Designation: E 758 – 98 (Reapproved 2002)

Standard Test Method for Mammalian Acute Percutaneous Toxicity[1]

This standard is issued under the fixed designation E 758; 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 laboratory test method is designed to test the acute toxicity of single applications of material(s) applied to the skin of mammals.

1.2 This test method is applicable to individual chemicals, simple and complex mixtures, and finished products, such as pesticides, repellents, and biocides.

1.3 *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.*

2. Summary of Test Method

2.1 This test method is designed to assess both the sublethal and lethal effects of materials applied to skin that has been clipped free of hair.

2.2 The test animals shall be suitably restrained and exposed to the material only by means of the cutaneous route. If inhalation of the test material is likely, the experiments should be carried out in an exhaust hood or similar environment.

2.3 The toxicity of a test material is determined by using occluded application on normal skin. Unoccluded application on normal or abraded skin, or occluded on abraded skin, may also be used if deemed desirable for the intended use of the data obtained.

2.4 The subjects remain in contact with the substance being tested for 4 to 6 h. The toxicity of the test material can be estimated from either the percent mortality or percent of animals exhibiting signs of intoxication.

3. Significance and Use

3.1 Generally, direct extrapolation of the results of animal to human toxicity cannot be made. This test method can be used for initial percutaneous toxicity studies on a single species. Ideally, the assessment of potential hazard to man requires the use of several mammalian species, such as rabbit, rat, guinea pig, and swine.

3.2 This method can be used to determine the dermal toxicity of technical chemicals, manufacturing use products or formulations.

NOTE 1—This test method is not intended to limit innovative approaches or modifications deemed desirable by individual investigators.

3.3 Some materials are not sufficiently toxic to mammals to be accurately measurable by this method. This may also be true when testing low concentrations of otherwise toxic formulations. Because of practical limitations in the amount of surface area of an animal that can be exposed and exposure times, only semiquantitative data can be obtained for such materials using this test. However, sufficient data should be generated by the use of the method to permit a reasonable estimate of the level of risk to humans.

3.4 Test materials that are a gas or highly volatile do not need to be tested.

4. Test Animals

4.1 Albino animals are generally used for percutaneous tests because direct effects on skin can be more easily observed than is the case when pigmented animals are used. Although lethality is the chief concern, other effects such as erythema, blanching, staining, etc., may occur and should be noted.

4.1.1 New Zealand White rabbits, the animal most commonly used for percutaneous studies, is the preferred species. Additional animal species may also be tested.

4.1.2 If rats are used, domesticated albino strains are preferred.

4.1.3 Guinea pigs from available stocks may be used.

4.1.4 Pigs are often used for percutaneous studies because their skin closely approximates that of man. Weanling Chester White or Landrace strains can be used for these tests.

4.2 All animals used shall be evenly divided as to sex.

4.3 All animals shall be young adults except for swine.

[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.

Current edition approved Oct. 10, 2002. Published March 2003. Originally approved in 1980. Last previous edition approved in 1998 as E 758 – 98.

5. Source of Test Animals

5.1 A reputable laboratory animal supplier must be used for any specified species or strain. A single constant source of supply for each species should be used by the individual testing laboratories to maintain genetically homogeneous test subjects.

5.1.1 Rabbits, rats, and guinea pigs are readily obtainable from licensed suppliers.

5.1.2 Swine can be obtained from local breeders in many areas. It is recommended that a relatively large supplier be used as a source to assure a steady supply from the same genetic background.

6. Condition of Animals

6.1 Only animals that show no signs of ill health shall be used for these studies.

6.2 Animals with cuts, abrasions, sores, or otherwise abnormal skins at or bordering on the application site shall not be used for this test. This restriction applies to studies that call for intentional abrasion of the intact skin as well as those that require intact skin.

6.3 Pregnant or lactating females will not be used.

6.4 No animal that has received medication within a 10-day period prior to the application of the test material shall be used for these studies, except as noted in 6.4.2. Test animals may not have been used for previous experimental procedures.

