Designation: E939 – 94 (Reapproved 2012)

Standard Test Method of Field Testing Topical Applications of Compounds as Repellents for Medically Important and Pest Arthropods (Including Insects, Ticks, and Mites):I Mosquitoes[1]

This standard is issued under the fixed designation E939; the number immediately following the designation indicates the year of original adoption or, in the case of revision, the year of last revision. A number in parentheses indicates the year of last reapproval. A superscript epsilon (ε) indicates an editorial change since the last revision or reapproval.

1. Scope

1.1 This test method is used to evaluate the repellency of promising compounds that have undergone primary laboratory studies and have been approved for skin application for secondary testing.

1.2 This test method is designed for the study of mosquito repellents, but with some modifications this test method can be used to determine the repellency of candidate compounds for other flying insects that attack humans.

1.3 The values stated in SI units are to be regarded as standard. No other units of measurement are included in this standard.

1.4 *This standard does not purport to address all of the safety concerns, if any, associated with its use. It is the responsibility of the user of this standard to establish appropriate safety and health practices and determine the applicability of regulatory limitations prior to use.*

2. Terminology

2.1 *Definitions of Terms Specific to This Standard:*

2.1.1 *complete protection time (CPT)*—the time from application of the repellent to the time of the first confirmed bite (a second bite by the same species within 30 min of the first). This permits any number of unconfirmed bites during the CPT.

3. Summary of Test Method

3.1 A measured amount of the candidate material is applied to the forearm or sometimes the lower leg. These areas are then protected from rubbing and are continuously exposed to mosquitoes in the field to determine the length of time the treatment provides either complete protection or a high level of protection.

4. Significance and Use

4.1 This test method is an important part of the final phase of study in the development of mosquito repellents for personal use.

4.2 This test method is primarily designed to simulate a situation in which a person treated with a repellent is exposed to natural populations of attacking mosquitoes.

4.3 The simplicity of the test offers flexibility under a relatively wide range of circumstances and geographical locations. By following this test method, international testing with a variety of vector mosquito populations is no more difficult to accomplish than tests with various domestic species.

4.4 A number of people test topical applications of a repellent for the following reasons:

4.4.1 To determine how long the repellent is effective;

4.4.2 To establish the effective dosage range;

4.4.3 To establish the range of effectiveness on several mosquito genera and species in a number of geographical areas; and

4.4.4 To identify the material in terms of odor, staining capability, plasticizing effect, and oiliness or greasiness.

4.5 No repellent should be tested on humans without the written consent of the test volunteers (hereafter referred to as test subjects) and prior approval of competent authority, as designated in applicable laws and regulations governing experimentation on humans.

5. Apparatus

5.1 *Insect collection vials.*

5.2 *Aspirator.*

5.3 *Stereoscope* (optional).

5.4 *Standard References for Mosquito Identification,* for determining species present in the field (optional).

5.5 *Temperature and Humidity Reading Equipment*—Ideally, a continuous recording device such as a hygrothermograph should be used to record conditions during tests. If such

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equipment is not available, readings should be made immediately before, midway during, and immediately after the tests are made, using a sling psychrometer.

5.6 *Air Speed Indicator and Light Meter,* optional but preferred if equipment is available.

5.7 *Watch.*

5.8 *Headnets.*

5.9 *Cotton Gloves.*

5.10 *Battery-Operated Head Lamps,* with red filters for tests with nocturnally active species.

5.11 *Notebook, Test Sheets, and Pencils,* for recording species, test data, date, and locality of the test. A sample work sheet is attached with recorded results (see Annex A1.).

5.12 *Water Supply, Mild Soap, and Paper Towels,* for washing treated skin.

5.13 *Clothing,* should be appropriate to the season and geographical area.

NOTE 1—There are differences of opinion as to whether the clothing worn should be uniform in color and type, however, data available do not reject or confirm this contention.

