

STANDARD HOUSE MOUSE ACUTE DRY BAIT

LABORATORY TEST METHOD

OPP Designation: 1.210

Revision No. 9

Revised:

1-1-75

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1. Scope

1.1 This method is designed to determine effectiveness of ready-to-use acute dry bait rodenticide products used for mouse control. The "one-day" version of this test also may be used for establishing "single-feeding" claims for "second-generation" anticoagulants. This method is applicable in connection with registration and enforcement procedures under the Federal Insecticide, Fungicide, and Rodenticide Act, as amended. The conduct of, reporting of, and recordkeeping for studies conducted according to this method must conform with the U.S. Environmental Protection Agency's "Good Laboratory Practice Standards" (40 CFR, Part 160).

2. Test Animals

2.1 All mice used in this test shall be house mice (*Mus musculus*), wild-type (wild-caught or from a wild mouse colony) or albinos (Swiss-Webster strain preferred). They shall be healthy, active, sexually mature, and fall within the following weight classes in grams within seven days prior to start of test:

	<u>Minimum</u>	<u>Maximum</u>	<u>Maximum acceptable differences in average weights between sexes</u>
Laboratory mice	15	35	5
Wild-type house mice	10	25	3

Animals must be weighed within three days of the start of the baiting period, at the end of the follow-up period (survivors only), or at the time of death.

2.2 Ectoparasite control with registered insecticide (or acaricide) products labeled for use on laboratory mice is permissible if applied externally to both test and control animals not less than seven days prior to start of test, if applied at rates not exceeding those permitted by the registered label, and if the pesticide used is not known or believed to potentiate the effects of the rodenticide used as the toxicant in this laboratory test.

3. Apparatus

3.1 Mice may be housed individually or in single-sex groups of 5 or 10 mice per group. The mice should be placed in solid-bottom, all-metal cages designed to hold laboratory mice or in specially constructed or modified cages suitable for maintaining house mice for this type of study. If mice are housed singly, cages must have a bottom surface area of at least 500 cm<sup>2</sup> (0.538 ft<sup>2</sup>). If mice are group-caged, enclosures must have a bottom surface area of at least 2000 cm<sup>2</sup> (2.15 ft<sup>2</sup>).

3.2 If subjects are group-caged, provide shelters in both test and control cages. Empty soup or beverage cans, with one end removed, slightly flattened to prevent rolling, have been found satisfactory for this purpose. Use at least four shelters if 10-mouse subgroupings are used. Use at least three shelters for subgroupings of five mice.

3.3 Metal or ceramic feeders, designed so that test mice may not nestle or wallow in diet, should be used.

4. Pretest Holding Conditions

4.1 All mice used in this test method must be held, sexes separate, for observation in the laboratory for a period of at least one and not more than four weeks prior to testing, the last seven days of which shall be under laboratory conditions (i.e., temperature, humidity, lighting, etc.) comparable to those of the animal testing room if not actually in the testing room. The test animals must not be fasted prior to testing. Water and a commercial mouse diet must be available to them at all times. Do not use the standard EPA rat and mouse challenge diet for pretest feeding.

5. Holding and Test Conditions

- 5.1 Temperature 20 to 25° C. Strong air currents from heaters or air conditioners shall not blow directly onto test animals.
- Relative humidity 50 to 55%.
- Light 12 h artificial light per day, not to exceed 2153 lx (200 ft candles) at cage location. Total reversing of the natural photoperiods of the test animals by timed lighting is not recommended.

5.2 The standard EPA rat and mouse challenge diet shall be composed of:

- Cornmeal (whole yellow ground corn) 65% by weight
- Rolled oat groats (ground) 25% by weight
- Sugar (10X powdered or confectioners, 95% + purity) 5% by weight
- Corn oil (95% + purity) 5% by weight

Combine dry ingredients together, add oil, and mix thoroughly. Be certain that the mixing utensils are clean of contamination before preparing diet.

5.2.1 The whole (not degerminated) yellow ground corn shall be from the most recently available crop and shall be reasonably freshly ground. Seventy-five percent (+ 5%) shall pass through a No. 10 screen (10 meshes to the inch or 2.54 cm) and 50% (+ 10%) shall be retained by a No. 20 screen (20 meshes to the inch). The remainder may be either larger or smaller than the screens mentioned.

5.2.2 The oats shall be steam-rolled oat groats (oat seed with the hulls removed) coarsely ground after the rolling process. Seventy-five percent (+ 5%) of the ground oats shall pass through a No. 5 screen (5 meshes to the inch) and 50% (+ 10%) shall be retained by a No. 20 screen (20 meshes to the inch). The remainder may be either larger or smaller than the screens mentioned.

