG B 200-01 (modified)

#### **Test of spray can and pump sprays direct treatment of flying and crawling insects by manual spraying.**

Formulations: The method is suitable for pump sprays.

Test insects: The test is suitable for bed bugs.

Treatment of insects: The treatment is carried out in a fume hood in which the ventilation can be regulated so that the spray jet from the pump spray is not affected. Bed bugs, 5 at a time, are put on paper tissues in glass rings (talc-powdered) with a diameter of 95 mm. The test containers prepared in this way are placed in a holder. At a distance of 30 cm, measured from the centre of the paper tissues up to the pump spray head, the pump spray is inserted into the apparatus so that the jet strikes the bed bugs vertically. The pump spray is weighed before and after each test to permit the applied quantity to be exactly determined.

Evaluation: A stop watch is pressed simultaneously with the spraying, so that the knock down effect on the insects can be found accurately and observed for knock-down times for up to two hours, and for knock-down after two hours. The bed bugs are tested in the test containers. After 24 and 48 hours the mortality rate is determined.

Artificial light and additionally a little day light runs during working hours, the temperature and relative humidity is given in the tables.

5 replicates (with 5 insects each) are done.



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### 5 Results

Table 1

**Efficacy against Bed bugs *Cimex lectularius*, adults,  
by direct spray treatment.**

study: Mo3758

method: BioG B 200-01 (modified)

amount: 1.4 gram / replicate

temp.: 20°C

rel. hum.: 21%

product	replicates	amount product /stroke	knock down time in minutes (') and seconds (")	% knock down after	% mortality after	
				2h	24h	48h
Chrysamed Universal Insektenspray Charge Nr.: UN 001 0.06 % Esbiothrin 0.12 % Permethrin	1	1.4 g	2'10"	100	100	100
	2	1.4 g	2'25"	100	100	100
	3	1.4 g	2'45"	100	100	100
	4	1.4 g	3'50"	100	100	100
	5	1.4 g	3'25"	100	100	100
<b>mean</b>		<b>1.4 g</b>	<b>2'55"</b>	<b>100</b>	<b>100</b>	<b>100</b>
range		---	2'10" – 3'50"	---	---	---

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51429 Bergisch Gladbach, Germany

Study no. : Mo3727  
Report no : BIO012/09  
Date : 2009-02-17  
Study Director : K.-H. Lüpkes

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### 6 Appendix

#### Breeding Method Bed Bugs

##### *Cimex lectularius*

The Bed bugs are kept in plastic cylinders (20 cm high, 9.5 cm diameter), closed by perforated lids. Inside folded filter paper as harborages is placed. An artificial blood feeding system is used once or twice a week.

Every four weeks the filter paper with adhered eggs and first nymph stage of Bed bugs is replaced. The eggs and young nymphs are transferred in a new cylinder. In this way the specimens live in colonies in defined developmental stages.

The cylinders are kept under 12 hours : 12 hours light : dark regime at 25°C ±2°C and 60% ±10% relative humidity.