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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.

ε1 NOTE—Section 3 was editorially corrected in November 2005.

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*, *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 an extended period of time.

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

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

3.2.4 *nulliparous*, *adj*—having never borne an offspring.

3.2.5 *oncogenicity*, *n*—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.

[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.

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

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

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

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.

10.5.1 Compute the test substance food mixture concentrations for each dosage group using the following formula:

$$X = 100\,K/G \quad (1)$$

where:

X = percent of active test substance in the diet (grams of test substance/100 g of ground food),
K = dosage of substance that is desired, expressed as grams of test substance/kg of body weight/day, and
G = amount of food consumed per day over a one-week period, expressed as grams of food consumed/kg of body weight/day.

10.5.2 Record the food consumption (and water, if necessary) throughout the study.

10.5.3 An alternative to this test method would be to determine the concentration of test substance in the feed prior to study initiation and then have it remain constant throughout the study. This procedure is less desirable because the exposure of the animals to the test substance decreases with the age of the animal.

10.6 *Observations*—Make observations of each animal at least once per day, with appropriate actions taken to minimize loss of animals to the study (for example, necropsy or refrigeration of animals found dead and isolation or sacrifice of weak or moribund animals).

10.6.1 Record all signs of toxicity (by dosage group and sex) as they are observed, including the time of onset, degree, and duration. These signs include, but are not limited to, changes in skin and fur, eyes and mucous membranes, and also respiratory, circulatory, autonomic, and central nervous systems, somatomotor activity, and unusual behavior patterns. Pay special attention to potential lesion and tumor development.

10.6.2 Weigh all animals at least once per week, on the same day of each week, and record the weights.

10.6.3 Record the temperature and humidity continually throughout the study.

10.6.4 At the end of one year, perform an interim sacrifice on the pre-selected animals.

10.6.5 At the end of 18 months, sacrifice all surviving mice and perform an interim sacrifice on the preselected rats.

10.6.6 At the end of two years, sacrifice all surviving rats.

10.7 *Clinical Examinations*—Make the following clinical examinations:

10.7.1 *Hematology*—At 12 months, 18 months, and terminal sacrifice, obtain blood smears from all animals in the high-dosage and control groups. Perform a differential blood count on these blood smears. If these data or data from the pathological examinations indicate a need, then obtain and examine blood smears from the other test groups.

10.7.2 If there is a major discrepancy between the results for the high-dosage group and the control group, then obtain and examine blood smears for the next lower group.

10.8 *Necropsy*—Perform a gross necropsy on the preselected 12-month and 18-month animals for interim sacrifice and on all mortalities during the study. The sacrifice should be conducted by accepted humane methods.

10.8.1 At the end of 18 months, sacrifice all surviving mice by accepted humane methods and subject all to a full gross necropsy.

10.8.2 At the end of 24 months, sacrifice all surviving rats by accepted humane methods and subject all to a full gross necropsy.

10.8.3 The gross necropsy includes examination of the external surfaces of the body; all orifices; and the cranial, thoracic, and abdominal cavities and their contents.

10.8.4 Preserve the following organs and tissues, or representative samples, in a suitable medium for future histopathological examination as follows: a sample of all tissues containing gross lesions or tumors, brain (including sections of medulla/pons, cerebellar cortex, and cerebral cortex), pituitary, thyroid/parathyroid, thymus, lungs and trachea, heart, bone marrow (either femur, sternum, or rib at the costochondral junction), salivary glands, liver, spleen, kidneys, adrenals, pancreas, gonads, uterus, accessory genital organs (epididymis, prostrate, and, if present, seminal vesicles), ovaries, aorta, skin, esophagus, stomach, duodenum, jejunum, ileum, caecum, colon, rectum, urinary bladder, representative lymph node, peripheral nerve, mammary gland, thigh musculature, eyes, femur (including articular surface), and spinal cord at three levels (cervical, midthoracic, and lumbar).

10.8.5 In addition, weigh the following organs: liver, kidneys, adrenals, gonads, and brain. Prior to weighing, dissect organs carefully and trim to remove fat and other tissue in a uniform manner. Weigh organs as soon as possible to avoid weight loss due to drying.

10.9 *Histopathology*—Perform full histopathological examinations on the tissues and organs of all animals in the control and high-dosage groups and all animals that died or were killed during the study.

10.9.1 Perform histopathological examinations on all gross lesions and tumors, lungs, liver, and kidneys of all animals.

10.9.2 For the interim sacrifice groups, perform histopathological examinations on all tissues and organs showing effects in other treated groups.

10.9.3 Conduct further histopathological examinations in other groups on organs that show lesions or tumors in the high-dosage group or for which clinical observations indicate such a need.

11. Interpretation of Results

11.1 Evaluate the findings of this study with the findings of previous studies with consideration for the toxic effects, necropsy, and pathological findings. The evaluation shall include the relationship between the dose of the test substance and the presence, incidence, and severity of abnormalities (including behavioral and clinical abnormalities); gross lesions, identified organs; body weight changes; effects on mortality; and any general or specific toxic effects.

11.2 For a negative test to be acceptable, the test shall meet the following criteria: no more than 10 % of any group is lost due to autolysis, cannibalism, or management problems; and survival in each group should be no less than 50 % at 18 months for mice and 24 months for rats.

11.3 Compare statistically the test group data (animal body weights, organ-to-body weight, and organ-to-brain weight ratios or appropriate alternate means of correction) for any given group with the control group for the same period. Any acceptable statistical method may generally be used.

11.4 Choose the statistical method during design of the study. Perform supplementary statistical tests as needed. Base the need and nature of these supplementary statistical tests on initial statistical analysis data.

12. Report

12.1 Report the following information:

12.1.1 Name of the investigator(s), laboratory, laboratory address, location of raw data, and date of initiation and termination of the test;

12.1.2 Name of the species and strain of animals tested, including the scientific name, source, and age of the animals at the beginning of the test;

12.1.3 Detailed description of the test substance, including its chemical name, Chemical Abstracts Services (CAS) number, synonyms, formulations, purity, source batch, lot number, physical/chemical properties, and the name of solvent or carrier, if used;

12.1.4 Description of the test facilities and housing conditions, including the test cages, temperature, humidity, and photoperiod;

12.1.5 Name and source of the feed, including description and analysis of the diet;

12.1.6 Concentration of the test substance in food or water and predicted and calculated doses for each test group when the tested substance is mixed in food or water;

12.1.7 Number of animals (male and female) per dosage group, body weights, food consumption (water consumption, if necessary), signs of toxicity (the numbers affected and dose by sex and dosage group), abnormal behavior, and percent mortality (by sex and dosage group);

12.1.8 Anything unusual regarding the test, any deviations from the protocol, and any other relevant information;

12.1.9 Statistical methods used; and

12.1.10 Significant necropsy findings, organ weights (liver, kidneys, gonads, and brain), organ-to-weight weight ratios (or appropriate alternate means of correlation).

13. Quality Assurance

13.1 Use good laboratory practices to ensure the quality and reliability of data developed using this test method (see Title 21 CFR, Part 58 and Title 40 CFR, Parts 160 and 792).

14. Precision and Bias

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

15. Keywords

15.1 chronic toxicity; feed efficiency; gavage; hematology; mice; necropsy; oncogenicity; oral; pesticide; rat; toxicity; tumor