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LABORATORY ASSESSMENT OF AN INSECTICIDE SPECIALITY INTENDED TO CONTROL BEDBUGS – Space treatment + Residual spray treatment

Product:

OXYPY +

SEPTEMBRE 2011
Report n°1457b/0711R

Sponsor:

OXY'PHARM
917, rue Marcel Paul
94500 Champigny-sur-Marne
France

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I, hereby Bruno Serrano, T.E.C. Director certify that the trials presented in this issue were done according to the Good Experiment Practices (G.E.P.) – French Agriculture Ministry agreement 94-021.

Anglet, 8th Septembre 2011

GOOD EXPERIMENT PRACTICES

STUDY TEC N°: 1457b/0711

SPONSOR : OXY'PHARM – 94500 Champigny-sur-Marne (France)

PRODUCT : OXYPY+

FACILITIES : T.E.C. 1 rue Jules Védrières 64600 Anglet (France)

TIMING : Start 25th July 2011

STUDY DIRECTOR : Bruno Serrano / Agronomist engineer

STUDY ENGINEER : Martine Falquier / Agronomist engineer

QUALITY INSURANCE RESPONSIBLE : Bruno Serrano /Agronomist engineer

METHODOLOGY :

Adapted French registration standard methodology C.E.B. N°135bis (1st edition: 1989 - Revised Mai 1996) in accordance with the E.P.P.O. Guideline n°135 (bulletin 18, 817-836 1988).

The trial is conducted in accordance with the procedures required to conduct Officially Recognized Trials (EOR), from the European Directive 91/414/CE and according to the laboratory agreement by the French Ministry of Agriculture.

ARCHIVAGE : 10 years, papers and electronic files

There were no circumstances which can have affected the reliability of the data presented in this report.

Bruno Serrano / Date : 8th Septembre 2011

LABORATORY ASSESSMENT OF AN INSECTICIDE SPECIALITY INTENDED TO CONTROL BED BUGS

PURPOSE:

The purpose of the study is to assess the global efficacy of the product applied in two ways:

- spot on treatment on the main bed bugs locations (head of the bed, under mattress) with a hand-held sprayer
- complete space treatment in order to reach hidden insects: curative + preventive effect by the deposit of the product onto cracks and crevices.

The trial was conducted according to an adaptation of the French registration standard:

- Méthode C.E.B. 135bis (1^{ère} édition : 1989 Révision : Mai 1996) : "Méthode d'étude de l'efficacité des préparations insecticides et/ou acaricides destinées aux traitements de volumes des locaux de stockage, de transformation industrielle et de commercialisation des produits d'origine animale ou végétale"

Beside of the direct insecticide efficacy, the trial is also measuring the residual activity until 6 weeks on surfaces exposed to the treatment (as the product contains an Insect Growth Regulator, this persistence trial is conducted on nymphs).

This issue follows the standard method design and relates any deviations.

1. EXPERIMENTAL CONDITIONS

1.1. Treatment chamber

The treatment is done in a closed 60 m³ chamber (8 m long x 3 m wide x 2.50 m high) in accordance with the CEB 135bis method and to be close to the reality of a bedroom.

The chamber represents the average conditions of a bedroom.

Some big cardboard boxes are set to represent furnitures and bed.

There is no ventilation.

The treatment room is kept in controlled climatic conditions:

20°C±1°C / 65%HR±5%RH / light 1200 lux.

1.2. Insects

Target organism:

The bedbugs *Cimex lectularius*, are from a wild strain retrieved in hotel rooms by a PCO (Biarritz – 64 France).

The insects are acclimatized for 24 hours before testing.
The insects used are 20 mixed sex adults per replicate.

Untreated control:

Some batches of insects are handled in the same conditions to check the natural mortality which must be lower than 10% to validate the trial.

2. TREATMENTS

2.1. Product and dosis

The experimental product is provided by OXYPHARM :

OXYPY + ready to use – Lot 040511 EXP 05/2013

Dose: 2 ml /m³

2.2. Application of the treatments

The purpose of the study is to assess the global efficacy of the product applied in two ways:

- spot on treatment on the main bed bugs locations (head of the bed, under mattress) with a hand-held sprayer
- complete space treatment in order to reach hidden insects: curative + preventive effect by the deposit of the product onto cracks and crevices

SPACE TREATMENT:

The treatments are done by using the fogger device provided by OXYPHARM (brand name : NOCOSPRAY) and set into a corner of the test chamber.

Space treatment: 2 ml/m³ i.e. 120 ml in the 60 m³ test chamber.

There was no ventilation after the treatment.

The insects are kept 4 hours in the test chamber.

2 replicates are conducted by factor.

RESIDUAL SURFACE TREATMENT:

Some tiles of materials (mattress ticking PES/viscose + carpet) are treated into the test chamber in order to assess the residual activity of the product 6 weeks later: as the product contains an Insect Growth Regulator, this persistence trial is conducted on adults + nymphs.

The treatments are done by using a pressurized hand-held sprayer provided with the product.

The droplets are thin enough to wet the surfaces without leaking and without excessive vapourization in the air.

Rate of application of the mixture product: 50 ml par m².

The materials are treated flat and the actual treated area is 10 times the area of the materials.

The treated plates are randomly assigned among the total treated area and not handled before complete drying.

The untreated materials are treated with water.

4 replicates are conducted.

