

Testreport

Laboratory study on the bite-reducing & repellent efficacy of
five treated textile samples
by Feelway Corporation

in comparison to five untreated samples
in cage tests with the yellow-fever mosquito
Aedes aegypti

- CONFIDENTIAL -

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1. Executive Summary

The bite-preventing effect of five treated textile samples from Feelway Corporation was compared to five untreated sample of the same fabrics in a cage test study with yellow-fever mosquitoes (*Aedes aegypti*) and two volunteers.

The textile samples were attached to the forearms of the volunteers and exposed to a blood-hungry cage population of yellow-fever mosquitoes. During a test period of 2 minutes, the number of probing or biting mosquitoes was documented. In addition, five untreated control samples were evaluated, in order to determine the biting permeability of the used fabrics.

The untreated 100% Cotton and 100% Poly samples (#3 and #5) could easily be pierced by the test mosquitoes, both volunteers received between 5 and 8 control bites within a maximum of 60 seconds. The untreated 100% Poly sample #2 received fewer bites and could not easily be pierced by the test mosquitoes, now both volunteers counted 2 bites in 2 minutes. During tests of the untreated 70% Cotton 30% Nylon sample, volunteer A received 4 bites in 2 minutes while volunteer B counted 2 bites in 2 minutes.

Compared to the control tests, the treated samples 100% Poly #2, 100% Poly #3, 100% Poly #5 and 70% Cotton 30% Nylon gave a 100% bite protection during tests of both volunteers. When volunteer A tested the treated 100% Cotton sample 2 bites occurred in 2 minutes while volunteer B received 4 bites in 2 minutes, which corresponds to a 85,7% and 76,7% bite protection, respectively.

2. General Information

Sponsor

FEELWAY CORPORATION
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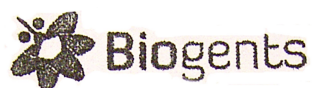
**Principal Investigator**

signed
Ulla Obermayr, Dipl. Biol.

date: 12.4.2012

Time Frame

Receipt of the testsubstances:	5.4.2012
Experimental start date:	11.4.2012
Experimental termination date:	12.4.2012
Data analysis and Testreport.	12.4.2012



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3. Material and Methods

3.1. Test Samples

The test samples were provided by Feelway Corporation, table 1 lists the different fabrics and provides further information.

Tab.1: Test Samples

N°	Sample Description
1	100% Cotton, untreated
2	100% Cotton, treated
3	100% Poly #2, untreated
4	100% Poly #2, treated
5	100% Poly #3, untreated
6	100% Poly #3, treated
7	100% Poly #5, untreated
8	100% Poly #5, treated
9	70% Cotton 30% Nylon, untreated
10	70% Cotton 30% Nylon, treated

All samples were kept in their original plastic packings at 20 – 22°C until the start of the tests.

3.2. Test Insects *Aedes aegypti*

Female mosquitoes of the genera *Aedes* 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 7-8 days after hatching from the pupae were used for the rapid cage tests.

3.3. Test Room

The tests were performed in an air-conditioned room (4,6 x 3,4 x 2,6 m) without windows. The temperature and relative humidity of the room air were 26 ±1°C and 60 ± 5% r.H. The light intensity was 350 Lux.

3.4. Test Cages

The test cages have a volume of 27.000 cm³ (41 x 41 x 16 cm). Four sides of the cage are made of acrylic glass, the floor of the cage is made of metal sheet and the rear side is

covered by a gauze sleeve. The floor sheet is equipped with a test window (size: 56 cm²; 14,8 x 3,8 cm).

Between tests, the cages are connected to a ventilation system that supplies the cages with warm and humid air ($26 \pm 1^\circ\text{C}$, $70 \pm 10\%$ r.H.) to remove remaining host odours and test substances from the air in the test cage (see fig. 1 for details). Test cages were filled with populations of 30 mosquitoes, those were lured out of their cages by a natural stimulus (humand hand) to ensure that only blood-hungry females were used for the test.

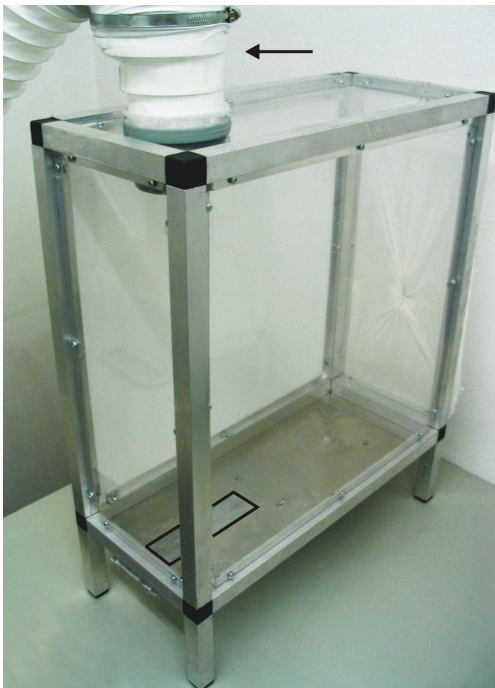


Fig. 1: BG-Test-Cage. The air ventilation system is connected to the cage (black arrow). The arm is exposed at the test window in the metal floor sheet (rectangle). The rear side is covered by gauze.

