Designation: E 652 – 91 (Reapproved 2003)

Standard Test Method for Nonresidual Liquid Household Insecticides Against Flying Insects[1]

This standard is issued under the fixed designation E 652; 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 covers the determination of the relative efficiency of household and industrial-use, contact insecticides dissolved in base oils.

1.2 *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:*

2.1.1 *culture*, *n*—all adult flies resulting from the seeding of eggs collected at one time on a given date.

2.1.2 *knocked-down*—pertaining to all test flies incapable of coordinated movement (moribund).

3. Summary of Test Method

3.1 Two methods for evaluating liquid household insecticides are permitted as follows:

3.1.1 For the small group method,[2] a minimum of 10 replicates of approximately 100 flies each are exposed to a total of 12 cm^3 of test insecticide per replicate.

3.1.2 For the large group procedure, use two separate fly cultures, four randomized tests with 500 flies per replicate using 10 replicates.

3.2 The difference in percentage mortality of the Official Test Insecticide (OTI) (see 8.2.1) and the test insecticide is the basis for evaluating the efficacy of the test insecticide by the small and large group test methods.

4. Significance and Use

4.1 This test method provides a satisfactory means of determining the relative efficacy of spray formulations against house flies (*Musca domestica*, L).

4.2 Test data obtained by this test method may also be adequate to support label claims for the use of the product against mosquitoes, gnats, flying moths, wasps, and certain other small flying insects. This test method is not designed to measure the residual action of the spray formulation.

4.3 As a biological test, it is subject to the variations that accompany the reactions of living organisms. It should be employed under the supervision of personnel familiar with the biological testing of insecticides.

5. Apparatus

5.1 *CSMA Pesticide Atomizer*, fitted with a No. 631 cut off and a glass reservoir.[3]

5.2 *Rearing Room*—A room of any convenient size, free of strong drafts, and maintained at 80 ± 2°F (27 ± 1°C) with a relative humidity of 50 ± 5 %. This room must be separate from the testing room and ventilated to minimize odors.

5.3 *Testing Room*, maintained at 80 ± 2°F (27 ± 1°C) and a relative humidity of 50± 5 %. This room may be of any convenient size capable of holding the standard Peet-Grady chamber with adequate additional space to permit efficient performance of the test.

5.4 *Peet-Grady Test Chamber* (see Annex A1.).

5.5 *Cylindrical Glass Battery Jars*, 6 in. (150 mm) in diameter and 9 in. (230 mm) high, or other suitable containers, to be used as fly larval medium containers.

5.6 *Calibrated Pipet*, or graduate with 0.1-cm^3 graduations.

5.7 *Electric Fan.*

5.8 *Air Separation Apparatus for Recovering Puparia*, constructed according to the specifications of Goodhue and Linnard.[4]

5.9 *Fly Cages*, providing at least 1 $in.^3$ (16.4 cm^3) of space per fly with a minimum of two sides and the top screened. Cages shall be constructed of metal or other suitable material and fitted with a sleeve opening, rubber membrane, or a door.

[1] This test method is under the jurisdiction of ASTM Committee E35 on Pesticides and is the direct responsibility of Subcommittee E 35.12 on Insect Control Agents.

Current edition approved July 15, 1991. Published September 1991. Originally published as E 652 – 78. Last previous edition E 652 – 84.

[2] "Peet-Grady Method," Official Method of the Chemical Specialties Manufacturers Association for Evaluating Liquid Household Insecticides.

[3] Available from Chemical Specialties Manufacturers Assn. (CSMA), 1913 Eye St., N.W., Washington, DC 20006.

[4] Goodhue, L. D., and Linnard, C. E., "Air Separation Apparatus for Cleaning Fly Pupae," *Journal of Economic Entomology,* Vol 43, 1950, p. 228.

A detachable floor is preferable to facilitate cleaning and insertion of a paper floor covering.[5]

6. Reagents and Materials

6.1 *Adult Fly Food*—5 % spray-dried (or instant) nonfat milk solids and 2 % granulated sugar dissolved in water (40 % formalin solution may be added at the rate of 1 + 1500 to delay spoiling). Each cage requires 15 cm^3 of food per 100 flies per day.

6.2 *Larval Medium*—340 g of CSMA Standard Fly Medium[6] added to 750 cm^3 of an aqueous suspension containing 15 g of moist cake yeast[7] (or 5 g of dry yeast[7]) and 10 cm^3 of nondiastatic Diamalt[7] per container (see 5.5). Some modifications in liquid content may be needed to give maximum larval production.

6.3 *Puparial Medium*—An added 2-in. (51-mm) layer of vermiculite on the dry top surface of the fly larval medium.

7. Test Specimen and Sample

7.1 The test insect must be the adult house fly (*Musca domestica L*) reared from the current CSMA official resistant house-fly strain.

