CONFIDENTIAL

LABORATORY ASSESSMENT OF THE ABILITY OF AN INSULATING MATERIAL TO ALLOW BY ITSELF THE DEVELOPMENT OF SEVERAL INSECTS AND MITES

TERMOKOMFORT BioFoamPearls
Sampling number: IG.MN.10 – 11/09/2015

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Warning

The results described in this report are produced by a laboratory test on the samples provided which have not suffered any damage related to the reality of use or of storage (humidity for example). TEC provides test results only on samples received and may in no event be liable regarding finished products in production or sale.

We have to keep in mind that it is possible that the material can be a natural harborage for the pests as a physical shelter or because it protects them from light and cold. The trial was only intended to check if the sample could be enough by itself to allow long-term installation and food survival of the pests, and at least if it does not encourage their development.

The trial has been conducted on laboratory strains of “model” insects and the susceptibility of the local insect’s strains can be different in other labs or in the real conditions of use. As such the results should be taken only as an indication of the potential for activity of the formulations or products under test. 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. Test variability on bioassays implies that the results of test given by TEC shall only be taken as one of the elements that contribute to the development of a product, but cannot be the sole support of product knowledge leading to its production and marketing/sale, and TEC strongly encourages the client to carry out further studies to consolidate the knowledge of the product's properties.

LABORATORY ASSESSMENT OF THE ABILITY OF AN INSULATING MATERIAL TO ALLOW BY ITSELF THE DEVELOPMENT OF SEVERAL INSECTS AND MITES

1. PURPOSE

Due to their components which can be a source of food, the insulating materials can be infested with numerous pests.

The purpose fo this trial was to assess the ability of several species or notorious pests to proliferate in the material alone without any other food source.

2. MATERIALS AND METHOD

The methodology was adapted from the requirements of the CUAP, Appendix D (cf. appendix at the end of this issue).

The principle was to infest the materials with a strong artificial infestation and to let in incubation in controlled climatic conditions, without any other food source, then to check if the insects survive, reproduct, lay eggs, if there are some larvae or new adults alive and if they damaged the materials.

2.1. Pests

In relation with the destination of the products (household use), the pest species choosen are:

- crawling insects: ants (*Lasius niger*), cockroaches (*Blattella germanica*), dark beetle (*Alphitobius diaperinus*)
- flying insects: food moths (*Plodia interpunctella* and *Ephestia kuehniella*), flies *(Musca domestica*)
- house dust mites (*Dermatophagoides pteronyssinus*)
- keratophagous insects: clothe moths (*Tineola bisselliella*) and carpet *beetles (Anthrenus flavipes*)
- termites (*Reticulitermes santonensis*)

Instar, age, strains and number of pests used in the trial (numbers are per replicate):

Ants: 50+/-2 adult workers of the black garden ant *Lasius niger*, retrieved from a wild nest (Louhossoa - France).

German Cockroaches: 20 from1 to 2 weeks old adult males of *Blattella germanica*, from a laboratory colony breeding (TEC – french official strain INA).

Darkling beetle: 20 from 1 to 2 weeks old adult males of *Alphitobius diaperinus*, from a laboratory colony breeding (french official strain INRA)

Food moths: 15 from 2 to 4 days old adult males of both *Plodia interpunctella* and *Ephestia kuehniella*, from a laboratory colony breeding (french official strains INRA)

Houseflies: 100+/5 mixed sex adults 4 to 6 days old + 50+/-2 last instar larvae of *Musca domestica*, from a laboratory colony breeding (TEC – strain Wellcome).

House dust mites: 300+/-30 mixed instar/sex of *Dermatophagoides pteronyssinus* from a laboratory colony breeding (french official strain INRA)

Clothe moths: 20 from 2 to 6 days old adults + 20 third instar nymphs + 30 eggs *of Tineola bisselliella* from a colony breeding (TEC – strain University of Rennes-France)

Carpet beetles: 20 from 2 to 6 days old adults + 20 third instar nymphs of *Anthrenus flavipes* from a colony breeding (french official strain INRA)

Termites: 20 adult workers of *Reticulitermes santonensis*, from a TEC laboratory colony breeding (strain Chiberta – 64 France).

