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Standard Test Method for Estimating Acute Oral Toxicity in Rats[1]

This standard is issued under the fixed designation E1163; 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 test method determines the lethality (LD50 value, slope and 95 % confidence interval (CI)) and signs of acute toxicity from a material using a limited number of rats. The technique used in this test method is referred to as the "Stagewise, Adaptive Dose Method."[2] This test method is an alternative to the classical LD50 test and is applicable to both liquids and solids.

1.2 This test method is not recommended for test materials which typically produce deaths beyond two days postdosing.

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:*[3]

E609 Terminology Relating to Pesticides

IEEE/ASTM SI 10 Standard for Use of the International System of Units (SI) (the Modernized Metric System)

3. Terminology

3.1 *Definitions:*

3.1.1 *delayed death*—an animal which does not die or appear moribund within 24 h but dies later during the observation period.

3.1.2 *gavage*—forced oral dosing, as by a tube that is passed down the throat to the stomach.

3.1.3 *LD50*—the statistically derived estimate of the dose of a test substance that would be expected to cause 50 % mortality to the test population under the specified test conditions.

3.1.4 *moribund*—at the point of death or extinction.

3.1.5 *pharmacotoxic*—gross physiological signs in response to a toxic material.

3.1.6 *signs of toxicity*—objective, observable evidence of toxicity.

3.1.7 *suspension*—a mixture in which very small particles remain suspended without dissolving.

3.1.8 *toxicity*—poisonous quality.

4. Summary of Test Method

4.1 Three to five different doses of the target compound are selected such that the doses span the entire dose response curve, with separation between the doses to be equal log intervals. One to two animals are given each dose as the first stage of the study. After 24 to 48 h, the responses to each dose are observed and used in determining the doses and animal numbers in the next stage of dosing.

4.2 The second and subsequent stages have one to four doses with one to three animals at each dose. Doses for subsequent stages are selected based on the estimates of the dose response distribution parameters and the uncertainties of these estimates. The dose response curve and its parameters are updated after each stage and dosing will stop when the 95 % confidence interval for the LD50 satisfies the following stopping rule: (upper 95 % CI – lower 95 % CI)/ (2 × LD50) < = 0.40).

4.3 The slope, LD50 and its 95 % confidence interval are calculated using the methods Feder.[2] No more than the use of 30 animals is recommended and shall constitute an additional stopping rule.

5. Significance and Use

5.1 This test method is of principal value in minimizing the number of animals required to estimate the acute oral toxicity (LD50). It also incorporates measures of variance (95 % CI) and a slope from which to make relative toxicity comparisons.

5.2 This test method is inappropriate for materials typically producing death two or more days after administration of the test compound unless the observation time between dosages is increased. This test method can be successfully applied,

[1] This test method is under the jurisdiction of ASTM Committee E50 on Environmental Assessment, Risk Management and Corrective Action and is the direct responsibility of Subcommittee E50.47 on Biological Effects and Environmental Fate.

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[2] Feder, P.I., Statistical Design Considerations for Stagewise, stagewise, adaptive dose allocation in dose responsive studies. In: Peace, Karl E., ed. Biopharmaceutical sequential statistical applications. New York: Marcel Dekker 1992.

[3] 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.

however, for materials producing only an occasional death two or more days after administration.

5.3 The LD50 is valuable as a measure of the relative acute toxicity of a material and can be used to make an estimate of potential hazard to humans when pesticides, other chemicals, or mixtures are ingested.

5.4 This test method allows for observation of signs of toxicity in addition to mortality. This information can be useful in planning additional toxicity testing.

6. Apparatus

6.1 *Syringe,* and an oral dosing needle or rubberized catheter to gavage the test compound is required.

7. Test Animals

7.1 Albino female rats weighing 190 to 300 g prefasted are used. A non in-bred rat such as the Sprague-Dawley strain is generally preferred. Female rats are preferred because historical data indicate that females in most instances have lower LD50 values than males.[4]

7.2 An additional test may be conducted with male rats, but it is not necessary, unless it is suspected that the substance is more toxic to males than females.

8. Pretest Conditioning

8.1 Examine each test animal on arrival for overt signs of disease, and condition to the environment for a minimum of seven days. Select animals that have not been used for any other tests.

8.2 Maintain animals during pretest and test periods in accordance with accepted laboratory practices for the care and handling of test animals.

8.3 Identify each animal with an ear tag or other suitable means.

8.4 During acclimation, observe the animals for adverse health effects. Eliminate any animal(s) demonstrating signs of spontaneous disease prior to the start of the study. Use only animals judged to be healthy.

8.5 The animals are housed individually. Rat chow or the equivalent and water are to be available *ad libitum* after dosing.

9. Sample Preparation

9.1 Because of the great variety of physical characteristics and formulations of chemicals and pesticides, it is not possible to stipulate how the test material should be prepared. The only criterion that can be specified is that the material must be in liquid form, that is liquid, solution, suspension, or emulsion, suitable for administration by gavage.

