Designation: E 757 – 80 (Reapproved 1995)

Standard Test Method for Efficacy of Canine Reproduction Inhibitors[1]

This standard is issued under the fixed designation E 757; the number immediately following the designation indicates the year of original adoption or, in the case of revision, the year of last revision. A number in parentheses indicates the year of last reapproval. A superscript epsilon (ε) indicates an editorial change since the last revision or reapproval.

INTRODUCTION

Vertebrate animal control is an art as well as a science. The development and effective application of control methods both require skills gained from extensive training and field experience. This is particularly true in dealing with the life forms which are highly adaptable and capable of elementary reasoning and thus develop widely varied individual behavior patterns. With these species, efficacy is often unusually difficult to attain. Subcommittee E35.17 recognizes, therefore, that standard test methods must be developed and control methods improved to advance the science and to provide reasonable safeguards for legitimate environmental concerns.

1. Scope

1.1 This test method covers the effectiveness of canine reproduction inhibitors. Any method for evaluating the use of a canine reproduction inhibitor should include recognition that the ultimate test for efficacy is whether it functions as an effective population control method under field conditions. While laboratory or pen test data are essential, final efficacy testing and determination must be accomplished under actual field conditions. No suitable standard laboratory test is available. The test method described here attempts to balance the need for and the feasibility of securing efficacy data.

1.2 This test method is intended for use primarily with monestrous members of the family Canidae. Because of great variation in reproductive physiology, (that is, delayed implantation in mustelids, delayed gestation in bats, uterine structural differences, estrous cycle variation, etc.) this method may not be readily applicable to other families and orders of mammals.

2. Referenced Documents

2.1 *ASTM Standards:*

E 552 Test Method for Efficacy of Acute Mammalian Predacides[2]

E 555 Practice for Determining Acute Oral LD50 for Testing Vertebrate Control Agents[2]

3. Laboratory Testing

3.1 All target specimens must be tested (see Practice E 555).

3.1.1 Efforts must be made to establish routine procedures and approaches for all test animals. All undue stress should be avoided since stress may cause or contribute to reproductive aberrations particularly in wild-caught canids.

3.2 *Test Animals*:

3.2.1 Test animals should be laboratory-reared or captured from wild environments.

3.2.2 Test animals should be reproductively mature adults except where juvenile sex hormones might be employed. The age and weight of test animals will vary but should not include very old, emaciated, obese, or seriously injured specimens. Any injuries from capture should be stabilized. The general condition of the test animals should be verified by a competent individual, preferably a veterinarian.

3.2.3 The sex and reproductive condition of animals used will depend upon the type of gametocide, hormone-affector, or other compound to be employed. In some instances, evaluation of a compound or technique may require testing with both sexes to determine actual effects in the field. In some cases, the opposite sex should be tested as a nontarget organism. For example, evaluation of diethylstilbestrol (DES) would also require testing of males since they are nontarget organisms with DES.

3.3 *Reference Animals*:

3.3.1 The terms “reference animals” and “reference group” are used to denote a group of animals maintained similarly to the test animals for the purpose of determining mortality due to illness, injuries, or other factors not related to test compounds.

3.3.2 Reference animals shall be of the same species and sex as the test animals. When domestic dogs are used as test animals, the reference animals shall be of the same breed and preferably of the same strain for uniformity. When wild species are used as test animals, the proportional numbers of laboratory-reared or wild-caught animals, or both, in the reference group shall be similar to those in the test group. The number of reference animals shall be the same as the number of test animals in each test group.

[1] This test method is under the jurisdiction of ASTM Committee E-35 on Pesticides and is the direct responsibility of Subcommittee E35.17 on Vertebrate Control Agents.

Current edition approved Aug. 29, 1980. Published November 1980.

[2] *Annual Book of ASTM Standards*, Vol 11.05.