3.5. Test Procedure

3.5.1. Zero Control

Prior to the efficacy test, the biting activity of the test mosquitoes is verified with the untreated forearm of the test person. A minimum of 10 probings or bites have to be observed in 30 seconds, otherwise 30 new mosquitoes are used.

3.5.2. Test Proper

The test samples are attached to the forearms of two test person (females, 33 and 35 years) by metal clips in a way that it is not stretched tight to the skin. Short after attaching the sample the treated surface is exposed to the test mosquitoes at the test window in the floor of the test cage. During a testing time of 2 minutes, the number of mosquitoes landing and biting is documented and compared to the test of the untreated control samples. At the end of each test, the test situation is digitally recorded.

The bite-protection/repellency is calculated according to the following formula:

$$\text{Protection \%} = 100 - \frac{\Sigma \text{ of bites on treated fabric per test time}}{\Sigma \text{ of bites on control fabric per test time}}$$

4. Results & Discussion

4.1. Efficacy of the treated fabrics

Table 2 summarizes the results from all single tests of the untreated and treated textile samples.

Tab. 2: Efficacy test of the fabrics. The numbers of landings and bites is given for each tested fabric. The tests were performed with *Ae.aegypti* and two test persons (n=2).

Sample	Volunteer	Number of Landings	Number of Bites / Time	Protection
1 100% Cotton, untreated	A	12	7 / 60 Sec	0%
3 100% Poly #2, untreated		35	2 / 120 Sec	0%
5 100% Poly #3, untreated		13	5 / 55 Sec	0%
7 100% Poly #5, untreated		10	7 / 25 Sec	0%
9 70% Cotton 30% Nylon, untreated		42	4 / 120 Sec	0%
2 100% Cotton, treated		5	2 / 120 Sec	85,7%
4 100% Poly #2, treated		37	0 / 120 Sec	100%
6 100% Poly #3, treated		24	0 / 120 Sec	100%
8 100% Poly #5, treated		16	0 / 120 Sec	100%
10 70% Cotton 30% Nylon, treated		17	0 / 120 Sec	100%
1 100% Cotton, untreated	B	15	5 / 35 Sec	0%
3 100% Poly #2, untreated		40	2 / 120 Sec	0%
5 100% Poly #3, untreated		14	8 / 60 Sec	0%
7 100% Poly #5, untreated		8	5 / 35 Sec	0%
9 70% Cotton 30% Nylon, untreated		20	2 / 120 Sec	0%
2 100% Cotton, treated		10	4 / 120 Sec	76,7%
4 100% Poly #2, treated		2	0 / 120 Sec	100%
6 100% Poly #3, treated		12	0 / 120 Sec	100%
8 100% Poly #5, treated		3	0 / 120 Sec	100%
10 70% Cotton 30% Nylon, treated		8	0 / 120 Sec	100%

The presented cage tests were performed in order to gather information on the repellent- or bite-preventing potential of five treated fabrics within a first screening process.

The diurnal yellow-fever mosquito *Ae. aegypti* was used as the standard test mosquito for the repellent efficacy tests, as it is reliably aggressive and easy to breed under laboratory conditions. The mosquito-density in the test cage and the short distance to the test area lead to a high biting pressure, compared to natural conditions in the field, but a qualitative evaluation of the treated article in comparison to an untreated control can easily be drawn.

The untreated 100% Cotton and 100% Poly samples (#3 and #5) could easily be pierced by the test mosquitoes, both volunteers received between 5 and 8 control bites within a maximum of 60 seconds. The untreated 100% Poly sample #2 received fewer bites and could not easily be pierced by the test mosquitoes, now both volunteers counted 2 bites in 2 minutes. During tests of the untreated 70% Cotton 30% Nylon sample, volunteer A received 4 bites in 2 minutes while volunteer B counted 2 bites in 2 minutes.

