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LABORATORY ASSESSMENT OF AN INSECTICIDE SPECIALITY

Speciality:

All Insects Killer CHRYSAMED

NOVEMBER 2012
Report n°1523c/0712R

Sponsor:

EUROMEDITRADE
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13015 Marseille
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, le 22 November 2012

Disclaimer

The results described in this report were generated *in vitro* and on the provided samples.
The samples tested were accepted in good faith that they were representative of the intended final formulation(s)/products and the test methods employed were the ones agreed by the sponsor.
As such the results should be taken only as an indication of the potential for activity of the formulations or products under test.
The trial has been conducted on a laboratory strain of a “model” insect and the susceptibility of the local insects strains can be different in other labs or in the real conditions of use.
Then, these results cannot be considered as confirmation that a formulation or product will work in a clinical or field application.
Evidence for such activity can only be obtained from properly constructed and executed clinical or local field trials.

GOOD PRACTICES

STUDY TEC N°: 1523c/0712

NUMBER OF PAGES: 17

SPONSOR: EUROMEDITRADE – Marseille (France)

PRODUCT: All Insects Killer CHRYSAMED, experimental sample / liquid in a hand-held pump sprayer 500 ml, received the 16th July 2012, Batch n° 0905121014 DP : 05/2014

FACILITIES: T.E.C. 1, rue Jules Védrines, ZAC Maignon 64600 Anglet (France)

DATE OF TESTING: Start 23rd July 2012 - End 23rd October 2012

REPLICATES: 4

STUDY DIRECTOR: Bruno Serrano / Agronomist engineer

STUDY ENGINEER: Martine Falquier / Agronomist engineer

QUALITY INSURANCE RESPONSIBLE: Bruno Serrano / Agronomist engineer

METHODOLOGY:

The test system was adapted from the standard method C.E.B. n°135/159.
This method is part of the Appendix of approved methodologies for PT1 Biocides registration, in the "DRAFT Guideline to replace part of Appendices to chapter 7 (page 187 to 200) from TNsG on Product evaluation".
The trial is conducted in accordance with the procedures of Good Practices required to conduct Officially Recognised Trials (EOR), from the European directive 91/414/CE, according to the laboratory agreement by the French Ministry of Agriculture.

ARCHIVING: 10 years, hard and electronic copies

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

Bruno Serrano

Date: 21st November 2012

LABORATORY ASSESSMENT OF AN INSECTICIDE SPECIALITY

PURPOSE:

The purpose of this study is to assess the efficacy of an insecticide speciality applied as a residual spray onto surfaces and intended to control insect pests in household environment.

The trial was conducted using a method adapted from the French registration standard:

- Method C.E.B. 135 (First edition: april 1987 Revised: March 2007) : “Méthode d’étude de l’efficacité des préparations insecticides et/ou acaricides destinées aux traitements de surface des locaux de stockage, de transformation industrielle et de commercialisation des produits d’origine animale ou végétale”

The residual effect of the product is assessed until 3 months after treatment.

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 to be close to the reality of use.
The test chamber has no ventilation and it is separated form the storage room where the treated tiles are kept for ageing.
The treatment room allows to keep controlled temperatures between 20 and 25°C (±2°C).

1.2. Treated materials

1.2.1. Plates

A “plate” is defined as a plate of a material usually found in construction of food storage premises, which can be more or less sorbent and porous.

1.2.1.1. Choice of the treated materials

The materials used for the trial have been checked as non effective against the pest species by a preliminary biological testing.

Two types of materials are treated in order to assess the efficacy of the product in relation with the physical properties of the materials found in the actual conditions of use (sorbent and unsorbent).

- ceramic tiles (non porous),
- concrete (500 kg/m3).

1.2.1.2. Dimension and preparation of the plates

The plates are squares of 15 cm x 15 cm in order to be covered by a Petri box adapted to the species (e.g. 14 cm for insects and 5,5 cm for dust mites).