6.4.1 Application to the animals or their quarters of an insecticide, whether liquid, aerosol, vapor, or dust, shall be considered medication for purposes of this method.

6.4.2 In order to maintain healthy animal stocks, prophylactic medication is often required. However, if such medication is routinely used by the laboratory conducting the tests and all animals on test receive the same treatment, the restrictions in 6.4 need not apply. Examples of prophylactic treatment are antibiotics for the control of salmonella infections in rabbits, and the use of ascorbic acid as a dietary supplement for guinea pigs.

7. Pretest Conditioning

7.1 All animals to be used in these studies shall be quarantined to ensure their good health before use.

7.2 All animals shall be quarantined for a period of one week before use to acclimate them to changed quarters and diet.

7.3 Animals must be fed an adequate diet for the species and shall be allowed free access to food.

7.4 All species must be provided fresh water *ad libitum.*

7.5 Each animal shall be identified.

7.6 It is advisable to weigh each animal 1 week prior to testing and again just prior to the test.

7.6.1 Food consumption should also be noted.

7.7 Laboratory animal housing and test environments shall be maintained according to acceptable animal care accreditation organization requirements; significant deviations therefrom shall be noted and reported.

8. Animal Housing

8.1 All animals shall be housed in cages that are thoroughly cleaned and sanitized prior to use.

TABLE 1 Weights of Young Adult Test Animals

Species	Weight	
	Male	Female
Rat	180 to 300 g	175 to 250 g
Guinea pig	350 to 450 g	375 to 450 g
Rabbit	2 to 3 kg	2 to 3 kg
Swine[A]	15 to 18 kg	15 to 18 kg

[A] Not an adult animal, but a weanling.

8.2 Cage sizes for each species shall meet the requirements specified in the *Guide For The Care and Use of Laboratory Animals* **(1)**.[2]

8.2.1 For swine sufficient space must be allowed for the animals to turn about easily and stand, sit, and lie in a normal position.

8.3 If possible, bedding should not be used in the cages during the test, because stuporous animals may suffocate. Cages with wire mesh bottoms are recommended.

8.4 Optimally, each species should be housed in separate rooms. Where this is not possible, rats and mice may be kept in the same room, but all others must be housed in separate rooms.

9. Weight of Test Animals

9.1 Pretest weights of the test animals shall be taken 1 week before and just prior to application of the test material. Only animals that either maintain their weight or show an increase equivalent to others of the same age, sex, and starting weight shall be used for the studies.

9.2 All animals used shall be young adults, with the exception of swine. The swine used for these studies shall be weanlings. All of either sex must be within ± 20 % of the same weight.

9.3 The various species are considered to be young adults when they are within the weight ranges given in Table 1.

9.3.1 In the selection of test subjects an attempt should be made to produce as nearly identical groups as possible.

10. Number of Test Animals

10.1 All test groups shall be evenly divided by sex.

10.2 For valid estimates of error and a test of the significance of differences between the sexes, five animals of each sex per dose are required. No fewer than three dose levels shall be used for these estimates **(2)**.

10.2.1 Range finding may be helpful to determine dose levels. A statistical method such as that of Thompson and Weil **(3)** may be used for small animals. For the larger animals, the method proposed by Deichmann and LeBlanc **(4)** is a better choice, since it requires the use of fewer animals.

10.3 Where the volume of the materials applied is equivalent (that is, equal volume per kilogram) for all doses tested, a single control group of four animals may be used.

11. Animal Preparation

11.1 Remove each animal from its cage 24 h prior to test and examine for gross defects of the skin, diarrhea, or other

[2] The boldface numerals in parentheses refer to the list of references at the end of this test method.

visible signs of ill health. No animals in questionable health will be used for the test.

11.1.1 Weigh the animals. Reject those which have lost appreciable weight during the holding period.

11.1.2 Carefully remove the hair from the backs and sides of the test animals by closely clipping with an electric animal clipper. Take care to clip closely yet do not allow the clipper to abrade the skin.

