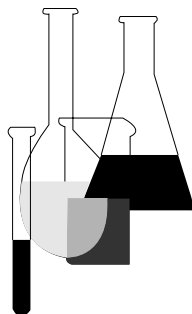




Health Effects Test Guidelines

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 OPPTS Harmonized Test Guidelines Series 870, Guideline 870.2600 Skin Sensitization, dated August 1998; 40 CFR 798.4100 Dermal Sensitization; OECD 406 Skin Sensitization (adopted July 1992); and OECD 429 Skin Sensitization: Local Lymph Node Assay (adopted April 2002).

(b) **Purpose.** The purpose of the selected test is to identify substances with skin sensitization potential. 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. Testing is not required if the test material is a known skin sensitizer. If it is suspected that the test material is a strong dermal irritant, see OPPTS 870.1000, paragraph (d)(2)(iii).

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

Challenge exposure is an exposure of a previously treated subject to a test substance following an induction period to elicit a contact hypersensitivity response.

Induction exposure is the administration of a test substance to the test subject with the intention of inducing contact sensitization.

Induction period is a period of at least 1 week following an induction exposure during which sensitization 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 mammalian species, the reactions may differ and only erythema and edema may be seen.

Stimulation index (SI) is the ratio of ³H-methyl thymidine or ¹²⁵I-iododeoxyuridine (¹²⁵IU) incorporation into test group lymph nodes relative to that recorded for solvent/vehicle control group lymph nodes.

(d) **Test procedures**—(1) **Methods.** Any of the following test methods is considered to be acceptable:

- (i) Local Lymph Node Assay (LLNA) test, or
- (ii) Guinea-Pig Maximization Test (GPMT), or
- (iii) Buehler test.

(2) **Choice of assays.** See OPPTS 870.1000 for a general discussion of factors to be considered prior to performing the test. In addition, the following considerations apply:

(i) The LLNA (see references in paragraphs (g)(1) through (g)(6) of this guideline) is a preferred alternative method, where applicable, to the traditional guinea pig test because it demonstrates an equivalent prediction of human allergic contact dermatitis as compared to the other sensitization tests, provides quantitative data and an assessment of dose-response, gives consideration to animal welfare concerns, and is suitable for testing colored substances. It should be recognized that there are certain testing situations that may necessitate the use of traditional guinea pig tests. The tester should note that the LLNA may not be appropriate for all types of test materials, such as certain metallic compounds, high molecular weight proteins, strong dermal irritants and materials that do not sufficiently adhere to the ear for an acceptable period of time during treatment. When using the LLNA, particular care should be taken to ensure that hydrophilic materials are incorporated into a vehicle system that wets the skin and does not immediately run off. Thus, wholly aqueous vehicles or test materials and runny liquids are to be avoided. In all instances, the tester must document that appropriate techniques were used to facilitate adherence to the mouse ear for an adequate exposure duration. It may be possible to use the LLNA to test some of these materials if appropriate techniques are used to facilitate adherence.

(ii) In situations for test materials where the LLNA is not applicable or may provide unreliable or problematic results, the GPMT or Buehler tests are recommended (see references in paragraphs (g)(7) through (g)(14) of this guideline).

(iii) Although the LLNA, GPMT, or Buehler tests are considered to be acceptable tests, it is recognized that other tests may give useful results. If other tests are used, the investigator must provide justification/reasoning for use of other procedures and methods and protocols must be provided. A positive and negative control group must be included in each test.

(e) **Test methods—(1) LLNA method—(i) Principle of the method.** The basic principle underlying the LLNA is that skin sensitizers induce proliferation of lymphocytes in the lymph nodes draining the site of chemical application. Generally, under appropriate test conditions, this proliferation is proportional to the dose applied, and provides a means of obtaining an objective, quantitative measurement of sensitization. The test measures cellular proliferation as a function of *in vivo* radioisotope incorporation

into the DNA of dividing lymphocytes. The LLNA assesses this proliferation in the draining auricular lymph nodes located in the cervical region at the bifurcation of the jugular vein. Lymphocyte proliferation in test groups is compared to that in concurrent solvent/vehicle-treated controls. A positive control is added to each assay to provide an indication of appropriate assay performance.

