

## ADVANCED METHOD FOR EVALUATION OF THE RESIDUAL EFFICACY OF PRODUCTS TO CONTROL *CIMEX LECTULARIUS* (HEMIPTERA: CIMICIDAE)

**BIRGIT HABEDANK, BIRGIT SNELINSKI, MICHAEL REINSCH,  
AND JUTTA KLASSEN**

Federal Environment Agency (FEA), Sect. IV 1.4, Corrensplatz 1,  
14195 Berlin, Germany

**Abstract** In order to avoid treatment failures in bed bug control and to prevent the development of insecticide-resistant bed bug strains, laboratory efficacy tests of products for bed bug control must be adapted more to practical conditions. For our study, *Cimex lectularius* of the insecticide susceptible laboratory strain of the FEA served as test animals. The bed bugs were fed on rabbits once a week. Juvenile *Cimex lectularius* after 4 and adults after 6 blood feedings were used for the efficacy tests. For efficacy evaluation, insecticidal formulations containing pyrethroids, carbamate or pyrrol were used according to the instructions for use. The test products were applied to 3 different types of surfaces: Hornitex™ (non-sorptive surface), plywood and wallpaper (surfaces of different sorption). Test animals were exposed to treated surfaces for at minimum five different exposure times (e.g. 5, 15, 30, 60 and 180min) to simulate different exposure conditions. The efficacy of several insecticides declined at shorter exposure times. Only one pyrethroid formulation showed 100% mortality of adult and juvenile bed bugs at all tested exposure times on all test surfaces. Standard rearing conditions and defined age of test animals revealed reproducible efficacy results. The test results showed that it is important for the practical use of tested products, even if they are used according to the instructions for use, to know the range of efficacy in different application conditions, e.g. in dependence of the exposure time of bed bugs and the types of surfaces. Thus, application of a product can be optimized and treatment failures can be avoided.

**Key Words** Insecticides, pyrethroid, carbamate

### INTRODUCTION

*Cimex lectularius* are often not immediately recognized, because they are hiding within luggage, furniture or other objects that are brought into human dwellings. Because these haematophagous parasites need a blood meal only at intervals of about a week or longer, stitches are often overlooked during the first weeks of infestation, or other blood-sucking insects are assumed to be the cause of it. After a blood meal lasting only a few minutes, the nocturnal parasites hide quickly in the immediate vicinity of the host (e.g. in beds, wooden furniture, wooden moldings, wallpaper, picture frames, electrical outlets, upholstered furniture). Typically the parasites can develop, reproduce and spread unnoticed for several weeks before a bed bug infestation is diagnosed. The eradication of a bed bug infestation, once introduced and spread in human habitation, is particularly difficult. All the hiding places of bed bugs must be found and all the cracks and crevices must be treated with highly effective insecticides. However, under practical conditions in most cases not all the hiding places are found. Hence, residual insecticide spraying is necessary for bed bug control.

Different classes of insecticides are used for bed bug control, predominantly pyrethroids. Studies on the efficacy evaluation of products or active ingredients for bed bug control are published e.g. by Busvine and Lien, 1961; Fletcher and Axtell, 1993; Moore and Miller, 2006; Turner and Brigham, 2008; Barile et al., 2008; Lilly et al. 2009.

As a consequence of increasing numbers of bed bug infestations recorded in many countries, of treatment failures after the application of insecticides and of increasing numbers of evidently insecticide-resistant bed bug populations (e.g. Hwang et al, 2005; Boase, 2008; Doggett and Russel, 2008; CDC and U.S.EPA 2010), there is a need to revise and optimize the laboratory test methods for the evaluation of insecticides for bed bug control.

In order to use insecticidal products effectively against a target organism, it is important to consider the life cycle and behaviour of the target organism and to know the qualities and limits of the products used. Therefore the method of exposing target insects to residual deposits of insecticides on surfaces (Busvine, 1971) at our laboratory has been adapted to simulate more practical conditions under consideration of short exposure times of the bed bugs to insecticidal products and different types of test surfaces. To date, various products containing pyrethroids, carbamate or pyrrol were tested in our laboratory with the presented test method.

## MATERIAL AND METHODS

### Test Products

Test objects were products that are registered as biocides for bed bug control. Aim of our studies was to evaluate the efficacy of these products, when they are applied according to their instructions for use. With respect to owners' rights, the brand names and exact active ingredients have been anonymized. All products tested were for professional use only and had to be dissolved in water before use. We present detailed results of 3 tested products, products I and II containing pyrethroids and product III a carbamate.