The residual efficacy is assessed on the tiles stored 90 days in climatic controlled conditions.

1.2.1.3. Storage of the treated plates

The treated plates are stored at a temperature of 25 °C +/- 2 °C and a relative humidity of 70 +/- 5 %, without contact between each other to avoid any transfert of product.

The plates are stored flat on the floor of a special chamber with an homogeneous lighting by fluorescent tubes placed at 2.5m from the floor.

The lights used are typical fluorescent tubes found in the food industry: PHILIPS MASTER TL-D Xtra Secura 58W/840 (color 840, power 58 W, 1.50 m long and 26 mm diameter).

The plates are exposed to the light with a photoperiod of 16 hours, alterning with 8 hours of darkness.

Ventilation : smooth, passive (< 1 m3/h).

1.3. Target insect species

In relation with the use, the species choosen are:

Blattella germanica (German cockroach)
Lasius niger (black ant)
Ctenocephalides felis (flea)
Musca domestica (fly)
Dermatophagoides pteronyssinus (house dust mite)
Tineola bisselliella (clothes moth)
Reticulitermes santonensis (termite)
Aedes aegypti (mosquito)
Aedes albopictus (Tiger mosquito)
Cimex lectularius (bed bug)

The insects ae from laboratory colony breedings from french official specialized institutes (INRA, TEC, ORSTOM, INA Paris-Grignon).
The strain are susceptible strains.

AGE AND INSTAR OF TARGET ORGANISMS

Scientific name	Common name	Instar	Age
Blattella germanica	blatte germanique	adulte, sexe mâle	de 1 à 3 semaines
Lasius niger	fourmi noire	adulte	de 1 à 2 semaines
Ctenocephalides felis	puce	adulte+larve	2 sem.+ 3ème stade
Musca domestica	mouche	adulte	de 4 à 6 jours
Derm. Pteronyssinus	acarien	mix ad+larve	de 0 à 2 semaines
Tineola bisselliella	mite vêtements	larve	3ème stade
Reticulitermes santonensis	termite	adulte	de 2 à 4 semaines
Aedes aegypti	moustique	adulte	de 4 à 6 jours
Aedes albopictus	moustique Tigre	adulte	de 4 à 6 jours
Cimex lectularius	punaise de lits	adulte	de 1 à 3 semaines

For each replicates, 25 insects are exposed.

Untreated control:

Some batches of insects are placed onto the same materials treated with water and handled in the same conditions than the materials treated with the product.

2. TREATMENTS

2.1. Standard product and dosis

No standard product was used in the trial design.

2.2. Experimental product and dosis

The experimental sample is provided by EUROMEDITRADE :

All Insects Killer CHRYSAMED, experimental sample / liquid in a hand-held pump sprayer 500 ml, received the 16th July 2012, Batch n°0905121014 DP : 05/2014

Dosis: 10 sprays per m², i.e. between 13 and 15 ml per m².

2.3. Application of the treatments

The treatments are done by using the provied hand-held pump-up sprayer.

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

Average rate of application of the product: 14 ml/m².

The materials are treated falt 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 by factor. A factor is a combination of the date of persistence, the insect species and the type of material.

3. Assessments

3.1. Principle

The day of the treatment (after drying), the insects are placed onto the plates during 4 hours.

The residual activity is assessed after 90 days.

The experimenter records the mortality at regular time intervals.
After the 4 hours exposure time, the insects are withdrawn from the plates by gentle succion and placed onto untreated plates with food and water sources, in breeding climatic conditions.

3.2. Mortality assessments

3.2.1. Knockdown and mortality

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, soem recoveries can occur after a knockdown effect lasting more or less longer.

3.2.2. Assessments

- knockdown effect: during the 4 hours exposure time. A compulsory assessment is done after 4 hours.
- Lethal effect: after 24 hours of exposure. An insect unable to move (fly) properly is classified as dead.

3.3. Dates of assessments

3.3.1. Instant effect

Until 24 hours after exposure.

