Revision No. 9
Revised:
7-23-74
1-1-75
9-1-76
1-21-77
2-17-78
8-15-80
6-18-91

STANDARD NORWAY RAT/ROOF RAT ANTICOAGULANT DRY BAIT

LABORATORY TEST METHOD

OPP Designation: 1.203 (2-25-74)

1. Scope

1.1 This method is designed to determine effectiveness of ready-to-use dry bait anticoagulant rodenticide products used for rat control. 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 rats used in this test shall be Norway rats (Rattus norvegicus), wild-type (wild-caught or from a wild-type Norway rat colony) or albinos (Wistar strain preferred), or wild-type roof rats (R. rattus). Subjects shall be healthy, active, sexually mature, and 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
Laboratory rats	150	300	50
Norway rats	150	400	65
Roof Rats	100	225	40

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.

2.2 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.

3. Apparatus

3.1 The rats should be placed in screen-bottom all-metal cages designed to hold laboratory rats and having a bottom surface area of 500 to 2000 cm^2 (0.538 to 2.15 ft^2).

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

4. Pretest Holding Conditions

4.1 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. During the last seven days of this period, animals must be held 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 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 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.

5.2.1 The whole (not degerminated) yellow ground corn shall be from the most recently available crop and be reasonably fresh 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 OPP 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.

6. Procedure

6.1 A test group consists of a minimum of 20 rats (10 males, 10 females), individually caged. Include one untreated control test group of 20 rats (10 males, 10 females), individually caged, in each test. If a series of tests is being conducted at the same time on the same species, only one untreated control test group need be included. 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 bait and the standard OPP rat and mouse challenge diet are each offered to test rats in separate containers (3.2) on opposite sides of the front of the cage. The two containers must be identical in type and size. At least 40 grams of bait and 40 grams of challenge diet must be available, in separate containers, to each test group animal per day. The control group must be offered only the EPA rat and mouse challenge diet. At least 40 grams of challenge diet should be available in each food container per day for control group subjects. The food offered in each container should be equal and consistent throughout the test. The gross weight of each container and its contained food must be 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 by each rat during the preceding 24 h. Weighing accuracy must be at least to the nearest 0.5 gram. Spilled food shall be recovered 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 rat and mouse challenge diet containers in the cage every 24 h to offset possible feeding position preferences. The test rats 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 animals in test group(s) die within less than 15 days.

7.2 Remove dead rats 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 rats for a minimum of five days following the bait-exposure period.

8.2 Continue feeding OPP rat and mouse challenge diet and recording amounts consumed daily.

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

9. Calculation and Evaluation of Results

9.1 Record date, weight, and sex of each rat 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 sheet as well as typed numerical summaries of test results.

9.2 The product is considered to have satisfactory bait acceptance if a minimum of 33 percent of the food consumed by the test group animals was the toxic bait, if at least 90% of the test group subjects die during the 20-day test, and if no more than 10% of control group subjects die during the 20-day test.

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.