All insects/mites were acclimatized for 48 hours before the trial..

2.2. Procedure

The principle was to infest the materials and to let it in optimal development conditions during 6 weeks.
This was done in comparison with standard breeding foods to compare the dynamic of populations, hatching, reproduction, this was the "Untreated Control".

A percentage of reduction of development was calculated by the formula below:

$$\frac{\text{Population found on the breeding food} - \text{Population found on the material}}{\text{Population found on the breeding food}} \times 100$$

The damage to the materials was also recorded.

4 replicates were conducted per material and per species of pest.

The climatic conditions were 27±1°C, 70±5% RH, photoperiod 8h light 700 lux/ 16h dark.

3. EXPERIMENTAL SAMPLE

The tested sample was an insulation material made of beads aggregated together by a glue and provided by SECO cvba:

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The samples left are kept available for 3 motnhs for any further analysis.

4. RESULTS

4.1. Presentation

The data are given in appendix.

4.2. Reliability

The viability and development in the Untreated Control series were as expected (mortality < 5% and usual nymph development ratios), wd deduce that:

- the living material was viable in the test conditions
- the larvae developed normally in the test conditions

The test was validated and the results obtained on the materials were usable as they are.

4.3. Comments

MITES: no living mites were observed during the inspection conducted after three weeks that corresponds to one development cycle, which means that the mites have not found enough food source to survive and reproduce.

INSECT ADULTS:
Ants, termites, cockroaches, darkling beetles, flies, carpet beetles, food moths and clothes moths were recorded as dead from the observation carried out after three weeks. This result was confirmed during the +6 weeks observation.
No nesting was observed, insects were obviously starved to death without having had time to reproduce.
No degradation was observed visually.

INSECT LARVAE:
The larvae of flies, darkling beetles, moths and carpet beetles all died during the observation carried out after three weeks.
No moulting was observed, their development has therefore interrupted.
No degradation was observed

5. CONCLUSION

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

The insulating material **" TERMOKOMFORT BioFoamPearls Sampling number: IG.MN.10 – 11/09/2015"** did not allow, by itself, the settling and the development of the insects and mite in testing.

This means that the components were not favorable to the consumption by the pests and then, they died by starving without continuing their developement/reproduction, so there was no further insects' generation.

APPENDIX

- CUAP methodology
- climatic conditions and food sources of the Control series
- results

ANNEX D
Determination of resistance to attack by vermin's

D.1 Principle
Conditioned test specimens and test control specimens are placed in contact with eggs and larvae of selected vermin's for 6 months (maximum).
It is verified whether the protective effect of chemical additives on the wool will kill all vermin's during development of the first generation or prohibit the development of a second generation. Eggs and larvae of clothes moth (*Tineola biselliella*) as well as carpet beetle (Anthrenus flavipes) are used as test vermin's.

D.2 Specimens
Test specimens: 6000 ± 500 mg or at least 200 cm^3 of insulation material
Test control specimens: 6000 ± 500 mg or at least 200 cm^3 of woollen material without chemical additives for improvement of resistance to attack by vermin's

Note*: A test control specimen is used to check that the test has been done correctly and that the test vermin's are viable.*

D.3 Conditioning
Conditioning of test specimens and of test control specimens is achieved by storage at a temperature of (27±1)°C and (70±10)% air humidity for 3 days.

D.4 Procedure
Four tests with test specimens and four tests with test control specimens are performed for each test insect at a temperature of (27±1)°C and (70±10)% air humidity.
Each of the specimens shall be placed in a separate glass container (r=5cm, h=8cm) with a metal screw top. The screw top includes a ventilating opening (r=0,5 cm, covered with gauze).
On each of the specimens the following number of insects shall be placed:
larvae of clothes moth (Tineola biselliella) 20
larvae of carpet beetle (Anthrenus flavipes) 20
eggs of clothes moth (Tineola biselliella) 30
carpet beetle (Anthrenus flavipes) 20 adult beetles for laying eggs, after 14 days the beetles are removed
Each test is performed for 6 months (maximum) or until death of the larvae.
If more than 20% of the insects at the test control specimens die, the test is invalid.