3.3.2. Residual activity

The residual actvity is assessed by exposing the insects on the plates treated 90 days before.

4. RESULTS

4.1. PRESENTATION

The synthesis of data is given in Table I.

The raw data by species/date/replicate/materials are given in APPENDIX.

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

TRIAL AT DAY0 (day of application):
KT100 = time of exposure to knockdown/kill 100% insects/mites

Target	*Material*	*KT100*	*Death rate*	*Mortality 24h*
German cockroach *Blattella germanica*	Ceramic	15 min	100%	100%
	Concrete	30 min	100%	100%
Black ant *Lasius niger*	Ceramic	5 min	100%	100%
	Concrete	10 min	100%	100%
Flea (adult) *Ctenocephalides felis*	Ceramic	10 min	100%	100%
	Concrete	15 min	100%	100%
Flea (larvae) *Ctenocephalides felis*	Ceramic	5 min	100%	100%
	Concrete	10 min	100%	100%
Fly *Musca domestica*	Ceramic	5 min	100%	100%
	Concrete	5 min	100%	100%
House dust mite *Dermatophagoides pteronyssinus*	Ceramic	10 min	100%	100%
	Concrete	20 min	100%	100%
Clothes moth (larvae) *Tineola bisselliella*	Ceramic	15 min	100%	100%
	Concrete	30 min	100%	100%
Termite (adult worker) *Reticulitermes santonensis*	Ceramic	15 min	100%	100%
	Concrete	30 min	100%	100%
Mosquito *Aedes aegypti*	Ceramic	5 min	100%	100%
	Concrete	5 min	100%	100%
Tiger mosquito *Aedes albopictus*	Ceramic	5 min	100%	100%
	Concrete	5 min	100%	100%
Bed bug *Cimex lectularius*	Ceramic	10 min	100%	100%
	Concrete	20 min	100%	100%

TRIAL AT DAY0 + 3 months:

KT100 = time of exposure to knockdown/kill 100% insects/mites

Target	*Material*	*KT100*	*Death rate*	*Mortality 24h*
German cockroach *Blattella germanica*	Ceramic	1 h	100%	100%
	Concrete	1 h 15 min	100%	100%
Black ant *Lasius niger*	Ceramic	20 min	100%	100%
	Concrete	30 min	100%	100%
Flea (adult) *Ctenocephalides felis*	Ceramic	30 min	100%	100%
	Concrete	45 min	100%	100%
Flea (larvae) *Ctenocephalides felis*	Ceramic	20 min	100%	100%
	Concrete	30 min	100%	100%
Fly *Musca domestica*	Ceramic	15 min	100%	100%
	Concrete	15 min	100%	100%
House dust mite *Dermatophagoides pteronyssinus*	Ceramic	15 min	100%	100%
	Concrete	30 min	100%	100%
Clothes moth (larvae) *Tineola bisselliella*	Ceramic	30 min	100%	100%
	Concrete	45 min	100%	100%
Termite (adult worker) *Reticulitermes santonensis*	Ceramic	30 min	100%	100%
	Concrete	45 min	100%	100%
Mosquito *Aedes aegypti*	Ceramic	10 min	100%	100%
	Concrete	10 min	100%	100%
Tiger mosquito *Aedes albopictus*	Ceramic	10 min	100%	100%
	Concrete	10 min	100%	100%
Bed bug *Cimex lectularius*	Ceramic	45 min	100%	100%
	Concrete	1 h	100%	100%

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 product gave a complete and definitive mortality (no recoveriies after 24 hours).

The efficacy remains effective until 90 days after application.

The product gave slower efficacy on the porous material.

5. CONCLUSION

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

The product **All insect Killer CHRYSAMED**, applied as a surface treatment at a rate of 14 ml/m², has proved:

- A fast and definitive insecticide efficacy against the following pests:
  - crawling insects: the German cockroach, the black ant and the flea
  - hidden insects: house dust mites, clothes moths, termites and bed bugs
  - flying insects: flies, mosquitos and Tiger mosquitoes.