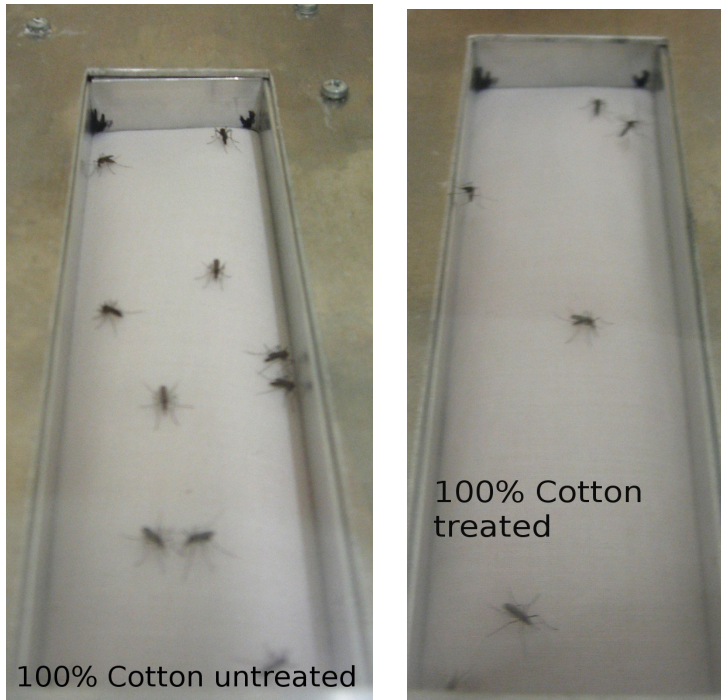
Compared to the control tests, the treated samples 100% Poly #2, 100% Poly #3, 100% Poly #5 and 70% Cotton 30% Nylon gave a 100% bite protection during tests of both volunteers. When volunteer A tested the treated 100% Cotton sample 2 bites occurred in 2 minutes while volunteer B received 4 bites in 2 minutes, which corresponds to a 85,7% and 76,7% bite protection, respectively.

The presented cage tests give a good indication on the bite protection potential of the treated samples, however samples were tested only once against one mosquito species, following the sponsor's request.

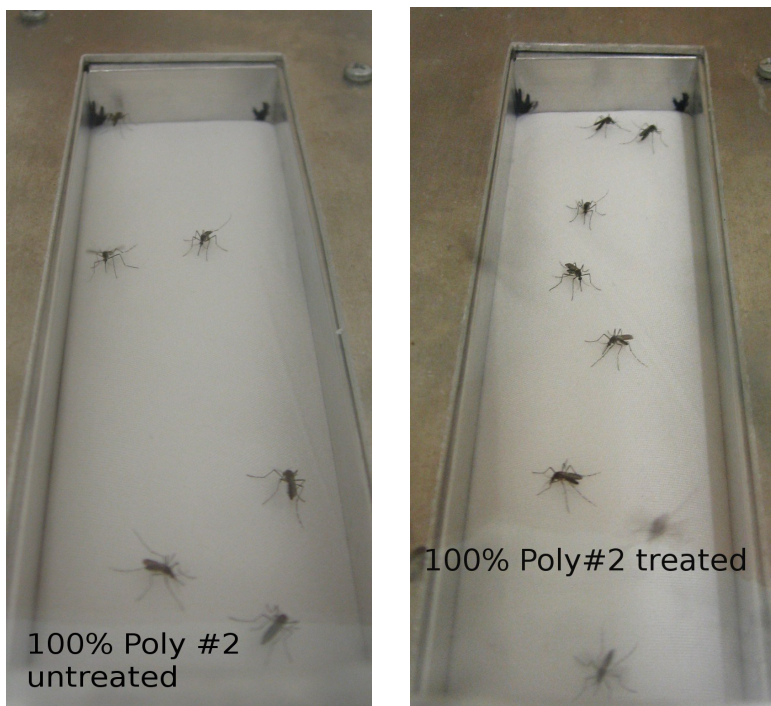
A larger study with efficacy tests after different wearing times, with more than 2 volunteers and against different mosquito species would generate data which can be statistically analysed and thereby provide significant and more meaningful results.

