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.