Revision No. 9

# STANDARD PEROMYSCUS SPECIES ANTICOAGULANT DRY BAIT LABORATORY TEST METHOD

Revised 9-1-76, 2-2-76, 11-1-80, 7-4-91

OPP Designation: 1.216 (1-1-75)

## 1. Scope

1.1 This method is designed to determine effectiveness of ready-to-use anticoagulant dry bait rodenticide products used for control of Peromyscus spp. (e.g., white-footed or deer mice). It 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 Peromyscus spp. of a species claimed or to be claimed on the product label. Mice shall be either wild-caught or from an outbred colony of captive animals. Mice shall be healthy, active, sexually mature, and weight 15-40 g. The maximum acceptable difference in average weights between the sexes is 5 g.

2.2 Ectoparasite control with registered insecticide (or acaricide) products labeled for use on laboratory rodents 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 in the product being tested.

## 3. Apparatus

3.1 The mice may be housed individually or in single-sex groups of 5 or 10 mice per group. 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 Peromyscus spp. mice for this type of study. If mice are housed singly, cages must have a bottom surface area of at least 500 $cm^2$ (0.538 $ft^2$). If mice are group-caged, each enclosure must have a bottom surface area of at least 2,000 $cm^2$ (2.15 $ft^2$).

3.2 If subjects are group-caged, provide shelters in both test- and control-group cages. Empty soup or beverage cans with one end removed, slightly flattened to prevent rolling, have been found satisfactory for this purpose. Use two cans for every five mice in the enclosure.

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 field rodent 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 field rodent challenge diet shall be composed of:

| | |
|---|---|
| Commercial rodent laboratory diet | 50% by weight |
| Rolled oat groats (ground) | 50% by weight |

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

5.2.1 The commercial rodent laboratory diet shall be ground to conform to certain specifications. Seventy-five percent (± 5 percent) of the ground diet shall be small enough to pass through a No. 10 screen (10 meshes to the inch) and 50 percent (± 10 percent) large enough to be retained by a No. 20 screen (20 meshes to the inch). The remainder may either be 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 percent) of the ground oats shall pass through a No. 5 screen (5 meshes to the inch) and 50 percent (± 10 percent) 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 standard EPA field rodent 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° 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.

6. Procedure

6.1 A test group consists of a minimum of 20 mice (10 males, 10 females), individually-caged or caged in subgroups of 5 or 10 animals of the same sex. For each test or series of similar tests conducted at the same time on the same species, include one untreated control test group of 20 mice (10 males, 10 females) caged in the same manner as the group(s) exposed to the 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 Ready-to-use rodenticide-treated food and the standard EPA field rodent challenge diet must be each offered to test mice in separate containers (3.3) on opposite sides of the front of the cage to provide at least 10 grams per diet per animal per day. If mice are caged individually, one container of each food must be used. The two containers must be identical in type and size and must be placed equidistant from the rodent's point of access to water. If mice are group-caged, at least one container must be used per diet for every 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 field rodent 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. The control group is offered only the EPA field rodent 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 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 during the preceding 24 h. For individually-caged subjects, weighing accuracy must be at least to the nearest 0.1 gram. For group-caged subjects, weighing accuracy should be to the nearest 0.1 gram and must be at least to the nearest 0.5 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 Reverse the position of the bait and standard EPA field rodent challenge diet containers in the cages every 24 h to offset possible feeding position preferences of the mice. 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 15 days, even if all mice exposed to toxic bait die in less than 15 days.

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

7.3 Remove, toxicant-treated food at the end of the 15-day 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 mice in toxic-bait and control groups for a minimum of five days following test period.

8.2 Continue feeding EPA field rodent 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 amount 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 test-group animals die during the bait-exposure and post-exposure observation periods, if the toxic bait comprises at least 33% of the total food consumption by the test group during the bait-exposure period, and if no more than 10% of the control group subjects die during the study.

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