



Standard Test Method for Evaluation of Delayed Contact Hypersensitivity¹

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1. Scope

1.1 This test method determines whether a test substance will elicit delayed-contact hypersensitivity in guinea pigs. The test method is applicable to individual chemical compounds, simple and complex mixtures, and also finished products.

1.2 *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. Referenced Documents

2.1 Federal Standard:

OPPTS 870.2600 Health Effects Test Guidelines, Skin Sensitization. US Environmental Protection Agency Office of Prevention, Pesticides and Toxic Substances²

3. Terminology

3.1 *skin sensitization*—a delayed immunologically mediated cutaneous reaction to a substance as a result of prior exposure.

3.2 *induction concentration*—the concentration of a test substance which will produce minimal irritating effects. In case of non-irritating materials, neat concentration will be used.

3.3 *challenge dose*—a dose of the highest concentration of test substance which shows no irritation.

4. Summary of Test Method

4.1 The test material or a solution of the material is placed on the shaved backs of 20 guinea pigs under occlusive patches. Six hours later, the patches and test material are removed from the animals. This procedure is repeated at the same site once a week for two more weeks for a total of three 6-h exposures. These are called the induction exposures.

4.2 Two weeks after the last induction exposure, the test material is applied to a freshly clipped skin site on each test animal that has not been exposed previously. The same

patching procedure for the induction exposures is used. Ten additional untreated animals are patched in the same manner to serve as controls. This treatment is called the primary challenge.

4.3 Twenty-four hours after the primary challenge application, the animals are depilated, and a minimum of 2 h later, the test sites are graded on a scale of zero to three. This grading is repeated 24 h later and is called a 48-h grade.

4.4 Grades of one or greater in the test group indicate sensitization, provided grades of less than one are noted on control animals.

4.5 Test animals may be rechallenged 7 to 15 days after primary challenge. Test material is applied to freshly clipped, previously unexposed skin sites (same procedure as challenge). Ten previously untreated animals serve as controls.

5. Significance and Use

5.1 This test method can be used to determine whether a test material will elicit skin sensitization or delayed contact hypersensitivity in guinea pigs.^{3,4,5,6}

5.2 The results of this test method are a good indication of the potential for a given test material to produce delayed contact hypersensitivity in human beings under the appropriate conditions.

5.3 This test method can be used as a screening technique before conducting a similar test with human subjects.

6. Reagents and Materials

6.1 *Bandage*, a 20 by 20 mm pad on a 37 by 40 mm adhesive square⁷, or a chamber.⁸

³ Buehler, E. V., *Arch. Dermat.*, Vol 91, 1965, pp. 171–177.

⁴ Griffith, J. F. and Buehler, E. V., “Prediction of Skin Irritancy and Sensitizing Potential with Animals and Man”, *Cutaneous Toxicity*, Academic Press, NY, 1977, pp. 162–173.

⁵ Ritz, H. L., and Buehler, E. V., “Planning, Conduct, and Interpretation of Guinea Pig Sensitization Patch Tests”, *Cutaneous Toxicity*, Academic Press, NY, 1980, pp. 25–40.

⁶ “Delayed-type Contact Sensitization”, *Principles for Evaluation of the Toxicity of Household Substances*, National Academy of Sciences, Washington, DC, 1977, pp. 36–44.

⁷ Parke-Davis Read Bandage available from Professional Medical Products, Inc., P.O. Box 3288, Greenwood, SC 29648.

⁸ Hill Top chamber is available from Hill Top Research Inc., Miamiville, OH 45147, and has been found suitable for this purpose.

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 Available from the Environmental Protection Agency Ariel Rios Building 1200 Pennsylvania Avenue, N.W. Washington, DC 20460 (202) 260-2090