4. Repellents

Repellents are either synthetic chemical or plant-based compounds. They are used for personal protection against mosquitoes and other haematophagous insects. Candidate compounds should be evaluated simultaneously in laboratory and field.

4.1. Laboratory evaluation

4.1.1. Objectives

- To test the repellent activity of the candidate compound against mosquitoes
- To determine the average protection time rendered by the repellent
- To test the safety to volunteers

4.1.2. Repellency against mosquitoes

These studies will be carried out in the laboratory maintained at 27 $\pm$ 2^{o}C and 60–70% relative humidity using laboratory-reared strains of vectors in cloth cages (2 cu ft). Studies will be carried out against each individual species in replicates of three. In each of the replicate cage, one hundred 3 to 5-day old sugar-fed female mosquitoes will be held. Mosquitoes that are pre-starved for 4 h or more prior to testing will be used for the experiment. These conditions and format of experiment given below need standardisation for different species. Use of known repellent as control is optional.

In the cage, five plastic bowls with sugar-soaked cotton (10% in water) should be placed at four opposing corners and one in the middle. These bowls should be treated separately with three different specified concentrations of the formulation, one specified concentration of DEET (known synthetic repellent compound as positive control) and with only sugar-soaked cotton (negative control). The four treated bowls will be placed in four opposing corners while the untreated negative control in the middle on the floor of the cage. Five minute landing counts will be made at 0, 1, 2, 4 and 6 h. The cups will be removed between the exposure intervals. Mean percent repellency for each percent formulation and species will be calculated based on the data of the three replicates at the given times of observation. Percent repellency will be calculated using the formula given below.

(a) $$\frac{\text{No. landing on negative control} - \text{No. landing on treated with repellent}}{\text{No. landing on negative control}} \times 100$$

(b) $$\frac{\text{No. landing on negative control} - \text{No. landing on treated with DEET}}{\text{No. landing on negative control}} \times 100$$

Where, **(a)** provides % repellency of the candidate repellent; and **(b)** % repellency of DEET. Efficacy of the candidate repellent can be assessed relative to DEET.

4.1.3. Determination of effective dose of repellency and protection time

Testing should be done for *Anopheles, Aedes* and *Culex* mosquitoes and other disease vectors. Evaluations should be carried out in the laboratory maintained at standard temperature and humidity. Approximately 25 cm area of one arm of human volunteer should be marked on the skin with marker and test repellent dissolved in a suitable solvent should be applied on the marked portion. Repellent should be applied in different concentrations on the arms of different persons. Remaining exposed area should be covered with a paper sleeve. Exposures are made sequentially of an arm with application of solvent alone followed by progressively increased doses of repellent. Hands are put into cages containing approximately 100–150 three day old 4-hour starved mosquitoes in a 2 x 2 x 2 ft cloth cage. These conditions and the format of experiment need standardisation for different species. The experiments on humans should be attempted only on toxicologically safe compounds. The propensity of mosquito for biting should be ensured by inserting an untreated hand into the cage. However, before the start of each exposure, the bare hand used as control (only solvent) of volunteer was exposed for 30 seconds. Observations are made for the first 3 minutes of every half an hour exposure. If at least two mosquitoes landed on or bit the hand, the repellency test should then be continued. The test should be continued until at least two bites occur and followed by a confirmatory bite (second bite) in the following exposure period. The time between application of the repellent and the second successive bite should be recorded as the protec-

tion time. The dose of repellent providing at least 6–8 h of protection in mosquito cage experiments may be consdered to be an ideal compound for use in field as repellent for field evaluation. Calculate the effective dose (ED_{90}) by probit analysis. After reaching a dose, which gives 100% repellency, the arm is re-exposed hourly until repellency declines to 50% compared with contemporary counts on the untreated arm. Emphasis should be on percent protection in relation to dose and time after treatment. The experiment will have two positive controls with known synthetic repellents, e.g. DEET, and negative control only solvent that is used as base for preaparing the test repellent. Data should be recorded in the format given in Table 36.

Table 36. Laboratory evaluation of repellents on humans (Cage method)

Name of the repellent…….....Date…….....Time …….....…....
Temp…….....Relative humidity.....…….Dose of application……..…

Replicate number*	Time of first bite	Time of confirmatory bite	Protection time	Percent protection
Dose 1				
Dose 2				
Dose 3				
Dose 4				
Positive control				
Negative control				

* Separate row for each replicate.

4.1.4. Human safety evaluation

A semi-structured questionnaire should be used to record the perceptions of human volunteers about the positive and/or negative side effects of the repellents (Annexure 3).

4.2. Field evaluation

Human subjects should be selected randomly without bias. Prior informed consent should be obtained for participation in the evaluation. The effective dose determined in the laboratory should be used in the field evaluation. Use of known repellent compound and is optional. The experiments on human subjects should be attempted on toxicologically safe compounds.

Repellent should be applied to arm or leg of the volunteer laid down on a cot and areas with application should be exposed. Positive control (with DEET) and negative control (solvent alone) should be included for assessing the repellency of the candidate repellent. Five volunteers, two for test repellent, two for positive control and one for negative control should be employed for the evaluation. Pre informed and free consent should be obtained from the volunteers (Annexure 6). Human volunteers should be placed at least 10 metres away from each other. Mosquitoes landing on the volunteers should be collected from dusk to dawn and examined for species identification. Insect collectors should be rotated every four hours to avoid slack-

ness and bias. Both complete protection and percent repellency should be determined as described in laboratory evaluation. Data should be recorded in the format given in Table 37.

Table 37. Mosquito landing collections

Name of the repellent.....Date..... Time.....Temp.....
Relative humidity.....Repellent and concentration.....Species.....

Time (hrs)	Number of bites					Percent protection	Average protection time
	Test 1	Test 2	Positive control 1	Positive control 2	Negative control		
1800							
1900							
2000							
2100							
2200							
2300							
2400							
0100							
0200							
0300							
0400							
0500							
0600							
Total							