7.2 Adult house flies in test groups must be between 3 and 6 days of age at the time of testing.

8. Calibration and Standardization

8.1 *Apparatus*:

8.1.1 *Atomizer*—Maintain pressure at a constant 12.5 ± 0.5 psi (86.2 ± 3.4 kPa) as measured by a gage of not more than 30-psi (207-kPa) capacity or a manometer. Calibrate the atomizer at 80 ± 2°F (27 ± 1°C) to deliver 12 cm^3 of OTI in 24 ± 1 s.

8.1.2 *Test Chamber Contamination*—Consider chambers contaminated and unsatisfactory for use when test flies (3 to 6 days old) held in the chamber for a 12 to 16-h period with food, but without insecticide treatment, show mortalities greater than 10 %, or when over 10 % of the flies are paralyzed within 30 min after liberation.

8.2 *Reference Standards*:

8.2.1 *Current Official Test Insecticide* (OTI).[3]

9. Procedure

9.1 *House Fly Rearing Technique*:

9.1.1 *Larval Medium*—Mix the larval medium (see 6.2) thoroughly until a loose, fluffy consistency is obtained, transfer it to the battery jar (or other container) without packing, cover with a suitable cover, and place in the insectary. The amount of suspension required for best rearing results will need to be determined in each laboratory and it may be varied to prevent mold growth. It is suggested that the medium be prepared in the late afternoon of the day before egg collection.

9.1.2 *Eggs*—Collect eggs for a period not longer than 16 h from food dishes or other oviposition medium in cages containing mature flies not more than 8 days old. It is suggested that fresh oviposition medium be placed in fly cages in the late afternoon for egg collection early on the following morning. Measure and seed the collected eggs without delay. Wash all the eggs together in tap water at room temperature and measure groups of 2000 as accurately as possible. This may be done by allowing the eggs to settle in a calibrated pipet or graduate (0.1 cm^3 of settled eggs is approximately 700), or the eggs can be filtered and measured in calibrated pits or cells. Use 10 cm^3 of tap water to measure and to scatter the eggs in a pit or trench 0.5 in. (13 mm) deep which is located in the center of the surface of the larval medium. Cover the eggs with loose medium and place the covered containers in the insectary with at least 1.5-in. (38-mm) separation to permit free air circulation. The maximum temperature in the jar (about 3 days later) must not exceed 130°F (54.4°C). Under normal conditions more than 85 % of the eggs should hatch within 36 h of the time they are laid.

9.1.3 *Pupae*—Approximately 3 to 4 days after the eggs have been seeded, a 2-in. (51-mm) layer of vermiculite may be added on the surface of the larval medium to aid in pupae recovery.[8] Mature larvae migrate to the top portion of the medium or to the vermiculite layer, and normally all larvae will have pupated about 9 days after seeding the eggs. When this occurs, the portion containing pupae may be removed, poured into a shallow tray, and air-dried at room temperature. An electric fan may be used to hasten drying. Then separate the pupae from the dry medium or the vermiculite. Handle gently and as little as possible to avoid injury to the pupae. Any method that permits at least 90 % of the flies to emerge is considered satisfactory.

9.1.3.1 *Air-Separation Apparatus*—An air-separation apparatus (see 5.8) is used by several laboratories for cleaning pupae and has been found to be more rapid than the indicated tray method. The device employs a blower, a cyclone collector, and a suction pipe to separate the heavier pupae from a layer of vermiculite placed on the surface of the fly larval medium.[8]

9.1.3.2 Combine all of the pupae maturing on a given day into one lot, mix, and measure into test unit groups. Each group is held in a shallow dish and placed in a cage that provides at least 1 in.^{3} (16.4 cm^3) of space per pupae.

9.1.4 *Adults*—If the large group procedure is used, the test unit consists of approximately 500 pupae. If the small group procedure is used, more than 500 pupae are placed in stock cages and adult flies are sampled prior to testing. Under normal rearing conditions, obtain at least 80 adult flies for each 100 eggs seeded. Daily supply each cage of adult flies with 15 cm^3 of adult fly food for each 100 flies and prepare so as to prevent the flies from drowning.

9.2 *Test Procedure*:

9.2.1 Before a fly spray test is started, the Peet-Grady Chamber must be clean and have clean paper on the floor, all ports and other openings must be closed, the temperature must

[5] Cages available from American Biological Supply Co., 1330 Dillon Heights Ave., Baltimore, MD 21228, have been found suitable for this purpose.

[6] The CSMA Standard Fly Medium is a product of Ralston Purina Company, P.O. Box 337, Richmond, IN 47374, and has been found suitable for this purpose.

[7] The yeasts and Diamalt are products of Standard Brands, Inc., and have been found suitable for this purpose.

[8] Incho, H. H., "A Rapid Method for Obtaining Clean House Fly Pupae," *Journal of Economic Entomology,* Vol 47, 1954, p. 938.