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StandardTest Method for Chronic Oral Toxicity Study in Rats[1]

This standard is issued under the fixed designation E1619; 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 covers a long-term study to determine the effects of a substance in a mammalian species such as the rat following prolonged and repeated oral exposure. Under the conditions of the chronic toxicity test, effects that require a long latency period or that are cumulative should become manifest.

1.2 This test method assumes that the user is knowledgeable in mammalian toxicology and related pertinent areas, and relies heavily on the judgment of the evaluator.

1.3 The values stated in SI units are to be regarded as the standard. The inch-pound units given in parentheses are for information only.

1.4 *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.* For specific hazard statements, see Section 6.

2. Referenced Documents

2.1 *ASTM Standards:*[2]

E609 Terminology Relating to Pesticides

E943 Terminology Relating to Biological Effects and Environmental Fate

2.2 *Federal Standards:*[3]

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

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

Title 40, CFR, Toxic Substance Control Act, Part 792, Good Laboratory Practice Standards

Title 40, CFR, Environmental Protection Agency, Part 798, Health Effects Testing Guidelines, Subpart D, Chronic Exposure, Chronic Toxicity

3. Terminology

3.1 *Definitions*—See Terminology E609 and Terminology E943.

3.2 *Definitions of Terms Specific to This Standard:*

3.2.1 *chronic toxicity, n*—the adverse effects occurring as a result of the daily exposure of mammalian species to a test substance by diet, water, capsule, or gavage for a one-year period.

3.2.2 *concentration, n*—the weight of test substance per unit weight of the diet (expressed as milligrams per kilogram of diet). The weight of test substance per volume of H_2O (expressed as milligrams per millilitre of water), or at a constant rate in the diet (expressed as parts per million).

3.2.3 *feed efficiency, n*—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.

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

3.2.5 *test substance, n*—pesticide or other material (element, chemical compound, formulation, known mixture) administered orally for the purpose of determining chronic toxicity.

4. Summary of Test Method[4]

4.1 One mammalian species, a rodent, is required; the rat is the preferred rodent. Forty rats (twenty females and twenty males) are used at each of the five dose levels (control-, low-, two intermediate levels-, and high-dosage groups). If it is determined that an interim sacrifice is necessary, the number

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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.

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

[4] Benitz, K. F., "Measurement of Chronic Toxicity," *Methods of Toxicology*, ed. G. E. Paget, Blackwell Scientific Publications, Oxford, England, 1970, pp. 32-131.

should be increased by the number of animals scheduled to be sacrificed during the course of the study (see CFR, Title 40, Part 798).

4.2 The high-dose level in rats should elicit some signs of toxicity without causing excessive lethality. The lowest dosage level should be one that does not induce any evidence of toxicity. This level should be higher (if possible) than that expected for human exposure. The intermediate-dosage level should produce a minimal observable effect. 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.

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

4.4 After one year, prior to necropsy, urine, hematology, and blood samples are collected for analysis and then test animals are sacrificed.

4.5 Data collected from treatment and control groups are compared statistically to detect changes in food or water consumption, or both, 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 should generate data to identify the majority of chronic effects and shall serve to define long-term dose response relationships. In addition the test should allow for the detection of general toxic effects including neurological, physiological, biochemical, and hematological effects and exposure-related morphological (pathology) effects.

5.2 This test method should provide information on target organs, the possibilities of accumulation, and may be used for establishing safety criteria for human exposure. It provides information on potential health hazards likely to arise from repeated exposure over a long period of time.

6. Hazards

6.1 Minimize contact with all test substances and solutions 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 concerning acute mammalian toxicity and special handling procedures should be known before this test method is used.

6.2 Dispose excess test substance, solutions, diets, excreta, and treated animals with consideration for health and environmental safety, and in accordance with all federal, state, and local regulations.

7. Facilities

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

7.2 *Environment*—House test and control animals in cages designed to hold laboratory animals. Provide for appropriate water and food consumption. Maintain all animals in a temperature-, humidity-, and light-controlled room. The conditions should be 18 to 26°C (64.4 to 78.8°F) for temperature, 40 to 70 % for humidity, and a 12-h light, 12-h dark lighting cycle.

8. Test Animals

8.1 Perform the test with one mammalian species; the rat is the preferred rodent species. If another mammalian species is used, justification or reasoning for the selection must be recorded.

8.2 Obtain rats three weeks post-weaning. The Sprague-Dawley (COBS/CD) rat is an example of a strain frequently used. The females should be nulliparious and nonpregnant. Acclimate the animals for a period of no less than seven days. Dosing of rats should begin ideally before six weeks old, but no later than eight weeks of age.

8.3 All animals for a given test must come from one source and strain and be 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.

9. Diets

9.1 The preferred administration of test substance is incorporated into a diet. However, the test substance may be administered dissolved in drinking water or a solvent, or given by gavage or capsule for a period of at least twelve months. The choice of route of administration depends upon the physical and chemical characteristics of the test substance.

9.1.1 If the test substance is administered by gavage, a five-day/week dosing regimen is acceptable.

9.1.2 When necessary, dissolve or suspend the test substance in a suitable solvent. If a vehicle or diluent is needed, it should not elicit toxic effects itself nor substantially alter the chemical or toxicological properties of the test substance.

9.2 Formulate diets in accordance with the nutrient requirements of the test species. Any unmedicated commercial diet that meets the minimum nutritional standards of the test species is acceptable.

10. Range-Finding Study

10.1 Previous data or a range-finding study should be used/conducted to assist in the selection of the appropriate doses for the chronic study.

10.2 Use groups of six male and six female rats between six and eight weeks of age. Randomize, number, and place all