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STANDARD PEROMYSCUS SPECIES ANTICOAGULANT TECHNICAL AND CONCENTRATED DRY BAIT LABORATORY TEST METHOD

OPP Designation: 1.223 (5-6-76)

1. Scope

1.1 This method is designed to determine effectiveness of ready-to-use dry bait anticoagulant technical and concentrated 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 of a species claimed or to be claimed on the product label. Subjects shall be healthy, sexually mature, and shall weigh 15-40 g. The maximum acceptable difference in average weights between 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 the start of the 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 anticoagulant rodenticides.

3. Apparatus

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

3.2 If subjects are group-caged, provide shelters in both the 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 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. During the last seven days of this period, mice 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 OPP 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 OPP field rodent challenge diet shall be composed of:

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

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

5.2.1 The commercial laboratory rodent diet shall be ground to conform to certain specifications. Seventy-five percent (± 5%) of the ground diet shall pass through a No. 10 screen (10 meshes to the inch) and 50% (± 10%) shall be large enough to 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 standard OPP 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. Challenge diet shall be at room temperature when offered to test or control animals. Challenge diet shall not be prepared and stored for longer than six months.

5.3 Test baits made from technical or concentrated products should be formulated by adding the toxicant to a mixture of ingredients suitable for comprising the "non-toxic" portion of baits used to control Peromyscus spp. Baits meeting criteria in tests run according to this method should be prescribed on end-use product labels which bear bait mixing and application directions. The bait recipe provided in 5.3.1. may be used in tests with Peromyscus spp. if the concentrate product tested is to be mixed with "non-toxic" materials at a ratio of 1 part concentrate to 19 parts other bait materials. If the product to be tested contains the active ingredient at a concentration different from 20 times the desired bait concentration, adjust the relative amounts of oats and active ingredient source (technical or concentrate) appropriately. Concentrations of active ingredients in test baits should conform to levels previously accepted for dry baits made from the same active ingredient.

5.3.1

Rolled oat groats (ground)	60% by weight
Cornmeal (whole yellow ground corn)	20% by weight
Sugar (10X powdered or confectioners, 95%+ purity)	10% by weight
Corn oil (95%+ purity)	5% by weight
Anticoagulant concentrate	5% by weight

5.3.2 When technical anticoagulants are being tested with baits made according to the recipes prescribed above, dilute and mix toxicant with cornstarch to prepare an intermediate concentrate of the appropriate active ingredient strength for a 1:19 dilution with the remaining bait materials.

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

5.3.4 The oats shall comply to the specifications given in subsections 5.2.2 OPP field rodent challenge diet.

5.3.5 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%) of the cornmeal shall pass through a No. 10 screen (10 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.

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

5.3.7 When chlorophacinone or any other anticoagulant normally used at 0.005% is available in a mineral oil solution at a concentration of 0.28%, mix 20 cc of concentrated oil per kilogram of bait. Delete the oils and increase the oats by 8 percent.

5.3.8. Technical grade toxicants normally used at a 0.005% concentration should be premixed at the rate of 0.1 g of technical to 99.9 g of cornstarch, or other appropriate diluent, using the dry dilution method. These newly formed concentrates can then be further mixed by combining them with the baits such as the one described in 5.3.1 at the rate recommended on the label (usually 1:19).

6. Procedure

6.1 A test group consists of a minimum of 20 mice (10 males, 10 females), individually-caged or group-caged in single-sex subgroups of 5 or 10 animals each. For each test or series of tests conducted at the same time, 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-treated food and the standard OPP field rodent challenge diet must be offered to test mice in separate containers (3.2). If mice are caged individually, one container of each food must be used. Containers must be identical in type and size and must be placed on opposite sides of the front of the cage, equidistant from the sides of the cage, and 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 the enclosure walls and the water source(s). If two or more containers are used per diet, containers shall be presented in pairs (one of test 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 15 grams per container, per animal, per day. The control group is offered only the OPP 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 the 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. Weighing accuracy must be at least to the nearest 0.5 gram for tests with group-caged subjects. Individual caging may not be used unless consumption can be established to the nearest 0.1 gram. Spilled food shall be receovered and weighed to establish exact food consumption data. Where 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 OPP field rodent challenge diet containers in the cage 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 bait-exposure 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 test group and control group mice for a minimum of five days following test period.

8.2 Continue feeding OPP field rodent challenge diet and recording 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 the date, weight, and sex of each mouse dying during the test and of survivors in both the test and control groups. Record the amounts of treated and untreated food consumed during the test and posttest periods. Report all data collected, including initial and final weights of test and control group subjects. Include copies of all "raw" data sheets as well as typed numerical summaries of test results. Indicate the composition of the bait prepared for the test, including the amount of each ingredient that was used and the percent of the bait comprised by each ingredient.

9.2 The product is considered to have performed satisfactorily if at least 33% of the food consumed by the test animals was the bait treated with the toxicant, if at least 90% of the test group animals die during the 20-day test, and if no more than 10% of the control group subjects die during the 20-day test.

9.3 The test report must include reports of chemical analyses of the technical or concentrate product used, the test bait made from the technical or concentrate product used, and the challenge diet for the active ingredient claimed to be in the technical or concentrate product. These tests must be conducted according to methods acceptable to the U.S. Environmental Protection Agency.