



Designation: E 593 – 95

## Standard Test Method for Efficacy of a Multiple-Dose Rodenticide Under Laboratory Conditions<sup>1</sup>

This standard is issued under the fixed designation E 593; 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 laboratory test method is designed to measure efficacy of ready-to-use dry bait formulations prepared with multiple-dose (anticoagulant) lethal rodenticides for the control of commensal rodents, including the Norway rat (*Rattus norvegicus*), roof rat (*R. rattus*), and the house mouse (*Mus musculus*), but may also be applicable to some other species of rodents having behavioral patterns, physiology, and feeding preferences similar to those mentioned.

1.2 This test method is for the use of those wishing to develop efficacy data.

1.3 This test method may also prove valuable to manufacturers, or formulators, or both and others by providing a test procedure for monitoring product quality.

1.4 This method, within limits, will enable users of large quantities of commercial rodenticides to evaluate the efficacy of specific lots, and either evaluate or compare the efficacy of different formulations, or different toxicants, or any combination thereof.

1.5 The details of this test method are fairly specific in order to reduce many variables and make it possible to replicate or duplicate 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, provided such change does not give an unrealistic efficacy advantage to the test bait.

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

1.8 This test method represents the combined, several-years efforts, of more than 70 scientists, users, and laboratory specialists from several countries and has not been superseded by better documentation (1995); therefore, it is the view of the committee that it be continued for the reference benefit of any new concerns in the United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 *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 1372 Test Method for Conducting a 90-Day Oral Toxicity Study in Rats<sup>2</sup>

### 3. Summary of Test Method

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

3.2 The test animals shall be exposed to the toxic formulation and challenge diet for a specified period of time and the efficacy measured by the percent mortality achieved.

### 4. Test Animals

4.1 Ready-to-use bait formulations containing multiple-dose (anticoagulant) lethal toxicants shall be tested on all species for which they are to be used.

4.2 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.

4.3 Wild rodents are to be used in the final testing of a bait (6.3).

4.3.1 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.

4.4 Where laboratory rats are used, Wistar or Sprague-Dawley derived albino rats have been selected as the strains of choice for the sake of uniformity.

4.5 Where laboratory mice are used, any recognized commercially available strain of albino mice is acceptable.

### 5. Reference Animals

5.1 The term “reference animals” is used to denote a group

<sup>1</sup> 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.

Current edition approved Nov. 10, 1995. Published January 1996. Originally published as E 593 – 77. Last previous edition E 593 – 77 (1989).

<sup>2</sup> *Annual Book of ASTM Standards*, Vol 11.05.

of animals maintained very similarly to the test animals for the purpose of determining mortality due to illness, injury in handling or shipment, or other factors unrelated to the toxicant. They are not sufficiently maintained comparable to the test animals to be considered control animals in the true sense of the definition.

5.2 A minimum of 20 animals of the same species (sexes equal) of comparable physical condition and weight as the test group shall be maintained as a reference group and will serve to monitor mortality which might occur in a group not receiving the toxicant (6.4).

5.3 The reference animals shall be caged individually in cages of the same or similar type and size as those used for the actual test group.

5.4 The reference animals may be placed on the same or a separate cage rack from the test animals and housed in the same animal room, or they may be maintained in another animal room under the same environmental conditions (that is, temperature, humidity, light, etc.) as the test animals.

5.5 The reference group shall be maintained concurrently with the test. One group of reference rodents may be used for one or more test groups in laboratories equipped to conduct several tests at one time. This assumes all tests are with the same species and the rodents obtained from the same source.

5.6 Reference animals shall be maintained on a nutritionally balanced commercial rodent diet with water available *ad libitum*.

## 6. Source of Test Animals

6.1 Laboratory strains of albino rats and mice may be reared by the testing laboratory or purchased from commercial suppliers.

6.2 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.

6.3 Wild stock of Norway or roof rats, or house mice, may be live-trapped from the wild, reared in outdoor colonies, or reared under laboratory conditions which, hopefully, 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 drastically alter their natural wild tendencies.

## 7. Condition of Animals

7.1 All animals shall be in apparent good health with no obvious external open wounds or fresh scars as determined by the presence of scabs.

7.2 Preferably, females should not be pregnant.

7.3 Test and reference animals shall not knowingly have been previously exposed to any pesticide (that is, rodenticide, etc.). (For exceptions, see 7.3.1 and 7.3.2.)

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

7.3.2 Ectoparasite control with appropriate concentrations of Sevin, malathion, or pyrethrum dusts is not considered medication and is permissible if applied externally to both test

and reference animals not less than 3 days prior to the start of the test.

## 8. Weight of Test Animals

8.1 Pretest weights of the animals shall be taken no earlier than 7 days prior to the start of the test.

8.2 Rodents at the initiation of the test should fall within the weight class described in Table 1. This may facilitate data comparisons by reducing in-group variation. However, specific test objectives may require other weight group patterns.

8.3 As a general rule, the males of most rodent species become heavier than females; therefore, the average weight of the males used in a test may exceed that of the females.

8.4 To avoid biasing a test where one sex or age group (that is, weight group) may be more susceptible than another to a specific toxicant on a milligram per kilogram basis, a maximum acceptable difference between the average weight of the females and males for each test has been provided in Table 1. This difference generally should not exceed 30 % of the minimum acceptable weight.

## 9. Number of Test Animals

9.1 Twenty test rodents (ten male and ten female) are the minimum number to be used for conducting a single acceptable laboratory efficacy test.

9.2 Although optional, additional laboratory tests may be conducted to provide a statistically more sound base of efficacy.

9.3 Ten rodents may prove adequate for manufacturers or formulators monitoring for quality control or for range-finding in screening experimental compounds.

9.4 Twenty animals (sexes equal) shall be used for the reference group that is maintained for each test group.

9.5 The reference group(s) may be used as a test group in subsequent tests, providing they have not been exposed to the challenge diet and they meet initial specified criteria.

## 10. Test Cages

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

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

10.2 Preferably, these cages will be suspended in racks.

10.3 Cages shall have a wire mesh or screen bottom to permit droppings to pass through.

TABLE 1 Weight Classes for Test Rodents

| Test Animal      | Weight, g |     | Acceptable Difference in Average Weights Between Sexes (8.4) max, g |
|------------------|-----------|-----|---------------------------------------------------------------------|
|                  | min       | max |                                                                     |
| Laboratory mice  | 15        | 30  | 5                                                                   |
| Wild house mice  | 10        | 25  | 3                                                                   |
| Laboratory rats  | 200       | 400 | 50                                                                  |
| Wild Norway rats | 150       | 400 | 50                                                                  |
| Wild roof rats   | 100       | 200 | 33                                                                  |