### Test Insects

*Cimex lectularius* of an insecticide susceptible laboratory strain of the FEA served as test animals for the investigations. The *Cimex lectularius* strain has been reared at the FEA and formerly the Institute for Water-, Soil- and Air-Hygiene of the Federal Health Agency since 1947. The bed bugs are kept in glass Petri dishes (diameter 10 cm) containing filter paper and are maintained at standard rearing conditions in an incubator with a temperature of  $25\pm 3^\circ\text{C}$  and a relative humidity of  $45\pm 10\%$ . The eggs are stored in an incubator at  $32\pm 2^\circ\text{C}$  and  $45\pm 10\%$ , respectively. The bed bugs of all developmental stages get a blood meal on rabbits every 14 days. For efficacy tests, the bed bugs beginning with the first instar were fed weekly on rabbits. Juvenile *Cimex lectularius* got 4 and adults got 6 blood feedings prior to the tests and were used 5 to 6 days after their last blood meal.

### Test Surfaces

All tested insecticidal products were applied as a standard to 3 different types of surfaces: Hornitex™ (pressboard with a plastic surface) as a sample for a plain and not sorptive surface, as well as plywood and wallpaper with surfaces of different sorptivity. The test surfaces had a size of 10 cm x 10 cm.

### Procedure of the Efficacy Test

The test is designed as a no-choice residual film contact test for the target organisms, i.e. the test animals are not able to avoid the contact to the insecticide-treated surface.

The test surfaces were laid on large plates (size of 50 x 50 cm). During the spraying application of the dissolved products to every large plate, the turning points were outside of the plate (total spraying area: 70 x 50 cm) in order to ensure a homogeneous insecticidal covering of the test surfaces. Identical surfaces sprayed with water served as controls. After a drying time of about 1 hour the bed bugs were exposed to the treated test surfaces in batches of 12 animals (for adults: males and females in ratio 1:1). The test animals were held on the surfaces with glass rings (diameter 8 cm), covered with a glass lid to prevent escape.

Each product was tested for at least 5 predetermined exposure times (e.g. 2 min, 5 min, 15 min, 30 min and 60 min) during that the bed bugs had contact to the insecticide treated surfaces. The demonstrated product samples I-III were tested with a minimal exposure time of 5 min, and additional exposure times of 3 h and 24 h to demonstrate an overall picture of the efficacy of the sample products, though in general our focus was to study product efficacy at the given short exposure times.

Two replicates were conducted for each type of surface and duration of exposure. At the end of the exposure each batch of bed bugs was transferred into a glass petri dish containing filter paper. The animals are observed as a standard 2 h, 6 h, 24 h, 48 h, 72 h, 96 h and 8 days after the start of exposure. The observation repeatedly lasted until day 21 (about 4 weeks after the last blood feeding of the bugs) to ensure that no test animal would recover. Temperature and relative humidity were measured during the tests.

The observed status of the test animals was divided into 5 categories with A and B for vital or only slightly affected animals, and C, D and E for affected animals, that were not able to move forward and to turn ventral-dorsal, that were knocked down, moribund and dead. The percentage of affected test animals was defined as the percentage of the number of animals rated in categories C, D and E in relation to the total number of animals in the test batch. The mortality was determined at day 8 after begin of the exposure, and included bugs of the categories C, D and E that did not recover to A and B till the end of the test (see Figure 1a and 3a). The data are presented as mean  $\pm$

standard deviation (SD). Abbott’s correction (1925) was applied if natural mortality in the control groups exceeded 5% .A test was considered as valid if the mortality of control animals did not exceed 10%.

## RESULTS

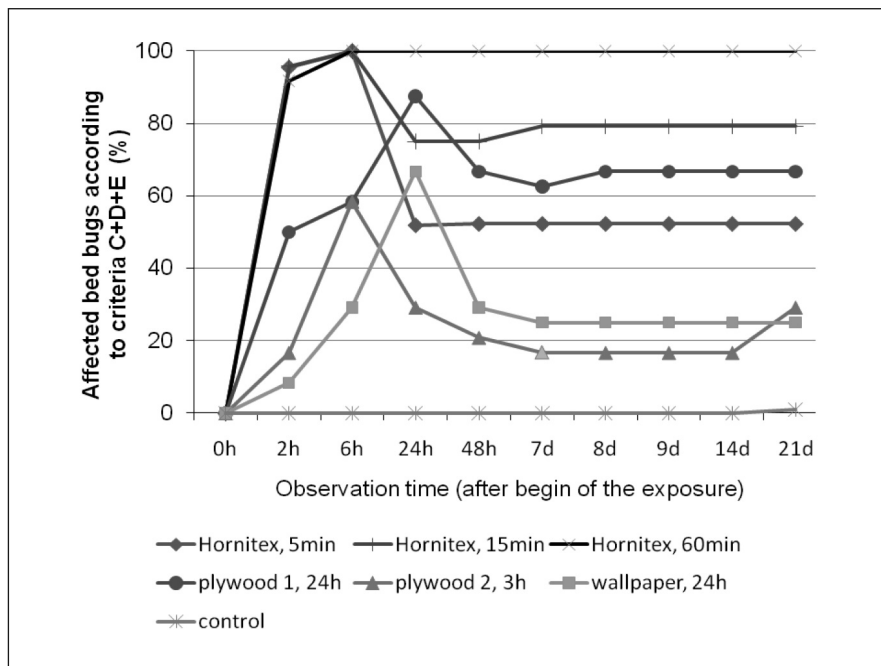
### Examples For the Effect of a Prolonged Observation Period, Short Exposure Times and Different Types of Surfaces

