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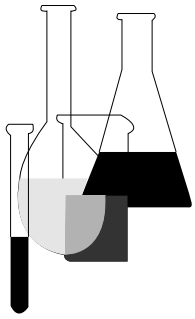
Health Effects Test Guidelines

OPPTS 870.2600
Skin Sensitization



INTRODUCTION

This guideline is one of a series of test guidelines that have been developed by the Office of Prevention, Pesticides and Toxic Substances, United States Environmental Protection Agency for use in the testing of pesticides and toxic substances, and the development of test data that must be submitted to the Agency for review under Federal regulations.

The Office of Prevention, Pesticides and Toxic Substances (OPPTS) has developed this guideline through a process of harmonization that blended the testing guidance and requirements that existed in the Office of Pollution Prevention and Toxics (OPPT) and appeared in Title 40, Chapter I, Subchapter R of the Code of Federal Regulations (CFR), the Office of Pesticide Programs (OPP) which appeared in publications of the National Technical Information Service (NTIS) and the guidelines published by the Organization for Economic Cooperation and Development (OECD).

The purpose of harmonizing these guidelines into a single set of OPPTS guidelines is to minimize variations among the testing procedures that must be performed to meet the data requirements of the U. S. Environmental Protection Agency under the Toxic Substances Control Act (15 U.S.C. 2601) and the Federal Insecticide, Fungicide and Rodenticide Act (7 U.S.C. 136, *et seq.*).

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OPPTS 870.2600 Skin sensitization.

(a) **Scope**—(1) **Applicability.** This guideline is intended to meet testing requirements of both the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) (7 U.S.C. 136, *et seq.*) and the Toxic Substances Control Act (TSCA) (15 U.S.C. 2601).

(2) **Background.** The source materials used in developing this harmonized OPPTS test guideline are the OPPT 40 CFR 798.4100 Dermal Sensitization; OPP 81–6 Dermal Sensitization (Pesticide Assessment Guidelines, Subdivision F—Hazard Evaluation; Human and Domestic Animals) EPA report 540/09–82–025, 1982; and OECD 406 Skin Sensitization.

(b) **Purpose.** Determination of the potential to cause or elicit skin-sensitization reactions (allergic contact dermatitis) is an important element in evaluating a substance's toxicity. Information derived from skin-sensitization tests serves to identify possible hazards to a population exposed repeatedly to a test substance. The test selected should identify substances with significant allergenic potential and minimize false negative results.

(c) **Definitions.** The definitions in section 3 of TSCA and in 40 CFR Part 792—Good Laboratory Practice Standards (GLP) apply to this test guideline. The following definitions also apply to this test guideline.

Challenge exposure is an experimental exposure of a previously treated subject to a test substance following an induction period, to determine if the subject will react in a hypersensitive manner.

Induction exposure is an experimental exposure of a subject to a test substance with the intention of inducing a hypersensitive state.

Induction period is a period of a least 1 week following an induction exposure during which a hypersensitive state may develop.

Skin sensitization (allergic contact dermatitis) is an immunologically mediated cutaneous reaction to a substance. In the human, the responses may be characterized by pruritis, erythema, edema, papules, vesicles, bullae, or a combination of these. In other species, the reactions may differ and only erythema and edema may be seen.

(d) **Principle of the test method.** Following initial exposure to a test substance, the animals are subjected, after a period of not less than 1 week, to a challenge exposure with the test substance to establish whether a hypersensitive state has been induced. Sensitization is determined by examining the reaction to the challenge exposure and comparing this reaction with that of the initial induction exposure. The test animals are initially exposed to the test substance by intradermal and/or epidermal application (induction exposure). Following a rest period of 10 to 14 days (the induction period), during which an immune response may develop, the

animals are exposed to a challenge dose. The extent and degree of skin reaction to the challenge exposure is compared with that demonstrated by control animals that undergo sham treatment during induction and then receive the challenge exposure.

(e) **Test procedures.** (1) Any of the following test methods is considered to be acceptable.

(i) Buehler test.

(ii) Guinea-pig maximization test (GPMT).

(iii) Other.

(A) Open epicutaneous test.

(B) Maurer optimization test.

(C) Split adjuvant technique.

(D) Freund's complete adjuvant test.

(E) Draize sensitization test.

