

**Toxicity evaluation of a textile sample  
“Gewebe CF, marine”  
against *Ixodes ricinus* according to the  
TL 8305\_0331 guideline**

**Final report**

## 1. Summary

The knock down effect of a textile sample “Gewebe CF, marine” after 100 wash cycles was evaluated against nymphs of the tick species *Ixodes ricinus*. Tests were performed according to the guideline TL 8305-0331 of the German armed forces (2009) using WHO test tubes (Faulde et al. 2003).

The textile samples were attached to the inner wall of the test tubes and ten test animals were released into the tubes. The time until knock down of all test ticks was recorded, whereby knock down was defined as the inability of the animals to walk. In addition, the time until the first tick and the fifth tick was knocked down was also documented. A total of 10 repetitions of sample tests were performed using 10 ticks each, giving a total of 100 individuals. Additionally 3 negative control runs were performed using 10 ticks each.

On textile samples that underwent 100 wash cycles the first tick was knocked down after an average of 13:45 minutes and the fifth tick after 21:26 minutes. Total knock down of all ticks occurred after an average of 27:46 minutes. During the test period no ticks of the controls showed a knock down.

Begin of knock down of *I. ricinus* ticks has to start within 27:10 minutes, to meet the criteria of the guideline mentioned above. The 10 % knock down rate is clearly below this cut off time. Considering the standard deviation, also 100 % knock down is still in the range.

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## 2. General Information

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Test conduction	Dipl. Biol. Juliane Bendin
Item description	“Gewebe CF, marine” smartpolymer GmbH Breitscheidstr. 97 07407 Rudolstadt
Time schedule	Receipt of products: 30.07.2014 Start of tests: 06.08.2014 End of tests: 07.08.2014 Study report: 27.08.2014

### Aim of investigation

Evaluation of the knock down effect of a treated, prewashed (100 times) fabric against nymphs of *Ixodes ricinus* according to the technical guideline TL 8305-0331 of the German armed forces (2009).

### 3. Material and Methods

#### 3.1 Test organisms

*Ixodes ricinus* nymphs (F2 generation, breeding line of Insect Services) were used in the test 7-9 month after moulting. Ticks were kept under the following conditions: long-day (16 h light: 8 h darkness) at 20 °C and 90 % r.h. for 2-3 month post moulting, thereafter at 10 °C, complete darkness and 90 % r.h.. One day prior to the tests ticks were again acclimated to long-day conditions at 20 °C. The constant humidity of 90 % was provided by a saturated MgSO<sub>4</sub> solution (Winston & Bates 1960).

#### 3.2 Test product

The tested fabric Gewebe CF marine (Figure 1) provided by smartpolymer GmbH was stored at room temperature. For testing the darker side was used. The sample was prewashed by the client: 100 wash cycles.



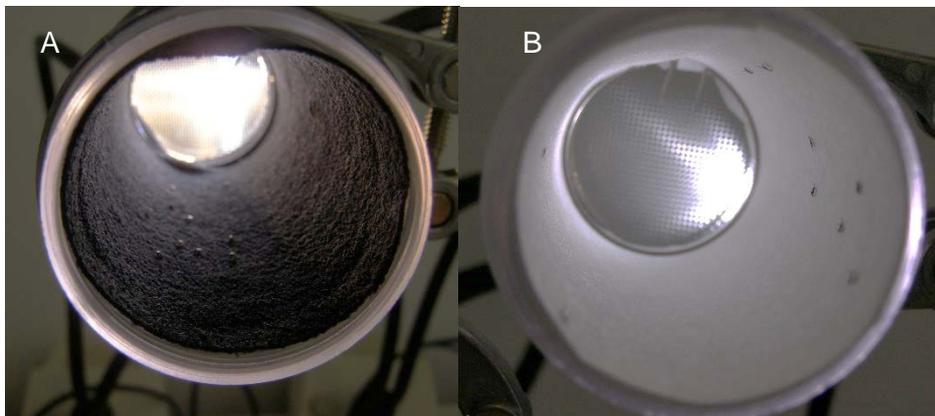
Figure 1: Example of a used test product (dimensions 12 x 12.6 cm).

### 3.3 Technical Guideline TL 8305-0331

The tests were performed according to the technical guideline TL 8305-0331 of the German armed forces (2009), a procedure published by Faulde et al. (2003) which was based on the recommendations of the WHO (1981). According to the guideline, knock down has to commence before  $27.1 \pm 8.5$  minutes in *I. ricinus* nymphs.

