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Testreport

Screening of an insecticide-impregnated fabric
for its toxicity on the yellow fever mosquito *Aedes aegypti*
using WHO-Susceptibility-Tubes

- CONFIDENTIAL -

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1. Executive Summary

The toxicity of a permethrin impregnated textile sample provided by Cognis GmbH was evaluated within WHO-Tubes. An untreated fabric sample served as a control, in order to determine the natural mortality rate of the test mosquitoes.

Test mosquitoes were exposed to the treated surface for 5 minutes, afterwards they were removed from the WHO-Tubes by an aspirator and kept in small cages in insecticide-free air for further observation. The number of knocked-down mosquitoes was documented after 60 minutes and the mortality rate was calculated after 24 hours. Each sample was tested in ten repetitions on 50 mosquitoes.

The natural mortality rate was 4% in 24 hours, therefore the test mortality rate did not need to be corrected using Abbott's formula (see page 6).

The treated PGI blanket caused a 100% knock-down one hour after the exposition. The 24 hours mortality rate was also 100%.

2. General Information

Sponsor

Cognis GmbH
Henkelstraße 67
40589 Düsseldorf

Testing Facility

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Principal Investigator

signed
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date: 1.4.2011

Time Frame

Receipt of the testsubstances:	30.3.2011
Experimental start date:	31.3.2011
Experimental termination date:	1.4.2011
Data analysis and Testreport.	1.4.2011



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3. Material and Methods

3.1. Test Samples

The test samples, a treated and an untreated PGI blanket, were provided by Cognis GmbH. Both textile samples remained in their original plastic packing and were kept at room temperature until the start of the tests.

3.2. Test Insects *Aedes aegypti*

Female yellow-fever mosquitoes of the species *Aedes aegypti* were reared according to the standard protocol at a temperature of 27° C, a relative humidity of 60 – 80 % and a 12:12 hour photoperiod. The light period (150 Lux) was set from 8:00 to 20:00. After hatching of the eggs, larvae were kept in a water basin (30 x 30 x 10 cm) filled with a 1:1 mixture of tap- and deionised water and fed with fishfood flakes (Tetra Min[®]). Before hatching the pupae were transferred to a cage (40 x 30 x 20 cm) and provided with sugar solution (10% dextrose). Mosquitoes at an age of 8 days after emergence from the pupae were used for the toxicity tests.

3.3. Test Room

The WHO exposition tests were performed in a test room (4,60 x 3,40 x 2,60 m) without windows under standard conditions at 27 ±1 °C and a relative humidity of 60 ±5 %.

3.4. Procedure

The exposition tests were performed on the basis of a guideline published by the World Health Organization (WHO, 1998):

A test sample is inserted into a standard WHO plastic tube and fastened in position with two spring-wire clips, in a way that it lines with the inner wall of the tube (see fig. 1). Ten mosquitoes are collected into a transfer tube with an aspirator, the transfer tube is then closed by a plastic slide and connected to the exposure tube, containing the textile sample. The slide is opened and test mosquitoes are gently blown into the exposure tube and the slide is closed again (see the Appendix section for a detailed illustration). After an exposition time of 5 minutes, mosquitoes are blown back into the transfer tube, removed with an aspirator and placed in a 500 mL plastic cup for further observation (see fig. 2). The plastic cup is covered by a nylon net fastened with rubber band. As a nutrition source sugar soaked cotton wool is placed on top of the nylon net. Mosquitoes are now kept in insecticide free air at a temperature of 28 ±1 °C and a relative humidity of 80 ± 5%. Each fabric is tested in 5 repetitions on 50 mosquitoes. In addition an untreated control cloth is also tested on 50 mosquitoes to calculate the natural mortality rate.



Fig. 1: *Standard-WHO-Tubes*. The textile sample is inserted into the plastic tube. The transfer tube is not shown (see the Appendix section for further information).



Fig. 2: *Plastic-Cup*. Through the whole at the bottom mosquitoes are transferred into the cup.

3.5. Data Analysis

One hour after the exposition the number of knocked down mosquitoes is counted. The percent mortality (rate of dead test mosquitoes) is determined after 24 hours:

$$\% \text{ Mortality} = 100 \times \frac{\text{Number of dead or knocked-down test mosquitoes}}{\text{Total number of test mosquitoes}}$$

In case of a control mortality between 5 and 20%, Abbott's formula is used to correct the observed average mortality:

$$\text{Corrected \% Mortality} = 100 \times \frac{\% \text{ Mortality in test-replicates} - \% \text{ Mortality in Controls}}{100 - \% \text{ Control Mortality}}$$

4. Results

4.1. Knock-down and mortality rates

Table 1 summarizes the *knock-down*- and mortality-rates from all tests. The control mortality was 4% in 24 hours, therefore test mortality rates did not need to be corrected using Abbott's formula.