11.1.3 Establish an area of sufficient size to allow application of the test material. Preliminary studies or past experience are required to determine the surface area required, which may vary considerably, depending on the active ingredient and formulation. As a first approximation, an area equivalent to 10 % of the body surface is suggested. An area greater than 30 % of the body surface is not practical. Spector **(5)** gives a convenient equation for calculating surface area (*A*), in square centimetres, from body weight (*W*), in grams, for various species of animals. This equation is $k = A/W^{2/3}$ The following constants (*k* values) shall be used for purposes of this method:

Species	*k* Value
Rabbit	9.0
Rat	10.0
Guinea pig	9.0
Swine	9.5

To assure uniform areas of application, semiflexible clear plastic templates of appropriate sizes may be used in demarcating the area, and removed. In no case will application be made directly over the vertebral column.

11.1.4 All animals to be used in a given test will have identical surface areas demarcated in as nearly the same anatomical position as possible.

11.1.5 The test animals will be returned to their cages after pretest preparation.

12. Sample Preparation

12.1 Because of the great variety of physical characteristics and formulations of pesticides, it is not possible to stipulate how the test material should be prepared. The only criterion that can be specified is that the material be formulated as it will be for field use. In most cases, this will result in liquid or finely ground solid formulations.

12.2 The test material shall be at the same temperature as that of the room in which the test is conducted at the time of application to the animals.

13. Test Facilities and Supplies

13.1 The test procedure outlined herein is for rabbits, but can be modified to accommodate the other animal species listed. Place Elizabethan collars around the necks of the animals or other appropriate retainers to prevent ingestion of the test material. Restrain swine by placing them in a sling which is raised sufficiently high to allow the animals' feet to touch the floor but not to allow sufficient flexing of the legs for escape.

13.2 If inhalation of the test material is likely, equip the room in which the tests are conducted so as to provide sufficient ventilation. Face velocity of the hoods or other test facilities should be from 50 to 75 ft/min to assure that only percutaneous effects are observed. Higher face velocities may tend to evaporate the test material, while lower velocities may be insufficient to prevent inhalation.

13.3 Maintain the room in which the test is conducted within the limits of temperature and humidity specified for the animals' housing.

13.4 Occlusive dressings, when deemed necessary by the test supervisor, shall be of suitable impervious material. Nonirritating surgical tape shall be used to seal the sides of the dressing after application of the test material.

14. Limit Test

14.1 If existing acute toxicity information on the test material or chemical substances that are structurally similar indicate little or no toxicity involved, then a single limit test is appropriate.

14.2 Select a single group of six rabbits consisting of three males and three females.

14.3 Place the clipped animals, suitably restrained so as to prevent them from licking their backs but otherwise allowed freedom of movement, on a table or bench of appropriate height for convenient application.

14.4 Place a large dose (2 g per kg body weight) of test material on the backs of the rabbits. Expose them for 4 to 6 h and continually monitor for signs of systemic toxicity.

14.5 At the end of the exposure period, remove any remaining material from the backs and return the rabbits to their cages where food and water are available,

14.6 If no lethality is demonstrated, no further testing for acute dermal toxicity is required. No pathological examination is required.

15. Procedure

15.1 Select groups of each sex of test animals, as evenly divided as to weight as possible, and randomize to determine dosage to be applied, room position, and order of application to assure statistical validity of the results. It is preferable that randomization be done without preselection; for practical reasons, however, this is essentially limited to the smaller rodents.

15.2 Place the clipped animals, suitably restrained so as to prevent them from licking their backs but otherwise allowed freedom of movement, on tables or benches of appropriate height for convenient application. When hoods are used, place the animals facing out.