### 3.4 Experimental set-up

For experiments, the test kits of the WHO were used (WHO/VBC/81.806). These kits consist of a transparent synthetic tube (length: 12 cm, diameter: 4 cm) with a screw thread on each end. One piece of fabric (size: 12 x 12.6 cm) was put inside the tube such that the wall of the tube was completely covered by fabric, the darker fabric side thereby inward-looking (figure 2 A). For control runs one piece of filter paper of the same size as the fabric was placed inside a clean tube. The fabric or filter paper (NeoLab Art. no. E1422, Heidelberg) was fixed with a metal clamp at the end of the tube. One end of the tube was closed by a piece of gauze (mesh size: 250  $\mu\text{m}$ ) which was fixed with a synthetic lid with a circular window (diameter: 3.7 cm). The other end of the synthetic tube was kept open for better observations. During the experiments the tube was held in a horizontal position and it was lightened through the end with the gauze lid using a cold light source.



**Figure 2: A) WHO tube containing the test product face fabric inward-looking. B) WHO tube containing filter paper as control.**

### 3.5 Test procedure

Ten individuals were transferred into the tube using a paintbrush and the time monitoring started. The test individuals were observed through the open end of the tube. Time until knock down of the first, fifth and tenth tick was documented. Knock down is defined as the inability to walk. The tests were stopped when all individuals were knocked down. Ticks walking close to the lids or to the open end of the tube were transferred to the centre of the tube again. Each test was repeated 10 times with 10 new test animals and a new piece of fabric. The control runs were stopped after 32 minutes. The whole test was conducted on two days, whereby 3 control runs with 10 *I. ricinus* nymphs each were performed. Overall 100 individuals were used for the test and 30 for control.

### 3.6 Test conditions

During the tests, temperature and relative humidity inside the test room were measured by a Testostor 175 data logger (Testo AG, Lenzkirch, Germany). Mean temperature and humidity ( $\pm$  standard deviation) were  $23.6 \pm 0.6$  °C and  $57.2 \pm 3.3$  % r.h.

## 4. Results

The prewashed test samples induced a knock down effect in all ticks.

