Designation: E 1103 – 96 (Reapproved 2004)ε1

Standard Test Method for Determining Subchronic Dermal Toxicity[1]

This standard is issued under the fixed designation E 1103; 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—Editorial changes were made throughout in February 2005.

1. Scope

1.1 This test method describes a procedure for the assessment and evaluation of the toxic characteristics of a test substance that is applied daily to the skin of experimental animals for 90 days.

1.2 This test method is not capable of determining effects that have a long latency period (for example, carcinogenicity) or are life shortening.

1.3 This test method is intended primarily to be used with rats, guinea pigs, or rabbits. Other species may be used with appropriate modifications.

1.4 *This standard does not purport to address 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:* [2]

E 609 Terminology Relating to Pesticides

E 943 Terminology Relating to Biological Effects and Environmental Fate

2.2 *Federal Standards:*

Title 21, Code of Federal Regulations (CFR), Food and Drug Administration, Part 58, Good Laboratory Practice for Nonclinical Laboratory Studies[3]

Title 40, Code of Federal Regulations (CFR), Environmental Protection Agency, Part 798, Health Effects Testing Guidelines, Subpart C, Subchronic Exposure, Oral Toxicity[3]

Title 40, Code of Federal Regulations (CFR), Environmental Protection Agency, Part 798, Health Effects Testing Guidelines, Subpart B, General Toxicity Testing, Acute Dermal Toxicity[3]

Title 40, Code of Federal Regulations (CFR), Environmental Protection Agency, Subchapter E, Pesticide Programs; Part 160, Good Laboratory Practice Standards[3]

Title 40, Code of Federal Regulations (CFR), Subchapter R, Toxic Substance Control Act, Part 792, Good Laboratory Practice Standards[3]

3. Terminology

3.1 *Definitions*—For definitions of terms used in this test method, see Terminology E 609 and E 943.

3.2 *Definitions of Terms Specific to This Standard:*

3.2.1 *dose, dosage, n*—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).

3.2.2 *no observed adverse effect dose (NOAED), n*—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.

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

3.2.4 *test substance, n*—pesticide or other material (element, chemical compound, formulation, known mixture) administered dermally for the purpose of determining subchronic dermal toxicity.

4. Summary of Test Method

4.1 The test substance is applied daily to the skin in geometrically graduated doses to several groups of animals, one dose per group, for a period of 90 days. Generally at least three dose levels with a control and, where appropriate, a vehicle control group are employed.

4.2 Animals that die during the test are necropsied. At the conclusion of the test, the surviving animals are sacrificed and necropsied and appropriate histopathological examinations performed.

[1] This test method is under the jurisdiction of ASTM Committee E35 on Pesticides and Alternative Control Agents 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.

4.3 A limit test with one dose of at least 1000 mg/kg body weight can be conducted using the procedures described for this method if no toxicity would be expected based upon data of structurally related compounds. If this test produces no observable toxic effects, then a full study using three doses is not necessary.

5. Significance and Use

5.1 This test method provides information on health hazards likely to arise from repeated exposure to a pesticide or other chemical by the dermal route over a short time period. It may provide information needed to establish safety criteria for human exposure.

5.2 Signs of toxicity other than lethality can be observed.

5.3 This method can provide information on target organs and possible cumulative effects. It may aid in the selection of doses for chronic studies.

6. Hazards

6.1 Minimize contact with all test substances, solutions, and mixed diets with appropriate protective clothing, gloves, eye protection, and so forth. The use of fume hoods and increased ventilation in test rooms is necessary when handling volatile substances. Know information on acute mammalian toxicity and special handling procedures before this method is used.

6.2 Ensure health and environmental safety prior to disposal of excess test substances, solutions, mixed diets, excreta, and treated animals, and in accordance with all federal, state, and local regulations.

6.3 Clean and rinse glassware, feeders, and other equipment with volatile solvents only in well ventilated areas. The use of fume hoods may be necessary when handling volatile substances.

6.4 Consider periodic medical examinations for all personnel caring for animals or handling test substances.

7. Facilities Required

7.1 Test animals shall be individually housed in cages meeting the requirements specified in the *Guide for The Care and Use of Laboratory Animals*.[4]

7.2 Animal holding rooms should be maintained at 70 ± 3°F (21.1 ± 2°C), with 50 ± 5 % relative humidity for guinea pigs or rats and 43 ± 3 % for rabbits, and a 12-h light-dark cycle.