5.2.3 The corn oil shall be of the type available as cooking oil, undiluted with other oils, and shall not be rancid.

5.2.4 The standard EPA rat and mouse challenge diet may be stored under refrigeration if it is to be used within three days of preparation. If it is to be held for longer periods the diet shall be packaged in plastic containers [2.2 to 4.5 kg (5 to 10 lb) per container], tightly closed or sealed, and maintained at  $-18^{\circ}$  C or below until it is to be used. It shall be at room temperature when offered to test or control animals. Challenge diets shall not be prepared and stored for longer than six months.

### Procedures

6.1 A test group consists of a minimum of 20 mice (10 males, 10 females), individually-caged or group-caged in single-sex groups of 5 or 10 animals each. For each test or series of tests conducted at the same time on the same species, include one untreated control group of 20 mice (10 males, 10 females), caged in the same manner as the group(s) to be exposed to toxic bait. Acclimate all animals to test conditions for three days prior to exposure to toxicant, immediately following pretest holding period (4.1).

6.2 Water must be available to each animal at all times. Glass water bottles equipped with ball-type watering tubes are recommended. Gravity fed automatic or open-cup type waterers are not recommended.

6.3 The rodenticide and the standard EPA rat and mouse challenge diet must be offered to test mice in separate containers on opposite sides of the front of the cage. If mice are caged individually, one container of each food must be used. Containers must be identical in size and type and must be placed equidistant from the sides of the cage and equidistant from the rodents' point of access to water. If mice are group-caged, at least one container must be used per diet for each five animals in the cage. If one container is used per diet, the containers must be equidistant from enclosure walls and the water source(s). If two or more containers are used per diet per enclosure, containers shall be presented in pairs (one of bait and one of challenge diet). Container pairs shall be deployed such that proximity to walls, shelter, or water sources dictates no clear advantage to either container position. The food offered in each container should be equal and consistent throughout the test and must be at least 10 grams per container per animal per day. The control group is offered only the EPA rat and mouse challenge diet, which shall be presented in amounts and numbers of containers equivalent to those used for the test group. The gross weight of each container and its contained food are determined daily and, for a "two-day" test, returned to starting weight by addition of the given food. If food becomes fouled by urine or feces, replace food in each container. Record each day the quantity of each food consumed by each rat during the preceding 24 h. Weighing accuracy must be at least to the nearest 0.5 gram for group-caged

tests. Individual caging may not be used unless consumption can be determined to the nearest 0.1 gram. Spilled food must be recovered and weighed to establish exact food consumption data. Where the food spillage is damp it shall be dried to approximately its original moisture content before weighing.

6.4 In "two-day" tests, reverse the position of the bait and standard EPA rat and mouse challenge diet containers in the cage every 24 h to offset possible feeding position preferences of the mice. In "one-day" tests with individually-caged mice, place container holding bait to the left for half of the toxic-bait-group subjects and to the right for the remaining toxic-bait-group subjects. Container positions may be reversed after 12 hours. The test mice must have a free choice between treated and untreated food.

6.5 Animals on test should not be subjected to undue or unnecessary stress from noise or human activities (i.e., movement). Human activity within the animal test room shall be minimal.

## 7. Test Period

7.1 Maintain test period for one or two days for acute rodenticide baits. If "single-feeding" claims for anticoagulant baits are being tested, a one-day bait-exposure period must be used.

7.2 Remove dead mice daily, or more frequently as observed.

7.3 Remove toxicant-treated food at the end of the test period, leaving and maintaining the untreated food.

7.4 More than a 10% mortality in the control group negates the test, even if a 100% mortality had been achieved in the test group.

7.5 This laboratory efficacy test should be replicated at least once.

## 8. Test Period Follow-Up

8.1 Maintain observation on surviving toxic-bait and control group mice for a minimum of five days following test period. For "single-feeding" tests of anticoagulant baits, the post-exposure observation period must be at least 10 days.

8.2 Continue feeding EPA rat and mouse challenge diet and record amounts consumed daily.

8.3 Describe unusual activities of test and control mice in report of test and posttest periods.

## 9. Calculation and Evaluation of Results

9.1 Record date, weight, and sex of each mouse dying during the test and of survivors in both the test and control groups, and amounts of treated and untreated food consumed during the test and posttest periods. Retain original laboratory test records for future reference. Report all data collected,

including initial and final weights of test subjects. Include copies of all "raw" data sheets as well as typed numerical summaries of test results.

9.2 The product is considered satisfactory if at least 90% of subjects in the test group die during the bait-exposure and post-exposure observation periods, and if no more than 10% of control-group subjects die during the study.

9.3 The test report must include reports of chemical analyses of the test bait and the EPA challenge diet for the active ingredient claimed to be in the test bait. These analyses must be conducted using methods that are acceptable to the U. S. Environmental Protection Agency.