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Standard Practice for Conducting Subacute Dietary Toxicity Tests with Avian Species[1]

This standard is issued under the fixed designation E857; 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.

1. Scope

1.1 This practice describes a procedure for determining the subacute dietary toxicity of a test substance administered to birds in their daily diet. The LC_{50} value time to mortality and slope of the dose response curve may also be derived.

1.2 This practice is applicable to substances that can be mixed uniformly into the diet.

1.3 This practice is intended primarily to be used with the young of the following species: northern bobwhite (*Colinus virginianus*), Japanese quail (*Coturnix japonica*), mallard (*Anas platyrhynchos*), and ring-necked pheasant (*Phasianus colchicus*). Other species or age groups, for example, with wild-trapped birds, may be used with appropriate husbandry modifications to the practice.

1.4 This standard is used routinely to address avian regulatory testing requirements. Modifications to the procedures described in this standard have been proposed and are being evaluated to better address the needs of the latest risk assessment procedures. Specifically, the latest procedures call for individual bird feed consumption measurements so that a more precise dose can be determined. While such procedures may replace procedures described in the current standard, there is no certainty that the newest procedures will work as anticipated, and validation is not complete. Therefore, the current guideline has utility prior to validation and acceptance of a modified standard.

1.5 *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, health, and environmental practices and determine the applicability of regulatory limitations prior to use.* For specific precautionary statements see Section 6.

1.6 *This international standard was developed in accordance with internationally recognized principles on standardization established in the Decision on Principles for the Development of International Standards, Guides and Recommendations issued by the World Trade Organization Technical Barriers to Trade (TBT) Committee.*

2. Referenced Documents

2.1 *ASTM Standards:*[2]

IEEE/ASTM SI 10 American National Standard for Use of the International System of Units (SI): The Modern Metric System

3. Terminology

3.1 *Definitions of Terms Specific to This Standard:*

3.1.1 *LC_{50}*—the statistically derived estimate of the concentration of a test substance in the diet that would be expected to cause 50 % mortality to the test population under the specified test conditions.

3.1.2 *concentration*—the weight of test substance per unit weight of diet.

3.1.3 *substance or test substance*—the element, chemical compound, formulation, known mixture, or material mixed in diets and fed to birds for the purpose of determining an LC_{50}.

3.1.4 *negative control*—a group of birds maintained under conditions identical to the test birds except for the absence of the test substance in their diet.

3.1.5 *positive control*—a group of birds maintained under conditions identical to the test birds except for the replacement of the test substance in the diet with a substance known to elicit a consistent toxic response.

3.2 *Units and Symbols:*

3.2.1 Refer to IEEE/ASTM SI 10.

4. Summary of Practice

4.1 This practice describes how to determine the subacute dietary toxicity of a test substance when administered to birds in their daily diet. The median lethal concentration (LC_{50}) in

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[2] For referenced ASTM standards, visit the ASTM website, www.astm.org, or contact ASTM Customer Service at service@astm.org. For *Annual Book of ASTM Standards* volume information, refer to the standard's Document Summary page on the ASTM website.

the diet is a measure of a specific toxic effect (that is, lethality). The LC_{50} has been used as a comparable index of toxicity. However, other expressions of toxicity also may be appropriate.

4.2 Groups of birds of the same species are fed diets containing a test substance or mixture of substances at selected concentrations for 5 days. This is followed by a minimum of 3 days (or for as long as the birds continue to exhibit toxic signs) on untreated food. The test substance is mixed into the diets, usually in a geometric series of concentrations.

4.3 General observations of the signs of toxicity and the acceptance of the test substance in the diet also must be reported.

4.4 Concurrent negative controls must be maintained throughout the test. A positive control also may be used.

5. Significance and Use

5.1 This practice provides a means of measuring the susceptibility of an avian species to a test substance in its diet under controlled conditions. The LC_{50} obtained in this test is a conditional measure of subacute toxicity because consumption is voluntary, and because the dietary route may introduce metabolic transformations of the test substance that might be absent in other exposure techniques.

5.2 Use of this practice contributes to the evaluation of the hazards of chemicals to birds because exposure is analogous to most field exposures, that is, through dietary intake.

