Designation: E 652 – 91 (Reapproved 1996)ε1

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.

ε1 NOTE—Editorial changes were made throughout in October 1996.

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 E-35 on Pesticides and is the direct responsibility of Subcommittee E 35.12 on Insect Control Agents.

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[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 be 80 ± 2°F (27 ± 1°C), and all windows must be shaded equally. In both the large-group and small-group procedures,

[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.

only flies that are capable of flying may be liberated into the Peet-Grady chamber. In the large-group procedure, all flies in one cage are used in a single test; but in the small-group method, a sample of approximately 100 flies, ±5, is used in each test. Samples may be taken by liberating the flies directly into the chamber and continuing until about 10 % of the flies remain in the stock cage to be discarded. The order of spray treatments must be randomized.

9.2.2 Immediately after liberation of the flies in the chamber, spray a total of 12 cm^3 of insecticide in equal quantities through each spray hole. Slowly oscillate the nozzle of the atomizer in a horizontal plane to avoid spraying walls and ceilings and to effect uniform distribution of the spray. Complete this procedure within 1 min from the time the spraying was started and keep the chamber closed at a constant temperature in the range of 80 ± 2°F (27 ± 1°C) for a total of 10 min. At the end of this period, open the ports and ventilate the chamber with the exhaust fan while the flies are collected.

9.2.3 Pick up the knocked-down flies (see section 2.2) in any manner that will not appreciably disturb or harm them and immediately transfer them to the clean cages (see 5.9). These flies may be counted when they are picked up or later, depending upon which time is most convenient. During the subsequent 24-h recovery period, maintain the cage under rearing room conditions of temperature and humidity and supply the treated flies with an adequate quantity of 5 % sugar solution arranged so they cannot drown in it. A gauze-wrapped ball of cotton saturated with the 5 % sugar solution is satisfactory.

9.2.4 Count the unaffected “up” flies in the chamber at the end of the 10-min exposure period and discard.

9.2.5 After a test is completed, remove all toxic residues from the chamber. Renew the paper on the floor and thoroughly clean the inside walls and ceiling. Wipe with a clean cloth saturated with alcohol containing 10 % acetone, or wash with soap and water to remove most toxic residues. Special cleaning may be required to remove insoluble toxic residues from certain chemical compounds. It is recommended that laboratories make a standard practice of taking periodic contamination observations, employing a normal test fly group (see 8.1.2).

10. Report

10.1 *Assembling the Data*—Count and record the number of unaffected flies at the end of the 10-min exposure period. Count the dead flies 16 to 24 h later, preferably by removing them from the recovery cage. Only flies that show no sign of life upon being touched may be counted as dead. If knocked-down flies were counted as they were collected, the sum of knocked-down and unaffected flies yields the total number of flies in the test. If knocked-down flies were not counted as collected, the recovered flies may be killed by a suitable method, after which they are counted. The sum of recovered and dead flies yields the knocked-down flies, and this sum added to the unaffected flies yields the total number of flies used in the test. The mortality is the percent dead of total flies and the knockdown is the percent knocked-down of total flies.

10.2 *Test Format*:

10.2.1 *Small Group Procedure*—Run ten parallel tests on the OTI and on each of the unknowns. Test each spray the same number of times on flies of the same culture (see 2.1) and test all sprays the same number of times on any one day, with the unknown samples and the OTI randomized as to order of testing. After the mortality data are obtained, calculate the average percent kills and determine the differences between the unknowns and the OTI.

10.2.2 *Large Group Procedure*—This evaluation is based on differences in mortality determined by a minimum of four randomized tests of the OTI and the unknowns. Select at least two cultures of flies, using 500 flies per replicate in the four randomized tests.

10.3 *Evaluation*:

10.3.1 Conduct the tests in accordance with the procedure previously described.

10.3.2 Use at least two cultures (see 2.1) of flies in making an official evaluation.

10.3.3 Do not use cages showing a combined mortality and crippling greater than 15 % on the day of the test.

10.3.4 An unknown insecticide must have a 10-min knockdown percentage equal to that of the OTI with a tolerance of −2. The kill by the OTI shall fall between 30 % and 55 % in all tests.

11. Precision and Bias

11.1 No statement is made about either the precision or the bias of Test Method E 652 for measuring the efficacy of the insecticide spray formulation since the result merely states whether there is conformance to the criteria for success specified in the procedure.

ANNEX

(Mandatory Information)

A1. PEET-GRADY CHAMBER

A1.1 The test chamber shall be rigidly constructed of wood, metal, or other suitable material. The inner surface shall be smooth, impervious to the usual household-type insecticides, and as free of cracks, projections, ledges, etc., as possible. The chamber shall be a cube having internal measurements of 72 ± 1 in. (1.83 ± 0.025 m). One wall shall contain a tight-fitting door large enough for a person to enter conveniently, with the interior side flush with the wall when closed. At least one wall, or the ceiling, must contain an observation window. It is preferable to have windows on opposite walls. Illumination is provided through a glass window in the ceiling, above which is placed an electric light of such intensity as to permit flies to be observed easily.

A1.1.1 An opening covered with 10 or 12-mesh wire screen is connected to an exhaust fan duct. The size and the location of this opening in relation to ventilation openings in the wall shall be such that thorough ventilation of the chamber is obtained. Preferably, the exhaust opening should be 1 ft^2 (0.09 m^2) or larger and located in or near the ceiling. Air inlet openings may be 6 by 6-in. (150 by 150-mm) ports covered with screen on the inside and provided with tight-fitting covers on the outside. Four ports located near the lower corners, or eight ports located near both the upper and lower corners, are satisfactory; but the ventilation ports should not be on the same level as the exhaust port. The entrance door may be used alone or in conjunction with the ventilation ports if a screen door is provided and thorough ventilation of the chamber is obtained. If the temperature of the air used to ventilate the chamber is lower than 80°F (27°C) heaters may be used to obtain the temperature 80 ± 2°F (27 ± 1°C) required during the test period. Such heaters must be removed before a test is started.

A1.1.2 Openings for the introduction of the insecticide shall be constructed and located, so that uniform distribution of the spray is effected without undue ventilation of the chamber. These openings may be round 1-in. (25-mm) holes located not less than 6 in. (152 mm) nor more than 12 in. (305 mm) from the ceiling and 18 in. (457 mm) from the nearest corner on each wall. A single hole may be provided in the center of each wall 6 to 12 in. (152 to 305 mm) from the ceiling.

A1.2 An exhaust fan moving air at not less than 1000 ft^3 (28.3 m^3)/min through the chamber shall be used to ventilate the chamber after each test. The exhaust fan shall be arranged with adequate piping to exhaust the chamber vapors in a safe manner.

A1.3 Unsized, nonglazed, absorbent paper, such as brown kraft or gray bogus, shall be used to cover the chamber floor. Two overlapping sheets of 36 to 40 in. (0.9 to 1 m) in width or one sheet of 6 ft (1.8 m) in width may be employed. No special weight is specified, although 60 to 80-lb (27 to 36-kg) gray bogus paper has been found excellent.