Table 1: Exposition Tests in WHO-Tubes. The number of *knocked-down* and *-dead* mosquitoes is given for each tested fabric as well as the mortality rate after 24 hours.

Sample	Number exposed	Number <i>knocked-down</i> after 1 hour	Number <i>dead</i> after 24 hours	Mortality after 24 hours
PGI blanket untreated	50	0	2	4%
PGI blanket treated	50	50	50	100%

The treated PGI blanket caused total knock-down within one hour after the exposition, the 24 hours mortality rate was also 100%.

Diagrams 1 displays the results.

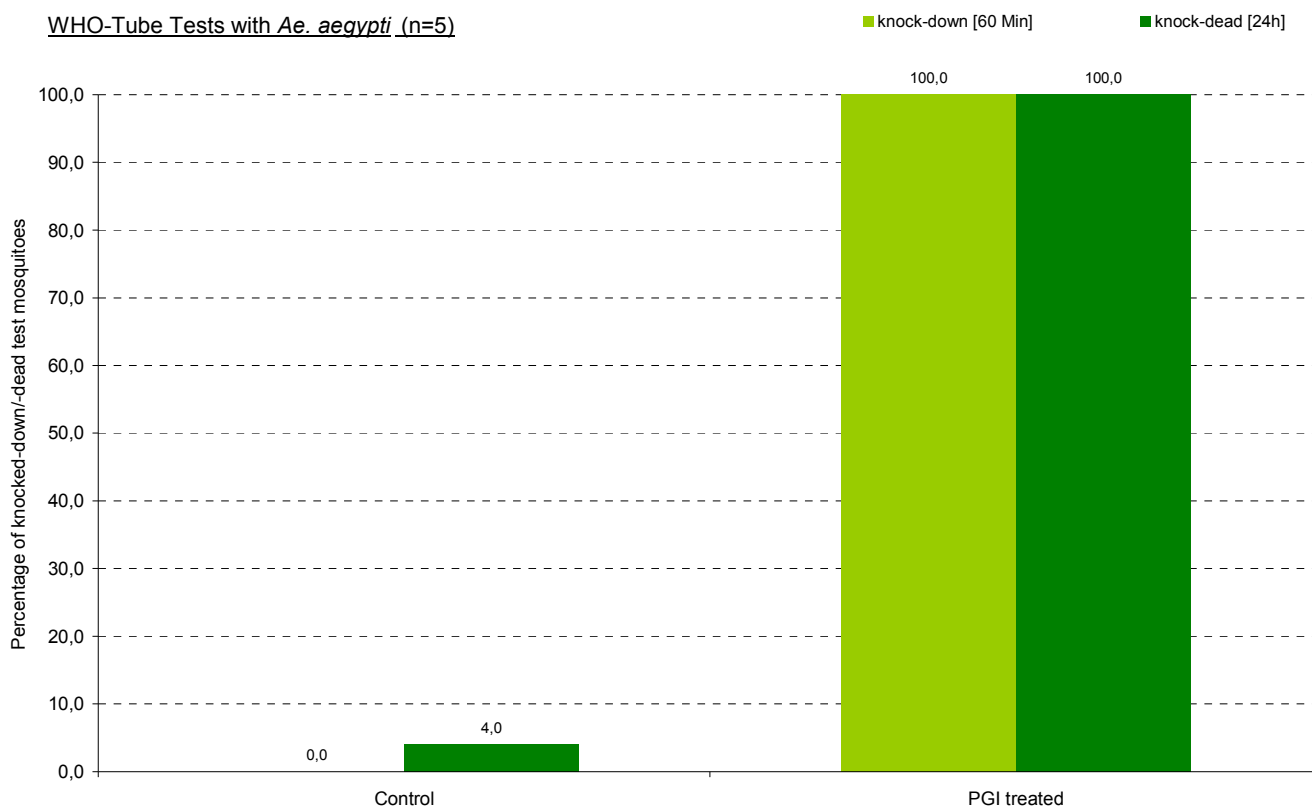


Diagram 1: *Knock-down and Mortality-Rates during WHO-Tube-Tests with Ae.aegypti.* The x-axis shows the test samples, the y-axis gives the mean number of knocked-down or dead test mosquitoes (in %), this number is also shown above each column. **Light green columns:** *knock-down* after 60 minutes, **green columns:** mortality rate after 24 hour. Number of repetitions: n=5.