5.3 The use of this practice allows for observation of signs of toxicity in addition to mortality.

5.4 The dose-response curve provides additional information about the response of birds to a test substance.

5.5 This practice can be used to study the effects of test substances in combination in order to simulate situations where birds may be exposed to more than one substance simultaneously **(1)**.[3]

5.6 This practice provides one basis for deciding whether additional toxicity testing should be conducted with birds.

6. Precautions

6.1 Contact with all test substances, solutions, and mixed diets should be minimized with appropriate protective clothing, gloves, eye protection, etc. The use of fume hoods and increased ventilation in test rooms is necessary when handling volatile substances. Mammalian toxicity and special handling procedures should be known before this practice is used.

6.2 Disposal of excess test substances, solutions, mixed diets, excreta, and treated birds should be done with consideration for health and environmental safety, and in accordance with all federal, state, and local regulations.

6.3 Cleaning and rinsing of glassware, feeders, and other equipment with volatile solvents should be performed only in well-ventilated areas.

6.4 Periodic medical examinations should be considered for all personnel caring for birds or handling test substances.

7. Facilities

7.1 Species requirements will vary, but pens and cages should include adequate room, clean food and water, heated areas for young birds, and protection from excessive disturbance. Space requirements have not been standardized for species normally used in this test. However, adherence to the general guidelines and principles found in the National Institutes of Health and the National Academy of Science publications **(2, 3, 4)** in addition to literature published on individual species should provide a basis for a humane approach to space requirements. Pens or cages must be placed so as to prevent cross-contamination **(5).**

7.2 Construction materials in contact with birds should not be toxic, nor be capable of adsorbing or absorbing test substances. Materials that can be dissolved by water or loosened by pecking should not be used. Stainless or galvanized steel, or materials coated with plastics are acceptable, but other construction materials may also be useful. Any material or pen shape is acceptable provided the birds are able to move about freely and that pens can be kept clean.

7.3 Ventilation, photoperiod, and relative humidity requirements vary little among test species, and these factors are particularly critical to the well-being of young birds. Relative humidity should be maintained at 45 to 70 %. Higher humidities may be appropriate for waterfowl. Photoperiod should be a minimum of 14 h of light. The amount and duration of heat for brooding is species specific **(6, 7).** A temperature gradient from approximately 38 °C to approximately 22 °C from an appropriate heat source should be established in brooders in order to allow the birds to seek a proper temperature. Ventilation should follow guidelines in *Guide for the Care and Use of Laboratory Animals.* Ventilation should be sufficient to supply 10 to 15 air changes per hour **(2).**

8. Diets

8.1 Dietary requirements vary according to the species and age of the test birds. Any unmedicated commercial diet that meets the minimum nutritional standards of the test species **(8)** is sufficient.

8.2 Contaminated feed may compromise a study **(9, 10, 11)**; therefore, feed should be analyzed periodically to identify background contaminants. Analysis may be especially important if the substance being tested is known or suspected of synergistic or antagonistic action with possible contaminants. Maximum allowable levels of heavy metals, pesticides, and other contaminants in feed have not been established.

8.3 Test diets should always be fresh and clean. The frequency that the diet is changed during a study is dependent upon the physical and chemical properties of the test substance, and the speed with which a test animal contaminates the feed with fecal matter or water, or both.

8.4 Test diets should be fed *ad libitum*.

8.5 Feed should not be used past its normal shelf life (usually 90 days).

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

8.6 Treated test diets should be stored so as to maintain the stability of the test substance in the diet.

9. Test Substance and Diet Preparation

9.1 Knowledge of the physical, chemical, and biological properties of the test substance is important in test diet preparation.

9.2 Test diets can be prepared by mixing the test substance directly into the feed or by dissolving or suspending the test substance in a solvent or carrier prior to mixing with the feed. The use of solvents or carriers may be necessary to achieve a uniform mix of the test substance in the feed.

9.3 The test substance is uniformly mixed into the diet. The physical and chemical properties of a test substance may cause variation in test diet concentrations and it is important to ensure that the test substance is available in the diet at the same concentration throughout the treatment period.

