



STUDY REPORT NUMBER	C0078/2526/01/ET/0001
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STUDY REPORT

EFFICACY TESTING OF MOSQUITO REPELLENT SAMPLE

SPONSOR	TEST FACILITY
MosquitNo B.V. Weegschaalstraat 3, 5632 CW Eindhoven, The Netherlands.	Reseda Life Sciences Pvt Ltd, No. 28, 1st Floor, 16th Main, 38th Cross, 4th T Block, Jayanagar, Bangalore - 560041, Karnataka, India.

STUDY DIRECTOR	Anulya A.
DATES	Starting date: 22-12-2025 Experimental phase starting date: 23-12-2025 Experimental phase completion date: 05-01-2026 Study completion date: 06-01-2026



TABLE OF CONTENTS

S. No.	CONTENTS	PAGE NO.
	COMPLIANCE STATEMENT	3
1	OBJECTIVE OF THE STUDY	4
2	TEST ITEM	4
3	BACKGROUND	4
4	TEST SPECIFICATIONS	5
4.1	TECHNIQUE	5
4.2	TEST PARAMETERS	5
4.3	ENVIRONMENTAL CONDITIONS	5
4.4	EVALUATION METHOD	5
5	DEFINITIONS	5
6	PARAMETERS OF EVALUATION	6
7	EXPERIMENTAL DETAILS	6
7.1	APPARATUS	6
7.2	REARING TECHNIQUE FOR MOSQUITOES	7
7.3	TEST SUBJECTS	7
7.4	SAMPLE STORAGE	7
8	PROCEDURE	7
8.1	MOSQUITOES PROPENSITY TO BITE ASSESSMENT	7
8.2	REPELLENCE TEST	8
8.3	ACCELERATED STABILITY STUDY	8
9	RESULTS	9
10	CONCLUSION	10



COMPLIANCE STATEMENT

This study was performed by *Reseda Plant Sciences Pvt Ltd* test facility under my direction (undersigned) according to the protocols based on the indications supplied by the following guidelines:

- Documentation issued by the European Commission: Technical Notes for Guidance. PRODUCT TYPE 18 – INSECTICIDES, ACARICIDES AND PRODUCTS TO CONTROL OTHER ARTHROPODS and PRODUCT TYPE 19 – REPELLENTS AND ATTRACTANTS (only concerning arthropods). CA-Dec12-Doc 6.2. a – Final.
- USA EPA (USA Environmental Protection Agency), EPA Product performance test guidelines OPPTS 810-3700: insect repellents to be applied on human skin, July 7, 2010.
- WHO Guidelines for efficacy testing mosquito repellents for human skin (WHO/CDS/NTD/WHOPES/2009.4).
- Accelerated temperature stability test.

The results of this report completely and faithfully reflect the raw data generated by the Study.

Date: 06-01-2026

Study director

Anulya A.



1.0.0 OBJECTIVE OF THE STUDY

The study's objective was to evaluate the mosquito repellency of samples impregnated with insect repellent using the “Hand in Cage technique”.

2.0.0 TEST ITEM DETAILS

2.1.0 Sample name/ID:

1. MosquitNo Bracelet – Untreated
2. MosquitNo Citronella Bracelet (EAN Code: 8718164111945) – Treated sample after 1-week incubation at 50 °C.
3. MosquitNo Citridiol Bracelet (EAN Code: 8718164115103) – Treated sample after 1-week incubation at 50 °C.

2.2.0 Sample image:



MosquitNo Bracelet –
Untreated



MosquitNo Citronella Bracelet
(EAN Code: 8718164111945) –
Treated sample after 1-week
incubation at 50 °C



MosquitNo Citridiol Bracelet
(EAN Code: 8718164115103) –
Treated sample after 1-week
incubation at 50 °C

2.3.0 Sample received date: 08-12-2025

3.0.0 BACKGROUND

The hand-in-cage test method for evaluating mosquito repellency is a standard laboratory procedure designed to measure the effectiveness of mosquito repellent samples and topical repellents through controlled human exposure. In this test, a volunteer's hand or forearm, typically covered by the treated sample, is placed inside a cage containing host-seeking female mosquitoes of species such as Aedes, Anopheles, or Culex. The cage environment simulates natural mosquito



behavior, allowing observation and quantification of mosquito landings, probing, or bites on the treated skin or sample over a set period. The primary outcomes measured include the complete protection time (CPT), the duration from repellent application until the first confirmed bite, and the overall reduction in mosquito contact compared to untreated controls. The test provides critical data on the level and duration of mosquito repellency, helping validate the protective performance of repellent-treated textiles essential for reducing mosquito-borne disease transmission risk. The hand-in-cage test is widely accepted and forms the basis of regulatory and product quality evaluation frameworks for mosquito repellent textiles.

