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STANDARD NORWAY RAT AND ROOF RAT ANTICOAGULANT LIQUID BAIT

LABORATORY TEST METHOD

OPP Designation: 1.201 (1-3-73)

1. Scope

1.1 This method is designed to determine effectiveness of anticoagulant rodenticide products used to make liquid baits for controlling commensal rats. 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 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
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 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 Rats shall be placed in solid-bottom all-metal cages designed to hold laboratory rats and having a bottom surface area of 500 to 2,000 cm^2 (0.538 to 2.15 ft^2).

3.2 Food shall be provided in metal or ceramic feeders designed so that rats may not nestle or wallow in diets.

3.3 Graduated 100-ml no-drip waterers fitted with ball-type watering tube should be used to dispense liquids to rats.

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 three 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 from graduated no-drip waterers 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.

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 (sexes equal), 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 Provide each cage with one or two feeders filled daily with a commercially available laboratory rat diet. Provide at least 40 grams of feed per animal per day.

6.3 Provide each cage with two 100-ml graduated no-drip waterers (3.4). Fill one waterer with tap water and the other with with the test liquid bait formulation diluted with tap water according to the mixing directions on the product's label. This procedure should provide each subject with access to amounts of liquid from each waterer that exceed the daily minimum requirement. Replenish both liquids as necessary so that waterers do not become less than approximately one-third filled. Reverse positions of the waterers daily.

6.4 Provide each control group animal with two 100-ml graduated no-drip waterers filled only with tap water. Replenish as necessary so that waterers do not become less than approximately one-third filled.

6.5 Each day, record the total quantity of each liquid consumed during the preceding 24 h for both the test and control groups.

6.6 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 minimized.

7. Test Period

7.1 Maintain test period for 15 days, even if 100% mortality occurs in all bait-exposed groups prior to that time.

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

7.3 In test group, remove toxicant-containing waterers at the end of the 15-day test period, leaving tap water waterers. In control group, remove one of the two waterers after the 15-day test period.

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 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 (test) period.

8.2 Continue feeding commercial rodent diet as in 6.2.

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 toxic and nontoxic liquids 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 mortality of 90% of test animals is obtained during the bait-exposure and post-exposure observation periods, if at least 33% of the volume of liquid consumed by test-group subjects was the liquid bait, 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 tap water 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.