15.2.1 For abraded skin studies, scratch the stratum corneum with a sharp hypodermic needle. Make two parallel sets of scratches in a tick-tack-toe fashion such that approximately 6.5 cm^2 (1 $in.^2$) squares are produced. Take care to avoid deep scratches that cause bleeding; only superficial scratches are desired.

15.3 Evenly apply the test material or solvent control to the previously delineated skin area by means of a blunted needle and syringe. If the test includes an occlusive dressing, apply the dressing and tape securely immediately after the test material has been delivered to the skin.

15.4 It is desirable that all test animals be treated in a minimal period of time.

15.5 Expose the animals to the test material for 4 to 6 h and continually monitor for signs of systemic toxicity. This exposure interval is more representative of potential human exposures than the standard 24-h interval. Furthermore, it places less stress on restrained animals, and is a convenient length of time to be accommodated in a normal working day. Weil et al **(6)** have reported that the results of a 4-h exposure period correlate with those of a 24-h period.

15.6 At the end of the exposure period, remove any remaining test material. Note and report the presence of any detectable material on the skin at this time.

15.6.1 Initially remove the excess material from the test site by blotting, followed by washing with soapy room temperature water or other appropriate solvent which will not alter the test results. If necessary, an additional rinse with plenty of room temperature water and blotting until dry will complete the removal.

15.7 Return test animals to their cages where feed and water are available at the conclusion of the exposure.

15.8 Observe the test animals twice daily for nonlethal signs of systemic toxicity, skin damage, and for death for an additional 13 days.

15.9 To preclude the possibility of undetected irreversible effects or delayed deaths, hold animals that do not show normal growth or show any other grossly detectable abnormality, and observe for an additional week.

15.10 Pathological examination is not required, but is a useful procedure in case of unexpected results with known materials or when new materials are being evaluated.

16. Signs of Intoxication and Skin Damage

16.1 The signs of intoxication will vary depending on the active ingredient(s) used and the pH, presence or absence of surfactant, volatility, concentration, oil-water partition coefficient and corrosivity of the formulation.

16.1.1 Organophosphorus and carbanate materials, if sufficiently toxic to mammals, will produce signs typical of cholinesterase inhibition. These are muscle fasciculations (frequently at the site of application), generalized tremors, miosis, salivation, lacrimation, muscular weakness (often most pronounced posteriorally), convulsions, progressive signs of respiratory malfunction, cyanosis and death. In rats, chromodacryorrhea (bloody tears) is commonly observed.

16.1.2 The polyhalogenated compounds produce much fewer distinctly separable signs of intoxication. Central nervous system stimulation, followed by generalized depression, convulsions, respiratory depression and death are the major sequelae noted.

16.1.3 Nicotine formulations produce generalized CNS stimulation, tremors, convulsions, and increased respiratory rate, followed by generalized depression and respiratory arrest.

16.1.4 Careful observation is required to accurately determine the onset time of minimal signs of intoxication. Signs of adverse effects from most test materials are usually observed far in advance of death, although this is not always the case.

16.2 Signs of skin irritation or damage such as erythema, edema and necrosis may also be observed. These are important effects that indicate damage to the skin, which usually allows increased penetration. For this reason, these signs must also be carefully noted.

17. Data Analysis

17.1 The method of analysis chosen will depend on the number of animals used for the study.

17.2 The LD50 and 95 % confidence limits shall be estimated.

17.3 Where only a few animals per dose are used, the LD50 may be conveniently estimated by several methods. Perhaps the most commonly used are the methods of Thompson and Weil **(3, 7, 8)** and of Litchfield and Wilcoxon **(9)**, although there are a variety of others that may be used as well. Horn **(10)** has prepared a table of the values obtained with various possible response combinations using the Thompson and Weil method, which requires no calculations and can be conveniently used.

