

STANDARD NORWAY RAT/ROOF RAT ACUTE DRY BAIT

LABORATORY TEST METHOD

OPP Designation: 1.209

Revision No. 10
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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 rat control. The one-day version of this test may also 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 this study 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:

	<u>Minimum</u>	<u>Maximum</u>	<u>Maximum acceptable differences in average weights between sexes</u>
Norway Rats			
Laboratory rats	150	300	50
Wild-type	150	400	65
Roof Rats	100	225	40

Animals shall be weighed no more than three days before the start of the test phase of the study. Animals that survive the study shall be weighed again at the end of the post-test 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 the rodenticide used as the toxicant in this laboratory test.

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 cm² (0.538 to 2.15 ft²).

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 EPA rat and mouse challenge diet for pretest feeding.

5. Holding and Test Conditions

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| 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:

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| 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 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° 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 waterers are not recommended.

6.3 The rodenticide bait and the standard EPA 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 this material should be available in each food container per day to 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, for a "two-day" test, 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 In two-day tests, reverse the position of the bait and standard EPA rat and mouse challenge diet containers in the cage after 24 h to offset possible feeding position preferences of test subjects. In one-day tests, place container holding bait to the left of half of the test group subjects of each sex and to

the right of the remaining test group subjects. Container positions may be switched after twelve hours. The test rats must have a free choice between treated and untreated food.

6.5 Animals on test must 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 rats daily, or more frequently as observed.

7.3 Remove toxicant-treated food at the end of the 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 rats and control group rats 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 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 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 a minimum total mortality of 90% of test animals is obtained 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.