



Designation: E 658 – 78 (Reapproved 1995)

Standard Test Method for Efficacy of Dog Repellents Designed for Direct Application to Garbage Bags¹

This standard is issued under the fixed designation E 658; 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 evaluation of the efficacy of candidate dog repellents that are intended to be applied to the surface of garbage bags. It is designed to stimulate the conditions of the daily encounters of solitary dogs with repellent-treated bags in a residential environment.

1.2 The values stated in SI units are to be regarded as the standard. The values given in parentheses are for information only.

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. Terminology

2.1 Definitions:

2.1.1 *efficacy test*—the exposure of at least eight dogs to repellent test period conditions on each of 4 days and reference test period conditions on 4 days (see 5.8).

2.1.2 *reference test period*—a single 1-h interval during which dogs are being exposed to reference test conditions (see 5.1).

2.1.3 *repellent*—the technical material or formulation being evaluated for possible repellent activity.

2.1.4 *repellent test period*—a single 1-h interval during which dogs are being exposed to repellent test conditions (see 5.1).

2.1.5 *storage period*—the length of time from application of repellent to bags until their use in a repellent test period.

2.1.6 *test period*—a single 1-h interval during which dogs are being exposed to repellent or reference test conditions.

3. Test Animals

3.1 A minimum of eight and preferably twelve dogs shall be used. An equal number of males and females is preferred. Mixed breeds or pure breeds, or both, may be used. However, if only eight or twelve dogs are used, the selection of a single breed, such as the beagle, is recommended.

3.2 If numerous efficacy tests are to be performed on a single repellent, it is recommended that several breeds be used. It is desirable in selecting breeds that representative breeds from some of the following American Kennel Club classifications be chosen: hounds, working dogs, sporting dogs, terriers, toys, and nonsporting dogs. It is recommended that a minimum of eight dogs per breed be used. All eight dogs of a breed should be used in the same test.

3.3 The regulations **(1)**² as set forth under The Animal Welfare Act of 1970 (Public Laws 89-544 and 91-579) for care of experimental animals should be followed. General guidelines for care of dogs are also contained in *Basic Care of Experimental Animals* **(2)**.

3.4 Pregnant or lactating females should be excluded from testing because of large increases in food consumption and possible behavioral changes. Sexually experienced male beagles can readily detect females in estrus by olfactory cues **(3)**. Because females in proestrus or estrus may disturb the reaction of males to the test, such females should not be used as test animals and should be housed at least 400 m (437 yd) from the test arenas.

3.5 A dog can be trained to tear bags open by offering a bag containing newspaper and the test ration (Note 1) each day. When the dog learns to tear the bag open within 30 min, it is ready for use in preliminary reference test periods.

NOTE 1—It is recommended that a good grade of horsemeat dog food be mixed 60 + 40 with a pelleted dry dog food that has been ground to a coarse powder. This ration can then be shaped into spherical units that comprise one third of the “adequate diet,” and refrigerated in airtight containers until just prior to the test. At this time, one unit can be placed in each bag.

3.6 A dog’s initial response to the test may be to damage more or fewer bags than is indicative of its subsequent performances. Therefore, dogs should undergo a series of preliminary reference test periods (all bags untreated) to become settled into a definite behavior pattern before actual testing begins. If a dog fails to damage a minimum of one bag each day in preliminary reference test periods, it should undergo further training or be eliminated from testing. Dogs with previous repellent experience should also be required to meet this criterion.

¹ This test method is under the jurisdiction of ASTM Committee E-35 on Pesticides and is the direct responsibility of Subcommittee E35.17 on Vertebrate Control Agents.

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² The boldface numbers in parentheses refer to list of references at the end of this method.

4. Test Arenas and Materials

4.1 The test arenas shall be a minimum of 7 by 15 m (23 by 49 ft). This arena size is considered large enough to prevent the treated bags from perceptibly affecting the dogs in the adjacent arenas and to allow the dog the freedom to move outside the repellent's effective range. It is advisable that arenas be no wider or longer than 45 m (148 ft).

4.2 The test arenas shall not be used to house animals.

4.3 Plastic (polyethylene) garbage bags of 37.9-litre (10-gal) capacity and 25.4 to 50.8 μm (1 to 2 mils) thickness should be used. Defective bags are not acceptable.

5. Test Design

5.1 There are two types of test periods in this test method: reference test periods and repellent test periods. The reference test periods acclimate the dogs to the test layout and serve as a check on the level of tearing in the repellent test periods. The repellent test periods evaluate the repellent. In both tests, three plastic bags are located at each end of each arena. All six bags are left untreated in reference test periods. In the repellent test periods, the bags located at one end of the arena are treated, and the others are not treated (see Fig. 1).