D.5 Expression of the results
The test is passed if no adult vermins (beetles or moths) develop from the eggs.
The damage caused by the vermins is to be stated.

BREEDING FOOD SOURCES FOR THE UNTREATED CONTROLS

Species	**Time to obtain:** eggs	other instar	**Temperature**	**R.H.**	**Food source**	**Cycle duration**
Blattella germanica	-	-	25°C (±1°C)	70 %	Dry bread + water source	-
Lasius niger	5 j	7 j	30°C (±2°C)	75 %	Water + 5% saccharose	35 d
Alphitobius diaperinus	10 j	15 j	30°C (±2°C)	75 %	20 g wheat powder + polystyren	84 d
Plodia interpunctella Ephestia kuehniella	3 j	7 j	25°C (±1°C)	70 %	50 g brushed corn + 5% glycerol	35 d
Musca domestica	3 j	7 j	25°C (±1°C)	70 %	Fermented milk + bran	10 - 20 d
Dermatophagoides pteronyssinus	-	-	25°C (±1°C)	75% min	50% wheat germen + 50% brewer's yeast	3 weeks

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Factor	Pest	Replicate	+3 weeks observations	+6 weeks observations
Test product	Ants	1	100% mortality	100% mortality
		2	100% mortality	100% mortality
		3	100% mortality	100% mortality
		4	100% mortality	100% mortality
		mean	**100% mortality**	**100% mortality**
	Cockroaches	1	100% mortality	100% mortality
		2	100% mortality	100% mortality
		3	100% mortality	100% mortality
		4	100% mortality	100% mortality
		mean	**100% mortality**	**100% mortality**
	Dark beetle	1	100% mortality	100% mortality
		2	100% mortality	100% mortality
		3	100% mortality	100% mortality
		4	100% mortality	100% mortality
		mean	**100% mortality**	**100% mortality**
	Food moth 1 *(P.interpunctella)*	1	100% mortality	100% mortality
		2	100% mortality	100% mortality
		3	100% mortality	100% mortality
		4	100% mortality	100% mortality
		mean	**100% mortality**	**100% mortality**
	Food moth 2 *(E.kuehniella)*	1	100% mortality	100% mortality
		2	100% mortality	100% mortality
		3	100% mortality	100% mortality
		4	100% mortality	100% mortality
		mean	**100% mortality**	**100% mortality**
	Flies Adults	1	100% mortality	100% mortality
		2	100% mortality	100% mortality
		3	100% mortality	100% mortality
		4	100% mortality	100% mortality
		mean	**100% mortality**	**100% mortality**
	Flies Larvae	1	100% mortality	100% mortality
		2	100% mortality	100% mortality
		3	100% mortality	100% mortality
		4	100% mortality	100% mortality
		mean	**100% mortality**	**100% mortality**
	House dust mites	1	100% mortality	100% mortality
		2	100% mortality	100% mortality
		3	100% mortality	100% mortality
		4	100% mortality	100% mortality
		mean	**100% mortality**	**100% mortality**
	Termites	1	100% mortality	100% mortality
		2	100% mortality	100% mortality
		3	100% mortality	100% mortality
		4	100% mortality	100% mortality
		mean	**100% mortality**	**100% mortality**

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Factor	Pest	Replicate	+3 weeks observations	+6 weeks observations
Test product	Clothe moths adults	1	100% mortality	100% mortality
		2	100% mortality	100% mortality
		3	100% mortality	100% mortality
		4	100% mortality	100% mortality
		mean	**100% mortality**	**100% mortality**
	Clothe moths larvae	1	100% mortality	100% mortality
		2	100% mortality	100% mortality
		3	100% mortality	100% mortality
		4	100% mortality	100% mortality
		mean	**100% mortality**	**100% mortality**
	Clothe moths eggs	1	100% mortality	100% mortality
		2	100% mortality	100% mortality
		3	100% mortality	100% mortality
		4	100% mortality	100% mortality
		mean	**100% mortality**	**100% mortality**
	Carpet beetles adults	1	100% mortality	100% mortality
		2	100% mortality	100% mortality
		3	100% mortality	100% mortality
		4	100% mortality	100% mortality
		mean	**100% mortality**	**100% mortality**
	Carpet beetles larvae	1	100% mortality	100% mortality
		2	100% mortality	100% mortality
		3	100% mortality	100% mortality
		4	100% mortality	100% mortality
		mean	**100% mortality**	**100% mortality**