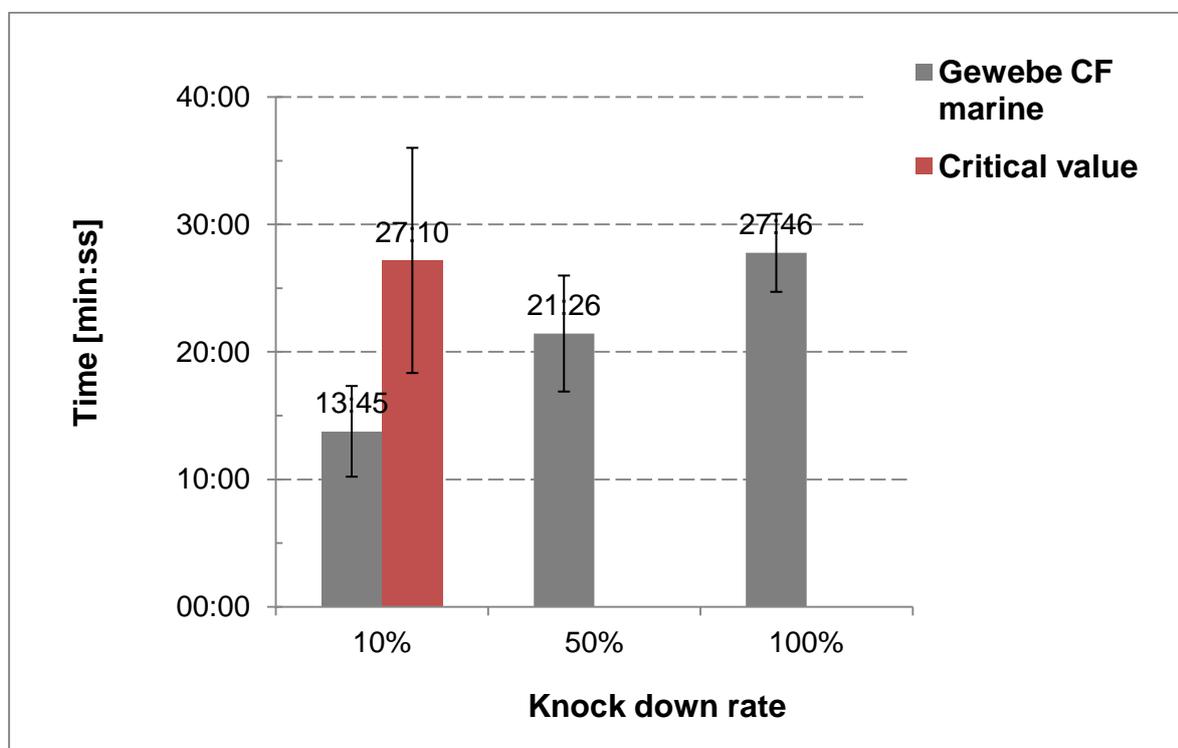


Figure 3: Mean time and standard deviation of 10 runs with 10 ticks each are diagrammed for the tested fabric "Gewebe CF marine". The equivalent (critical) value from the guideline TL 8305-0331 is also illustrated.

The average times until the first, the fifth and the tenth individual were knocked down are summarized in figure 3. On the fabric, the first *I. ricinus* nymphs were knocked down after an average of 13:45 minutes. 50 % and 100 % knock down was observed after a mean of 21:26 minutes, and 27:46 minutes, respectively. The data of every single test run is contained in table 2 (appendix). The ticks of the control runs showed no knock down within 32 minutes observation time.

## 5. Discussion

The test is designed for toxicity evaluation of textiles impregnated for protection against vectors by the German armed forces according to the technical guideline TL 8305-0331 (2009). Specific protocols were published by Faulde et al. (2003), and Faulde & Uedelhoven (2006) using ticks (*I. ricinus*) as test organisms.

The exposure tests performed with ticks met the criteria of the guideline for the tested fabric Gewebe CF, marine, prewashed 100 wash cycles. To meet this guideline, knock down of ticks has to commence within  $27.1 \pm 8.5$  min in *I. ricinus* nymphs. The determined knock down times of 10 % fell below the indicated times in the guideline. Considering the standard deviation 100 % knock down is still in the range.

## 6. Literatur

Faulde M., Uedelhoven W., Robbins R. (2003). Contact toxicity and residual activity of different Permethrin-based fabric impregnation methods for *Aedes aegypti* (Diptera: Culicidae), *Ixodes ricinus* (Acari: Ixodidae), and *Lepisma saccharina* (Thysanura: Lepismatidae). Journal of Medical Entomology 40: 935-941.

Faulde M., Uedelhoven W. (2006). A new clothing impregnation method for personal protection against ticks and biting insects. International Journal of Medical Microbiology 296 (S1): 225-229.

TL 8305-0331: Bundesamt für Wehrtechnik und Beschaffung (2009). Vektorenschutzausrüstung für textile Flächengebilde. Technische Lieferbedingungen

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Berlin, 27.08.2014

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## 7. Appendix

Table 2: Raw data of the performed test runs with 10 *I. ricinus* nymphs each on fabric Gewebe CF marine that underwent 100 wash cycles as well as control runs on filter paper. Time [hh:mm:ss] until knock down was measured. Controls were stopped after 32 minutes.

1) Fabric				2) Control			
Test No.:	time [hh:mm:ss]			Test No.:	time [hh:mm:ss]		
	10 % knock-down	50 % knock-down	100 % knock-down		10 % knock-down	50 % knock-down	100 % knock-down
1	00:13:01	00:26:41	00:30:47	1	00:00:00	00:00:00	00:00:00
2	00:11:48	00:18:34	00:24:30	2	00:00:00	00:00:00	00:00:00
3	00:19:32	00:23:20	00:31:15	3	00:00:00	00:00:00	00:00:00
4	00:13:03	00:18:16	00:27:22				
5	00:12:33	00:18:54	00:27:52				
6	00:15:25	00:22:17	00:26:40				
7	00:11:03	00:20:55	00:26:34				
8	00:19:41	00:26:12	00:31:04				
9	00:08:25	00:12:30	00:21:51				
10	00:13:03	00:26:38	00:29:42				
<b>Mean</b>	<b>00:13:45</b>	<b>00:21:26</b>	<b>00:27:46</b>		00:00:00	00:00:00	00:00:00
Standard deviation	00:03:33	00:04:33	00:03:04		00:00:00	00:00:00	00:00:00