5.2 The treated and untreated bags are not mixed at each location for two reasons: (a) this practice may cause dogs to be repelled from untreated bags; and (b) garbage bags from separate residences are not intermingled in many neighborhoods where garbage is collected.

5.3 Only one dog is tested in an arena at a time.

5.4 The amount of ration placed in each bag is calculated to give the dog one third of an adequate daily diet (see 6.1). Thus, the dog receives a free choice between treated and untreated bags and is not forced by hunger to open any of the treated bags.

5.5 Each dog should be randomly assigned (see Note 2) to a group of four dogs, and a 4 by 4 Latin square should be constructed for repellent test periods. A separate Latin square should be constructed for each group of dogs. Each square should contain four days (columns), four arenas (rows), and four dogs (denoted by Latin letters).

NOTE 2—A table of random numbers can be used for all randomly determined situations mentioned in this protocol.

5.6 The same groups of dogs should be used in constructing 4 by 4 Latin squares for reference test periods. The purpose of these reference tests is to establish whether the total number of

bags “opened” in repellent tests is lowered by the presence of the repellent.

5.7 A group of dogs shall be tested at the same time of day each day of the efficacy test. All the dogs shall be tested on each test day. If arenas are available, all the dogs should be tested simultaneously.

5.8 The following is a description of the sequence of events in an efficacy test. Begin with 4 days of reference test periods, and follow with 4 days of repellent test periods. Statistically analyze the total number of bags “opened” and “unopened” (see 8.1) during reference test periods against those in repellent test periods using a chi-square 2 by 2 contingency table. If the ratio of bags “opened” to bags “unopened” is significantly lower ($\alpha = 0.05$) in repellent test periods than in reference test periods, then the total number of bags damaged in the 4 days of reference test periods should be used for “T” in calculating repellency (see 9.1). Refer to 9.3 for a description of the use of a chi-square table for conducting this test (4).

6. Pretest Procedure

6.1 The allotment of ration to each dog should be sufficient to assure that the dog will obtain an adequate diet by feeding from three bags. Ideally, most dogs will damage three bags, yet few will damage more. Nutrient requirements may be obtained from the *National Academy of Science's Nutrient Requirements of Dogs* (5). Energy needs vary with climate, characteristics of coat, condition, activity levels, and age (5). Therefore, energy requirements should be determined for each dog using a modified version of the method used by Cowgill (6). In this method, each dog is fed the test ration ad libitum for 1 h at the same time each day. The dogs are weighed each day just prior to feeding. When a dog's weight is stable ($\pm 2\%$) on the first and last day of any 8-day period, the study is terminated. The adequate diet is determined by reducing the mean amount of food consumed during this 8-day period by 10 %. This reduction will assure complete consumption of the ration and the maintenance of good nutrition (6). The adequate diet should be determined during each of the four seasons.

6.2 Immediately prior to an efficacy test, each dog shall be given individual access to the adequate diet (see 6.1) on each of 8 consecutive days. It should be fed for 2 h each day beginning at the same time as the test periods which will be initiated. This period of acclimation will help assure a stabilized ration intake during testing. This schedule shall be maintained daily for each dog during weekends or any other

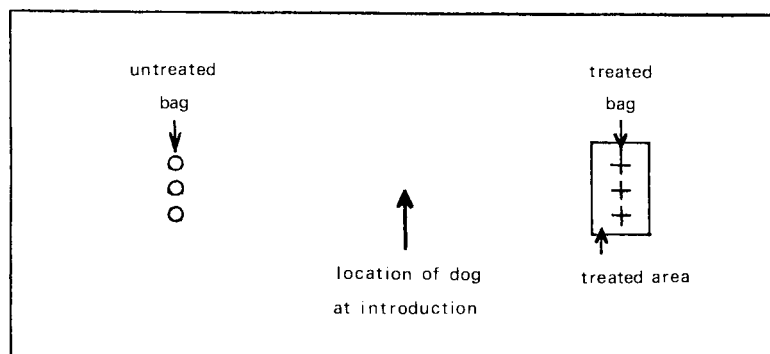


FIG. 1 Diagram of an Arena Arranged for a Repellent Test Period.

interruption in testing.

6.3 The preparation for an efficacy test may become excessive, since the dogs must be trained to tear bags (see 3.5), the adequate diet must be determined by ad libitum feeding (see 6.1), the dogs must be acclimated to the adequate diet to assure a stabilized intake during testing (see 6.2), and the dogs must be exposed to preliminary reference tests (see 3.6). Hence, it is acceptable to streamline this procedure in the following ways:

6.3.1 The determination of an adequate diet can be done concurrently with training to tear open bags. A garbage bag containing a small amount of test diet must be presented to each dog just prior to the 2-h feeding period. The amount consumed by each dog from this bag must be included when calculating the amount of test ration eaten that day.

