



Designation: E 654 – 96 (Reapproved 2003)

Standard Test Method for Effectiveness of Aerosol and Pressurized Spray Insecticides Against Cockroaches¹

This standard is issued under the fixed designation E 654; 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 effectiveness of aerosol and pressurized spray insecticides against cockroaches.

1.2 Test data by this test method may also be adequate to support claims for use of the product to control the exposed or accessible stages of silverfish, ants, centipedes, spiders, and certain stored product pests.

1.3 The values stated in inch-pound units are to be regarded as the standard. The values given in parentheses are for information only.

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 *aerosols*—for this method, pressurized formulations generally containing 20 % or less of low-volatile ingredients (insecticides, base oils, solvents, etc.) and 80 % or more of propellant (fluorinated hydrocarbons, liquefied petroleum gases, etc.).

2.1.2 *pressurized sprays*—for this method, formulations generally containing more than 20 % low-volatile ingredients (insecticides, base oils, solvents, etc.) and 3 to 80 % propellant (fluorinated hydrocarbons, liquefied petroleum gases, compressed gases, etc.).

2.1.3 *moribund*—pertaining to any insect that shows signs of life, but is incapable of normal locomotion.

3. Significance and Use

3.1 This test method provides a satisfactory means of determining the relative efficiency of aerosol formulations when applied as direct sprays to cockroaches. It is not designed to measure the residual action.

3.2 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.

3.3 In order to measure the effectiveness with reasonable tolerance, the test is run in conjunction with the Official Test Aerosol II (OTA II)² as the standard basis of comparison. Another standard that may be used with this test method is the Tentative Official Aqueous Pressurized Spray (TOAPS).²

4. Apparatus

4.1 *Official Test Aerosol II (OTA II)/Tentative Official Aqueous Pressurized Spray (TOAPS)*,² to be dispensed from the container in which it is supplied. Ensure that the OTA II/TOAPS dispenser meets the specifications designated on its label.

4.2 *Test Specimen Dispenser*—No restrictions are made on the dispenser. However, the test results apply only to the particular test specimen as dispensed from the particular unit employed.

4.3 *Cockroaches*—The test insects shall be healthy, normal, undeformed last nymphal instars or young males of the German cockroach, (*Blattella germanica* L). Wild cockroaches shall not be used.

4.3.1 Recently emerged last nymphal instars (for example, those whose pigmentation is not dark) shall not be used for testing purposes. It is recommended that the last nymphal-instar stage shall have been attained at least 3 days prior to testing.

4.3.2 Any suitable method permitting the production of large numbers of test insects under controlled temperature and humidity conditions may be employed. The rearing technique described by Woodbury and Barnhart,³ which makes good use of a brood chamber containing adult females from which large numbers of first instar nymphs may be collected at frequent intervals, has been successfully used.

4.3.3 All test insects shall be reared under uniform conditions.

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.

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² Available from the Chemical Specialties Manufacturers Assn. 1001 Connecticut Ave. S.W., Washington, DC 20036.

³ Woodbury, E. N., and Barnhart, C. S., "Tests on Crawling Insects," Soap and Sanitary Chemicals, SSCHA, Vol 15, No. 9, 1939.

4.4 *Rearing Room*, of any convenient size, free of strong drafts and maintained at a temperature of $80 \pm 2^{\circ}\text{F}$ ($27 \pm 1^{\circ}\text{C}$) and a relative humidity of $50 \pm 5\%$. This room should be separate from the testing room and should be ventilated to minimize odors.

4.5 *Test Room*, maintained at a temperature of $80 \pm 2^{\circ}\text{F}$ ($27 \pm 1^{\circ}\text{C}$) and a relative humidity of $50 \pm 5\%$, of a convenient size to permit the operator to conduct the test efficiently.

4.6 *Spray Chamber*, with walls of solid material, 18 in. (457 mm) square, 25 to 30 in. (635 to 762 mm) high, with an open top and floor.

4.6.1 The floor of the chamber shall be covered with $\frac{1}{2}$ -in. (13-mm) mesh, wire hardware cloth, with suitable guides fastened to permit the centering of the treatment container in a definite position with respect to the OTA II/TOAPS nozzle and the test specimen dispenser. The front wall may be a sliding door to permit convenient access to the chamber interior. An adjustable hinged shelf shall be attached to the outside of the center of the back upper edge of the chamber. A satisfactory shelf that will permit the OTA II/TOAPS and test specimen to be held in a standard position is constructed of aluminum sheet, fitted with adjustable guides that permit adjustment for different dispenser sizes. An adjustable, metal-support rod (casement window adjuster) can be used to regulate the shelf angle. Markings may be made on the rod and shelf to permit rapid adjustment for different dispensers. The chamber shall rest on a stand at the proper height for convenient test operation.

