Designation: E1619 – 11 (Reapproved 2016)

Standard Test 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

[1] This test method is under the jurisdiction of ASTM Committee E50 on Environmental Assessment, Risk Management and Corrective Action and is the direct responsibility of Subcommittee E50.47 on Biological Effects and Environmental Fate.

Current edition approved Feb. 1, 2016. Published May 2016. Originally approved in 1994. Last previous edition approved in 2011 as E1619 – 11. DOI: 10.1520/E1619-11R16.

[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 Publishing Office, 732 N. Capitol St., NW, Washington DC 20401-0001, http://www.gpo.gov.

[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

animals in appropriate cages for a five-day acclimation period. During this period, all rats will receive rodent diet minus the test substance. Dietary levels of the test substance to be administered may approximate the acute oral LD_{50} dosages and fractions thereof (such as 1X, 0.5X, 0.25X, 0.125X, 0.0625X, 0.03125X of the LD_{50}). One additional group of each sex will serve as a solvent or untreated control.

10.3 It is strongly recommended that a dietary group be removed from testing for humane purposes when food consumption is markedly reduced. If consumption as compared to controls or acclimation period values, or both, is reduced by more than 90 %, continued exposure will result in mortality of test animals in that group.

10.4 Base the no-effect and effect levels on the following parameters: body weight, organ-to-body weight ratios, hematology, clinical chemistry, gross necropsy, and food or water consumption, or both, if necessary. Histology on a limited selection of organs may be necessary in some instances.

10.5 If a lethal dose is not found, set the highest dietary dosage at 1000 mg/kg, since dosage below this value is assumed to be nontoxic.

11. Procedure[5]

11.1 Select four dosage levels (low, two intermediate concentrations, and high) plus an untreated or solvent control. Dose all animals by the same method during the entire experimental period.

11.2 Randomize, number, and assign at least 40 rats (20 females and 20 males) to each dosage group. If additional sacrifices are planned, increase the number of animals by the number scheduled to be sacrificed during the course of the study.

11.3 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 at least weekly.

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

11.5 *Diet Preparation*—Calculate test substance food mixtures 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 previous two-week period.

11.5.1 Compute test substance food-mixture concentrations for each dosage using the following formula:

$$X = 100K/G$$

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 and is expressed as grams of test substance/kilogram of body weight/day,

G = amount of food consumed/day over a one-week period and is expressed as grams of food consumed per kilogram of body weight/day.

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

11.5.2 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.

11.6 *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).

11.6.1 Record signs of toxicity (by dosage group and sex) as they are observed, including 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.

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

11.6.3 Record temperature and humidity continually throughout the study.

11.6.4 At the end of the one year, sacrifice all surviving animals.

11.7 *Clinical Examinations*—Make the following clinical examinations on at least five of each sex in each group of rats.

11.7.1 *Urinalysis*—Perform urinalysis at the termination of the testing period. Place randomly selected animals in from each group and from each sex in metabolism cages for urine collection. Evaluate each urine sample individually and include the following measurements: specific gravity, pH, protein, glucose, ketones, bilirubins, urobilinogen, as well as microscopic examination of formed elements.

11.7.2 *Hematology*—Make the following hematology determinations at least twice during the test period on all groups of animals including concurrent controls (at six months into the study and just prior to the terminal sacrifice at the end of the study) as follows: hematocrit, hemoglobin concentration, erythrocyte count, total and differential leukocyte counts, and a measure of clotting potential such as prothrombin time or platelet count, and reticulocyte count, if signs of anemia are present.

11.7.3 *Blood Chemistry*—Make clinical biochemical tests that are considered appropriate to all studies, at least twice during the test period on all groups of animals including concurrent controls (at six months and just prior to the terminal sacrifice at the end of the test period) as follows: electrolyte balance, carbohydrate metabolism, and liver and kidney function. The selection of specific tests will be influenced by observations on the mode of action of the test substance. Suggested determinations are as follows: calcium, phosphorus, chloride, sodium, potassium, fasting glucose with period of

[5] Fitzhugh, O. G., "Chronic Oral Toxicity", *Appraisal of the Safety of Chemicals in Food, Drugs and Cosmetics*, The Association of Food and Drug Officials of the United States, Washington, DC, 1959, 3rd printing 1975, pp. 36-45.

fasting appropriate to the species/breed, serum glutamic-pyruvic transaminase (now known as serum alanine aminotransferase), serum glutamic oxaloacetic transaminase (now known as serum aspartate aminotransferase), ornithine decarboxylase, gamma glutamyl transferase (now known as gamma glutamyl transpeptidase), urea nitrogen, albumen, blood creatinine, total bilirubin, and total serum protein measurements. Other determinations may be necessary for adequate toxicological evaluation include: analyses of lipids, hormones, acid/base balance, methemoglobin, and cholinesterase activity. Additional clinical biochemistry may be employed, where necessary, to extend investigation of observed effects.

11.8 *Necropsy*—Perform necropsy on all mortalities and any group withdrawn from the study. At the termination of the test, sacrifice all surviving animals by accepted humane methods and subject all to a full gross necropsy. This includes examination of the external surface of the body, all orifices, and the cranial, thoracic, and abdominal cavities and their contents.

11.8.1 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, brain (including sections of medulla/pons, cerebellar cortex, 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, and peripheral nerve.

11.8.2 Preserve the following tissues only if indicated by signs of toxicity or target organ involvement: mammary gland, thigh musculature, eyes, femur (including articular surface), spinal cord at three levels (cervical, midthoracic, and lumbar), and exorbital lachrymal glands.

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

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

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

11.9.2 For the interim sacrifice group, perform histopathological examination on all tissues and organs showing effects in other treated groups.

11.9.3 Carry out further histopathology in other groups on organs that show lesions in the high-dosage group or for which clinical observations indicate such a need.

12. Interpretation of Results

12.1 Compare statistically test group data (animal body weights, organ-to-body weight, and organ-to-brain weight ratios or appropriate alternate means of correction), food consumption, (water consumption, if necessary), feed efficiency, hematology, and clinical chemistry and urinalysis for any given group with the control group for the same period. Generally any acceptable statistical method may be used.

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

13. Report

13.1 Report the following information:

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

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

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

13.1.4 Description of test facilities and housing conditions, including test cages, temperature, humidity, and photoperiod,

13.1.5 Name and source of feed including description and analysis of diet,

13.1.6 The concentration of test substance in food or water, predicted and calculated doses for each test group when tested substance is mixed in food or water,

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

13.1.8 Anything unusual about the test, any deviations from the protocol and any other relevant information,

13.1.9 Statistical methods employed, and

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

14. Quality Assurance

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

15. Precision and Bias

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

16. Keywords

16.1 blood chemistry; chronic toxicity; feed efficiency; gavage; hematology; LD_{50}; necropsy; oral; pesticide; rat; toxicity; urinalysis