(ii) **Animal selection**—(A) **Sex and strain of animals.** Young adult female mice (nulliparous and non-pregnant) of the CBA/Ca or CBA/J strain should be used at age 8–12 weeks. All animals are to be age-matched (preferably within a one-week time frame). Females are used because the existing database is predominantly based on this gender. Males and other strains of mice should not be used until it is sufficiently demonstrated that significant strain-specific and/or gender-specific differences in the LLNA response do not exist.

(B) **Housing and feeding.** The temperature of the experimental animal room should be 21 ± 3 °C and the relative humidity 30–70%. When artificial lighting is used, the light cycle should be 12 hours light: 12 hours dark. For feeding, standard laboratory mouse diets are to be used with an unlimited supply of drinking water. The mice must be acclimatized for at least 5 days prior to the start of the test. Animals must be housed individually. Healthy animals are randomly assigned to control and treatment groups having statistically homogeneous body weights. The animals are uniquely identified prior to being placed on study. Although a variety of techniques exist to uniquely mark mice, any method that involves identification via ear marking (e.g., ear tags) must not be used.

(iii) **Test conditions**—(A) **Preparation of doses.** Solid test substances are to be dissolved in appropriate solvents or vehicles and diluted, if appropriate, prior to dosing of the animals. Stable suspensions might also be acceptable. Liquid test substances may be dosed directly or diluted prior to dosing. Fresh preparations of the test substance are to be prepared daily unless stability data demonstrate the acceptability of storage.

(B) **Solvent/vehicle.** The solvent/vehicle is to be selected on the basis of maximizing the test concentration while producing a solution/suspension suitable for application of the test substance. In order of preference, recommended solvents/vehicles are acetone/olive oil (4:1 v/v), *N,N*-dimethylformamide, methyl ethyl ketone, propylene glycol, and dimethyl sulfoxide, but others may be used if appropriately justified. The selected solvent/vehicle must not interfere with or bias the test result and should be selected to achieve the maximum concentration/skin exposure of the test substance. Ensure that hydrophilic materials are incorporated into a vehicle system that wets the skin and does not immediately run off. Thus, wholly aqueous vehicles are to be avoided.

(C) **Controls.** (1) Concurrent negative (solvent/vehicle) and positive controls are to be included in each test. In some circumstances, it may be useful to include a naive control. Except for treatment with the test substance, animals in the control groups are to be handled in an identical manner to animals of the treatment groups.

(2) Positive controls are used to ensure the appropriate performance of the assay. The positive control must produce a positive LLNA response at an exposure level expected to give an increase in the stimulation index (SI) of three or greater ($SI \geq 3$) over the solvent or vehicle control group. The positive control dose is to be chosen such that the induction is clear but not excessive. Preferred positive control substances are hexyl cinnamic aldehyde (HCA) and mercaptobenzothiazole. There may be circumstances where, given adequate justification, other positive control substances may be used. However, benzocaine should not be used as a positive control in the LLNA.

(3) The positive control substance is tested in the vehicle that is known to elicit a consistent response (i.e., acetone/olive oil). If a non-standard vehicle (chemically relevant formulation) is used with a positive control, the non-standard vehicle (chemically relevant formulation) must be tested for a local lymph node response prior to the initiation of the study and the results reported.