Product I was exactly used in concentration and application rate as described in the instruction for use (application rate of the dissolved product: 50 ml/m<sup>2</sup>). Identical application rates of the dissolved product were applied to all types of surfaces (50 ml/m<sup>2</sup> to Hornitex™, plywood 1, wallpaper). We tested 100 ml/m<sup>2</sup> on plywood 2, in order to determine the effect of the double of the advised application rate. The effect on adult and juvenile bugs was observed over a period of 21 days. The test results of the adults are shown in Figures 1a and 1b. To demonstrate the changing status of the test animals, we present only data from test batches with a mean percentage of affected animals below 100% in these figures. Some test animals in these batches were only knocked down during the first hours, and started to recover within 6 to 24 hours. The mortality of control animals on Hornitex™, plywood 1, plywood 2 and wallpaper altogether (n=96) on day 21 post exposure (27 d after the last bloodmeal) amounted to at mean 1%.

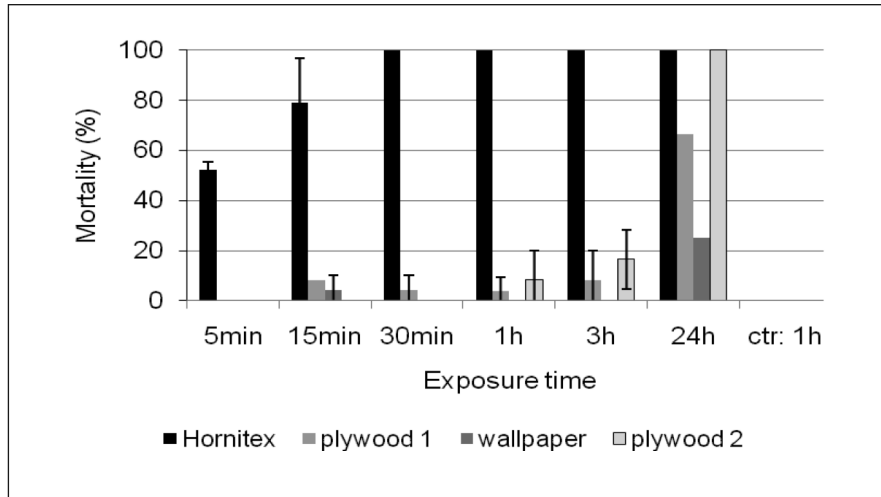
Product I killed adult bed bugs on Hornitex™ at an exposure time of 30 min or more. The mean mortality of bugs that were exposed to plywood 1 and 2 or wallpaper up to 3 hours, was less than 20% (Figure 2). Even for an exposure time of 24 hours, all adult bugs were killed only when exposed to plywood 2 (application rate 100 ml/m<sup>2</sup>), but not when exposed to plywood 1 and wallpaper. Similar results have also been observed for juvenile bed bugs.

Figure 2 shows the results for product II (advised application rate of the dissolved product: 50-100 ml/m<sup>2</sup>), where 50ml/m<sup>2</sup> was applied to Hornitex™ and 100 ml/m<sup>2</sup> was applied to plywood and wallpaper. At all exposure times (5 min to 24 h) the adult and juvenile test animals were knocked down completely, none of the test animals recovered, and the bugs died within the following 10-14 days. The residual effect remained high in all exposure times (5 min to 24 h) up to 12 weeks.

We tested product III in a first step exactly according to the instructions for use (advised application rate of the dissolved product: 40 ml/m<sup>2</sup>), and with exposure times of 5 min to 60 min, but observed a very low effect to the juvenile bed bugs. In the presented example (Figure 3a and b) we doubled the concentration and applied the advised application rate to Hornitex™ (40 ml/m<sup>2</sup>), and additionally used the double of the advised



**Figure 1a.** Product I. Mean percentage of affected adult bed bugs after different exposure times. Each line presents n=24 test animals.