9.4 In addition to homogeneity and stability testing required by GLPs, it is recommended that concentrations of the test substance in the diet be confirmed by analysis at the beginning of the test.

10. Test Organisms

10.1 This practice is intended primarily to be used with the young of the following species: northern bobwhite (*Colinus virginianus*), Japanese quail (*Coturnix japonica*), mallard (*Anas platyrhynchos*), and ring-necked pheasant (*Phasianus colchicus*). Other species may be used, but changes in diet, caging, and other factors may be necessary **(12, 13)**.

10.2 If laboratory or commercially reared birds are used in this practice they must come from the same source, and be of the same age, because different strains or age cohorts can introduce variability into the test. These birds should be similar in appearance to a wild species. The parentage and dietary history of purchased birds should be known. If captured wild birds are used, they should come from the same source and be of similar maturity.

10.3 Birds that are deformed, injured, emaciated, or phenotypically different from normal birds must not be used as test animals. The population of birds from which the test animals (treated and control) are selected shall be considered unsuitable for testing if mortality exceeds 5 % during the 3 days prior to testing.

10.4 The preferred age for Japanese quail and northern bobwhite is 14 days; for ring-necked pheasants, 10 days; and for mallards, 5 days **(14).** The preferred ages are based on the probability that test birds of these ages will not survive for 5 days without eating (see 12.1.4). Tests with younger or older birds also can be used to determine the LC_{50} **(15, 16, 17).** If data from one test are to be considered comparable with data from another test, the ages of birds between the two tests should deviate no more than one or two days.

10.5 Young birds of the species listed in 1.3 shall be conditioned to the test parameters of caging, food, water, and photoperiod from the time they hatch or are acquired until the initiation of the test. An acclimation period of at least 3 days is required (see 10.3). Older birds shall be conditioned for at least 7 days.

11. Procedure

11.1 *Range-Finding Test:*

11.1.1 To determine the test concentrations to be used in a definitive test, a range-finding test may be conducted for 5 days using three to five widely spaced concentrations.

11.1.2 One procedure is to use an initial concentration of at least 5000 ppm with two to four geometrically spaced lower concentrations. If there is no mortality at the 5000-ppm level, and test procedures and numbers of birds per concentration are the same as would be used in a definitive test, then the range-finding test may provide sufficient information to negate the need for a definitive test. If mortality does occur, then range-finding will suggest the approximate test concentrations to be used in a definitive test.

11.2 *Definitive Test:*

11.2.1 Individual test birds should be randomly assigned to groups and to control and test diet concentrations. Assignment to groups and initial weighing of the test birds should be done at the same time to avoid needless handling stress.

11.2.2 Water, and treated or untreated diets, should be available *ad libitum*.

11.2.3 The experimental (test and control) diets are available for 5 days after which they are replaced with untreated feed. Birds are held for a minimum of 3 days following treatment. In some situations, it may be necessary to extend the observation period in order to investigate prolonged or delayed effects.

11.2.4 Body weight must be recorded at the initiation and conclusion of the treatment and observation phases. Feed consumption must be recorded for both the treatment and observation phases; it is recommended that consumption during the treatment phase be recorded separately for the first two days and the last three days. Additional information may be gathered by measuring feed consumption daily. If the study continues beyond 8 days, body weight and feed consumption should be recorded weekly. Mortality, behavioral abnormalities, and other signs of toxicity should be recorded each day during the test.

11.2.5 Photoperiod during the test should be the same as during the conditioning period.

11.2.6 A minimum of 10 birds for each test concentration constitutes a treatment group, but groups may be subdivided into replicates with a minimum of five birds per replicate. The test concentrations should be geometrically spaced so as to result in 10 to 90 % mortality. Acceptable test results should have one concentration that kills more than 0 % but less than 50 % and one that kills more than 50 % but less than 100 %. These results usually can be obtained with four to six treatment levels. If it is necessary to extrapolate above or below the LC_{50} then three or more concentrations having partial mortality are desirable. However, test substances having steep dose response curves may make it difficult to obtain such results. Depending