3.3.3 Reference animals shall be maintained concurrently with test animals in cages or pens similar in size and type to those used for test animals. Reference animals shall be maintained under similar environmental conditions (temperature, humidity, lighting, etc.) to those under which test animals are maintained. Test and reference animals shall be maintained on similar nutritionally balanced diets.

3.4 *Pretest Conditioning*:

3.4.1 Various stages of the reproduction cycle may be affected by extrinsic biochemicals. Therefore, the test period will normally include those specific stages during which the test compounds are expected to function.

3.4.2 Wild-caught animals should be maintained in captivity for a period sufficient to acclimate them to captive conditions prior to application of the test gametocide, hormone affector, or other test compound. The diet and general condition of test animals should be stabilized for a minimum of 28 days prior to testing.

3.4.3 Laboratory-reared and acclimated wild-caught animals should be maintained for 7 days prior to the test in the type of pen or cage used in the test.

3.5 *Animal Facilities*—Cage or pen specifications may vary, but the type of cage or pen used should permit freedom of movement sufficient to prevent undue stress of test animals. The animal facilities shall meet the established standards which are required by law or regulations. It is desirable that they meet the guidelines suggested by the Institute of Laboratory Animal Resources, or approved by such organizations as the American Association of Accreditation of Laboratory Animal Care.

3.6 *Number of Test Animals*—The number of test animals will vary according to the statistical methods employed, and availability of animals. The number of test animals used in each group shall be the same as the number of reference animals. Extrapolation of data between species is not acceptable; therefore, laboratory tests must be made with each target species. However, due to potential reproductive aberrations under laboratory confinement, particularly in wild-caught animals, laboratory test data must be confirmed by data gained under actual field conditions.

3.7 *Analysis of Data*—Data from all species should be presented with accompanying narrative. Statistical treatment alone may convey invalid conclusions.

4. Toxicity and Effective Dose Levels

4.1 *Acute Toxicity and Effective Dose Levels*:

4.1.1 The acute oral LD50 in male laboratory rats should be established by standard toxicological procedures (see Practice E 555) and should precede intensive testing on the target species. The target species and sex should be considered as the standard laboratory animal.

4.1.2 Establish the effective oral dose (ED50) of test chemicals by administration to a minimum of six animals of each target species and sex. These should be sexually mature adults except where juvenile sex hormones are employed. Administer the chemicals after the upper digestive tract is void of food. In carnivores, this generally requires a minimum of 4 h after feeding.

4.1.3 The stages in the reproductive cycle during which test compounds should be administered may vary, depending on the biochemical nature of the compounds employed and the physiological responses anticipated. For example, compounds expected or known to alter estrus, ovulation, or fertilization might normally be administered immediately prior to or during estrus. Those expected or known to alter implantation of embryological development might be administered prior to or during estrus, or during gestation.

4.1.4 Observations should be made of all parameters likely to be affected by the test compounds but should always include mortality, intoxication symptoms, induced behavioral abnormalities, and alteration of the gestation period and parturition.

4.1.5 All animals that are affected or die as a result of treatment should be examined for gross pathological and histological changes. Similar examinations of unaffected survivors and reference animals are also desirable.

4.2 *Chronic Toxicity*—Administer doses of the test chemical to adult rats and six adult target species of the appropriate sex daily for a 30-day period (see Practice E 555). This must be done at the appropriate time during the reproduction cycle. Routes of administration should be identical with those used in field application. Use three or more dose levels. The highest dose used should produce a measurable level of effectiveness. The lowest dose should not produce any measurable adverse physiological or morphological effects. Following the test period, maintain the animals on a normal diet for an additional 60 days. Necropsy all animals that die during the test and the 60-day observation period. Observe, describe, and record organ changes, gross pathology, and histopathology. Sacrifice all animals on the 91st day and evaluate gross anatomical changes or abnormalities.

4.3 *Secondary Toxicity*:

4.3.1 Test for secondary toxicity in the following way: feed a nontarget or scavenger species prey animals containing a known quantity of the chemical and observe whether the chemical causes any adverse effects.