3. Assessments

3.1. Principle

The experimenter records the mortality at regular time intervals.

After the 4 hours exposure time, the insects (dead and/or alive) are withdrawn from the test and placed into glass jars with food and water sources, in breeding climatic conditions.

The observations are recording two phenomenons:

- knockdown (KD),
- mortality, lethal effect.

Main insecticides are acting on the nervous system and give successive effects: excitation, uncoordination of moves, paralysis (knock down) and lethargy conducting to death.

The paralysis phase depends on the active ingredient and the dosis, some recoveries can occur after a knockdown effect lasting more or less longer.

- knockdown effect : assessed 4 hours or less after treatment
- lethal effect: assessed 24 hours, 48 hours and 7 days after treatment.

As it is not doable to check if insects are knockdown or actually dead, the insects in the tables data will be classified as "dead" or "alive":

- dead (or knockdown): insects unable to move properly
- alive: insects able to move properly

4. RESULTS

4.1. PRESENTATION

The synthesis of data is given in Table I.

The raw data are given in APPENDIX.

Table I: synthesis of data in KT100 (time of exposure to kill or knockdown 100% of the insects):

	DIRECT CURATIVE EFFICACY	RESIDUAL EFFICACY AFTER 6 WEEKS ON ADULTS	RESIDUAL EFFICACY AFTER 6 WEEKS ON NYMPHS
OXYPY+	< 1h	< 30% in 7 days	< 7 days

4.2. COMMENTS

During all the trial, the death rates of the Untreated control batches of insects are lower than 5%, the trial is then validated.

The treatment gave a complete and definitive mortality (no recoveries after 24 hours).

The efficacy remains effective until 6 weeks after application, the Insect Growth Regulator is killing all the bedbugs nymphs.

5. CONCLUSION

In the conditions of this trial, with the product sample provided, the insects strains and methodology used:

The product **OXYPY +**, applied as a space treatment , at a rate of 2 ml per m³ and as a residual spray treatment at a rate of 50 ml/m², has proved:

- A fast and definitive insecticide efficacy against bedbugs in simulated-use conditions,
 - A residual activity of at least 6 weeks after treatment in laboratory conditions (the IGR still control the bedbugs nymphs).
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A P P E N D I X

Raw data

Note: the standards CEB can not be reproduced, but it can be purchased from the AFPP website: <http://www.afpp.net/>

SPACE TREATMENT - 2 ml/m3

REPLICATE 1		1 h			4h			24h			48h			7 days			
		D	A	%M	D	A	%M	D	A	%M	D	A	%M	D	A	%M	
OXYPY+	<i>Cimex lectularius</i>	H1	25	0	100	25	0	100	25	0	100	25	0	100	25	0	100
		B1	25	0	100	25	0	100	25	0	100	25	0	100	25	0	100
		H2	25	0	100	25	0	100	25	0	100	25	0	100	25	0	100
		B2	25	0	100	25	0	100	25	0	100	25	0	100	25	0	100
			mean	100	mean	100	mean	100	mean	100	mean	100	mean	100	mean	100	mean

REPLICATE 2		1 h			4h			24h			48h			7 days			
		D	A	%M	D	A	%M	D	A	%M	D	A	%M	D	A	%M	
OXYPY+	<i>Cimex lectularius</i>	H1	25	0	100	25	0	100	25	0	100	25	0	100	25	0	100
		B1	25	0	100	25	0	100	25	0	100	25	0	100	25	0	100
		H2	25	0	100	25	0	100	25	0	100	25	0	100	25	0	100
		B2	25	0	100	25	0	100	25	0	100	25	0	100	25	0	100
			mean	100	mean	100	mean	100	mean	100	mean	100	mean	100	mean	100	mean

Untreated Control	<i>Cimex lectularius</i>	H1	0	25	0	0	25	0	0	25	0	0	25	0	1	24	4
		B1	0	25	0	0	25	0	1	24	4	1	24	4	2	23	8
		H2	0	25	0	0	25	0	0	25	0	1	24	4	2	23	8
		B2	0	25	0	0	25	0	0	25	0	1	24	4	1	24	4
			mean	0	mean	0	mean	1	mean	3	mean	6					

H1, H2, B1, B2 = pests locations inside the test chamber (H = high ; B = low)
 D = dead or knockdown A = alive %M = % mortality or knockdown

RESIDUAL SPRAY TREATMENT - 50 ml/m²

TRIAL ON *Cimex lectularius*

TRIAL AT DAY0 (after drying)

Treatment	Material	Mean of replicates in % knockdown/ mortality				Mortality After 24 h
		1 h	2 h	3 h	4 h	
OXYPY	Mattress ticking	100	100	100	100	100
	Carpet tile	100	100	100	100	100
Untreated Control	Mattress ticking	0	0	0	0	0
	Carpet tile	0	0	0	0	0

TRIAL ON *Cimex lectularius*

TRIAL AT DAY0 + 6 WEEKS

Treatment	Material	Mean of replicates in % knockdown/ mortality				Mortality After 7 days
		1 h	2 h	3 h	4 h	
OXYPY+ on ADULTS	Mattress ticking	0	1	2	5	26
	Carpet tile	0	0	0	0	19
OXYPY+ on NYMPHS	Mattress ticking	0	0	0	0	100
	Carpet tile	0	0	0	0	100
Untreated Control	Mattress ticking	0	0	0	0	2
	Carpet tile	0	0	0	0	1