Designation: E 552 – 95

An American National Standard

Standard Test Method for Efficacy of Acute Mammalian Predacides[1]

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INTRODUCTION

Vertebrate animal control as a science is very primitive, lacking many of the research tools and procedures which are well established in other biological areas. Indeed the field still remains more of an art or skill highly dependent upon field experience usually garnered by trial and error. This is particularly true in dealing with the higher forms that are neither domestic nor commensal. All are capable of elementary reason as well as learned behavior patterns. Thus pesticidal work with these forms has been very difficult and in some areas impossible to standardize. However, the committee recognizes that the effort to do so must be made both to improve the science related and to provide some reasonable safeguards for legitimate environmental concerns. A major concern relates to countries or regions with severe problems from depredating mammalian predators. While United States research may seem to exceed the care of this test method, the committee desires to continue to validate this basic document as a point of established beginning.

1. Scope

1.1 This test method covers the effectiveness of predacides. Any test method covering the use of a predacide should include recognition that the ultimate test for efficacy is whether it functions as an effective management tool under range conditions. While laboratory or pen data are both necessary and useful, vital determination must be under actual use conditions. There is no standard laboratory animal. This test method attempts to balance the necessary and the feasible in making such an evaluation.

1.2 Research in this field in the United States is primarily associated with government funding, because of low-economic incentives and intensive regulation. Such factors periodically change without reference to science.

1.3 *This standard does not purport to address all of the safety concerns, if any, associated with its use. It is the responsibility of the user of this standard to establish appropriate safety and health practices and determine the applicability of regulatory limitations prior to use.*

2. Referenced Documents

2.1 *ASTM Standards:*

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

E 758 Test Method for Mammalian Acute Percutaneous Toxicity[2]

E 1291 Test Method for Conducting a Saturated Vapor Inhalation Study with Rats[2]

E 1373 Test Method for Conducting a Subchronic Inhalation Toxicity Study in Rats[2]

3. Laboratory Testing

3.1 Efforts must be taken to establish routine procedures and approaches for all test animals. All undue stress should be avoided; since stress alone can cause mortality, particularly in wild-caught specimens.

3.2 All target species must be tested (see Practice E 555).

3.3 *Animal Selection*:

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

3.3.2 Test animals should be adult. The age and weight of test animals will vary, but should not include aged, emaciated, nor injured specimens. Leg injury from capture should be stabilized. The general condition of the test animals should be validated by a competent individual, preferably a veterinarian.

3.3.3 Behavioral and physiological differences between sexes may require the separation of test results by sex.

3.4 *Pretest Conditioning*:

3.4.1 Wild-caught animals should be maintained in captivity for a sufficient time to allow them to become acclimated to captive conditions.

3.4.2 Cage or pen specifications vary of necessity. The type of cage or pen used should allow sufficient freedom of movement so as not to provide additional stress to the individual or group of test animals prior to the test.

3.4.3 Diet and general condition should be stabilized for a

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

minimum of 28 days prior to testing.

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

3.5 *Animal Numbers Required for Testing*—The number of test animals will vary according to the test, statistical method, and availability. Juvenile test animals may be required in some tests, but should not replace adults, unless specified. Final laboratory tests must be made with all target species since interspecific data may not be transposable (7.1).

3.6 *Observations*—Observations should be made on all parameters likely to be affected by the test, but should always include mortality, intoxication symptoms, induced behavioral abnormalities, and recovery period.

3.7 *Pathological Examination*—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 are also usually desirable.

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

4. Toxicity

4.1 *Acute Oral*:

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

4.1.2 Establish oral toxicity (LD50) by administration of the chemical to a minimum of twelve animals of each target species, consisting of six males and six females, all sexually adult. None of the females should be observably pregnant. Administer the chemical after the upper digestive tract is void of food. In mammals, this generally requires a minimum of 4 h.

4.2 *Dermal*:

4.2.1 Establish dermal toxicity using laboratory rats, according to the methods described by Draize (1955). If the LD50 of the toxicant falls below 200 mg/kg on rats or is anticipated to be used against the target species as a dermal toxicant even if only as an adjunct, then conduct dermal toxicity studies on target species. Use a minimum of twelve test animals, six males and six females.

4.2.2 Test for irritant characteristics to eyes and skin on laboratory rabbits. If any evidence of skin or eye irritation develops, then test the target species. Methodology similar to tests with human cosmetics will suffice.

4.3 *Inhalation*—Test toxicants intended to act as fumigants for toxicity by inhalation. Use twelve test animals of each target species, six adult males and six adult females, following similar tests with male laboratory rats. Methyl bromide or HCN may be used as comparative standards in the same chamber.

4.3.1 Use a sealed or gas-tight test chamber of known and measured air volume in which the test animal(s) can live for 10 min without severe oxygen depletion. Introduce a known quantity of the intended fumigant into the chamber and hold for 10 min. At the end of 10 min, introduce fresh air and evacuate the fumigant-laden air. Then hold the animals 7 days for observation, if not moribund. Then sacrifice them and examine for both gross and microscopic degenerative changes in body organs. Through repetitive tests, an LD50 will be obtained either in separate or group cage tests. Fumigant chambers at humane societies may be adapted to fulfill the requirements for large animals.

4.4 *Chronic*—Administer sublethal doses of the acute toxicant to adult rats and twelve adult target species daily for a 30-day period. Routes of administration should be identical with the ones which are used under field application. Use three or more dose levels. The highest dose should produce definite signs of toxicity. A lower dose should not produce any significant adverse physiological, biochemical, or morphological effects. Use test animals which are evenly divided by sex, if possible. Post any animals dying during the course of the test for degenerative organ changes and gross pathology. Sacrifice all animals on the 31st day and evaluate gross anatomical changes or abnormalities.

4.5 *Secondary*:

4.5.1 Test for secondary hazards in the following way. Feed a predatory or scavenger animal a prey species given a known quantity of chemical to observe whether the chemical causes any deleterious effects on the predator or scavenger.

4.5.2 Expose individual animals of one or more target species to the chemical under simulated field conditions. Animals killed in this manner are then exclusively offered ad libitum to the predator or scavenger species for a period of at least 7 days. Use appropriate avian and mammalian predators which should include at least one species of bird raptor as well as the domestic dog or domestic cat, or both. Additionally, tests with prey species killed by measured doses of toxicant may be informative.

4.5.3 Conduct replications on all tests when evidence of secondary hazard exists. Sacrifice and autopsy all test predatory or scavenger animals for gross pathological change at the conclusion of the test period.

4.6 *Non-Target Species*—Test selected non-target species identically, with baits for each predacide. These routinely will include domestic dogs or cats, or both, and a scavenger bird.

5. Behavioral Modification

5.1 When testing for acute and chronic sublethal effects, make special observations to ascertain the possibility of behavioral modification that might serve as vocal, chemical, or postural communication to the individuals of the target species. The ability of vertebrate animals to communicate warnings is well documented. Such behavioral changes induced by sublethal or lethal amounts of toxicant could well affect the efficacy once it is tested under free-field conditions. Maintain notes on any such behavioral changes. Conduct these observations in conjunction with the preceding studies in Section 4.

6. Devices

6.1 If devices are required for toxicant delivery, they must be laboratory- and field-tested before being incorporated as part of a mortality-inducing system. Testing devices under