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# Standard Test Method for Oncogenicity Study in Rats and Mice[1]

This standard is issued under the fixed designation E 1811; 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 oncogenicity potential of a substance in mammalian species such as the rat and mouse. The objective is to observe the test animals for a major portion of their life span for neoplastic lesions during exposure to various doses of a test substance by oral administration.

1.2 This test may also provide some information on long-term general toxicity.

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

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

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 and health practices and determine the applicability of regulatory limitations prior to use.* Specific hazards statements are given in Section 6.

## 2. Referenced Documents

2.1 *ASTM Standards:* [2]

E 609 Definitions of Terms Relating to Pesticides

E 943 Terminology Relating to Biological Effects and Environmental Fate

2.2 *Federal Standards:*[3]

Title 21 CFR, Part 58, Good Laboratory Practice for Nonclinical Studies, Food and Drug Administration

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

Title 40 CFR, Subchapter E, Pesticide Program: Part 160, Good Laboratory Practice Standards, Environmental Protection Agency

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

## 3. Terminology

3.1 *Definitions*—See Definitions E 609 and Terminology E 943.

3.2 *Definitions of Terms Specific to This Standard:*

3.2.1 *chronic toxicity*—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 an extended period of time.

3.2.2 *gavage*—administration of the test substance by placement into the stomach through an orogastric tube.

3.2.3 *neoplastic*—capable of producing abnormal tissue growth.

3.2.4 *nulliparous*—having never borne an offspring.

3.2.5 *oncogenicity*—the condition of producing tumors.

## 4. Summary of Test Method (Title 40 CFR, Part 798)

4.1 Two mammalian species are required. Rats and mice are the species of choice because of their relatively short life spans, the limited cost of their maintenance, their widespread use in pharmacological and toxicological studies, and the availability of inbred strains.

4.2 One hundred twenty-four rats (62 females and 62 males) and 112 mice (56 females and 56 males) are used for each of the four dose levels (control-, low-, intermediate-, and high-dosage groups).

4.3 The test substance is administered either in the diet or water or by gavage at levels based on results from prior feeding studies. The administration of test substance is for two years for rats; it is 18 months for mice.

4.4 An interim sacrifice is made at the end of one year for both rats and mice; after 18 months the interim sacrifice is for rats only.

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

4.6 Hematology blood samples are collected for analysis prior to interim sacrifices and termination of the test.

[1] This test method is under the jurisdiction of ASTM Committee E35 on Pesticides and is the direct responsibility of Subcommittee E35.26 on Safety to Man.

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

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4.7 Data collected from treatment and control groups are compared statistically for changes and to note the formation of tumors. Histopathological examinations are performed on selected tissues.

## 5. Significance and Use

5.1 This test method should generate data to determine the presence, incidence, and severity of abnormalities (including behavioral and clinical abnormalities) and whether significant tumor production has occurred. In addition, the test should allow for the detection of general toxic effects.

5.2 This test method 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, and so forth. The use of fume hoods and increased ventilation 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 Animal Resources[4] 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 animals in cages designed to hold laboratory animals. Provide appropriate food and water 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 two mammalian species such as the rat and the mouse. Justification or reasoning must be recorded if another mammalian specie is used.

8.2 Obtain animals three weeks post-weaning. The females should be nulliparous and nonpregnant. Acclimate the animals for a period of no less than seven days. The dosing of animals 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. Obtain test animals from a commercial source or rear 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 the normal animals must not be used as test subjects.

## 9. Diets

9.1 The preferred administration of test substance is incorporation into the diet. However, the test substance may be administered dissolved in drinking water or a solvent or given by gavage or capsule for the duration of the study. The choice of route of administration depends on the physical and chemical characteristics of the test substance.

9.1.1 A five-day-week dosing regimen is acceptable if the test substance is administered by gavage.

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. Procedure

10.1 Appropriate toxicological data on acute, subchronic, and metabolic responses should have been conducted to permit selection of the dosage levels.

10.2 Select three dosage levels (low, intermediate, and high) plus an untreated or solvent control. Space the dose levels to produce a gradation of chronic effects. Dose all animals by the same method during the entire experimental period.

10.2.1 The high-dosage level should elicit signs of minimal toxicity without substantially altering the normal life span.

10.2.2 The lowest dosage level should not interfere with the normal growth, development, and longevity of the animal, and it should not otherwise cause any indication of toxicity.

10.2.3 Both untreated and vehicle control groups are required if the toxic properties of the vehicle are not known or cannot be made available.

10.3 Randomize, number, and assign at least 124 rats (62 females and 62 males) and 112 mice (56 females and 56 males) to each dosage group. Of these animals, select and assign 12 animals (6 females and 6 males) for a 12-month interim sacrifice for each dosage group. Select and assign 12 rats (6 female and 6 males) for an 18-month interim sacrifice for each dosage group.

10.4 Administer the test substance (if in the diet or drinking water) ad libitum throughout the study and, depending on the stability of the test material, replace it at least weekly.

10.4.1 Perform chemical analysis of the test mixtures (depending on the stability of the test substance) at least once on each new batch of test food or water prepared.

10.5 *Diet Preparation*—Calculate the test substance food mixture for the first two weeks using the mean body weights and mean food consumption weights computed during the acclimation period. Thereafter, prepare the mixtures from the mean body weights and mean food consumption weights computed from the first week of the two-week period.

[4] *Guide for the Care and Use of Laboratory Animals*, NIH Publication No. 85-23, Public Health Service, National Institutes of Health, Revised 1985.

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