6.3.2 The ad libitum feeding to determine the adequate diet (see 6.1) can be substituted for the first 4 days of the procedure for acclimating the dogs to the adequate diet (see 6.2), or the preliminary reference tests (see 3.6) can be substituted for the first 4 days of the procedure that acclimates the dogs to the adequate diet.

6.4 Feces should be removed from the arenas prior to the first reference test period and at least every fourth day thereafter.

7. Test Procedure

7.1 Provide any shade or water needed in the center of each arena.

7.2 Prepare each treated bag in the following manner:

7.2.1 Fold a full double sheet (four printed pages) of newspaper once and place it, uncrumpled, into the bottom of the bag.

7.2.2 Crumple the edges of a full double sheet of newspaper to fit the diameter of the bag and force it into the bag, thus forming a layer over the first sheet.

7.2.3 Add two more sheets as described in 7.2.2.

7.2.4 If consistent with label directions, apply the repellent (7.4-7.7) inside the bag.

7.2.5 Close the bags with a twist tie just above the newspaper, immediately after the repellent is applied inside.

7.2.6 If consistent with label directions, apply the repellent on the outside of the bag (7.4-7.7).

7.2.7 Store the bags as described in 7.6.

7.2.8 Just prior to the test, assign three treated bags to each dog, reopen each bag, and place one third of the dog's adequate diet on top of the bottom sheet of newspaper (see 7.11 to 7.12).

7.2.9 If appropriate, apply a small additional quantity of repellent inside the bag (see 7.12).

7.2.10 Close the bag by tying the excess portion of the bag in a knot. The steps in 7.2.8, 7.2.9, and 7.2.10 should be completed within a few minutes to prevent excessive loss of repellent from within.

7.3 Prepare each untreated bag in the following manner:

7.3.1 Follow the procedure in 7.2.1-7.2.3. These steps may be performed at anytime prior to testing.

7.3.2 Just prior to the test period, assign three untreated bags to each dog, insert one third of the dog's adequate diet between the first (bottom sheet) and second sheet of newspaper (see 7.11 to 7.12) and close the bag by tying the excess portion of the bag in a knot.

7.4 Spray the bags to be treated and then store for the maximum length of time (prior to testing) for which efficacy is claimed. It is permissible to run additional tests utilizing shorter storage periods, but each storage period should be subjected to a complete efficacy test. The longest storage period should be evaluated first, the next longest, second and so on until all storage periods have been evaluated.

7.5 If the repellent is applied outdoors, protect the area surrounding the bags being treated from the wind to reduce loss of repellent during application.

7.6 Immediately suspend the treated bags by the neck (portion above the knot) of the bag above the floor of the storage area and a minimum of 15 cm (5.9 in.) apart. The bags must be in a relatively open-air situation, yet protected from precipitation. This method of storage should allow the repellent to vaporize at a normal rate. Report the specific conditions of storage.

7.7 Base the amount of product applied to the bags on label directions. If a quantity of repellent is applied both inside and outside the bags, report both quantities for each bag. The amount applied to each bag should not vary more than $\pm 10\%$ of the predetermined dose.

7.8 Meticulous care should be taken to prevent repellent contamination of untreated bags. Therefore, handle the treated and untreated bags with separate pairs of rubberized or plastic gloves.

7.9 Store the treated and untreated bags in separate locations. The untreated bags may be stored inside and need not be separated from each other or suspended above the floor.

7.10 Take appropriate measures to ensure that food in all bags is of equal freshness. If the test ration is refrigerated overnight subsequent to its preparation, the length of time it is refrigerated must be approximately uniform throughout the efficacy test (± 3 h).

7.11 Do not place the ration in the bags until just prior to the test period.

7.12 Because vaporized repellent may escape from treated bags during the insertion of food, a small amount (one third of the original dose) may be applied inside the bag. Place the food underneath the top three sheets of newspaper; consequently, if the worker is careful, the repellent will not be applied directly to it.

7.13 Place three bags at each end of each arena. It is recommended that metal stakes be used to hold the bags in place by forcing the stake through the knot in the excess part of the bag and into the ground. This will help prevent the dogs and wind from moving the bags.

7.14 Without adequate protection, the ground surrounding the treated bags will become contaminated with repellents, thus possibly, biasing subsequent tests. Therefore, stake all test bags down on plastic sheets (minimum of 1.2 m by 1.8 m by 125 μ m or 4 ft by 6 ft by 4 mils) large enough to prevent the dogs from shifting the free end of the bags off the plastic. Secure the corners of these sheets with stakes. From the onset, assign each plastic sheet to either treated or untreated bags and then use only for the assigned type of bags throughout the efficacy test. Following each test, immediately remove any rain that collects on the plastic sheets during the test, allowing the least possible