Designation: E 653 – 91 (Reapproved 2003)

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.

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:* [2]

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

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.

[1] This test method is under the jurisdiction of ASTM Committee E35 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).

Current edition approved July 15, 1991. Published September 1991. Originally approved in 1978. Last previous edition approved in 1984 as E 653–84.

[2] For referenced ASTM standards, visit the ASTM website, www.astm.org, or contact ASTM Customer Service at service@astm.org. For *Annual Book of ASTM Standards* volume information, refer to the standard's Document Summary page on the ASTM website.

[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.

6. Apparatus

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

[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.