**Figure 1b.** Product I. Mortality (Mean  $\pm$  SD) of adult bed bugs after exposure times of 5min to 24h on Hornitex, plywood 1 and wallpaper (application rate 50 ml/m<sup>2</sup>) and plywood 2 (application rate 100 ml/m<sup>2</sup>). Each bar presents n=24 test animals.

application rate for the sorptive surfaces plywood and wallpaper (80 ml/m<sup>2</sup>). The results for status development of the juvenile test animals are shown in Figure 3a. The recovering period in this example started 24 hours and was finished 72 hours after begin of the exposure. Figure 3a does not show the results of plywood, because the status of the test bugs did not change after 6 hours post exposure. Detailed mean mortality results of the test groups are shown in Figure. 3b. Even in these conditions of high concentration of the active ingredient, the eradication of juvenile bed bugs was only possible at exposure for at least 60 min for Hornitex™, 3h for wallpaper and 24 h for plywood.

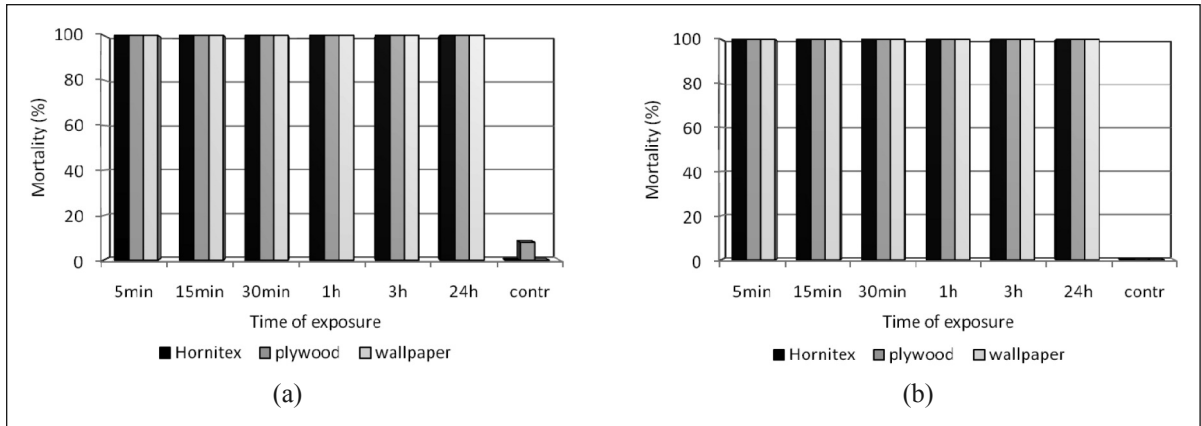
Altogether, only one product was assessed as able to eradicate bed bugs at short exposition times of 5min and 15 min under standardized laboratory conditions.

## DISCUSSION AND CONCLUSIONS

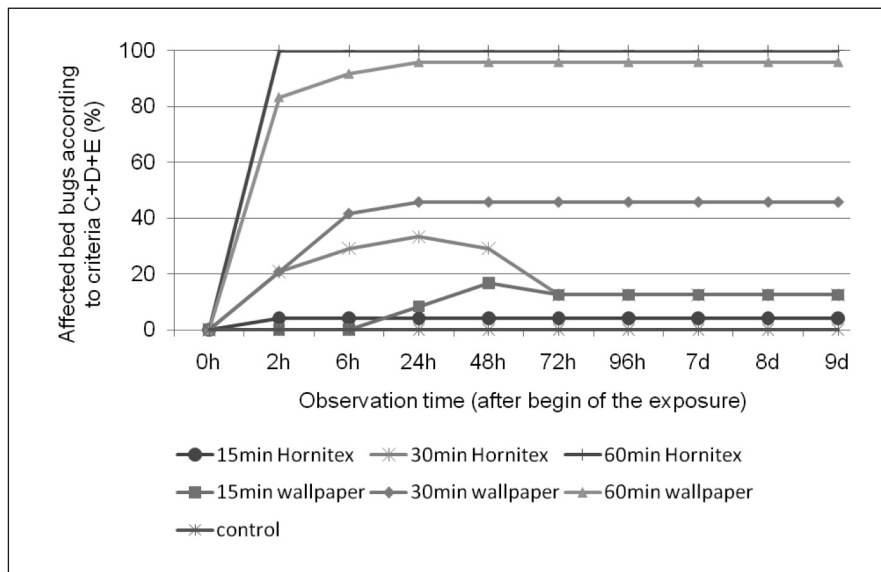
If the hiding places of bed bugs are found and treated during control measures, the bugs get in contact with large amounts of insecticidal products and will have insecticidal contact to more parts of the body. Because the bugs prefer cracks and crevices it is likely that a part of the bed bug population survives such spot treatments. These remaining bugs should be killed while they are searching for a host to get a blood meal. Therefore surfaces likely to be passed by these insects are treated with insecticides. If a fogger is used, almost all surfaces can have a residual insecticidal film. To lower the toxicological risk for the inhabitants in a dwelling the barrier spraying is practiced. In this case only insecticidal films of restricted size are applied on selected surfaces.

