Designation: E 565 – 95

Standard Test Method for Efficacy of a Single-Dose Acute Rodenticide Under Laboratory Conditions for Commensal Rodents[1]

This standard is issued under the fixed designation E 565; 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 measures the efficacy of ready-to-use dry bait formulations prepared with single-dose acute rodenticides for the control of commensal rodents, including the Norway rat (*Rattus norvegicus*), roof rat (*R. rattus*), and the house mouse (*Mus musculus*). This test method may also be applicable to some other species of rodents having similar behavioral patterns, physiology, and feeding preferences.

1.2 This test method is for use in developing efficacy data.

1.3 This test method also provides manufacturers, formulators, and other with a test procedure for monitoring product quality.

1.4 This test method, within limits, enables users of large quantities of commercial rodenticides to evaluate the efficacy of specific lots, or compare the efficacy of different formulations and different toxicants, or both.

1.5 This test method reduces many variables, thereby permitting replication or duplication of tests with reasonable accuracy.

1.6 This test method is not intended to be so restrictive that it will inhibit incentives towards the development of safer or more effective compounds or innovative approaches to bait formulation.

1.6.1 When justified by sound biological data or logical conclusions based on sound data, reasonable variations to some items in the test protocol may be made.

1.7 To ensure the quality and reliability of data developed using this test method, good laboratory practices should be followed (see 4.1).

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

1.9 This test method represents the combined, several-year efforts of more than 70 scientist, users, and laboratory specialists from several countries and has not been superseded by better documentation (1995); therefore, it is the view of the committee that it be continued for the reference benefit of any new concerns in the United States or abroad.

1.9.1 Attention is directed to related ASTM documents, Test Methods E 1163 and E 1372, that deal with similar situations; however, were developed for different purposes.

1.10 *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 Documents

2.1 *ASTM Standards:*

E 1163 Test Method for Estimating Acute Oral Toxicity in Rats[2]

E 1372 Test Method for Conducting A90-Day Oral Toxicity Study in Rats[2]

3. Summary of Test Method

3.1 This test method is based upon the principle of a free-feeding choice between a toxic bait formulation and a nontoxic, challenge diet offered to a selected group of test rodents.

3.2 The test animals are offered the toxic bait formulation and challenge diet formulation for a specified period of time and the efficacy is measured by the percent mortality achieved.

4. Animal Facilities

4.1 No precise physical requirements for animal accomodations are set forth. However, the animal facility shall (*a*) meet the established guidelines suggested by the Institute of Laboratory Animal Resources, or (*b*) be approved by such organizations as the American Association of Accreditation of Laboratory Animal Care (AAALAC).

4.2 Maintain temperatures within the range from 18 to 29°C (65 to 85°F) and record daily using a maximum-minimum thermometer, hygrothermograph, or other suitable equipment.

4.3 Strong air currents from heaters or air conditions shall not blow directly onto test or reference animals.

4.4 The normal recommended range of humidity for laboratory rats and mice is 40 to 70 %.

4.5 Natural lighting (through windows) is adequate. However, artificial lighting is acceptable and may be preferred when controlled by time clocks. Artificial lighting may be correlated

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

reasonably well to the natural day and night periods where the tests are being conducted, or may be set to provide a light regime anywhere from 12 to 16 h is recommended (see 1.6.1).

4.6 Artificial lighting may be of the incandescent or fluorescent types.

4.7 The total reversing of the natural photoperiods of the test animals by timed lighting is not recommended (see 1.6.1).

4.8 Light intensity is not specified. However, light intensity shall be relatively uniform for all test cages, whether natural or artificial lighting is used. In some instances, natural lighting may have to be supplemented with artificial lighting to achieve this. Light levels should not exceed 323 1× (30 ft-candles) about 1.0 m (3.3 ft) above the floor. This will reduce retinal lesions in albino rats and should be sufficient for performance of routine animal care.

4.9 Animals on test shall not be subjected to undue or unnecessary stress from noise or human activities (that is, movement). Human activity within the animal test room shall be restricted to performing test protocols (see 13.7.1).

