STANDARD NORWAY RAT AND ROOF RAT ACUTE PLACEPACK DRY BAIT LABORATORY TEST METHOD
OPP Designation: 1.219 (9-1-76)

Revised:
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1. Scope

1.1 This method is designed to determine effectiveness of acute dry bait rodenticide products used in placepacks for control of Norway rats and roof rats. This method is applicable 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 this study must conform with the U. S. Environmental Protection Agency's "Good Laboratory Practice Standards" (40 CFR, Part 160).

1.2 Tests run according to this method must be supplemented by a test run according to OPP 1.209, Standard Norway Rat and Roof Rat Acute Dry Bait Laboratory Test Method, in which the toxic bait is removed from the package and tested separately.

2. Definition

2.1 A placepack is a small amount of rodenticide bait wrapped in a protective covering and sold as a convenient method of placing baits; the expectation being that rodents will chew through the covering to consume the bait.

3. Test Animals

3.1 All rats used in this test shall be Norway rats (Rattus norvegicus), wild-type (wild-caught or from a wild Norway rat colony) or albinos (Wistar strain preferred), or wild-type roof rats (R. norvegicus). Subjects shall be healthy, active, sexually mature, and shall fall within the following weight classes in grams within seven days prior to start of test:

	Minimum	Maximum	Maximum acceptable differences in average weights between sexes
Norway Rats			
Laboratory strains	150	300	50
wild-type Norway rats	150	400	65

Animals shall be weighed no more than three days before the start of the bait-exposure phase of the study. Animals that survive the study shall be weighed again at the end of the post-exposure follow-up period. Animals dying during the study shall be weighed when they are found dead.

3.2 Ectoparasite control with registered insecticide (or acaricide) products labeled for use on laboratory rats 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 anticoagulant rodenticides.

4. Apparatus

4.1 The rats should be placed in single-sex groups of 5 or 10 animals in solid-bottom all-metal cages designed or adapted to hold laboratory rats and having a bottom surface area of 17,000 to 25,000 cm^2 (18.3 to 26.9 ft^2). The bottom of each cage should be covered to a depth of approximately 2.5 cm (1 in.) with clean wood shavings.

4.2 Each cage should contain two metal nest boxes approximately 36 by 36 by 10 cm (14 by 14 by 4 in.) in sizes and lacking bottoms.

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

5. Pretest Holding Conditions

5.1 All rats 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 rat diet must be available to them at all times. Do not use the standard OPP rat and mouse challenge diet for pre-test feeding.

6. Holding and Test Conditions

6.1	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.

6.2 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 thoroughly mix. Be certain that the mixing utensils are clean of contamination before preparing diet.

6.2.1 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%) be retained by a No. 20 screen (20 meshes to the inch). The remainder may be either larger or smaller than the screens mentioned.

6.2.2 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%) be retained by a No. 20 screen (20 meshes to the inch). The remainder may be either larger or smaller than the screens mentioned.

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

6.2.4 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 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.

7. Procedure

7.1 A test group consists of twenty rats (10 males, 10 females), 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 control group of 20 rats (10 males, 10 females) caged in the same manner as the group(s) to be exposed to the toxic bait. Acclimate all animals to test conditions for three days prior to exposure to toxicant, immediately following pre-test holding period.

7.2 The standard EPA rat and mouse challenge diet (6.2) shall be available in each cage at all time in two or more feeders. Fill each feeder daily with fresh standard EPA rat and mouse challenge diet sufficient to provide in excess of an average of 40 grams per animal per day, minimum.

7.3 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.

7.4 Provide each treated test group of rats with five placepacks each weighing at least 28 g (1 oz). Scatter the placepacks randomly on top of the wood shavings on the floor of the group cage. Replace each placepack only after the contents have been completely consumed or spilled. Each day record the number of placepacks that are chewed into and the number replaced.

7.5 Animals on test shall 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.

8. Test Period

8.1 Maintain test period for three days. If 100% mortality of bait-exposed rats occurs prior to three days, monitoring of control-group animals must continue for the 15-day test period plus the full follow-up period.

8.2 Remove dead rats daily, or more frequently as observed.

8.3 At the end of the 3-day bait-exposure period, remove placepacks along with any bait spilled from them, leaving and maintaining the EPA rat and mouse challenge diet.

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

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

9. Test Period Follow-Up

9.1 Maintain observation on surviving rats for a minimum of five days following test period.

9.2 Continue feeding EPA rat and mouse challenge diet.

9.3 Describe unusual activities of test and control rats in report of test and post-test periods.

9.4 Remove contaminated wood shavings and replace with fresh wood shavings to a depth of approximately 2.5 cm (1 in.).

10. Calculation and Evaluation of Results

10.1 Record date, weight, and sex of each rat dying during the test and of survivors in both the test and control groups. Record the number of place-packs chewed into and the total number of placepacks used. Retain original laboratory test records for future reference. Report all data collected, including initial and final weights of test-group and control-group subjects.

10.2 The product is considered satisfactory if at least 90% of subjects in the group exposed to the toxic bait die during the baiting and the post-baiting follow-up period and if no more than 10% of control-group rats dye during the entire test.

10.3 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.