(2) The GPMT of Magnusson and Kligman, which uses adjuvant, and the nonadjuvant Buehler test are given preference over other methods. Although strong preference is given to either the Buehler test or the GPMT, it is recognized that other tests may give useful results. If other tests are used, the tester should provide justification/reasoning for their use, methods and protocols must be provided, and each test should include a positive and a negative control group.

(f) **Screening tests.** The mouse ear swelling test (MEST) (see paragraphs (i)(9), (i)(10), (i)(11), and (i)(12) of this guideline) or the local (auricular) lymph node assay (LLNA) (see paragraphs (i)(13), (i)(14), (i)(15), and (i)(16) of this guideline) in the mouse may be used as screening tests to detect moderate to strong sensitizers. If a positive result is seen in either assay, the test substance may be designated a potential sensitizer, and it may not be necessary to conduct a further test in guinea pigs. If the LLNA or MEST does not indicate sensitization, the test substance should not be designated a nonsensitizer without confirmation in an accepted test using guinea pigs.

(g) **Animal selection**—(1) **Species and strain.** The young adult guinea pig is preferred. Commonly used laboratory strains should be employed. If other species are used, the tester should provide justification/reasoning for their selection.

(2) **Housing and feeding.** The temperature of the experimental animal room should be 20 ± 3 °C with the relative humidity 30–70 percent. Where the lighting is artificial, the sequence should be 12 h light/

12 h dark. Conventional laboratory diets may be used with an unlimited supply of drinking water. It is essential that guinea pigs receive an adequate amount of ascorbic acid.

(3) **Number and sex.** The number and sex will depend on the method chosen. Either sex may be used in the Buehler test and the GPMT. If females are used, they should be nulliparous and not pregnant. The Buehler test recommends using a minimum of 20 animals in the treatment and at least 10 as controls. At least 10 animals in the treatment group and 5 in the control group should be used with the GPMT, with the stipulation that if it is not possible to conclude that the test substance is a sensitizer after using fewer than 20 test and 10 control guinea pigs, the testing of additional animals to give a total of at least 20 test and 10 control animals is strongly recommended.

(4) **Control animals.** (i) The sensitivity and reliability of the experimental technique used should be assessed every 6 months in naive animals by the use of positive control substances known to have mild-to-moderate skin-sensitizing properties. In a properly conducted test, a response of at least 30 percent in an adjuvant test and at least 15 percent in a nonadjuvant test should be expected for mild-moderate sensitizers. Preferred substances are hexylcinnamic aldehyde (CAS No. 101–86–0), mercaptobenzothiazole (CAS No. 149–30–4), benzocaine (CAS No. 94–09–7), dinitro-chloro-benzene (CAS No. 97–00–7), or DER 331 epoxy resin. There may be circumstances where, given adequate justification, other control substances meeting the above criteria may be used.

(ii) Depending upon the test selected, animals may be used as their own controls, but usually there will be a separate group of sham-treated animals that are exposed to the test substance only after the induction period, whose reactions are compared to those of the animals that have received both induction and challenge exposures. Control groups which provide the best design should be used. Some cases may best be served by both naive and vehicle control groups.

(5) **Dose levels.** The dose level will depend on the test method selected. In the Buehler test, the concentration of the induction dose should be high enough to cause mild irritation, and the challenge dose should use the highest non-irritating concentration. In the GPMT, the concentration of the induction dose should be well tolerated systemically, and should be high enough to cause mild-to-moderate skin irritation; the GPMT challenge dose should use the highest non-irritating concentration.

(6) **Observation of animals.** (i) Skin reactions should be graded and recorded after the challenge exposures at the time specified by the methodology selected. This is usually at 24 and 48 hours. Additional notations should be made as necessary to fully describe unusual responses.

(ii) Regardless of the test method selected, initial and terminal body weights should be taken and recorded.

(7) **Procedures.** The procedures to be used are those described by the test method chosen. Brief summaries are given here, but the tester should refer to the original literature for more complete guidance on conducting the Buehler test (under paragraphs (i)(1), (i)(2), (i)(3), and (i)(4) of this guideline) or the GPMT (under paragraphs (i)(5), (i)(6), (i)(7), and (i)(8) of this guideline).

(i) The Buehler test uses topical administration via a closed patch on days 0, 6–8, and 13–15 for induction, with topical challenge of the untreated flank for 6 hours on day 27–28. Readings are made approximately 24 hours after removing the challenge patch, and again 24 hours after that. If the results are equivocal, the animals may be rechallenged one week later, using either the original control group or a new control group for comparison. See paragraphs (i)(1), (i)(2), (i)(3), and (i)(4) of this guideline.