4.2. Digital Documentation

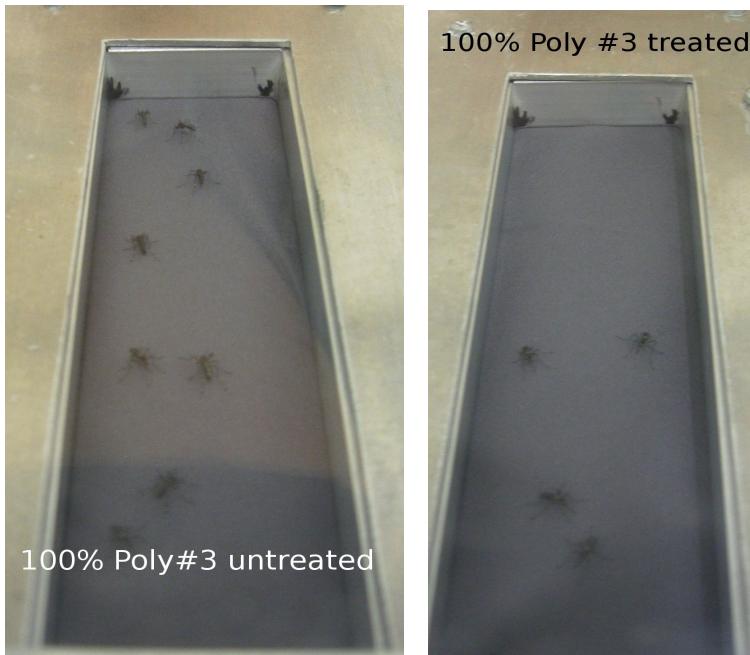
Figures 2 to 21 show all samples during efficacy cage tests, pictures were taken at the end of the testing time.



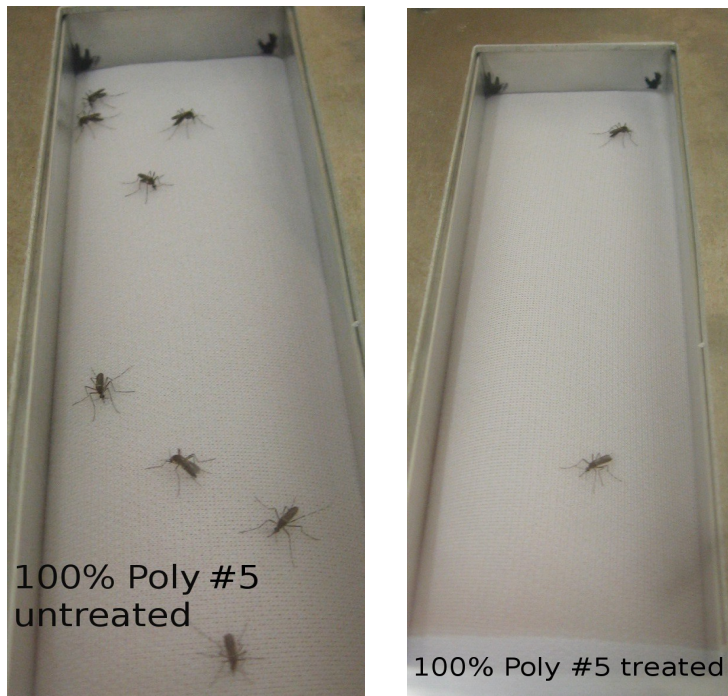
Figures 2 & 3 : Tests of untreated and treated 100% Cotton samples on volunteer A



Figures 4 & 5 : Tests of untreated and treated 100% Poly # 2 samples on volunteer A



Figures 6 & 7 : Tests of untreated and treated 100% Poly # 3 samples on volunteer A



Figures 8 & 9 : Tests of untreated and treated 100% Poly #5 samples on volunteer A



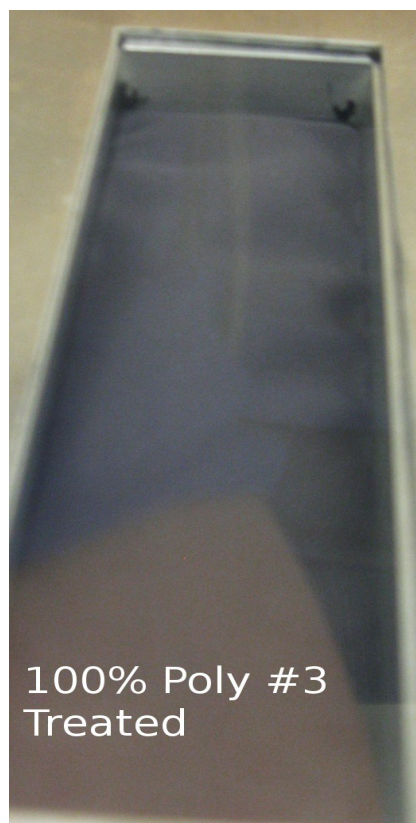
Figures 10 & 11 : Tests of untreated and treated 70% Co 30% Ny samples on volunteer A



Figures 12 & 13 : Tests of untreated and treated 100% Cotton samples on volunteer B



Figures 14 & 15 : Tests of untreated and treated 100% Poly #2 samples on volunteer B



Figures 16 & 17: Tests of untreated and treated 100% Poly #3 samples on volunteer B



Figures 18 & 19 : Tests of untreated and treated 100% Poly #5 samples on volunteer B



Figures 20 & 21 : Tests of untreated and treated 70% Co 30% Ny samples on volunteer B