



Standard Test Method for Estimating Acute Oral Toxicity in Rats¹

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1. Scope

1.1 This test method determines the lethality (LD50 value) 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 “Up-and-Down Procedure.”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 2 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:

- E 380 Practice for Use of the International System of Units (SI) (the Modernized Metric System)³
- E 609 Definitions of Terms Relating to Pesticides⁴

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

3.1.6 *toxicity*—poisonous quality.

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

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

4. Summary of Test Method

4.1 Female rats are dosed one at a time starting the first animal at the best estimate of the LD50. If this animal survives, then the next animal receives a higher dose; but if the first animal dies, the next animal receives a lower dose. If possible, subsequent doses are adjusted by a constant multiplicative factor, for example 1.3. The dose for each animal is adjusted up or down depending upon the outcome for the previous animal.

4.2 The dosing is repeated as above until four animals have been dosed after reversal of the initial outcome.

4.3 The LD50 is calculated using the maximum likelihood method.⁵

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).

5.2 This test method is inappropriate for materials typically producing death 2 or more days after administration of the test compound unless the observation time between dosages is increased. This test method can be successfully applied, however, for materials producing only an occasional death 2 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*,⁶ and an oral dosing needle or rubberized catheter to gavage the test compound are 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

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² Bruce, R. D., “An Up-and-Down Procedure for Acute Toxicity Testing,” *Fundamental and Applied Toxicology*, Vol 5, 1985, pp. 151–157.

³ *Annual Book of ASTM Standards*, Vol 14.02, excerpts in grey pages of Vol 11.04.

⁴ *Annual Book of ASTM Standards*, Vol 11.05.

⁵ Finney, D. J., *Probit Analysis*, 3rd ed., Cambridge University Press, Cambridge, England, 1971, pp. 50–80.

⁶ Hamilton Milliliter syringes available from Hamilton Co., Reno, NV have been found suitable for this purpose.

generally preferred. Female rats are preferred because historical data indicate that females in most instances have lower LD50 values than males.⁷

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 7 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 Weigh the animals and deprive of food for 18 to 20 h before administering the test substance.

10.2 Determine fasted body 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 Gavage one animal using an oral dosing needle or rubberized tubing. Return the animal to either *ad libitum* or 2 to 3-h feeding immediately after dosing.

10.5 Observe the animal for mortality and pharmacotoxic signs periodically for the first 4 h after dosing (at least once during the first 30 min) and daily thereafter for a total of 7 days.

10.6 Pharmacotoxic 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 Dose one animal at a time starting at the estimated LD50. Observe each animal for a minimum of 24 h. If the animal dies or appears moribund with symptoms such as shallow, labored or irregular respiration, muscular weakness or tremors, absence of voluntary responses to external stimuli, cyanosis and coma, decrease the dose for the next animal. If the animal survives and appears healthy, increase the dose for the next animal. If feasible, use a dose progression factor of 1.3.

10.8 After reaching the reversal of the initial outcome (that is the point where an increasing dose pattern is required to be decreased by a death or a decreasing dose pattern is required to be increased by a survival), dose an additional four animals in the up-down procedure and then stop.

10.9 If ten animals have been dosed with increasing dosages and no deaths have occurred within 24 h but delayed deaths are observed in three or more animals, stop the procedure. In this case, report that an LD50 could not be determined using this procedure.

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

10.11 Weigh all surviving animals on day 7 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:

11.2.1 If the number of decreased 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 up-and-down procedure described in this test method using male rats.

12. Calculation of Results

12.1 If all the dead animals have higher doses than all the live animals, then the LD50 is between the doses for the live and dead animals. If needed, the binomial test can be used to calculate the probability that the LD50 is between the lowest dose that killed all of the animals and the highest dose that killed none of the animals. If this probability is more than 95 %, then use the geometric mean of the two doses as an approximate LD50.

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 live and dead animals have only one dose in common and all other dead animals have higher doses and all other live animals have lower doses, then LD50 may approximate their common dose. Additional testing should only be

⁷ Dixon, W. J., "The Up-and-Down Method for Small Samples," *Journal of American Statistics Association*, Vol 60, 1965, pp. 967-978.



performed when it is determined that there is a practical need.

12.3 No estimate of the LD50 should be made when the slope of the maximum likelihood probit curve is zero. This event will happen when $(\sum N_A)(\sum AR_A) = (\sum R_A)(\sum AN_A)$, where A denotes the dose, N_A denotes the number of animals receiving dose A, R_A denotes the number of animals receiving dose A that die and the summations are over all administered doses.

12.4 If none of the above situations occur, then calculate the LD50 using the maximum likelihood method.⁵ This calculation may also be performed using the SAS⁸ or BMDP⁹ computer program packages or other suitable computer programs. Other methods, including nonparametric methods, can be used,

especially if probit and logit methods do not fit the data.

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

⁸ SAS(r) Proprietary Software is available from SAS Institute Inc., Cary, NC.

⁹ Dixon, W. J., ed., *BMDP Statistics Software*, University of California Press, Berkeley, CA.

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