7.3 Testing areas shall be maintained at the same temperature and humidity as animal holding rooms.

8. Test Animals

8.1 Rats, rabbits, or guinea pigs should be used. However albino rabbits are preferred because of their size, skin permeability, and extensive data base.

8.1.1 If another mammalian species is used, the investigator should record the justification for the selection.

8.2 Young adult animals should be used. The following weight ranges at the start of the test are recommended.

8.2.1 *Rats*—200 to 300 g.

8.2.2 *Rabbits*—2 to 3 kg.

8.2.3 *Guinea Pigs*—350 to 450 g.

8.3 Equal numbers of animals of each sex with healthy skin should be used at each dose. A minimum of ten animals per sex per group is recommended.

8.3.1 The females should be nulliparious and nonpregnant.

8.3.2 If interim or post sacrifices are planned additional animals should be included in the study.

9. Pretest Conditioning

9.1 Examine the animals on arrival for overt signs of disease and condition them to the environment for a minimum of 14 days. Select animals that have not been used on any other tests.

9.2 Maintain the animals during pretest and test periods according to accepted laboratory practices for the care and handling of animals.

9.3 Identify each animal with an ear tag or other suitable means.

9.4 During acclimation, observe the animals for respiratory distress, diarrhea, emaciation, ocular and nasal discharges, skin lesions, and eye defects. Eliminate any animal demonstrating signs of spontaneous disease prior to the start of the study. Use only animals judged to be healthy. Animals on test should be segregated in different rooms. Chow or the equivalent and water are to be available *ad libitum*.

10. Dose Level and Dose Selection

10.1 At least three doses plus a control must be used. Doses shall be spaced geometrically to produce test groups with a range of toxic effects and mortality rates. The data should be sufficient to produce a dose-response effect.

10.1.1 If no information is available for establishing doses, then a 14-day range-finding test should be conducted.

10.2 The highest dose should result in toxic effects but not produce severe skin irritation or an excessively high incidence of fatalities that would prevent a meaningful evaluation.

10.3 The lowest dose should not produce any evidence of toxicity. Where there is a reasonable estimate of human exposure, however, the lowest dose should exceed this value.

10.4 If more than one intermediate dose is used, then the doses should be spaced to provide a gradation of toxic effects.

11. Procedure

11.1 *Preparation of Animal Skin*:

11.1.1 Clip the fur from the dorsal area of the trunk of the test animals approximately 24 h before the test. Repeat clipping or shaving as needed, usually at approximately weekly intervals. When clipping or shaving the fur, take care to avoid abrading the skin which could alter the permeability of the skin.

11.1.2 Clear at least 10 % of the body surface for the application of the test substance.

11.2 *Application of the Test Substance*:

11.2.1 When testing liquids, apply the test substance as is or, if appropriate, diluted in a suitable solvent. If a solvent is employed, then include a solvent control group of treated animals. Consider the influence of the solvent on penetration of skin by the test substance.

[4] Available from the Institute of Laboratory Animal Resources, National Research Council, DHEW Publication No. (NIH) 80-23, 1980.

11.2.2 When testing solids, pulverize the test substance, if appropriate, and moisten with water or a suitable vehicle to ensure good contact with the skin. When a vehicle is used, consider the influence of the vehicle on penetration of skin by the test substance; include a vehicle control group of treated animals.

11.2.3 Apply the test substance uniformly over an area that is approximately 10 % of the total body surface area.

11.2.4 During the exposure period, hold the test substance in contact with the skin with a porous gauze and nonirritating tape. Place Elizabethan collars around the necks of the animals or other appropriate restrainers to prevent ingestion of the test material, but complete immobilization is not recommended.

11.2.5 Treat with the test substance for at least 6 h per day on a 7-day per week basis. Based on practical considerations, application on a 5-day per week basis may be acceptable.

11.2.6 If only a 6-h per day exposure is used, then wash the backs of the animals with tepid tap water to remove the test material. Dry the backs of the animals with a soft cloth.

11.2.7 The exposure period should last at least 13 weeks whether the test substance is applied 5 or 7 days per week.

11.3 *Observation of Animals*:

11.3.1 Observe the animals for at least 90 days.

11.3.2 Make careful cage-side observations for general health of each animal at least once per day. Make additional observations as needed with appropriate actions taken to minimize the loss of animals to the study (for example, necropsy or refrigeration of animals found moribund or dead).

11.3.3 Observe changes in skin and fur, eyes and mucous membranes, respiratory, circulatory, autonomic and central nervous systems, somatomotor activity and behavior pattern.

