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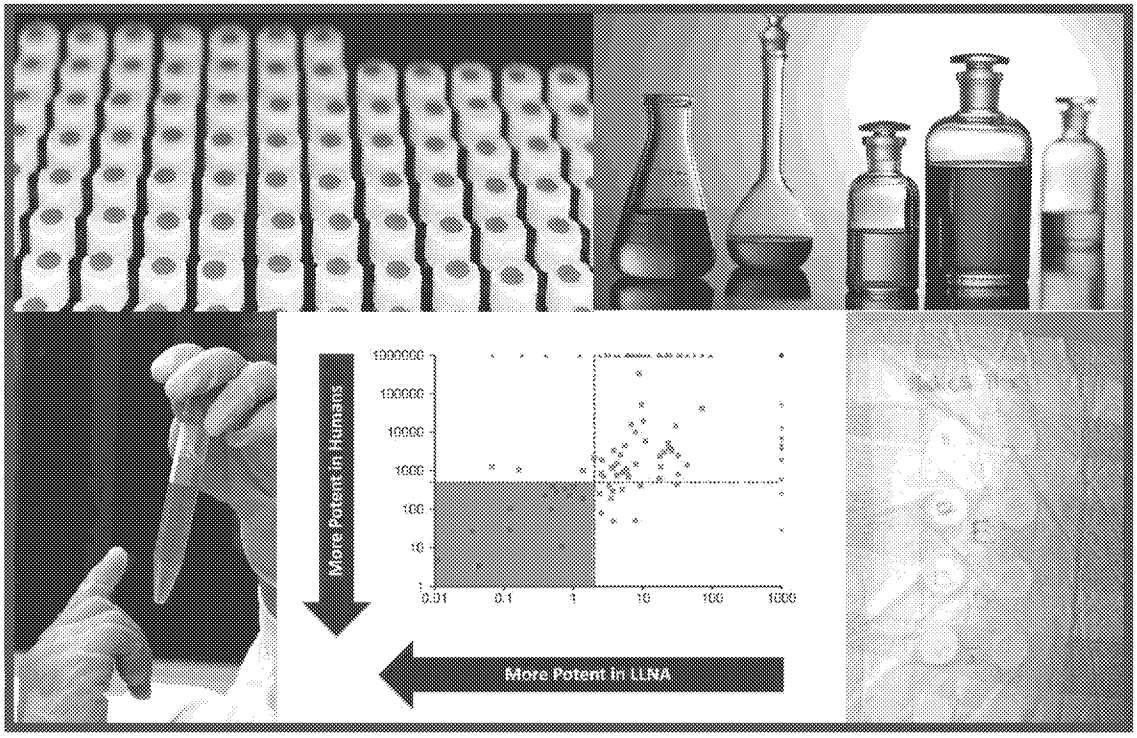

ICCVAM Test Method Evaluation Report: Usefulness and Limitations of the Murine Local Lymph Node Assay for Potency Categorization of Chemicals Causing Allergic Contact Dermatitis in Humans

Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM)

National Toxicology Program (NTP) Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM)

National Institute of Environmental Health Sciences
National Institutes of Health
U.S. Public Health Service
Department of Health and Human Services

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In 1997, the National Institute of Environmental Health Sciences (NIEHS), one of the National Institutes of Health, established the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) to:

- Coordinate interagency technical reviews of new and revised toxicological test methods, including alternative test methods that reduce, refine, or replace the use of animals
- Coordinate cross-agency issues relating to validation, acceptance, and national and international harmonization of new, modified, and alternative toxicological test methods

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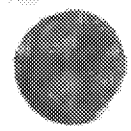

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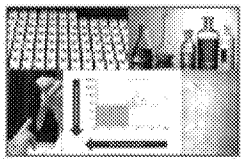

On the cover: This collage of pictures representing the use of the murine local lymph node assay for potency categorization of chemicals causing allergic contact dermatitis in humans includes (clockwise from top left): scintillation vials used to measure the quantity of radioactive tracer chemical incorporated into dividing lymph node cells; flasks and bottles containing liquids representing chemicals; a human clinical patch test for skin allergy; a graph of data used in the evaluation of the murine local lymph node assay for potency categorization; lab technician resuspending cells in a plastic tube.