5.14 *List of Chemical Names,* identifying the compounds or mixtures, or both, to be tested.

NOTE 2—In the event of a medical emergency, the chemical list along with pertinent toxicological data may be required.

6. Reagents and Materials

6.1 All test solutions are formulated on a weight to volume basis (usually with 95 % ethanol).

6.2 Test chemicals in 25 % ethanol solutions.

6.3 Test standard deet (*N*,*N*-diethyl-meta-toluamide) in a 25 % ethanol solution.

6.4 For final development studies, formulations of lotion, liquids, creams, solids, or pressurized aerosols containing ingredients for field evaluation are included.

7. Sampling

7.1 Each candidate repellent is paired with each other repellent or a standard on the arms of a subject and exposed simultaneously to the same insect population (See Annex A1).

7.2 A round-robin or paired test is used in the experiments, usually based on the number of experimental materials being evaluated.

7.3 Treatments are exposed to the mosquito population for as long as the repellents are effective, and the biting activity continues.

8. Procedure

8.1 Determine the identity of species of mosquito in the test area prior to the test. Determine the time to begin and end tests each day by the activity of the species to be tested. Diurnal nocturnal or other patterns of mosquito behavior will govern the scheduling of tests.

8.2 For field tests, make the initial studies with a 25 % ethanol solution of the candidate repellents (250 mg AI/mL). A compound or formulation may be retested at reduced or increased concentrations, or at full strength, if warranted.

NOTE 3—If the complete protection times (CPTs) of repellents are too long for the available testing period or if the CPTs are too short for evaluation, adjust the concentrations accordingly. The termination of an evaluation before a confirmed bite occurs is termed a "plus out" and should be avoided.

8.3 Spread 1 mL of the repellent formulation or repellent solution evenly over the forearm of the subject and compare directly with another repellent of the same concentration on the other arm (see Note 4). The behavior of some species necessitates the use of the legs instead of the arms as treatment sites. This can be determined by observation before the tests begin.

NOTE 4—The concentration of a compound being tested is not as important as the assurance that it is paired with another compound of equal concentration.

8.4 If legs are used as treatment sites, apply 1.5 mL on the skin between the ankle and knee.

8.5 Determine the surface area of the limbs of each test subject so that treatment rates of candidate repellents and the standard are uniformly applied. Adjust the application rates for differences in arm or leg size of different subjects.

8.6 Expose the treated arms or legs continuously to natural populations of the mosquito species being tested.

8.7 The duration of effectiveness of the repellent is indicated by the CPT.

8.8 Employ a balanced incomplete block (BIB) experimental design (round robin) when three to five chemicals are to be tested. With this design, test each repellent in the series on opposite arms of a given number of subjects.

NOTE 5—Ideally, the number of subjects should equal the number of candidate compounds, excluding the standard. Thus, if four compounds are to be tested, including the standard, three test subjects would be required. To illustrate, the pairings would be: AB, AC, AD, BC, BD, and CD. Subject 1 would test AB and CD; Subject 2, AC and BD, and Subject 3, AD and BC.

8.9 Because of their variability in attractiveness, assign repellents to test subjects in a randomized and balanced fashion so that each subject does not wear the same combination of materials more than once in a single BIB test series. For example, if compounds A, B, C, D, and E are tested with a standard F, then Subject 1 will test AB, CD, and EF; Subject 2 will test AC, BE, and DF; Subject 3 will test AD, BF, and CE; Subject 4 will test AE, BC, and CF; and Subject 5 will test AF, BC, and DE.

NOTE 6—If four or five compounds, excluding the standard, are tested in a BIB series, one replication of the BIB is sufficient for a statistical analysis. If three compounds are to be tested in this fashion, two replications of the round robin will be necessary.

8.10 A direct comparison of the candidate versus the standard repellent deet is used when fewer than three chemicals are to be tested. For these tests, four or more replications of tests with each chemical on at least three different subjects are necessary.