4.3.2 Expose individual animals of one or more prey species to the chemical under simulated field conditions. Animals dosed in this manner are then euthanized and exclusively offered no-choice ad libitum to the predator or scavenger species, which should include at least one species of bird (raptor or scavenger) as well as the domestic dog.

4.3.3 Conduct replications of all tests when evidence of secondary effects exists. Euthanize and necropsy all test predatory or scavenger animals for pathological organ changes at the conclusion of the test period.

4.4 *Toxicity to Nontarget Species*—Select appropriate nontarget species and sexes that might be affected and test identically with each gametocide, hormone-affector, or other test compound. These species routinely will include domestic dogs when evaluating the effects of test compounds on other canids.

5. Behavioral Modification

5.1 The ability of vertebrate animals to communicate warnings is well documented. Such behavioral changes induced by ingestion of chemicals could affect efficacy when tested under field conditions. When testing for acute effects and sublethal chronic effects, take special precautions to determine the possibility of behavioral changes that might serve as visual,

auditory, or other communication cues to individuals of the target species. Conduct these observations in conjunction with the studies described in Section 4 and maintain records on any such behavioral changes.

6. Application Methods

6.1 If devices or carrier baits are required for reproduction inhibitor delivery, they must be laboratory and field-tested before being adopted as part of a delivery system. Laboratory conditions are a restrictive environment for testing of devices or carrier baits and for developing efficacy data, although results of laboratory tests may be indicative of those which might be expected under field conditions. (See Ref. (**1**)[3] for one standardized baiting technique.)

7. Palatability (Acceptance)

7.1 A standard placebo for testing food acceptance by predatory or omnivorous animals does not exist because of the wide variation in species' food habits and available natural foods, and the disparity between naturally occurring foods and commercially prepared kennel rations. Preference, habituation, and opportunity to feed are as important as relative availability in wild canine food habits. Palatability should be tested using a suitable nutritionally balanced standard ration to which the test animals are accustomed. For each test, the standard ration will serve as the placebo for palatability tests of candidate test materials.

7.2 Palatability tests should be made with a minimum of six adult males, or six adult nonpregnant females, and six juveniles of the target species and sex if possible. The tests should be free choice between the placebo and the candidate test materials, with sufficient amount of each to be in excess of each animal's minimum daily requirement. Test animals should not be fasted; and the tests should be made at the normal, established feeding time.

7.3 Palatability studies with target species and sex are required for all candidate bait formulations which may be used as carriers for candidate test compounds and should be completed prior to field testing. Unpalatable active or inert ingredients may require encapsulation or "masking."

8. Initial Field Testing

8.1 Controlled field tests should be conducted in areas where relative or absolute numbers of the target population have been determined, depending on need and existing or developmental techniques. This entails development of a closely monitored area of appropriate and known size and, if possible, with recognizable physiographic or vegetative boundaries. Indices of relative abundance of other predatory species that may be affected should be obtained to the extent that available methodology permits. Where possible, a sample of the target species/sex shall be captured, marked, and released immediately prior to the test. An adequate sample can also be equipped with radio transmitters and monitored during the test period to acquire additional data. However, pretreatment and posttreatment indices or counts will afford a better means of determining effects on overall population densities. Such procedures may require data and observations for extended periods of time.

8.1.1 When chemicals are to be administered by food baits, a sample of baits and baiting locations should be monitored daily by preparation of the baiting sites so that visitation and consumption of baits can be determined on the basis of animal tracks left at these sites. Such sites should be distributed through appropriate representative portions of the study area. Data recorded should include, as a minimum, the species visiting and consuming baits, the number of baits consumed, and the rate of bait disappearance. This procedure may require weeks of baiting to overcome the wariness of some species and may require alteration of daily monitoring procedures.