ICCVAM Test Method Evaluation Report: Usefulness and Limitations of the Murine Local Lymph Node Assay for Potency Categorization of Chemicals Causing Allergic Contact Dermatitis in Humans

Interagency Coordinating Committee on the Validation of Alternative Methods

National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods

National Institute of Environmental Health Sciences
National Institutes of Health
U.S. Public Health Service
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ED_006472_00084346-00004

Table of Contents

List of Tables vi

List of Figures vii

List of Abbreviations and Acronyms viii

Interagency Coordinating Committee on the Validation of Alternative Methods: Agency Representatives ix

Acknowledgements x

Preface xv

Executive Summary xvii

1.0 Introduction 1

1.1 Background Information on the Murine Local Lymph Node Assay 1

1.2 ICCVAM, NICEATM, and Interagency Immunotoxicity Working Group 1

1.3 LLNA Background Review Document 1

1.4 Peer Review Panel 2

1.5 Scientific Advisory Committee on Alternative Toxicological Methods 2

1.6 Final ICCVAM Test Method Recommendations and Final Background Review Document 3

2.0 ICCVAM Recommendations: Usefulness and Limitations of the LLNA for Potency Categorization of Chemicals Causing Allergic Contact Dermatitis in Humans, Test Method Protocol, and Future Studies 4

2.1 ICCVAM Recommendations: Test Method Usefulness and Limitations 4

2.2 ICCVAM Recommendations: Test Method Protocol 5

2.3 ICCVAM Recommendations: Future Studies 5

3.0 Validation Status for Use of the LLNA to Determine Skin Sensitization Potency Categories 6

3.1 Test Method Description 6

3.1.1 General Test Method Procedures 6

3.2 Validation Database 6

3.3 Reference Test Method Data 8

3.4 Test Method Accuracy 8

3.4.1 DSA_{05} and EC3 Values 8

3.4.2 LLNA Classification of Strong and Other Than Strong Sensitizers in Humans 10

3.5 Test Method Reliability 13

3.5.1 Intra- and Interlaboratory Variability 13

3.5.2 Influence of LLNA Vehicle 13

3.6 Animal Welfare Considerations: Reduction, Refinement, and Replacement 14

ED_006472_00084346-00005

4.0 ICCVAM Consideration of Independent Peer Review Panel Report and Other Comments 15

4.1 ICCVAM Consideration of Independent Peer Review Panel Report 15

4.1.1 Comments on Draft ICCVAM Recommendations: Test Method Usefulness and Limitations 15

4.1.2 Comments on Draft ICCVAM Recommendations: Test Method Protocol 15

4.1.3 Comments on Revised Draft ICCVAM Recommendations: Future Studies 16

4.2 ICCVAM Consideration of Public and SACATM Comments 16

4.2.1 Public Comments in Response to 72 FR 27815 (May 17, 2007): The Murine Local Lymph Node Assay: Request for Comments, Nominations of Scientific Experts, and Submission of Data 17

4.2.2 Public Comments in Response to 72 FR 52130 (September 12, 2007): Draft Performance Standards for the Murine Local Lymph Node Assay: Request for Comments 19

4.2.3 Public Comments in Response to 73 FR 1360 (January 8, 2008): Announcement of an Independent Scientific Peer Review Panel Meeting on the Murine Local Lymph Node Assay; Availability of Draft Background Review Documents; Request for Comments 19

4.2.4 Public Comments in Response to 73 FR 25754 (May 7, 2008): Meeting of the Scientific Advisory Committee on Alternative Toxicological Methods (SACATM) 21

4.2.5 Public Comments in Response to 73 FR 29136 (May 20, 2008): Peer Review Panel Report on the Validation Status of New Versions and Applications of the Murine Local Lymph Node Assay (LLNA): A Test Method for Assessing the Allergic Contact Dermatitis Potential of Chemicals and Products: Notice of Availability and Request for Public Comments 22

4.2.6 Public and SACATM Comments: SACATM Meeting on June 18-19, 2008 22

5.0 References 23

Appendix A Timeline for ICCVAM Evaluation on Usefulness and Limitations of the Murine Local Lymph Node Assay for Potency Categorization of Chemicals Causing Allergic Contact Dermatitis in Humans A-1

Appendix B Updated ICCVAM-Recommended Protocol: The Murine Local Lymph Node Assay: A Test Method for Assessing the Allergic Contact Dermatitis Potential of Chemicals and Products B-1

Appendix C Final Background Review Document: Use of the Murine Local Lymph Node Assay for Potency Categorization of Chemicals Causing Allergic Contact Dermatitis in Humans C-1

Appendix D Independent Scientific Peer Review Panel Assessment D-1

D1 Summary Minutes from the Independent Scientific Peer Review Panel Meeting on March 4-6, 2008 ..... D-3

D2 Peer Review Panel Report: Validation Status of New Versions and Applications of the Murine Local Lymph Node Assay: A Test Method for Assessing the Allergic Contact Dermatitis Potential of Chemicals and Products D-33

ED_006472_00084346-00006

Appendix E GHS Materials on Skin Sensitization Potency E-1

E1 Meetings of the Sub-Committee of Experts on the Globally Harmonized System of Classification and Labelling of Chemicals Relevant to Subcategorization of Skin Sensitizers E-3

E2 ST/SG/AC.10/C.4.2006/16, Strong versus Weak Sensitizers E-9

E3 UN/SCEGHS/12/INF.16 (OECD), Progress Report on OECD Work Since the Last Sub-Committee Meeting E-23