17.4 Where five animals of each sex and three doses were used to establish the LD50 and 95 % confidence limits, the values will be estimated by an appropriate method, such as probit analysis **(11).**

18. Quality Assurance

18.1 To ensure the quality and reliability of data developed using this test method, good laboratory practices should be followed **(12, 13, 14).**

19. Report

19.1 The report shall include the following information:

19.1.1 Species and strain tested, number of animals per dose, sex and weights, LD50 and other calculated values, doses administered, toxic signs observed, and the onset and duration of such signs. Time to death shall be included.

19.1.2 Test conditions, that is, whether the test material was applied undiluted, in simple solution, in formulation, whether occluded or unoccluded, abraded or unabraded, etc.

19.1.3 Presence or absence of observable test material on the test animals at the termination of exposure and methods used for the removal of any remaining test material.

19.1.4 Any residual effect of any kind noted in survivors at the end of the 14-day test period, and the status of survivors at the end of 21 days.

19.1.5 Method used for statistical treatment of the data will be referenced. If probit analysis was used, and a linear regression model is valid, the slope of the line shall also be included.

19.1.6 Any prophylactic medication used to maintain the animals in good health (see Section 6).

19.2 Results of solvent/formulation control experiments, if conducted.

19.3 If animals are necropsied, include any pertinent data in the report.

20. Precision and Bias

20.1 No precision data are available for this standard at present.

21. Keywords

21.1 acute toxicity; chemicals; dermal; guinea pigs; percutaneous toxicity; pesticides; rabbits; rats; swine

REFERENCES

(**1**) *Guide for the Care and Use of Laboratory Animals*, Institute of Laboratory Animal Resources, National Research Council, DHEW Publication No. (NIH) 73-23, 1972, pp. 3–5.

(**2**) Title 40, CFR, Toxic Substances Control Act, Part 798.1100, Acute Dermal Toxicity, 1992.

(**3**) Thompson, W. R. and Weil, C. S. "On the Construction of Tables for Moving-Average Interpolation," *Biometrics*, Vol 8, 1952, pp. 51–54.

(**4**) Deichmann, W. B., and LeBlanc, I. J. "Determination of the Approximate Lethal Dose With About Six Animals," *Journal of Industrial Hygiene Toxicology*, Vol 25, 1943, pp. 415–417.

(**5**) Spector, W. S., ed., *Handbook of Biological Data*, W. B. Saunders and Co., Philadelphia, Pa., 1956, p. 175.

(**6**) Weil, C. S., Condra, N. I., and Carpenter, C. P., "Correlation of 4-Hour vs 24-Hour Contact Skin Penetration in the Rat and Rabbit and Use of the Former for Predictions of Relative Hazard of Pesticide Formulations," *Toxicology and Applied Pharmacology*, Vol 18, 1971, pp. 734–742.

(**7**) Thompson, W. R., "Use of Moving Averages and Interpolation to Estimate Median Effective Dose," *Bacteriological Review*, Vol 11, 1947, pp. 115–145.

(**8**) Weil, C. S., "Tables for Convenient Calculation of Median-Effective Dose (LD50 or ED50) and Instructions in Their Use," *Biometrics*, Vol 8, 1952, pp. 249–263.

(**9**) Litchfield, J. T., Jr., and Wilcoxon, F., "A Simplified Method of Evaluating Dose-Effect Experiments," *Journal of Pharmacology and Experimental Therapeutics*, Vol 96, 1949, pp. 99–117.

(**10**) Horn, H. J., "Simplified LD50 (or ED50) Calculations," *Biometrics*, Vol 12, 1956, pp. 311–322.

(**11**) Finney, D. J., *Probit Analysis*, 2nd Ed., London, Cambridge at the University Press, 1952.

(**12**) Title 40, Code of Federal Regulations (CFR), Environmental Protection Agency, Subchapter E, Pesticide Programs; Part 160, Good Laboratory Practice Standards, 1 July 1986.

(**13**) Title 21, CFR, Food and Drug Administration, Part 58, Laboratory Practice for Nonclinical Studies, 1987.

(**14**) Title 40, CFR, Toxic Substance Control Act, Part 792, Good Laboratory Practice Standards, 1987.