

Report prepared on behalf of  
Unisearch Limited

on

**IN-VITRO REPELLENCY  
TRIAL vs MOSQUITOS  
(1 formulation)**

**CERIT REFERENCE NUMBER:  
IRB/01/04**

by

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for

Insect Repellent Wrist Bands Pty Ltd

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**IN-VITRO REPELLENCY  
TRIAL vs MOSQUITOS**

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**AIM**

To evaluate by exposure to mosquitos (*Culex quinquefasciatus*), the repellency effects of a test formulation applied as an insect repelling wristband.

**FORMULATION**

The formulation evaluated in this report, were supplied by Bug Bam Insect Repellent Wrist Bands Pty Ltd, and was labelled as follows:

- Bug Bam Insect Repelling Wrist Bands  
Active Ingredients: Citronella, Lemongrass & Geranium oils

In this report the formulation is referred to as **Bug-Bam**. Each sample supplied was regarded as a blind sample; CERIT cannot verify the content of the samples.

The formulation was a plastic wrist band individually sealed in a "vapour barrier bag"; the samples were stored at room temperature (not exceeding 30°C).

**METHOD**

The methods used follow those described in the protocol **IN-VITRO REPELLENCY PROTOCOL USING HOUSEHOLD FLYING INSECTS CERIT/HF-HM/REP 1.0 27/11/91** and was carried out on 14/7/04 and 21/7/04.

**Modifications to Standard Protocol CERIT/HF-HM/REP 1.0 27/11/91**

**Formulations**

For these tests: plastic insect repelling wrist bands individually sealed in a vapour barrier packaging were used.

The standard protocol: allows for pump action spray-on formulations.

**In-vitro Repellency Trial vs Mosquitos  
(Bug Bam Insect Repellent Wrist Bands Pty Ltd: 1 formulation)**

**Treated Surface**

For these tests: the plastic insect repelling wrist bands were the treated surface.

The standard protocol: allows for a 10cm x 10cm square of absorbent paper towel to be treated with the formulations.

**Control**

For these tests: a white plastic rectangle 45mm x 115mm was used as an untreated control for the wrist bands. Additionally, a 10cm x 10cm square of untreated paper towel attached to a cardboard backing was used as a control for normal mosquito activity.

The standard protocol: allows for a 10cm x 10cm square of untreated paper towel to be used.

**Treatment Method**

For these tests: the insect repelling wrist bands were removed from their vapour barrier bags. The wrist bands were cut in half and stapled together to create a rectangular shape approximately 45mm x 115mm. Insect exposures commenced immediately.

The standard protocol: allows for the formulation to be applied to large sheets of paper towel, then cut into squares and attached treated side down to a cardboard square 10cm x 10cm.

**Analysis of Results**

For these tests: paired t-test was used to compare the insect repelling wrist band against the plastic control. This is more appropriate for a single treatment versus a control trial.

The standard protocol: allows for Analysis of Variance and Student-Newman Keuls test to allow multiple comparisons between formulations.

**IN-VITRO REPELLENCY PROTOCOL USING HOUSEHOLD FLYING INSECTS  
Protocol CERIT/HF-HM/REP 1.0 27/11/91 (Mod.)**

This protocol was devised by the Centre for Entomological Research and Insecticide Technology (CERIT),  
University of New South Wales.

**OBJECTIVE OF TEST**

The aim of the test was to evaluate by exposure to mosquitos the repellency effects of a test formulation applied as an insect repelling wrist band.

**INSECTS**

**Species:** The insect species tested was the mosquito (*Culex quinquefasciatus*). The strain used was laboratory bred and insecticide susceptible.

**Number of insects:** a minimum of 250 mosquitos per cage (20 x 20 x 25cm) were used.

**Sex and life stage of insects:** mixed sex adults aged between 5 and 10 days post emergence were used.

**Details of rearing:** Mosquitos were maintained at a temperature of 26.0±1.0°C and humidity of 75%±5%. Mosquito larvae were reared in deionised water and were fed fish food sticks. Adult mosquitos were kept in mesh cages with 10% sugar water provided *ad. lib.*

**FORMULATIONS**

The formulation, which was a plastic insect repelling wrist band, individually sealed in vapour barrier packaging, was supplied by the client. Formulation samples were stored at room temperature (not exceeding 30°C).

**TREATED SURFACE**

The plastic wrist bands measuring 22mm x 230mm were the treated surface.

**In-vitro Repellency Trial vs Mosquitos  
(Bug Bam Insect Repellent Wrist Bands Pty Ltd: 1 formulation)**

**CONTROL**

A white plastic rectangle 45mm x 115mm as well as an untreated paper towel 10 x 10cm square were used as a control.

**TREATMENT METHOD**

The insect repelling wrist bands were removed from their vapour barrier bags. The wrist bands were cut in half and stapled together to create a rectangular shape approximately 45mm x 115mm. Insect exposures commenced immediately.