8.1.2 Maximum effort should therefore by placed on application techniques with respect to: (*a*) differential movement and spatial characteristics of the target and nontarget species; (*b*) selectivity of the carrier (bait, mechanical device, etc.); and (*c*) specific placement of the carrier in relation to the ecological and behavioral characteristics of the target species. The selectivity of various application techniques can sometimes be determined prior to actual field testing of candidate compounds, by distribution of an appropriate carrier containing a dye or other marker, followed by sampling for the presence of the marker in collected animals or their droppings. Tracers such as chlortetracycline, or other inert compounds should be tested for acceptance prior to field trials since encapsulation or "masking" may be required.

8.1.3 Statistical evaluation of results should include the following:

8.1.3.1 Analysis of measurable variables,

8.1.3.2 Test for significance of efficiency,

8.1.3.3 Measurement of method specificity,

8.1.3.4 Deviation of field test results from laboratory test results.

8.2 *General Field Testing*:

8.2.1 Following initial field tests, general field tests should be conducted using the candidate canine reproduction inhibitors to determine the ultimate usefulness and safety of the compounds. All tests should be conducted by applicators who are adequately trained and qualified (FIFRA-1978).

8.2.2 Information required from general field tests includes the following:

8.2.2.1 Target species, indications of relative population densities, and their locations.

8.2.2.2 Size of the area involved, its appropriate legal description (section, township, range, etc.) and locations (county and state).

8.2.2.3 Nontarget species present and their relative abundance in the area.

8.2.2.4 Amount or number of baits/devices placed per acre, square mile, township, or other appropriate unit.

8.2.2.5 Type, location, and manner of placement of baits/devices.

8.2.2.6 Number and distribution of target and nontarget species/animals affected.

8.2.2.7 The impact on economic resource management and

[3] The boldface numerals in parentheses refer to the list of references at the end of this method.

health/disease problems should be determined in evaluating efficacy, by using such pre- and post-survey techniques as are available.

REFERENCES

(**1**) Oleyar, C. M. and McGinnes, B. S.," Field Evaluation of Diethylstilbestrol for Suppressing Reproduction in Foxes," *Journal of Wildlife Management*, Vol 38, No. 1, 1974, pp. 101–106.

(**2**) Ables, E. D.," Home Range Studies of Red Foxes (*Vulpes vulpes*)," *Journal of Mammalogy*, Vol 50, 1969, pp. 108–122.

(**3**) Balser, D. S., "Management of Predator Populations with Anti-fertility Agents", *Journal of Wildlife Management*, Vol 28, No. 2, 1964, pp. 352–358.

(**4**) Beck, J. R., and Jackson, W. B., "Consideration in Testing Vertebrate Pesticides to be Used in Disease-Control Activities," *ASTM STP 625*, Am. Soc. Testing Mats., 1977, pp. 195–205.

(**5**) Beck, J. R., and Stein, H. S., Jr., "Development and Extension of the Rationale for Field-Testing Vertebrate Pesticides and Devices Under Actual Conditions," *ASTM STP 680*, Am. Soc. Testing Mats., 1979.

(**6**) Buffington, C. D., and Giles, R. H., Jr.," Methods for Obtaining and Evaluating Inputs for Management Information Systems for Wildlife Agencies," *Proceedings*, 25th Conference of the Southeastern Game and Fish Commissioners, 1971.

(**7**) Cheal, M. L., and Sprott, R. L., "Social Olfaction: A Review of the Role of Olfaction in a Variety of Animal Behaviors," Psychological *Reports*, Vol 29, 1971, pp. 195–243.

(**8**) Connolly, G. E., and Longhurst, W. M., "The Effects of Control on Coyote Populations," *Bulletin 1872*, University of California, Division of Agricultural Sciences, Berkeley, California, Aug. 1975, pp. 37.

(**9**) Fuller, J. L., "Individual Differences in the Reactivity of Dogs," *Journal of Comparative and Physiological Psychology*, Vol 41, 1948, pp. 339–347.

(**10**) Gates, N. L., Card, C. S., Eroshenko, V., Hulet, C. V., "Insensitivity of the Coyote Testis to Orally Administered Cadmium," *Theriogenology*, Vol 5, No. 6, 1976, pp. 281–288.