4.0.0 TEST SPECIFICATIONS

4.1.0 Technique: Hand in cage study protocol for mosquito repellent products as per EPA OPPTS 810.3700 guidelines

4.2.0 Test parameters:

Species: *Aedes aegypti*

Age: 2 to 5 days old-female

Feeding status: Sugar-fed, non-blood-fed

Number of mosquitoes: 100 per test

4.3.0 Environmental conditions:

Temperature: 27 ± 2 °C

Humidity: $80 \pm 10\%$

Photoperiod: 16:8 Light:Dark cycle

4.4.0 Evaluation method: Percentage of protection and percentage of efficacy

5.0.0 DEFINITIONS

5.1.0 Bite (B): This term refers to an insect penetrating skin with its mouthparts and ingesting blood.

5.2.0 First bite (FB): This term refers to the first bite the volunteer received from the test insects on the exposed test area of the forearm.

5.3.0 Landing (L): This term refers to an insect that lands on the exposed area of the forearm but does not probe or bite.

5.4.0 Protection time (PT): This term refers to the time from the application of the repellent to the time until the first bite (FB). This is the period a repellent is expected to remain efficacious.



5.5.0 Repellence (R): This term refers to a lack of insect landing and probing human skin where repellent has been applied.

6.0.0 PARAMETERS OF EVALUATION

6.1.0 Percentage of efficacy (%E): Efficacy represents the repellency performance of the treated sample and indicates the extent to which mosquito landings are reduced in comparison with the untreated control. It reflects the repellent action of the treatment in discouraging mosquitoes from approaching and landing on the treated surface. The efficacy percentage is calculated as:

$$\% \text{ of efficacy } (\%E) = 100 - (\text{Landings on Treated Arm} \times 100 / \text{Landings on Control Arm})$$

6.2.0 Percentage of protection (%P): Protection represents the bite-preventive performance of the treated sample and indicates the extent to which mosquito bites on the skin are reduced in comparison with the untreated control. It reflects the ability of the treatment to prevent successful biting or blood feeding. The protection percentage is calculated as:

$$\% \text{ of protection } (\%P) = 100 - (\text{Bites on Treated Arm} \times 100 / \text{Bites on Control Arm})$$

7.0.0 EXPERIMENTAL DETAILS

7.1.0 Apparatus

Cage Size: Cubic, 20,000 cm³, rectangular, with 1 sleeved opening for inserting the subject's arm in the cage. The test chamber is made of a net (walls and top) and white fabric (underneath).



Figure 1 - Square test chamber.



7.2.0 Rearing technique for mosquitoes:

The larvae/pupae are reared under optimal conditions at $27 \pm 2^{\circ}\text{C}$, relative humidity $80 \pm 10\%$, and photoperiod 16: 8 hours (light: dark). Feeding of adults is done by providing a cotton pad soaked with a 10% sucrose solution. No blood meal is given before the test. Mosquitoes are starved for 12 hours before the test. The test insects are used only for one test and are then incinerated.

7.3.0 TEST SUBJECTS

Number of test subjects: Each test was conducted on six volunteers, comprising three males (M1 – M3) and three females (F1 – F3).

Configuration: Volunteers are not allowed to use perfume, drink alcoholic beverages or coffee, or smoke for 12 hours before the test and throughout the test's duration until its conclusion.

The test subject's forearm, from wrist to elbow, is used as the test area. A latex glove protects the test subject's hand. Before treatment, the area is washed with unscented (fragrance-free) soap, rinsed with water, then with a solution consisting of 70% ethanol or isopropyl-based rubbing alcohol and 30% water, and is dried with a towel.

7.4.0 SAMPLES STORAGE

Test samples are stored in a cool, dry laboratory area, protected from sunlight, heat, and humidity. Each sample is sealed in a clean aluminum foil, followed by an airtight plastic bag, clearly labeled with identification details.

Samples are handled with clean gloves to prevent contamination and tested within a defined period to maintain the integrity and reliability of results.