- A residual activity of at least 91 days after treatment in laboratory conditions.

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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/

Blattella germanica

TRIAL AT DAY0 (after application and drying)

Treatment	Material	Mean of replicates in % of knockdown/mortality				Mortality
		1 h	2 h	3 h	4 h	after 24 hours
Experimental product	Ceramic tiles	100	100	100	100	100
	Concrete					
Untreated Control	Ceramic tiles	0	0	0	0	0
	Concrete	0	0	0	0	0

TRIAL AT DAY0 + 90 days

Treatment	Material	Mean of replicates in % of knockdown/mortality				Mortality
		1 h	2 h	3 h	4 h	after 24 hours
Experimental product	Ceramic tiles	100	100	100	100	100
	Concrete					
Untreated Control	Ceramic tiles	0	0	0	0	0
	Concrete	0	0	0	0	0

Lasius niger

TRIAL AT DAY0 (after application and drying)

Treatment	Material	Mean of replicates in % of knockdown/mortality				Mortality
		1 h	2 h	3 h	4 h	after 24 hours
Experimental product	Ceramic tiles	100	100	100	100	100
	Concrete					
Untreated Control	Ceramic tiles	0	0	0	0	0
	Concrete	0	0	0	0	0

TRIAL AT DAY0 + 90 days

Treatment	Material	Mean of replicates in % of knockdown/mortality				Mortality
		1 h	2 h	3 h	4 h	after 24 hours
Experimental product	Ceramic tiles	100	100	100	100	100
	Concrete					
Untreated Control	Ceramic tiles	0	0	0	0	0
	Concrete	0	0	0	0	0

Ctenocephalides felis

TRIAL AT DAY0 (after application and drying)

Treatment	Material	Mean of replicates in % of knockdown/mortality				Mortality
		1 h	2 h	3 h	4 h	after 24 hours
Experimental product	Ceramic tiles	100	100	100	100	100
	Concrete					
Untreated Control	Ceramic tiles	0	0	0	0	0
	Concrete	0	0	0	0	0

TRIAL AT DAY0 + 90 days

Treatment	Material	Mean of replicates in % of knockdown/mortality				Mortality
		1 h	2 h	3 h	4 h	after 24 hours
Experimental product	Ceramic tiles	100	100	100	100	100
	Concrete					
Untreated Control	Ceramic tiles	0	0	0	0	0
	Concrete	0	0	0	0	0

Musca domestica

TRIAL AT DAY0 (after application and drying)

Treatment	Material	Mean of replicates in % of knockdown/mortality				Mortality
		1 h	2 h	3 h	4 h	after 24 hours
Experimental product	Ceramic tiles	100	100	100	100	100
	Concrete					
Untreated Control	Ceramic tiles	0	0	0	0	0
	Concrete	0	0	0	0	0

TRIAL AT DAY0 + 90 days

Treatment	Material	Mean of replicates in % of knockdown/mortality				Mortality
		1 h	2 h	3 h	4 h	after 24 hours
Experimental product	Ceramic tiles	100	100	100	100	100
	Concrete					
Untreated Control	Ceramic tiles	0	0	0	0	0
	Concrete	0	0	0	0	0

Dermatophagoides pteronyssinus

TRIAL AT DAY0 (after application and drying)

Treatment	Material	Mean of replicates in % of knockdown/mortality				Mortality
		1 h	2 h	3 h	4 h	after 24 hours
Experimental product	Ceramic tiles	100	100	100	100	100
	Concrete					
Untreated Control	Ceramic tiles	0	0	0	0	0
	Concrete	0	0	0	0	0

TRIAL AT DAY0 + 90 days

Treatment	Material	Mean of replicates in % of knockdown/mortality				Mortality
		1 h	2 h	3 h	4 h	after 24 hours
Experimental product	Ceramic tiles	100	100	100	100	100
	Concrete					
Untreated Control	Ceramic tiles	0	0	0	0	0
	Concrete	0	0	0	0	0