(**11**) Gates, N. L., "Chemosterilant Evaluation in Coyotes: Parameters for Animal Selection and Laboratory Methodology," *ASTM STP 625*, Am. Soc. Testing Mats., 1977, pp. 106–113.

(**12**) Giles, R. H., Jr., Ed., *Wildlife Management Techniques*, The Wildlife Society, 1969.

(**13**) Kolenosky, G. B., and Johnston, D. H., "Radio Tracking Timber Wolves in Ontario," *American Zoologist*, Vol 7, 1967, pp. 289–303.

(**14**) Leslie, P. H., and Gower, J. C., "The Properties of a Stochastic Model for the Predator-Prey Type of Interaction Between Two Species," *Biometrika*, Vol 47, 1960, p. 219.

(**15**) Levins, R., "The Strategy of Model Building of Population Biology," Americana Sciences, Vol 54, 1966, pp. 421–431.

(**16**) Linhart, S. B., Brusman, H. H., and Balser, D. S., "Field Evaluation of an Antifertility Agent, Stilbestrol, for Inhibiting Coyote Reproduction," *Proceedings*, 33rd North American Wildlife Conference, 1968, pp. 316–327.

(**17**) Linhart, S. B., Dash, G. J., Roberts, J. D., and Savarie, P. J., "Test Methods for Determining the Efficacy of Coyote Attractants and Repellents," *ASTM STP 625*, Am. Soc. Testing Mats., 1977, pp. 114–122.

(**18**) Linhart, S. B. and Enders, R. K., "Some Effects of Diethylstilbestrol on Reproduction in Captive Red Foxes," *Journal of Wildlife Management*, Vol 28, No. 2, 1964, pp. 358–363.

(**19**) Linhart, S. B., and Kennelly, J. J., "Fluorescent Bone Labeling of Coyotes with Dimethylchlortetracycline," *Journal of Wildlife Management*, Vol 31, 1964, pp. 317–321.

(**20**) Linhart, S. B., and Robinson, W. B., "Some Relative Carnivore Densities in Areas Under Sustained Coyote Control, *Journal of Wildlife Management*, Vol 31, 1972, pp. 362–365.

(**21**) Robinson, W. B., and Grand, E. F., "Comparative Movements of Bobcats and Coyotes as Disclosed by Tagging," *Journal of Wildlife Management*, Vol 22, 1958, pp. 117–122.

(**22**) Roughton, R. D. and Bowden, D. C., "An Experimental Design for Field Evaluation of Predator Attractants," *ASTM STP 680*, Am. Soc. Testing Mats., 1979.

(**23**) Scott, T. G., "A Method of Estimating the Red Fox Population," *Iowa State College Journal of Science*, Vol 15, 1941, pp. 155–159.

(**24**) Spencer, D. A., "Testing and Registration of New Control Materials," *Proceedings*, 2nd Vertebrate Pest Control Conference, Anaheim, Calif., 1967, pp. 3–8.

(**25**) Stellflug, J. N., Gates, N. L., and Sasser, R. G., "Reproductive Inhibitors for Coyote Population Control: Developments and Current Status," Proceedings, 8th Vertebrate Pest Conference, Sacramento, California, 1978, pp. 185–189.

(**26**) Timm, R. M., Howard, W. E., Monroe, M. W., Teranishi, R. and Murphy, E. L., "A Method for Evaluating Coyote Scent Baits," *ASTM STP 625*, Am. Soc. Testing Mats., 1977, pp. 151–156.

(**27**) Turkowski, F. J. and Popelka, M. L. "Testing the Responses of Coyotes and Other Wild Predators to Odor Attractants," *ASTM STP 680*, 1979, p. 255.

(**28**) Wadsworth, R. M., ed., "The Measurement of Environmental Factors in Terrestrial Ecology," *Symposium of the British Ecological Society*, Blackwell, Oxford, England, 1967, p. 248.