**CONDITIONS DURING TEST**

Tests were carried out in a large room with good ventilation to avoid saturation of formulation in the air. Temperature - 25.0±1.0°C; Humidity - Ambient

**TESTING PROCEDURE - REPELLENCY ASSAY**

The trial commenced at 0 hours when the newly prepared wrist band was clipped against one side of the cage. Before each count, mosquitos were 'activated' by disturbing the air and brushing the sides of the cage until a majority of the mosquitos were flying. The cage was left undisturbed for 30 seconds to allow the mosquitos to land. The number of mosquitos, which had landed upon the cage over the area where the treated surface was clipped, were recorded at 0, 4, 24, 40 and 48 hours post treatment.

After each count, the treated surface was removed and taken to another room to prevent saturation of the air with the formulation. The test was to be terminated prematurely if repellency action had expired.

The test was replicated eight times over a period of three days for the test formulation and the controls.

**ANALYSIS OF RESULTS**

A paired t-test was applied to the landing results for the test formulation and the plastic control, (Sokal and Rohlf "Biometry", Freeman 1981).

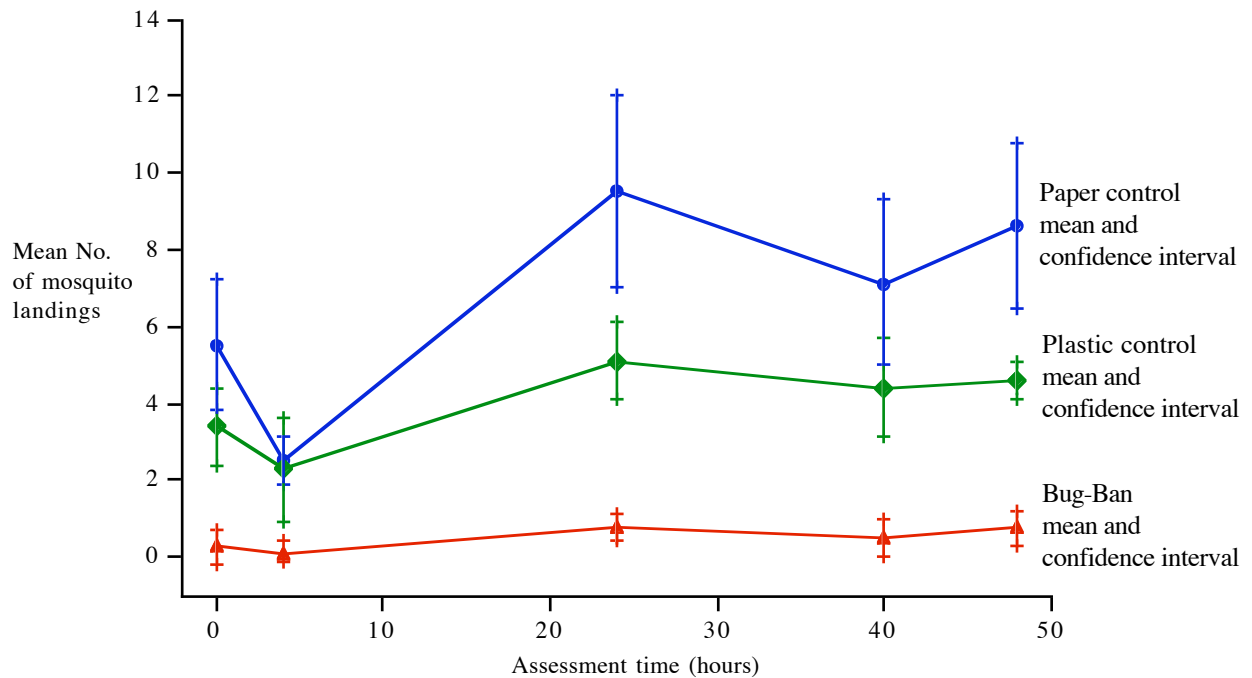
**RESULTS**

**Table 1:** Means and 95% Confidence Limits calculated from 'mosquito landings' results for eight replicates of each formulation at the indicated assessment times.

Treatment		Assessment time				
		0 hours	4 hours	24 hours	40 hours	48 hours
Bug-Bam	-95% CL	-0.2	-0.1	0.4	0.0	0.3
	Mean	0.3	0.1	0.8	0.5	0.8
	+95% CL	0.7	0.4	1.1	1.0	1.2
Untreated plastic control	-95% CL	2.4	0.9	4.1	3.1	4.1
	Mean	3.4	2.3	5.1	4.4	4.6
	+95% CL	4.4	3.6	6.1	5.7	5.1
Untreated paper control	-95% CL	3.8	1.9	7.0	5.0	6.5
	Mean	5.5	2.5	9.5	7.1	8.6
	+95% CL	7.2	3.1	12.0	9.3	10.8

**In-vitro Repellency Trial vs Mosquitos**  
**(Bug Bam Insect Repellent Wrist Bands Pty Ltd: 1 formulation)**

**Graph 1:** Means and 95% Confidence Limits calculated from 'mosquito landings' results for eight replicates of each formulation at the indicated assessment times.



**DISCUSSION**

Mosquitoes were deterred from landing on the cage area where the Bug-Bam treatment was attached, with little variation in mosquito landings over the 48 hour period for which the exposure continued. The t-test comparisons indicate that for every assessment time, the mosquito landings on the Bug-Bam treatment were significantly lower than the landings on the plastic control (5% level of significance). The landings on the paper control were within the range normally expected for this protocol, indicating normal mosquito activity

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