5. Discussion

The original purpose of the WHO Susceptibility Bioassay is to detect the presence of resistant individuals in an insect population to certain insecticides. Wild mosquitoes are collected in the field and exposed to standardized paper sheets treated with toxic substances (e.g. DDT, Dieldrin, Malathion, Permethrin, etc.) inside the standard WHO plastic tubes. The WHO also suggests the use of standard tubes as an alternative to WHO standard plastic cones.

One disadvantage of the standard cones is, that in case of evaluating an insecticide with repellent properties (like permethrin), test mosquitoes tend to fly-off the treated test surface, a behaviour that leads to a reduced exposition. Inside the WHO plastic tubes mosquitoes are forced to contact the treated surface, as the inner wall is completely covered with the sample, just the ends of the tube are closed by a plastic slide on one side and with a gauze cover for air ventilation on the other side. Nevertheless some test mosquitoes were also found to fly inside the tubes during permethrin testing.

The test procedure followed the guideline published by the World Health Organization (WHO, 1998): 10 test mosquitoes were transferred into a tube containing the test sample and exposed to the treated surface for 5 minutes, following the sponsor's request. The original protocol suggests an exposition time of 3 minutes (for the evaluation of treated bednets) but gives no recommendations for the tests of treated apparel. Afterwards test mosquitoes were removed and kept in insecticide free air for further observation. The number of *knocked-down* mosquitoes was counted after 60 minutes, the mortality rate was determined after 24 hours. The natural mortality rate was determined with an untreated fabric.

The WHO allows control mortality rates up to 20%, but in case of a control mortality rate greater than 5%, the test mortality rates need to be corrected by Abbott's formula. In the study presented, the natural mortality rate was 4%, the test mortality therefore needed to be corrected.

The treated PGI blanket had a strong toxic effect on the test mosquitoes, all were knocked-down within one hour after the exposition and could not recover within the consecutive 23 hours, leading to a mortality rate of 100%.

References

WHO (1998). Report of the WHO Informal Consultation. Test Procedures for Insecticide Resistance in Malaria Vectors, Bio Efficacy and Persistence of Insecticides on Treated Surfaces. WHO/CDS/CPC/MAL/98.12.

WHO (2005). Guidelines for Laboratory and Field Testing of Long-Lasting Insecticidal Mosquito Nets. WHO/CDS/WHOPES/GCDPP/2005.11.

Appendix

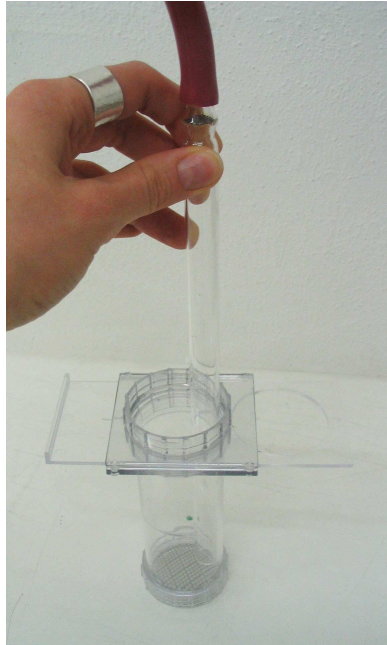


Fig. 3: 10 Test mosquitoes are transferred into a clear transfer tube by an aspirator

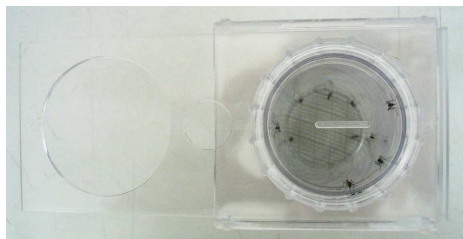


Fig. 4: Plastic slide is closed after the transfer, now the tube can be connected to the exposition tube.

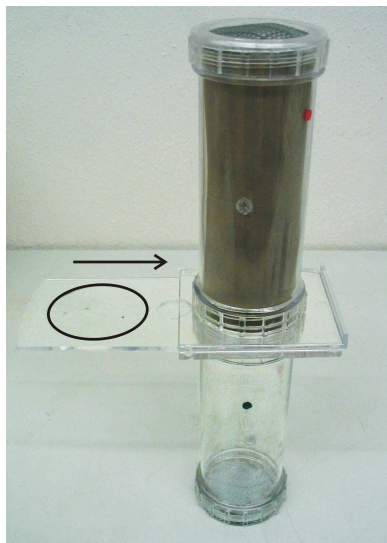


Fig. 5: Exposition and transfer tube are connected, plastic slide can be pulled into position (→) and mosquitoes are blown into the exposition tube through the opening.