Designation: E 653 – 91 (Reapproved 1996)ε1

Standard Test Method for Effectiveness of Aerosol and Pressurized Space Spray Insecticides Against Flying Insects[1]

This standard is issued under the fixed designation E 653; 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 determines the effectiveness of aerosol and pressurized space-spray insecticides against house flies (*Musca domestica L*) and, with modifications in dosage, other flying insects.

1.2 The test may be conducted using approximately 100 house flies per test (small group) or 500 flies per test (large group).

1.3 *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. Referenced Document

2.1 *ASTM Standards:*

E 652 Test Method for Nonresidual Liquid Household Insecticides Against Flying Insects[2]

3. Terminology

3.1 *Definitions of Terms Specific to This Standard:*

3.1.1 *aerosols*—for this test method, the spray from aerosol dispensers should be in finely divided form in which 80 % or more of the individual spray particles have an arithmetic mean diameter of 30 µm or less, and none of the spray particles have a diameter of more than 50 µm. Aerosols shall be no less effective than the selected reference standards when tested against house flies at the same dosage or less.

3.1.2 *fly culture*—all adults resulting from the seeding of eggs collected at one time on a given date.

3.1.3 *knocked-down flies*—all adult test flies incapable of coordinated movement (moribund).

3.1.4 *pressurized sprays*—these products deliver mist sprays intermediate between aerosols and sprays intended to deposit an insecticidal residue. They produce sprays in which less than 80 % of the particles have an arithmetic mean diameter of 30 µm and many are 50 µm to 100 µm in mean diameter. Pressurized sprays shall be no less effective than the selected reference standards when tested against house flies at no more than twice the dosage specified for the selected reference standard.

4. Summary of Test Method

4.1 If the small-group method is used, ten tests are run on the Official Test Aerosol (using the selected reference standard)[3] and on each of the specimens in parallel. The specimens of a series shall be randomized in the order of testing.

4.2 If the large-group method is used, the test is conducted as in 4.1, with the exception that five, rather than ten tests are required.

4.3 The average percentage mortality of the test insecticide compared with that of the selected reference standard is the basis for assigning either Grade A (aerosol or pressurized space spray) or Grade B (pressurized space spray) rating to the test specimen.

5. Significance and Use

5.1 This test method provides a satisfactory means of determining the relative efficacy of aerosol and pressurized space spray insecticide formulations against house flies (*Musca domestica*, L) strains.

5.2 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 activity.

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

6. Apparatus

6.1 *Reference Standard*[3]—The reference standard shall be one of the current selected reference standards from the

[1] This test method is under the jurisdiction of ASTM Committee E-35 on Pesticides and is the direct responsibility of Subcommittee E35.12 on Insect Control Agents. It was originally developed by the Chemical Specialties Manufacturers Association (CSMA).

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[2] *Annual Book of ASTM Standards*, Vol 11.05.

[3] The Official Test Aerosol (Selected Reference Standard) has been found suitable for this test and is available from CSMA, 1913 Eye Street N.W., Washington, DC 20006.

container in which it is supplied. The selected reference standards are (a) OTA-II to be used for oil-based aerosol products, or (b) TOAPS to be used for water-based aerosol products. When reporting results, the selected reference standard should be identified by its date.

6.2 *Test Specimen Dispenser*—No restriction is placed on the test specimen dispenser. However, it should be noted that the test results apply only to the test specimen as dispensed from the particular unit employed.

6.3 *Fly Cages*[4]—Cages of any convenient type may be used if they provide at least 1 in.^{3} (16 cm^3) of space per fly and have at least two sides and the top screened. The cages should be constructed of metal or other suitable material, and fitted with a sleeve opening, rubber membrane, or door. A detachable floor is preferable to facilitate cleaning and the insertion of a paper floor covering.

6.4 *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 %. The rearing room should be separate from the testing room and ventilated to minimize odors and gases from fermenting media.

6.5 *Testing Room*—A room of any convenient size capable of holding the test chamber, with adequate additional space to permit efficient performance of the tests. The room shall be maintained at 80 ± 2°F (27 ± 1°C), with a relative humidity of 50± 5 %.

6.6 *Test Chamber*—A standard Peet-Grady chamber meeting the general specifications given in Test Method E 652. If a larger chamber is used, it is recommended that its dimensions approximate a normal size room.