Tineola bisselliella

TRIAL AT DAY0 (after application and drying)

Treatment	Material	Mean of replicates in % of knockdown/mortality				Mortality
		1 h	2 h	3 h	4 h	after 24 hours
Experimental product	Ceramic tiles	100	100	100	100	100
	Concrete					
Untreated Control	Ceramic tiles	0	0	0	0	0
	Concrete	0	0	0	0	0

TRIAL AT DAY0 + 90 days

Treatment	Material	Mean of replicates in % of knockdown/mortality				Mortality
		1 h	2 h	3 h	4 h	after 24 hours
Experimental product	Ceramic tiles	100	100	100	100	100
	Concrete					
Untreated Control	Ceramic tiles	0	0	0	0	0
	Concrete	0	0	0	0	0

Reticulitermes santonensis

TRIAL AT DAY0 (after application and drying)

Treatment	Material	Mean of replicates in % of knockdown/mortality				Mortality
		1 h	2 h	3 h	4 h	after 24 hours
Experimental product	Ceramic tiles	100	100	100	100	100
	Concrete					
Untreated Control	Ceramic tiles	0	0	0	0	0
	Concrete	0	0	0	0	0

TRIAL AT DAY0 + 90 days

Treatment	Material	Mean of replicates in % of knockdown/mortality				Mortality
		1 h	2 h	3 h	4 h	after 24 hours
Experimental product	Ceramic tiles	100	100	100	100	100
	Concrete					
Untreated Control	Ceramic tiles	0	0	0	0	0
	Concrete	0	0	0	0	0

Aedes aegypti

TRIAL AT DAY0 (after application and drying)

Treatment	Material	Mean of replicates in % of knockdown/mortality				Mortality
		1 h	2 h	3 h	4 h	after 24 hours
Experimental product	Ceramic tiles	100	100	100	100	100
	Concrete					
Untreated Control	Ceramic tiles	0	0	0	0	0
	Concrete	0	0	0	0	0

TRIAL AT DAY0 + 90 days

Treatment	Material	Mean of replicates in % of knockdown/mortality				Mortality
		1 h	2 h	3 h	4 h	after 24 hours
Experimental product	Ceramic tiles	100	100	100	100	100
	Concrete					
Untreated Control	Ceramic tiles	0	0	0	0	0
	Concrete	0	0	0	0	0

Aedes albopictus

TRIAL AT DAY0 (after application and drying)

Treatment	Material	Mean of replicates in % of knockdown/mortality				Mortality
		1 h	2 h	3 h	4 h	after 24 hours
Experimental product	Ceramic tiles	100	100	100	100	100
	Concrete					
Untreated Control	Ceramic tiles	0	0	0	0	0
	Concrete	0	0	0	0	0

TRIAL AT DAY0 + 90 days

Treatment	Material	Mean of replicates in % of knockdown/mortality				Mortality
		1 h	2 h	3 h	4 h	after 24 hours
Experimental product	Ceramic tiles	100	100	100	100	100
	Concrete					
Untreated Control	Ceramic tiles	0	0	0	0	0
	Concrete	0	0	0	0	0

Cimex lectularius

TRIAL AT DAY0 (after application and drying)

Treatment	Material	Mean of replicates in % of knockdown/mortality				Mortality
		1 h	2 h	3 h	4 h	after 24 hours
Experimental product	Ceramic tiles	100	100	100	100	100
	Concrete					
Untreated Control	Ceramic tiles	0	0	0	0	0
	Concrete	0	0	0	0	0

TRIAL AT DAY0 + 90 days

Treatment	Material	Mean of replicates in % of knockdown/mortality				Mortality
		1 h	2 h	3 h	4 h	after 24 hours
Experimental product	Ceramic tiles	100	100	100	100	100
	Concrete					
Untreated Control	Ceramic tiles	0	0	0	0	0
	Concrete	0	0	0	0	0