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Factor	Pest	Replicate	+3 weeks observations	+6 weeks observations
untreated control	Ants	1	2% mortality	3% mortality
		2	2% mortality	5% mortality
		3	1% mortality	2% mortality
		4	3% mortality	4% mortality
		mean	**2% mortality**	**3.5% mortality**
	Cockroaches	1	0% mortality	x 3 population
		2	0% mortality	x 2 population
		3	0% mortality	x 3 population
		4	0% mortality	x 3 population
		mean	**0,0% mortality**	**x 2.8 population**
	Dark beetle	1	0% mortality	x 4 population
		2	1% mortality	x 4 population
		3	1% mortality	x 4 population
		4	0% mortality	x 5 population
		mean	**1% mortality**	**x 4,3 population**
	Food moth 1 *P.interpunctella*	1	92% egg-laying	x 4 population
		2	91% egg-laying	x 4 population
		3	90% egg-laying	x 3 population
		4	94% egg-laying	x 4 population
		mean	**91,8% egg-laying**	**x 3,8 population**
	Food moth 2 *E.kuehniella*	1	93% egg-laying	x 3 population
		2	88% egg-laying	x 4 population
		3	95% egg-laying	x 4 population
		4	94% egg-laying	x 4 population
		mean	**92,5% egg-laying**	**x 3,8 population**
	Flies Adults	1	99% egg-laying	N/A
		2	92% egg-laying	N/A
		3	93% egg-laying	N/A
		4	92% egg-laying	N/A
		mean	**94.0% egg-laying**	**N/A**
	Flies Larvae	1	87% giving adults	N/A
		2	91% giving adults	N/A
		3	86% giving adults	N/A
		4	93% giving adults	N/A
		mean	**89,3% giving adults**	**N/A**
	House dust mites	1	5% mortality	x 17 population
		2	1% mortality	x 20 population
		3	5% mortality	x 18 population
		4	4% mortality	x 16 population
		mean	**3,8% mortality**	**x 17,8 population**
	Termites	1	5% mortality	15% mortality
		2	5% mortality	5% mortality
		3	5% mortality	5% mortality
		4	5% mortality	10% mortality
		mean	**5.0% mortality**	**8.8% mortality**

Note: ants and termites were not allowed to give a next generation (social insects), so only the mortality was recorded

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Factor	Pest	Replicate	+3 weeks observations	+6 weeks observations
untreated control	Clothe moths adults	1	92% egg-laying	91% larvae alive
		2	90% egg-laying	93% larvae alive
		3	85% egg-laying	82% larvae alive
		4	87% egg-laying	86% larvae alive
		mean	**88,5 egg-laying**	**88.0% larvae alive**
	Clothe moths larvae	1	0% mortality	81% giving adults
		2	2% mortality	83% giving adults
		3	0% mortality	92% giving adults
		4	0% mortality	81% giving adults
		mean	**1% mortality**	**84.3% giving adults**
	Clothe moths eggs	1	79% giving larvae	91% larvae alive
		2	82% giving larvae	90% larvae alive
		3	84% giving larvae	95% larvae alive
		4	88% giving larvae	98% larvae alive
		mean	**83.3% giving larvae**	**93.5% larvae alive**
	Carpet beetles adults	1	0% mortality	x 3 population
		2	0% mortality	x 4 population
		3	0% mortality	x 3 population
		4	0% mortality	x 4 population
		mean	**1% mortality**	**x 3,5 population**
	Carpet beetles larvae	1	2% mortality	85% giving adults
		2	1% mortality	89% giving adults
		3	3% mortality	95% giving adults
		4	2% mortality	91% giving adults
		mean	**2% mortality**	**90.0% giving adults**