6.6.1 When a Peet-Grady chamber is used, the actuator nozzles should be directed so that the spray goes through a port.

6.6.2 Adjustable fixtures may be used to hold the dispensers and distribute the sprays from the same place and angle for each test. Since different adjustments may be required for various test dispensers, the spray pattern from new dispensers should be determined prior to testing. Successful use has been reported with a fixture adjusted to position the dispenser 8 in. (203 mm) from the ceiling and 10 in. (254 mm) from a corner of the Peet-Grady chamber.

6.7 *Exhaust Fan*—An exhaust fan, capable of moving air through the test chamber at not less than 1000 ft^3/min (0.5 m^3/s), shall be used to ventilate the chamber after each test. It shall be arranged with adequate piping to exhaust the chamber vapors in a safe manner.

6.8 *Paper*—Unsized, nonglazed, absorbent paper (such as brown kraft or gray bogus) shall be used to cover the test chamber floor. Two overlapping sheets of 36 to 40 in. (0.9 to 1.0 m) in width or one sheet of 6 ft (1.8 m) in width may be employed. No special weight is specified, but 60 to 80-lb (27 to 36-kg) gray bogus has been found to be satisfactory.

6.9 *Apparatus for Collecting Treated Flies*—Any convenient means of picking up the paralyzed flies without injuring or appreciably disturbing them may be used. If a vacuum device is used, it must produce gentle suction, have a sufficiently large receptacle to prevent crowding the flies, and be cleaned after each test.

6.10 *Adult Fly Food*—Dissolve 5 % of spray-dried (or instant) nonfat dry milk solids and 2 % granulated sugar in water. A40 % formalin solution may be added at the rate of 1 + 1500 to delay spoiling.

6.11 *Shallow Containers*—Containers shall not be more than 0.75 in. (19 mm) high, to hold 5 % sugar solution as food for paralyzed flies. A gauze-wrapped ball of cotton saturated with sugar solution is also satisfactory.

6.12 *Larval Medium Containers*, cylindrical glass battery jars, approximately 6 in. (152 mm) in diameter and 9 in. (229 mm) high, or other suitable containers.

6.13 *Larval Medium*—For each container, mix 340 g of CSMA Standard Fly Larval Medium[5] with approximately 750 cm^3 of an aqueous suspension containing 15 g of moist cake yeast[6] or 5 g of active dry yeast and 10 cm^3 of nondiastatic diamalt.[6] Thoroughly mix this combination until a loose, fluffy consistency is obtained, transfer it to the container without packing, cover the container with a cloth or other suitable cover, and set it in the rearing room. The amount of suspension required for best rearing results will need to be determined in each laboratory and 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.

6.14 *Calibrated Centrifuge Tube, Pipet, Pit, or Cell*, to be used for the measurement of 2000 eggs (0.1 cm^3 of settled eggs equals approximately 700 eggs).

6.15 *Air-Separation Apparatus*—An air-separation apparatus, constructed according to the specifications of Goodhue and Linnard,[7] will provide a rapid means of separating pupae from the larval-rearing medium. The apparatus employs a suction pipe, blower, and cyclone separator to remove dried vermiculite (placed on the fly larval medium prior to pupation) from the heavier pupae.

6.16 *Vermiculite.*[8]

6.17 *Shallow Tray*.

6.18 *Clean Cloths*.

6.19 *Ethyl Alcohol, Ethyl Alcohol Containing 10 % Acetone, Soap and Water, or Detergent and Water*.

6.20 *Oviposition Medium.*

6.21 *Test Insect*—The test insect shall be the adult house fly, *Musca domestica L*, reared from the current official CSMA non-resistant house fly strain.[9] Healthy test groups with an average age of 4 days shall be used and individual flies in the test groups shall not be less than 3 or more than 6 days old at the time of testing. The strain shall be of such susceptibility that the Official Test Insecticide (OTI)[9] will cause a 24-h

[4] Cages available from American Biological Supply Co., 1330 Dillon Heights Ave., Baltimore, MD 21228, have been found satisfactory for this method.

[5] The CSMA Standard Fly Larval Medium is available from the Ralston Purina Co., P.O. Box 337, Richmond, IN 47374.

[6] Yeasts and diamalt, manufactured by Standard Brands, Inc., are available from local distributors.

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

[8] Terra Lite Brand Vermiculite Soil Conditioner (No. 2 grade), available from most garden or farm supply stores, has been found to be satisfactory for use in this method.

[9] Available from CSMA, 1913 Eye Street N.W., Washington, DC 20006.

mortality of 30 to 55 %, with approximately 95 % of the flies paralyzed within 10 min following the spray application in accordance with Method E 652. At least two cultures of flies meeting these specifications shall be used in making an official evaluation.