(iv) **LLNA test procedure**—(A) **A minimum of five animals are used per dose group.** At least three consecutive doses of the test substance are to be used. A solvent/vehicle control group and a positive control group are also required. Doses are normally selected from within the concentration series 100%, 50%, 25%, 10%, 5%, 2.5%, 1%, 0.5%, 0.1%. In general, dose selection is based on factors such as toxicity, solubility, irritancy and any other available information such as the results of other testing and structure-activity relationships. To avoid false negatives, test as high a concentration as possible. Generally, the maximum concentration tested is the highest achievable level that avoids overt systemic toxicity and excessive local irritation. To identify the appropriate maximum test substance dose, an initial toxicity test, conducted under identical experimental conditions except for an assessment of lymph node proliferative activity, may be necessary. To support an ability to identify a dose-response relationship, data must be collected on at least three test substance treatment doses, in addition to the concurrent solvent/vehicle control group. Where the LLNA study results are negative, the concurrent positive control must induce a $SI \geq 3$ relative to its solvent/vehicle-treated control.

(B) **LLNA experimental procedure.** The LLNA experimental procedure is to be performed by appropriately trained staff as follows:

(1) Day 1. Record the body weight of each mouse prior to dermal applications. Apply 25 μ L/ear of the appropriate dilution of the test sub-

stance, or the positive control, or the solvent/vehicle control alone to the dorsum of both ears. A positive displacement pipettor may facilitate application of the test material.

(2) Days 2 and 3. Repeat the application procedure as carried out on day 1.

(3) Days 4 and 5. No treatment.

(4) Day 6. Record the body weight of each mouse. Inject 250 μ L of sterile phosphate buffered saline (PBS) containing 20 μ Ci of 3 H-methyl thymidine or 250 μ L PBS containing 2 μ Ci 125 IU and 10^{-5} M fluorodeoxyuridine into each experimental mouse via the tail vein. Five hours later, the draining (auricular) lymph node of each ear is excised and pooled in PBS for each animal. A single cell suspension of lymph node cells (LNC) is prepared for each mouse. The single cell suspension is prepared in PBS by either gentle mechanical separation through 200-mesh stainless steel gauze or another acceptable technique for generating a single cell suspension. The LNC are washed twice with an excess of PBS and the DNA precipitated with 5% trichloroacetic acid (TCA) at 4 $^{\circ}$ C for approximately 18h.

(5) For the 3 H-methyl thymidine method, pellets are resuspended in 1 mL TCA and transferred to 10 mL of scintillation fluid. Incorporation of 3 H-methyl thymidine is measured by B-scintillation counting as disintegrations per minute (dpm) for each mouse and expressed as dpm/mouse. For the 125 IU method, the 1 mL TCA pellet is transferred directly into gamma counting tubes. Incorporation of 125 IU is determined by gamma counting and also expressed as dpm/mouse.

(C) **Observations.** At a minimum, observe mice once daily for any clinical signs, either of local irritation at the application site or of systemic toxicity. Weighing mice prior to treatment and at the time of necropsy will aid in assessing systemic toxicity. All observations are systematically recorded, with records being maintained for each individual mouse.

(D) **Measurements and calculation of results.** (1) The proliferative response of lymph node cells from the pooled lymph nodes of each individual animal is expressed as the number of radioactive disintegrations per minute (dpm) per animal, subtracting out any background dpm. Then the group mean dpm, along with an appropriate measure of inter-animal variability (i.e., mean \pm standard deviation), is calculated for each test group (i.e., positive, solvent/vehicle, and any other control groups) and the solvent/vehicle group. Final results are expressed as the SI which is calculated as a ratio (i.e., SI = mean dpm of test group divided by mean dpm of solvent/vehicle control group).

(2) In addition to an assessment of the magnitude of the ratio estimate, SI, conduct statistical analyses which include both an overall assess-

ment (e.g. ANOVA) of the dose-response relationships and pairwise comparisons of the SIs of the test groups, positive control group and any other control group versus that of the solvent/vehicle control group. In choosing an appropriate method of statistical analysis, the investigator should be aware of possible inequality of variances and other related problems that may necessitate a data transformation or a nonparametric statistical analysis.