11.3.4 Record signs of toxicity as they are observed, noting the time of onset, severity, and the duration.

11.3.5 Weigh each animal weekly. Determine food and water consumption weekly, if abnormal body weight changes occur.

11.4 *Clinical Examinations*:

11.4.1 Conduct hematology determinations on at least 5 animals of each sex in each group three times during the test period: just prior to initiation of dosing, after approximately 30 days of test and just prior to terminal sacrifice at end of test.

11.4.1.1 Make hematology determinations as follows: hematocrit, hemoglobin concentration, erthrocyte count, total and differential leukocyte counts, and a measure of clotting potential such as prothrombin time, thromboplastin time or platelet count, and if signs of anemia are present, reticulocyte count.

11.4.2 Carry out clinical biochemistry determinations on at least 5 animals of each sex in each group three times during the test period. The selection of specific tests will be influenced by the mode of action of the test substance. Tests that are considered appropriate for all studies include: electrolyte balance, carbohydrate metabolism, and kidney and liver function. The selection of specific tests will be influenced by observations on the mode of action of the substance. Suggested determinations are as follows: calcium, phosphorus, chloride, sodium, potassium, fasting glucose with period of 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 as follows: analyses of lipids, hormones, acid/base balance, methemoglobin, and cholinesterase activity. Additional clinical biochemistry may be employed, where necessary, to extend the investigation of observed effects.

11.4.3 Conduct the following examinations on at least 5 animals of each sex in each group: ophthalmological examinations and urinalysis. Urinalysis is not required on a routine basis but only when there is an indication of need based on expected or observed toxicity.

11.4.4 If urinalysis is determined necessary, perform at the termination of the testing period. Randomly selected animals from each sex and each group may be placed 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.

11.5 *Gross Necropsy*:

11.5.1 At the end of the test, weigh and sacrifice all surviving animals.

11.5.2 Subject all animals to a full gross necropsy that includes examination of the external body surface, all orifices, and the cranial, thoracic, and abdominal cavities and their contents.

11.5.3 Weigh the liver, kidney, adrenals, brain, and gonads wet, as soon as possible after dissection to avoid drying.

11.5.4 Preserve the following organs and tissues in a suitable medium for possible future histopathological examination: normal and treated skin, all gross lesions; brain—including sections of medulla/pons, cerebellar cortex and cerebral cortex; pituitary; thyroid/parathyroid; thymus; trachea; lungs; heart; sternum with bone marrow; salivary glands; liver; spleen; kidneys; aorta; pancreas; adrenals; gonads; uterus; accessory genital organs; gall bladder (if present); stomach; esophagus; duodenum; jejunum; ileum; cecum; colon; rectum; urinary bladder; representative lymph node; peripheral nerve; eye; and mammary gland of female animals. Additional tissues may be taken as deemed appropriate for the study.

11.6 *Histopathology*:

11.6.1 Perform complete histopathology on normal and treated skin and on organs and tissues listed in 11.5.4 on all animals in the control and high dose groups.

11.6.2 Examine histologically all gross lesions in all animals.

11.6.3 Examine histologically expected target organs in all animals.

11.6.4 Conduct histopathological examination of lungs of animals for evidence of infection, since this provides a convenient assessment of the state of health of the animals.

12. Quality Assurance

12.1 To ensure the quality and reliability of data developed using this test method, good laboratory practices should be followed (see 2.2).

13. Interpretation of Results

13.1 Evaluate all observed results, both quantitative and incidental, by an appropriate statistical method.

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

13.3 Choose the statistical method during the design of the study. Supplementary statistical tests may be performed. The need for and the nature of these supplementary statistical tests may be necessary based upon initial statistical analysis of data.

13.4 Evaluate the findings of the subchronic dermal study in conjunction with the findings of previous studies in terms of the observed toxic effects and the necropsy and histopathological findings. Determine the no observed adverse effect dose (NOAED).

14. Report

14.1 Report the following information:

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

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

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

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

14.1.5 Name and source of feed, including description and analysis of diet;

14.1.6 The concentration of test material in food or water, predicted and calculated doses for each test group when tested material is mixed with food or water;

14.1.7 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);

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

14.1.9 Statistical methods employed; and

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

15. Precision and Bias

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

16. Keywords

16.1 dermal toxicity; guinea pig; hazard; necropsy; rabbit; rat; skin; subchronic toxicity