(ii) The GPMT uses intradermal injection with and without Freund's complete adjuvant (FCA) for induction, followed on days 5–8 by topical irritation/induction, followed by topical challenge for 24 hours on day 20–22. Readings are made approximately 24 hours after removal of the challenge dose, and again after another 24 hours. As with the Buehler test, if the results are equivocal, the animals may be rechallenged 1 week later. If only 10 animals were used initially and gave equivocal results, the use of an additional 10 experimental and 5 control animals is strongly recommended. See paragraphs (i)(5), (i)(6), (i)(7), and (i)(8) of this guideline.

(iii) Blind reading of both test and control animals is recommended.

(iv) Removal of the test material should be accomplished with water or an appropriate solvent, without altering the existing response or the integrity of the epidermis.

(v) Hair is removed from the site of application by clipping, shaving, or possibly by depilation, depending on the test selected.

(h) **Data and reporting.** Data should be summarized in tabular form, showing for each individual animal the skin reaction, results of the induction exposure, and the challenge exposure at times indicated by the method chosen. As a minimum, the erythema and edema should be graded and any unusual finding should be recorded.

(1) **Evaluation of the results.** The evaluation of results will provide information on the proportion of each group that became sensitized and the extent (slight, moderate, severe) of the sensitization reaction in each individual animal.

(2) **Test report.** In addition to the reporting requirements as specified under 40 CFR part 158 (for pesticides) and 40 CFR part 792, subpart J (for toxic substances), the following specific information should be reported:

(i) A description of the method used and the commonly accepted name.

(ii) Information on the positive control study, including the positive control substance used, the method used, and the time conducted.

(iii) The number, species, strain, age, source, and sex of the test animals.

(iv) Individual weights of the animals at the start of the test and at the conclusion of the test.

(v) A brief description of the grading system.

(vi) Each reading made on each individual animal.

(vii) The chemical identification and relevant physicochemical properties of the test substance.

(viii) The vehicles used for induction and challenge, and justification for their use, if other than water or physiological saline. Any material that might reasonably be expected to react with or enhance or retard absorption of the test substance should be reported.

(ix) The total amount of test substance applied for induction and challenge, and the technique of application in each case.

(x) Description of any pre-test conditioning, including diet, quarantine and treatment of disease.

(xi) Description of caging conditions including number (and any change in number) of animals per cage, bedding material, ambient temperature and humidity, photoperiod, and identification of diet of test animal.

(xii) Histopathological findings, if any.

(xiii) Discussion of results.

(xiv) Manufacturer, source, purity, and lot number of test substance.

(xv) Physical nature, and, where appropriate, concentration and pH value for the test substance.

(xvi) A list of references cited in the body of the report, i.e., references to any published literature used in developing the test protocol,

performing the testing, making and interpreting observations, and compiling and evaluating the results.

(i) **References.** The following references should be consulted for additional background information on this test guideline.

(1) Buehler, E.V. Delayed contact hypersensitivity in the guinea pig, *Archives of Dermatology* 91: 171–177 (1965).

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(4) Buehler, E.V. Prospective testing for delayed contact hypersensitivity in guinea pigs: the Buehler method; in *Methods in Immunotoxicology*, Vol. 2, ed. G. Burleson, A. Munson, and J. Dean. Wiley, NY, pp. 343–356 (1995).

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(7) Magnusson, B. Identification of contact sensitizers by animal assay. *Contact Dermatology* 6:46 (1980).

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(10) Maisey, J. and Miller, K., Assessment of the ability of mice fed on Vitamin-A supplemented diet to respond to a variety of potential contact sensitizers. *Contact Dermatitis* 15: 17–23 (1986).

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(14) Kimber, I. et al. Identification of contact allergens using the murine local lymph node assay: comparisons with the Buehler Occluded Patch Test in guinea pigs. *Journal of Applied Toxicology* 10: 173–180 (1990).

(15) Kimber, I. et al. The murine local lymph node assay: results of an interlaboratory trial. *Toxicology Letters* 55:203–213 (1991).

(16) Basketter, D.A. et al. Interlaboratory evaluation of the local lymph node assay with 25 chemicals and comparison with guinea pig test data. *Toxicology Methods* 1:30–43 (1991).