9.2 The test material shall be at the same temperature as that of the room in which the test is conducted at the time of administration to the animals.

10. Procedure

10.1 Deprive the animals of food for 12 to 18 h before administering the test substance.

10.2 Weight of each rat and calculate the dose according to this body weight to give the specified quantity of test substance per unit of body weight.

10.3 Record all information necessary to document animal weights and volume of test substance administered to each animal.

10.4 Dose four to six animals in the first stage each at a different dose spanning the estimated dose response curve (0 % lethality to 100 % lethality). Gavage animals using an oral dosing needle or rubberized tubing. Observe each animal for a minimum of 24 h. Use the methods of Feder et al (1992)[2] to estimate the dose response curve and its parameters. Check to see if the stopping rule (defined in 4.2) is met. If not, then proceed to the second stage of dosing. Return the animals to either ad libitum or 2 to 3-h feeding immediately after dosing. Return the animal to either ad libitum or 2 to 3-h feeding immediately after dosing.

10.5 Observe the animals for mortality and pharmatoxic signs periodically for the first 4 h after dosing (at least once during the first 30 min) and daily thereafter for a total of seven days.

10.6 Pharmatoxic signs most frequently seen are as follows: respiratory rate increase or decrease, hypoactivity, prostration, ataxia, unkept appearance, body tremors, blanching, gasping, diarrhea, lethargy, chromodacryorrhea, and red excretion around nares.

10.7 For the second and subsequent stages of dosing, select doses based on the previous stages(s) and the result of the analysis and estimates of the dose response curve and parameters. Select doses to fill in the gaps in the curve, adding higher doses if 100 % lethality has not been observed or lower doses if 0 % lethality has not been observed, or both. Partial responses (some lethality, some survival) for at least one dose are necessary to fit the dose response curve.

10.8 After each stage of dosing, estimate the dose response curve and its parameters to determine if the stopping rule (defined in 4.2) has been met. Continue stages of dosing until the stopping rule is met or 30 animals have been dosed, whichever comes first.

10.9 Record all pharmacotoxic symptoms and time of death. Perform a gross necropsy on all animals that die.

10.10 Weigh all surviving animals on day seven and perform a gross necropsy. The necropsy should entail a macroscopic inspection of all visceral organs. Record all findings.

11. Test Options

11.1 If deemed necessary, perform the test concurrently and independently in both sexes using the same strain and body weight.

11.2 To ascertain the lethality in males, dose six male rats at the determined LD50 value of the females. Report the LD50 as follows:

[4] Dixon, W. J., "The Up-and-Down Method for Small Samples," *Journal of American Statistics Association*, Vol 60, 1965, pp. 967–978.

11.2.1 If the number of deceased males is none, report that with 95 % confidence the LD50 value for males exceeds the administered dose.

11.2.2 If one or two animals die, report that the estimated LD50 value for males exceeds the administered dose.

11.2.3 If three animals die, report that the estimated LD50 value for males equals the administered dose.

11.2.4 If four or five animals die, report that the estimated LD50 value for males is less than the administered dose.

11.2.5 If six of six animals die, execute the this entire procedure described in this test method using male rats.

11.3 Generally, single oral exposures should not exceed volume requirements for the rat (10 mL/kg), nor should daily exposures exceed 5-g test compound/kg body mass. To satisfy volume requirements while reaching the maximum daily dose limit, two oral doses may be administered separated by at least 4 h. If mortality does not occur following a daily oral exposure of 5 g/kg, an LD50 cannot be calculated and the limit test requirements will have been met (that is, LD50 > 5 g/kg).

12. Calculation of Results

12.1 Calculate the dose response curve and its parameters (slope, LD50 and 95 % CI) using nonlinear regression on a two parameter (slope and intercept) probit model after each stage using the dose, number exposed at the dose, and number responding to the dose. As dosing continues with subsequent stages, all stages should be included in the determination of the dose response curve. Therefore, a stage effect should be included in the nonlinear regression to determine if responses differ with respect to stage. If stage effects are significant, a stage may need to be excluded from the analysis. More specific details of the analysis approach are found in Feder.[2]

12.1.1 Additional toxicity tests should not be conducted if the existing test provides answers to all of the practical questions that need to be answered concerning the LD50.

12.2 If the maximum number of animals has been used and an adequate LD50 and 95% CI cannot be estimated because the live and dead animals have no doses in common and all dead animals have higher doses and all live animals have lower doses, then LD50 may approximated by the average between the two doses were live and dead occur.

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13. Report

13.1 The report shall contain such information as test species and source and sex of animals.

13.2 The report should include study initiation and termination dates, individual body weights, dose levels, dose administered, return to feeding time, mortality, pharmacotoxic signs, gross necropsy results, and LD50, if determined.

14. Precision and Bias

14.1 A precision and bias statement cannot be made at this time.

15. Keywords

15.1 chemicals; LD50; oral toxicity; pesticides; rats