4.7 *Treatment Container*—A $3\frac{1}{2}$ -in. (89 mm) diameter, open-metal cylinder or other suitable material, 3 in. (76 mm) high, with a 16-mesh screened bottom soldered in place. Metal cups of the proper dimensions with the handles removed and the bottoms replaced with soldered on wire screen have been found satisfactory. The containers shall be free of insecticide traces and shall have the entire inner wall surface oiled, greased, or dusted to prevent the escape of cockroaches and to confine them to the container floor.

4.8 *Paper*, 5 by-5 in. (127 by 127-mm) squares, unsized, nonglazed, absorbent paper (such as brown kraft or gray bogus), beneath each treatment container during aerosol application. No specific weight is required, but 60 to 80-lb (27 to 36-kg) gray bogus paper has been suitable.

4.9 *Recovery Dishes* may be glass crystallizing dishes (125 mm in diameter and 65 mm in height) or other suitable containers. Covers of 16-mesh screen may be used during the 48-h holding period after treatment.

4.10 *Purina Laboratory Chow*,⁴ to be fed to the cockroaches until the time of testing.

4.11 *Water*, to be supplied to the cockroaches until the time of testing.

5. Test Specimen

5.1 Prior to use, calibrate the OTA II/TOAPS and the test specimen at $80 \pm 2^{\circ}\text{F}$ ($27 \pm 1^{\circ}\text{C}$) to determine their spray rates in grams per second.

5.2 Align the dispensers on the adjustable shelf and determine their settings to permit rapid handling during testing.

5.3 Run a minimum of ten test groups for the test specimen in conjunction with ten test groups using the OTA II/TOAPS. Make an equal number of replicates for members of any test series on a given test day.

5.4 The dosages shall be approximately the same throughout a given test series and shall be of such magnitude as to result in an average of 50 to 75 % of the insects dead and moribund at 24 h with the OTA II/TOAPS. Consider average dosages the same if they agree within 0.2 g.

5.5 Weigh the test dispensers both before and after the spraying of each test group and record the weight of the material used.

6. Procedure

6.1 Isolate the last instar nymphs in the recovery dishes in groups of 20 with a suction device, by immobilizing them with chilling or with carbon dioxide (CO_2) gas or by any other means that will permit handling the insects without injury.

6.2 Place a square of paper (see 4.8) in the center of the spray chamber floor, using the guides to aid in the placement. Change the paper after each spray application.

6.3 Align the test specimen dispenser so that the aerosol mist will be directed into the open top of the treatment container. A line may be drawn on the chamber wall and dispenser to assist in the alignment of the dispenser nozzle.

6.4 Immediately before spraying, transfer the cockroaches from the recovery dish to the treatment container and agitate the container to distribute the cockroaches evenly over the container floor.

6.5 Place the treatment container on top of the insecticide paper in the center of the spray chamber floor. Use the guides for aid in centering the container. The bottom of the treatment container should now be 24 in. (610 mm) from the dispenser nozzle.

6.6 Apply the spray. Remove the treatment container from the spray chamber 30 s after the start of the spray.

6.7 Immediately transfer the insects from the treatment container to the recovery dish and hold them under rearing-room conditions for a 48-h observation period. During this time, do not administer food or water to the cockroaches.

6.8 Repeat 6.2-6.7 for the remaining nine groups of the test specimen series and the ten groups of the OTA series.

6.9 At the end of the 48-h holding period, determine the percentage of insects dead and moribund (see 3.3).

6.10 Use the average percent dead and moribund from the test specimen compared with that from the OTA II/TOAPS as the basis for evaluation of the test specimen.

6.11 The test specimen shall meet the standard if its average percentage dead and moribund is equal to, greater than, or within 10 percentage points less than the average percentage of the OTA II/TOAPS series. Table 1 shows the results of a typical series of tests.

6.12 Results may be taken after a 24-h holding period or at intervals longer than 48 h for critical studies. If this is done, it is recommended that the insects be fed and watered at the end of the initial 48-h period.

⁴ Purina Laboratory Chow available from the Ralston Purina Co., St. Louis, MO or its equivalent, has been found suitable for the purpose.