(v) Data interpretation and reporting for LLNA—(A) Data Interpretation. (1) A substance is regarded as a skin sensitizer in the LLNA if at least one concentration of the test material results in a 3-fold or greater increase in ³H-methyl thymidine or ¹²⁵IU incorporation in the lymph node cells of test group lymph nodes relative to that recorded for solvent/vehicle control lymph nodes, as indicated by the SI. However, the magnitude of the SI should not be the sole factor used in determining the biological significance of a skin sensitization response. A quantitative assessment must be performed by statistical analysis of individual animal data in order to provide a more complete evaluation of the test substance (see paragraph (e)(1)(iv)(D)(2) of this guideline). Factors to be considered in evaluating the biological significance of a response or outcome of the test include the results of the SI determinations, statistical analyses, the strength of the dose-response relationship, chemical toxicity, solubility, and the consistency of the solvent/vehicle and positive control responses.

(2) Strong irritants may yield false positive results in the LLNA due to the initiation of a significant lymphocyte proliferation. However, the dose-response information from the assay may help to uncover a strong irritant response since, for instance, it has been shown that the proliferation induced by irritation usually results in a shallow dose-response relationship. Concurrent evaluation of ear swelling may also provide helpful information on differentiating weak sensitizers from strong irritants.

(B) Test report. The test report for LLNA must contain the following specific information:

(1) Test substance. (i) Identification data and CAS number, if known, and EPA registration number, if applicable;

(ii) Physical nature and purity;

(iii) Physicochemical properties relevant to the conduct of the study;

(iv) Stability of the test substance, if known; and

(v) Lot number of the test substance.

(2) Solvent/vehicle. (i) Solvent/vehicle used and its purity;

(ii) Justification for choice of solvent/vehicle, if appropriate; and

(iii) Solubility and stability of the test substance in the solvent/vehicle.

(3) Test animals. (i) Strain of mice used;

(ii) Acclimation information;

(iii) Number, age, and sex of mice;

(iv) Source, housing conditions, diet, etc.;

(v) Individual body weight of the animals at the start and end of the test, including body weight range, mean, and associated error term for each group;

(vi) Health and microbiological/pathogen status of the mouse; and

(vii) Details of animal food and water quality;

(4) Test conditions. (i) Details of test substance preparation;

(ii) Details of the administration of the test substance;

(iii) Detailed description of treatment and sampling schedules; and

(iv) Methods for measurement of toxicity.

(5) Results. (i) Positive and negative (solvent/vehicle) control data in tabular form;

(ii) Data from range-finding study, if conducted;

(iii) Doses used;

(iv) Rationale for dose level selection;

(v) Signs of toxicity;

(vi) Dpm/mouse values for each mouse within each treatment group and control group;

(vii) Group mean dpm/mouse and associated error term for each treatment group and control group;

(viii) The SI calculated, compared to the concurrent solvent/vehicle control group, for each test substance treatment dose group, the concurrent positive control group, and any other concurrent control group;

(ix) Individual mouse dpm data must be presented in tabular form, along with the group mean dpm, its associated error term and the SI for each dose group;

(x) Criteria for considering studies as positive or negative (including information on any qualitative or quantitative measure of ear swelling);

- (xi) Dose-response relationship;
 - (xii) Statistical analyses and method applied;
 - (xiii) Concurrent and negative control data as established in the tester's laboratory; and
 - (xiv) Concurrent positive control data.
- (6) Discussion of the results.
- (7) Conclusions.
- (8) The reporting requirements specified under 40 CFR Part 158 (for pesticides) and 40 CFR Part 792, Subpart J (for toxic substances) should be followed.

(2) **GPMT and Buehler Methods**—(i) **Principle of the test methods.** 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.

(ii) **Animal selection**—(A) **Species and strain.** The young adult guinea pig is preferred. Young adult commonly used laboratory strains must be employed.

(B) **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.

