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

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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.

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[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,