8.0.0 PROCEDURE

8.1.0 Mosquitoes propensity to bite assessment

100 adult female specimens of *Aedes aegypti*, at least 5 days old and showing trophic activity, are sucked from the breeding cages and moved to the test cages 30 minutes prior to each repetition. Mosquito propensity to bite is assessed by inserting a control forearm with the untreated sample into the cage, followed by the test sample. At every replication, a control forearm should be inserted through the sleeve into the cage and exposed to mosquitoes for up to 30 seconds to verify biting



pressure. A minimum of 10 mosquitoes should get attracted, land and bite in 30 seconds to use this set of mosquitoes for further studies; otherwise, either the subject arm or the mosquito population is changed till 10 mosquitoes get attracted, land, and bite in 30 seconds. This test is performed by each test subject.

8.2.0 REPELLENCE TEST

Hand in cage technique was used to evaluate the given samples by comparing the number of landings and bites made by mosquitoes during the 5 minutes of exposure to the forearm of volunteers wrapped with an insect repellent impregnated test sample and a test sample without any insecticide (control) in a cage containing 100 females of *Aedes aegypti* mosquitoes.

Forearms of the volunteers without any test sample were also inserted for 30 seconds to check the mosquito bite propensity and to use the data to extrapolate the number of bites for 2–5-minute exposure.

The percentage of protection offered by the test sample was calculated based on the number of bites per experiment, and the percentage of efficacy offered by the test sample was calculated based on the number of landings per experiment.

The number of landings and the number of bites is recorded. An identical procedure is followed for various configurations.

8.3.0 ACCELERATED STABILITY STUDY

The test samples were stored in their original sealed commercial packaging under accelerated stability conditions at $50 \pm 2^{\circ}\text{C}$ for a total test period. At the end of the test period, samples were removed from the incubator, allowed to equilibrate to laboratory conditions, and evaluated for mosquito repellency efficacy/protection.

One week of accelerated study at $50 \pm 2^{\circ}\text{C}$ typically corresponds to approximately 6 months of real-time efficacy/protection.

9.0.0 RESULTS

The average number of bites and landings of mosquitoes on the samples is as follows:

9.1.0 Table 1: Record of the number of landings and bites on test samples after a 1-week accelerated study (incubated at $50 \pm 2^{\circ}\text{C}$) is presented below.

SAMPLE	MosquitNo Bracelet – Untreated		MosquitNo Citronella Bracelet (EAN Code: 8718164111945) – Treated sample after 1-week incubation at 50 °C.		MosquitNo Citridiol Bracelet (EAN Code: 8718164115103) – Treated sample after 1-week incubation at 50 °C.	
	Vol	L	B	L	B	L
M1	28	14	5	1	4	1
M2	23	11	2	0	1	0
M3	22	10	2	0	2	0
F1	27	13	5	2	4	0
F2	31	15	9	3	6	2
F3	25	12	4	1	3	1

9.2.0 Table 2: The percentage of efficacy (% E) and percentage of protection (% P) of the treated sample after a 1-week accelerated study (incubated at $50 \pm 2^{\circ}\text{C}$) against the control sample are presented below.

SAMPLE	MosquitNo Citronella Bracelet (EAN Code: 8718164111945) – Treated sample after 1-week incubation at 50 °C.		MosquitNo Citridiol Bracelet (EAN Code: 8718164115103) – Treated sample after 1-week incubation at 50 °C.		
	Vol	% E	% P	% E	% P
M1		82.14	92.86	85.71	92.86
M2		91.30	100.00	95.65	100.00
M3		90.91	100.00	90.91	100.00
F1		81.48	84.62	85.19	100.00
F2		70.97	80.00	80.65	86.67
F3		84.00	91.67	88.00	91.67
Avg.		83.47	91.52	87.68	95.20



10.0.0 CONCLUSION

- The tested sample “**MosquitNo Citronella Bracelet (EAN Code: 8718164111945) – Treated sample after 1-week incubation at 50 °C**” has shown an **efficacy of 83.47%** and **protection of 91.52%** against mosquitoes (*Aedes aegypti*).
- The tested sample “**MosquitNo Citridiol Bracelet (EAN Code: 8718164115103) – Treated sample after 1-week incubation at 50 °C**” has shown an **efficacy of 87.68%** and **protection of 95.20%** against mosquitoes (*Aedes aegypti*).

Summary of the report: The test sample after 1-week incubation at 50 °C has shown excellent efficacy and bite protection against the *Aedes aegypti* mosquitoes.