(C) **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 must 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 must 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

(D) **Control animals.** (2) Every 6 months, assess the sensitivity and reliability of the experimental technique 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 is expected for mild-to-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 (CAS No. 25068–38–6). There may be circumstances where, given adequate justification, other control substances meeting the above criteria may be used.

(2) To ensure that the response to the challenge reaction in treated animals is truly of allergic origin and not due to skin irritancy, a sham-treated vehicle-only control is included in the test strategy. This sham-treated control group is treated in exactly the same manner as the test animals, except that during the induction phase the test article is omitted. The selected vehicle must not interfere or alter the test results.

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

(F) **Observation of animals.** (1) Skin reactions are to 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 are to be made as necessary to fully describe unusual responses.

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

(G) **Procedures.** The procedures to be used are those described by the test method chosen. Brief summaries are given here, but the tester is referred to the original literature for more complete guidance on conducting the Buehler test (see references in paragraphs (g)(7) through (g)(10) of this guideline) or the GPMT (see references in paragraphs (g)(11) through (g)(14) of this guideline).

(1) 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.

(2) 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.

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

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

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

(iii) **Data and reporting for GPMT and Buehler Methods.** Data must 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 must be graded and any unusual finding must be recorded.

(A) **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.

(B) The following specific information is to be reported for the GPMT and Buehler Methods.

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

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

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

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

(5) A brief description of the grading system.

(6) Each reading made on each individual animal.

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

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

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

(10) 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 must be reported.

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

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

(13) 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 animals.

(14) Histopathological findings, if any.

(15) Discussion of results.

(16) 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.

(17) The reporting requirements as specified under 40 CFR Part 158 (for pesticides) and 40 CFR Part 792, Subpart J (for toxic substances) should be followed

(f) **Screening tests.** The mouse ear swelling test (MEST) (see references in paragraphs (g)(15) through (g)(18) of this guideline) may be used as a screening test to detect moderate to strong sensitizers. If a positive result is seen in this 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 MEST does not indicate sensitization, the test substance should not be designated a nonsensitizer without confirmation in an accepted test using guinea pigs or LLNA if appropriate.

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

(1) *The Murine Local Lymph Node Assay: A Test Method for Assessing the Allergic Contact Dermatitis Potential of Chemicals/Compounds*. Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM), National Institutes of Environmental Health Sciences, NIH Publication No. 99-4494.3 (1999). (Document available at <http://iccvam.niehs.nih.gov/methods/llnadocs/llnarep.pdf>.) Description and picture of auricular lymph node dissection available at <http://iccvam.niehs.nih.gov/methods/llnadocs/LLNAProt.pdf>.

(2) Kimber, I. et al. The murine local lymph node assay for identification of contact allergens: a preliminary evaluation of in situ measurement of lymphocyte proliferation. *Contact Dermatitis* 21:215-220 (1989)

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(5) 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).

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

(8) Ritz, H.L. and Buehler, E.V. Planning, conduct and interpretation of guinea pig sensitization patch test in *Current Concepts in Cutaneous Toxicity*, ed. V. Drill and P. Lazar. Academic, New York, NY. pp. 25-42 (1980).

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- (13) Magnusson, B. Identification of contact sensitizers by animal assay. *Contact Dermatology* 6:46 (1980).
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- (15) Gad, S.C. et al. Development and validation of an alternative dermal sensitization test: the mouse ear swelling test (MEST). *Toxicology and Applied Pharmacology* 8 4:93–114 (1986).
- (16) 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).
- (17) Thorne, P.S. et al., The noninvasive mouse ear swelling assay, I. Refinements for detecting weak contact sensitizers. *Fundamental and Applied Toxicology* 7:790–806 (1991).
- (18) Thorne, P.S. et al. The noninvasive mouse ear swelling assay. II. Testing the contact sensitizing potency of fragrances. *Fundamental and Applied Toxicology* 7:807–820 (1991).