



Designation: E 1372 – 95 (Reapproved 1999)

Standard Test Method for Conducting a 90-Day Oral Toxicity Study in Rats¹

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1. Scope

1.1 This test method has been designed to examine the subchronic oral toxicity of a test substance during a 90-day period of continuous oral exposure to rats (*Rattus norvegicus*) following initial acute toxicity tests.

1.2 The values stated in SI units are to be regarded as the standard. The values given in parentheses are for information only.

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 609 Definitions of Terms Relating to Pesticides²

E 1163 Test Method for Estimating Acute Oral Toxicity in Rats²

2.2 Federal Standards:

Title 40, Code of Federal Regulations (CFR), Environmental Protection Agency, Part 798, Health Effects Testing Guidelines, Subpart C, Subchronic Exposure, Oral Toxicity³

Title 40, Code of Federal Regulations (CFR), Environmental Protection Agency, Part 798, Health Effects Testing Guidelines, Subpart B, General Toxicity Testing, Acute Oral Toxicity³

Title 40, Code of Federal Regulations (CFR), Environmental Protection Agency, Subchapter E, Pesticide Programs; Part 160, Good Laboratory Practice Standards³

Title 21, Code of Federal Regulations (CFR), Food and Drug Administration, Part 58, Good Laboratory Practice for Nonclinical Laboratory Studies³

Title 40, Code of Federal Regulations (CFR), Toxic Substance Control Act, Part 792, Good Laboratory Practice Standards³

3. Terminology (see Definitions E 609)

3.1 Definitions:

3.1.1 *concentration*—The weight of the test substance per unit weight of the diet (expressed as mg/kg of diet). The weight of the test substance per volume of H₂O (expressed as mg/ml of H₂O), or at a constant rate in the diet (expressed as ppm).

3.1.2 *dose, dosage*—The quantity of a substance applied per unit treated or applied to or entered into organism. This is expressed as the weight of the test substance per unit weight of test animal (mg/kg).

3.1.3 *feed efficiency*—This value is a measure of the efficiency with which the animals convert food to body weight. The calculation is the total body weight gain per total food consumed.

3.1.4 *gavage*—forced feeding, as by tube that is passed down the throat to the stomach.

3.1.5 *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.

3.1.6 *no observed effect dose (NOED)*—The highest tested dose of a substance at which the measured biological variables of a specific group under test conditions show no statistically significant dose-related adverse difference from the control treatment group.

3.1.7 *nulliparous*—Having never borne an offspring.

3.1.8 *subchronic oral toxicity*—The adverse effects occurring as a result of the daily exposure of experimental rats to a test substance by diet, water, capsule or gavage for a 90-day period.

3.1.9 *test substance*—Pesticide or other material (element, chemical compound, formulation, known mixture) administered orally for the purpose of determining subchronic oral toxicity.

4. Summary of Test Method

4.1 Equal numbers of rats (minimum of 80), 40 of each sex, of the same source, age, and strain are randomly divided into at least four test groups (control, low, medium, and high dosage groups). If it is determined that an interim sacrifice is necessary, the number of animals per test group shall be increased (at least 5 male and 5 female per dosage group), before the initiation of the study. The highest dosage level shall result in toxicological effects, but not cause more than 20 % fatalities. The lowest dosage level should be one that does not induce any

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

³ Available from U.S. Government Printing Office, Superintendent of Documents, Washington, DC 20402.

evidence of toxicity. This level should be (if possible) higher than that expected for human exposure. The intermediate dosage should produce a minimal observable effect. If more than one intermediate dose is used, the dose levels should be spaced to produce a gradation of toxic effects. Where appropriate, a vehicle control (the volume of which corresponds to the volume of vehicle at the highest exposure level) should be used. The selection of test substance dosages may be estimated from a preliminary 14-day range finding study (see Section 12).

4.2 Daily observations of all individual animals for signs of toxicity and mortality are recorded.

4.3 A sample of test animals may be sacrificed at 45 days and the remaining animals at 90 days into the study. Urine, hematology, and blood samples are collected for analysis prior to necropsy.

4.4 Data collected from treatment and control groups are compared statistically to detect changes in food or water consumption, body weights, organ-to-body weight and organ-to-brain weight ratios, hematology and clinical blood and urine values. Histopathological examinations are also performed on selected tissues.

5. Significance and Use

5.1 This test method is intended to permit the determination of a no observed effect level and the toxic effects associated with the repeated oral exposure to a substance for a period of 90 days. This subchronic test is not capable of determining toxic effects requiring an extended latency period to develop (for example, carcinogenicity and life shortening). It will provide information on target organs, the possibilities of accumulation, and can be used in selecting dosage levels for chronic studies and for establishing safety criteria for human exposure. It provides information on potential human health hazards likely to arise from repeated exposure over a limited period of time.

6. Hazards

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. Information on acute mammalian toxicity and special handling procedures should be known before this test method is used.

6.2 Disposal of excess test substances, solutions, mixed diets, excreta, and treated animals 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. The use of fume hoods may be necessary when handling volatile substances.

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

7. Test Animals

7.1 This test method is intended for use with young adult male and female rats. The females should be nulliparous and

nonpregnant. The Sprague-Dawley is an example of a strain frequently used. Rodents other than rats may be used with appropriate modifications and justifications.

7.2 At least 20 animals (10 male and 10 females) should be used at each dosage level. An additional group of rats (10 male and 10 female) can be treated with the high dosage level for 90 days and observed for reversibility, persistence, or delayed occurrence of toxic effects for a posttreatment period of appropriate length, normally not less than 28 days. If it is determined that interim sacrifices are necessary, the number of animals per test group shall be increased (at least 5 male and 5 female per dosage group), before the initiation of the study.

7.3 All animals for a given test must come from one source and strain and be of approximately the same age to minimize variability. Test animals may be obtained from commercial sources or reared in laboratory colonies, but they must not have been used in a previous test. Animals should be healthy and disease free and those that are deformed, injured, emaciated, or phenotypically different from normal animals must not be used as test subjects. The population of animals from which the test subjects (treated and control) are selected shall be considered unsuitable for testing if mortality exceeds 5 % during the acclimation period. At the beginning of the study the weight variation of the rats used should not exceed ± 20 % of the mean weight for each sex. Dosing should begin as soon as possible after weaning, ideally before the rats are 6-weeks old, and in any case, not more than 8-weeks old.

8. Facilities

8.1 No precise physical requirements concerning the facility are set forth. However, the animal facility shall meet the established standard that may be required by law or regulations. It is desirable that the animal facilities meet the guidelines suggested by the Institute of Laboratory Animals Resources or facilities that have been approved by such organizations as the American Association of Accreditation of Laboratory Animal Care (AAALAC).

TABLE 1 Minimum Space Recommendations for Laboratory Rats⁴

Weight of Rat (g)	Floor area/rat (cm ²) ^A
<100	109.68 (17.0 in. ²)
100–200	148.40 (23.0 in. ²)
200–300	187.11 (29.0 in. ²)
300–400	258.08 (40.0 in. ²)
400–500	387.15 (60.0 in. ²)
>500	451.64 (70.0 in. ²)

^A Height should be at least 17.8 cm (7 in.).

9. Test Cages

9.1 The test and control rats shall be housed individually in all metal cages designed to hold laboratory rats and should provide for proper water and food consumption. Table 1⁴ suggests the minimum cage sizes for rats.

⁴ Institute of Laboratory Animal Resources, National Research Council, "Guide for the Care and Use of Laboratory Animals," DHEW Publication No. (NIH) 85-23, 1985, pp. 13.