In both cases the bugs have to move across the insecticidal film and might get in contact with the insecticidal product only for a short duration. Considering this, it is necessary to include short exposure times to laboratory efficacy tests for the evaluation of products for bed bug control. We included 3 different types of surfaces with different sorptive properties as a test standard, as surfaces with such properties occur in human dwellings. The different mortality of the test bugs after contact to insecticidal films at Hornitex™, plywood and wallpaper demonstrates that it is important to consider these or similar surfaces in laboratory efficacy tests. Repeatedly, in laboratory conditions it was necessary to apply a higher application rate of the products to plywood and wallpaper, as it is shown for the given product examples.

In the presented efficacy investigations, the total percentage of test animals that were knocked down, moribund and dead (criteria C,D, E) varied during the first 3 days, because some test animals were able to recover, and remained constant in the further course of observations. During the following days up to the end of the trials the changes in these animals shifted from criteria C to D to E. In other tests that are not shown here, we observed after this period only in single cases a recovering of test animals on the one hand, and of impairment (B to C) on the other hand. Therefore we estimated the mortality after 8 days and use an observation period of a minimum of 8 days.



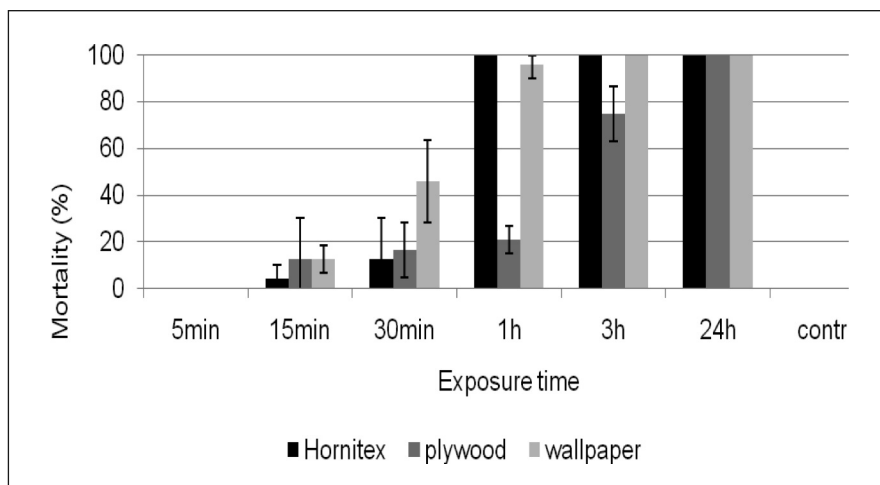
**Figure 2.** Product II. Mortality of adult (a) and juvenile (b) bed bugs after exposure times of 5 min to 24 h on Hornitex (application rate 50 ml/m<sup>2</sup>), plywood and wallpaper (application rate 100 ml/m<sup>2</sup>). Each bar presents n=24 test animals.



**Figure 3a.** Product III. Mean percentage of affected juvenile bed bugs after different exposure times. Each line presents n=24 test animals.

The described standard test method includes standard rearing conditions and defined age of the test animals, and simulates practical application conditions. Until now we found only one product with 100% mortality at all tested conditions, especially at a short exposure times of 5 min. On the other hand, some insecticidal products are highly effective only at long exposure times or at higher concentrations or application rates than stated on the label.

The results show that it is important for the practical use of tested products to know the range of efficacy in different application conditions (e.g. in dependence of the types of surfaces and the exposure time of the bed bugs). The information should be communicated and detailed information included into the users instructions, to ensure an effective use of insecticidal products and to avoid further developments of resistant bed bug populations.



**Figure 3b.** Product III. Mortality (Mean  $\pm$  SD) of juvenile bed bugs after exposure times of 5min to 24h on Hornitex (application rate 40ml/m<sup>2</sup>), plywood and wallpaper (application rate 80ml/m<sup>2</sup>). Each bar presents n=24 test animals.

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