7. Rearing of Test Insects

7.1 Collect eggs for a period of not longer than 16 h from food dishes or other oviposition media in cages containing mature flies not more than 8 days old. Fresh oviposition medium may be placed in the cages in the late afternoon, and the eggs collected early the following morning.

7.2 Measure and seed the collected eggs without delay, as follows:

7.2.1 Wash the eggs 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 centrifuge tube containing tap water, or the eggs can be filtered and measured in a calibrated pit or cell.

7.2.2 Use 10 cm^3 of tap water to measure and scatter the eggs in a pit or trench, 0.5 in. (13 mm) deep in the center of the larval medium (see 6.13).

7.2.3 Cover the eggs with loose medium, replace the container covers, and place the containers in the rearing room at least 1.5 in. (38 mm) apart to permit free air circulation.

7.2.4 The maximum temperature in the larval medium (about 3 days later) shall not exceed 130°F (54°C). Under normal conditions, more than 85 % of the eggs should hatch within 36 h of the time they were laid.

7.3 Mature larvae migrate to the top portion of the rearing medium or into a vermiculite layer, and normally all will have pupated by about the ninth day after seeding the eggs. When this occurs, the portion containing pupae is removed, poured into a shallow tray, and air-dried at room temperature. An electric fan may be used to hasten drying.

7.4 Then separate the pupae from the dry mixture, by handling as gently and as little as possible (90 % of the flies must be permitted to emerge). Either of the two following methods have been found to be satisfactory:

7.4.1 Sprinkle the dry, pupae-medium mixture on an inclined tray set in front of an air blast from an electric fan to blow off the dried medium, leaving the heavier pupae on the tray.

7.4.2 Employ an air-separation apparatus (see 6.15). If this method is used, place a 2-in. (51-mm) layer of vermiculite on the larval medium 3 or 4 days after seeding. Approximately 6 or 7 days after seeding, loosen the pupae-vermiculite mixture, pour it into a shallow tray, dry with the electric fan, and use the air-separation apparatus to separate the dry vermiculite from the heavier pupae.[10]

7.5 Combine all of the pupae maturing on a given day into one lot, mix, and measure into test unit groups. Hold each group in a shallow container and place in a cage with at least 1 $in.^3$ (16 cm^3) of space per pupae (see 6.3). If the large-group method is used, each test group shall consist of approximately 500 pupae. If the small-group method is used, more than 500 pupae are placed in stock cages, and adult flies are sampled prior to testing. Under normal rearing conditions, at least 80 adult flies should be obtained from each 100 eggs seeded. Daily supply each cage of flies with 15 cm^3 of adult fly food (see 6.10) for each 100 flies, and prepare so as to prevent the flies from drowning. Satisfactory food shall be available to the flies at all times until testing.

7.6 Hold the adult flies until they are about 4 days old (see 6.21). They will then be ready for testing.

8. Preparation of Apparatus

8.1 *Reference Standards (OTA II and TOAPS) and Test Specimen Dispensers*—Prior to use, calibrate the selected reference standards and test aerosols or pressurized space sprays (see 6.2) at 80 ± 2°F (27 ± 1°C) to determine the rate of delivery in grams per second.

8.2 *Test Chamber*—Before a test is started, clean the Peet-Grady chamber, place clean paper on the floor, close all ports and other openings, maintain the temperature at 80 ± 2°F (27 ± 1°C), and, equally shade all windows.

8.2.1 Chambers are considered to be contaminated and unsatisfactory for test purposes when test flies held for a 12 to 16-h period with food, but without insecticide treatment, show mortalities in excess of 10 %; or when more than 10 % of the flies are knocked-down within 30 min after liberation into the test chamber.

8.2.2 Periodic contamination observations, employing a normal fly test group, should be a standard practice.

9. Procedure

9.1 In both the small and large group procedures, liberate into the test chamber only those test insects that are capable of flying. Do not use cages showing a combined mortality and crippling greater than 15 % on the day of test.

9.1.1 In the small-group method, use a sample of approximately 100 ± 5 flies in each test. Small-group samples may be taken by liberating the flies directly into the chamber from stock-rearing cages and continuing until about 10 % remain. Discard the remaining flies.