5. Housing

5.1 The test and reference rats or mice shall be maintained in all metal-cages designed to hold laboratory rats or mice.

5.1.1 Cages shall have a minimum floor space of 387 cm^2(60 $in.^2$) for rats and 258 cm^2(40 $in.^2$) for mice. Maximum cage sizes shall be no more than three times the minimum floor space.

5.2 Preferably, these cages should be suspended in racks.

5.3 Cages shall have a wire mesh or screen bottom to permit feces and urine to pass through.

5.4 A tray or other arrangement under the cages shall be used to permit recover of spilled foods. Each spilled food must be separately weighed and these weights shall be added to the unconsumed bait or challenge diet to determine consumption.

5.4.1 Damp spilled food shall be dried to approximately its original moisture content before weighing.

5.5 Water shall be available ad libitum. Glass water bottles equipped with stainless steel or glass sipper tubes are recommended. Gravity-fed automatic or open-cut waterers are not recommended.

6. Test Animals

6.1 Ready-to-use bait formulations containing single-dose acute toxicants shall be tested on all species for which they are to be used.

6.2 Laboratory (domesticated) strains of albino rats and mice may be valuable for preliminary screening or for finding the range of appropriate toxicant concentrations, bait materials, and other useful additives, but they cannot be substituted for the wild strains in the final test.

6.3 Wild rats and mice shall be used in the final testing of a bait (see 7.3).

6.3.1 Some correlation established between the laboratory and the wild rodents of the same species may prove helpful for future tests with a particular bait formulation or rodenticide.

6.4 Where laboratory rats are used, Wistar or Sprague-Dawley derived albino or Long Evans (hooded) rats have been selected as the strains of choice for the sake of uniformity.

6.5 Where laboratory mice are used an recognized commercially available strain of albino mice is acceptable.

7. Source of Test Animals

7.1 For each test, laboratory rats or mice may be reared by the testing laboratory or purchased from the same commercial supplier at the same time (as one lot).

7.2 In all instances, the test rodents shall be selected on a random basis within the scope of the other limitations placed on the condition of the test animals described herein (see Section 8).

7.3 Wild stock of Norway rats, roof rats, or house mice may be live-trapped from the wild, reared in outdoor colonies, or reared under laboratory conditions that will permit them to retain much of their natural physiological and behavioral characteristics. Breeding stock used for rearing wild rodents shall not be selected for docile qualities or other characteristics that significantly alter their natural wild tendencies.

8. Condition of Animals

8.1 All animals shall be in apparent good health with no obvious external open wounds, scars, or evidence of rhinitis.

8.2 Females should not be pregnant (see 9.3).

8.3 Test and reference animals shall not knowingly have been previously exposed to any pesticide (such as rodenticide) except as follows:

8.3.1 Animals previously anesthetized or medicated either orally or by injection shall not be used for test purposes within the 7-day period following the last treatment.

8.3.2 Ectoparasite control with appropriate concentrations of sevin, malathion, or pyrethrin dusts is not considered medication and shall be applied externally to both test and reference wild animals on Day 1 of confinement.

9. Pretest Conditioning

9.1 Test and reference animals shall be caged individually in cages of the same type or kind that are to be used in the test.

9.2 Laboratory strains of rodents obtained from a commercial supplier or from other sources shall be conditioned (acclimated) to the test laboratory for a minimum of 3 days before the start of a test regime.

9.3 Wild-captured rodents or wild species from mixed sex colonies may be separated by sex and held caged in groups or individually for a period of time exceeding the gestation period to ensure that no females are pregnant.

9.4 As a minimum, the last 7 days of the holding period for wild rodents shall be under laboratory conditions (that is, temperature, humidity, and lighting) comparable to those of the testing animal room, if not actually in the testing room.

9.5 All rodents shall receive a nutritionally balanced commercial rodent diet and water ad libitum during the pretest period.

9.6 Food consumption shall be measured and recorded during this period. Water consumption could be monitored, if desired. Rodents failing to feed or drink normally should be removed from the test or reference groups, or both.

10. Number of Test Animals

10.1 Twenty test rodents (10 male, 10 female) shall be the minimum number to be used for conducting a single acceptable laboratory efficacy test.