

STANDARD HOUSE MOUSE ANTICOAGULANT PLACEPACK PENETRATION

LABORATORY TEST METHOD

OPP Designation: 1.218 (1-1-75)

Revision No. 9

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9-1-76

2-22-78

11-15-80

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1. Scope

1.1 This method is designed to determine effectiveness of anticoagulant dry bait rodenticide products used in placepacks for controlling house mice. This test must be run in addition to OPP 1.204, Standard House Mouse Anticoagulant Dry Bait Laboratory Test Method, in which the toxic bait is removed from the placepack and presented in food containers. 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. Definition

2.1 A placepack is a small packet containing rodenticide bait which is to be applied unopened to control rodents. The rodents are expected to chew through the packet's covering and consume the bait.

3. Test Animals

3.1 All mice used in this test shall be house mice (Mus musculus), wild-type (wild-caught or from a wild mouse colony) or albinos (Swiss-Webster strain preferred). They 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>
Laboratory mice	15	35	5
House mice	10	25	3

3.2 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 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 in the product being tested.

4. Apparatus

4.1 The 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 house mice for this type of study. If mice are housed

singly, cages must have a bottom surface area of at least 500 cm<sup>2</sup> (0.538 ft<sup>2</sup>). If mice are group-caged, each enclosure must have a bottom surface area of at least 2,000 cm<sup>2</sup>.

4.2 If mice are group-caged, provide shelters in both the test and control cages. Empty soup or beverage cans, with one end removed, slightly flattened to reduce rolling, have been found satisfactory for this purpose. Use one two cans for every five mice in the cage.

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

4.4 Graduated 100-ml no-drip waterers fitted with ball-type watering tubes should be used. Automatic or open-cup type waterers are not recommended.

## 5. Pretest Holding Conditions

5.1 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, 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 mouse diet must be available to them at all times. Do not use the standard OPP rat and mouse challenge diet for pretest 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 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 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 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%) 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 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. Challenge diet shall be at room temperature when offered to test or control animals. Challenge diet shall not be stored for longer than six months.

## 7. Procedure

7.1 A test group consists of 20 mice (10 males, 10 females), caged individually or in single-sex groups of 5 or 10 animals each. For each series of tests being conducted at the same time on the same species, include one control test group that will not be exposed to toxic bait. Acclimate all animals to test conditions for three days prior to exposure to toxicant.

7.2 Water must be available to all subjects at all times. One water bottle, replenished daily, should be more than adequate for subjects caged individually. Several water bottles should be used per cage for group-caged subjects. Deploy water bottles at locations which would be likely to bias animal's selection between bait and challenge diet.

7.3 At the start of the test, the standard OPP rat and mouse challenge diet (6.2) must be provided in each cage. For individually-caged subjects, use one container of challenge diet. For group-caged subjects, provide challenge diet in two or more feeders. Provide at least 10 grams of challenge diet per container per animal per day. The control group is offered only the challenge diet.

7.4 For individually caged subjects, provide each test-group animal one placepack at the start of the test. Deploy placepack on front of cage on the opposite side from the challenge diet container and about as far away from the water source as is the challenge diet container. Replace placepack when it appears to have less than 10 g of bait left in it or if bait becomes thoroughly fouled with urine, feces, or other debris. For group-caged subjects, provide each treated group with at least two placepacks at the start of the test, using more if necessary to provide 10 grams of bait per animal per day. Scatter

placepacks randomly on the floor of the group's cage and on top of any animal bedding used in the cage. Replace placepacks if after their contents have been largely consumed, spilled, or fouled. Each day record the number of placepacks that are chewed into and the number replaced.

7.4 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 should be minimized.

#### 8. Test Period

8.1 Maintain test period for 15 days, even if all mice exposed to toxic bait die in less than 15 days.

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

8.3 Remove placepacks and any remaining scattered bait at the end of the 15-day test period, leaving and maintaining the OPP 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 test- and control-group mice for a minimum of five days following test period.

9.2 Continue feeding OPP rat and mouse challenge diet.

9.3 Describe unusual activities of test and control mice in report of test and posttest periods.

9.4 Remove any contaminated animal bedding in cage and replace with bedding.

#### 10. Calculation and Evaluation of Results

10.1 Record the date, weight, and sex of each mouse killed. Record the number of placepacks used and the number chewed into. Retain original laboratory test records for future reference.

10.2 The product is considered satisfactory if at least 90% of subjects in the test group die during the bait-exposure and post-exposure observation periods, and if no more than 10% of control-group subjects die during either replication.

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.