9.1.2 In the large-group method, use all flies in one cage (approximately 500) in a single test.

9.2 Weigh the selected reference standard or the test specimen dispenser.

9.3 With the dispenser at 80 ± 2°F (27 ± 1°C), apply spray mixture of 3.0 ± 0.5 g/1000 ft^3 (28 m^3) in a continuous flow for a Grade A aerosol or pressurized spray evaluation. For a Grade B pressurized spray evaluation, apply a spray mixture of 6.0 ± 0.5 g/1000 ft^3 (28 m^3) in a continuous flow. In a Peet-Grady test chamber, this is 0.540 to 0.756 g for a Grade A spray and 1.188 to 1.404 g for Grade B. The selected reference standard dosage is 3.0 ± 0.5 g/1000 ft^3 (28 m^3), or 0.540 g to 0.756 g per Peet-Grady chamber for both Grade A and Grade B sprays. Slowly oscillate the dispenser nozzle to effect uniform distribution. Take care not to direct the spray mist onto interior surfaces of the test chamber.

9.4 The test chamber shall remain closed at a constant temperature of 80 ± 2°F (27 ± 1°C) for 15 min from the time the mist was introduced.

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

9.5 Weigh the selected reference standard or test specimen dispenser and record the actual weight of the introduced material.

9.6 Count the number of test insects knocked-down at 5 and 10-min intervals following spray application.

9.7 At the end of 15 min, open the ports and ventilate the chamber with the exhaust fan.

9.8 Pick up the knocked-down flies and transfer them immediately to clean cages (see 6.3). These flies may be counted when they are picked up or when the mortality counts are made.

9.9 During the subsequent 24-h recovery period, hold the cage under rearing room conditions of temperature and humidity, and supply the treated flies with an adequate quantity of 5 % sugar solution (see 6.11).

9.10 Count the unaffected ("up") flies in the chamber at the end of the 15-min exposure period and discard.

9.11 When the test is completed, remove all toxic residues from the test 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 or detergent and water to remove most toxic residues. Special cleaning procedures may be required to remove toxic residues after tests with some chemical compounds.

9.12 Count the number of dead flies at the end of a 16 to 24-h recovery period, preferably by removing them from the recovery cage. Only flies that show no sign of life upon being touched may be counted as dead.

9.12.1 If the knocked-down flies are counted as they are collected, the sum of the knocked-down ("down") and the unaffected ("up") flies yields the total flies in the test. If the knocked-down flies are not counted as they are collected, the recovered flies are killed and counted. The sum of the recovered flies and the dead flies yields the total knocked-down flies, and this sum added to the unaffected flies yields the total used in the test.

9.13 Follow the procedure in 9.1-9.12 with test specimens tested in conjunction with the selected reference standard. All applications are randomized in the order of testing. Test each sample the same number of times on flies of the same culture and test all samples on any one day.

10. Calculation

10.1 The "aerosol test knockdowns" are the percent knocked-down ("down") of the total flies at 5, 10 and 15-min after spray application. Calculate the "aerosol test knockdowns" as follows:

$$\text{Aerosol test knockdowns, \%} = (\text{knocked}-\text{down flies} \times 100)/\text{total flies}$$

10.2 The "aerosol test knockdown mortality" is the percent dead of total flies. The unaffected ("up") flies at the end of the 15-min exposure period are considered to be alive at the end of the 24-h observation period. Calculate the "aerosol test knockdown mortality" as follows:

$$\text{Aerosol test knockdown mortality, \%} = (\text{dead knocked}-\text{down flies} \times 100)/\text{total flies}$$

11. Report

11.1 The test specimen (aerosol or pressurized spray), tested at 3.0± 0.5 g/1000 ft^3 (28 m^3) dosage, shall be reported as meeting the standard if its average aerosol test knockdown and its average aerosol test knockdown mortality are equal to, greater than, or not more than 5 percentage points below those of the selected reference standard.

11.2 The test specimen (pressurized spray), tested at the 6.0 ± 0.5 g/1000 ft^3 (28 m^3) dosage, shall be reported as meeting the standard if its average aerosol test knockdown at 15 min and its average aerosol test knockdown mortality are equal to, greater than, or not more than 5 percentage points below those of the selected reference standard tested at 3.0 ± 0.5 g/1000 ft^3 (28 m^3).

11.3 The report shall also include:

11.3.1 Specifications on the specimen dispenser, including type container, valve, actuator, etc.

11.3.2 The selected reference standard, specified by its date.

12. Precision and Bias

12.1 No statement is made about either the precision or the bias of Test Method E 653 for measuring the effectiveness of aerosol and pressurized space spray insecticide formulations since the result merely states whether there is conformance to the criteria for success specified in the procedure.