E4 UN/SCEGHS/15/INF.14 (OECD), Proposal for Revising Chapter 3.4 with Respect to Strong versus Weak Sensitizers: Explanatory Notes and Revised Chapter with Visible Changes E-27

E5 ST/SG/AC.10/C.4/2008/18 (Secretariat), Revision of Chapter 3.4 with Respect to Strong versus Weak Sensitizers E-43

E6 ST/SG/AC.10/C.4/2008/18/Add.1 (Secretariat), Revision of Chapter 3.4 with Respect to Strong versus Weak Sensitizers, Addendum E-55

E7 UN/SCEGHS/16/INF.3 (Secretariat), Section 3.4.2 of Chapter 3.4 ..... E-63

Appendix F *Federal Register* Notices and Public Comments F-1

F1 *Federal Register* Notices F-3

F2 Public Comments Received in Response to *Federal Register* Notices F-17

F3 SACATM Comments: SACATM Meeting, June 18-19, 2008 ..... F-93

Appendix G Relevant Skin Sensitization Regulations and Testing Guidelines G-1

G1 Table of Relevant Skin Sensitization Test Regulations G-3

G2 EPA Health Effects Test Guidelines OPPTS 870.2600: Skin Sensitization (March 2003) G-7

G3 International Organization for Standardization – ISO 10993-10: Biological Evaluation of Medical Devices Part 10: Tests for Irritation and Skin Sensitization (July 2010) G-25

G4 OECD Test Guideline 429: Skin Sensitization – Local Lymph Node Assay (Adopted July 2010) G-27

G5 OECD Test Guideline 406: Skin Sensitisation (Adopted July 1992) G-49

ED_006472_00084346-00007

List of Tables

Table 3-1 Chemical Classes Represented in the LLNA Potency Database 7

Table 3-2 Distribution of 136 Substances for Classification Rate Analyses 9

Table 3-3 Concordance of LLNA and Human Data for Strong Sensitizer, Other Sensitizer, and Nonsensitizer Categories for 136 Substances at Selected LLNA EC3 Values 11

Table 3-4 Classification Rates for the Prediction of Human Potency Categories by Selected LLNA EC3 Cutoff Values for 136 Substances 12

Table 4-1 Opportunities for Public Comments 17

ED_006472_00084346-00008

List of Figures

Figure 3-1 LLNA EC3 and Human Results by GHS Potency Category for 136 Substances.....9

Figure 3-2 Classification Rates for the LLNA EC3 Prediction of 27 Strong Human Sensitizers.....12

ED_006472_00084346-00009

List of Abbreviations and Acronyms

ACD	Allergic contact dermatitis
BRD	Background review document
CPSC	U.S. Consumer Product Safety Commission
CV	Coefficient of variation
DSA	Dose per skin area
DSA_{05}	Induction dose per skin area, in $\mu g/cm^2$, in a human repeat-insult patch test or human maximization test that produces a positive response in 5% of the tested population
EC3	Estimated concentration of a substance expected to produce a stimulation index of 3, the threshold value for a substance to be considered a sensitizer in the LLNA
ECVAM	European Centre for the Validation of Alternative Methods
EPA	U.S. Environmental Protection Agency
ESAC	ECVAM Scientific Advisory Committee
FR	*Federal Register*
GHS	Globally Harmonized System of Classification and Labelling of Chemicals
HMT	Human maximization test
HRIPT	Human repeat-insult patch test
ICCVAM	Interagency Coordinating Committee on the Validation of Alternative Methods
IWG	Immunotoxicity Working Group
JaCVAM	Japanese Center for the Validation of Alternative Methods
LLNA	Murine local lymph node assay
LOEL	Lowest observed effect level
NICEATM	National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods
NIEHS	National Institute of Environmental Health Sciences
n	Number
NOEL	No observed effect level
OECD	Organisation for Economic Co-operation and Development
R^2	Coefficient of determination
SACATM	Scientific Advisory Committee on Alternative Toxicological Methods
SI	Stimulation index

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Preface

Allergic contact dermatitis (ACD) is an adverse health effect that frequently develops in workers and consumers following repeated exposure to skin sensitizing chemicals and products. ACD results in lost workdays[3] and can significantly diminish quality of life (Hutchings et al. 2001; Skoet et al. 2003). To minimize the occurrence of ACD, regulatory authorities require testing to identify substances that may cause skin sensitization (**Appendix G**). Sensitizing substances must be labeled with a description of the potential hazard and the precautions necessary for workers and consumers to avoid development of ACD.

Skin sensitization testing has typically required the use of guinea pigs (Buehler 1965; Magnusson and Kligman 1970). However, in 1998, the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) evaluated an alternative test method known as the murine (mouse) local lymph node assay (LLNA). Based on the validation database and performance, ICCVAM recommended the LLNA as an alternative test method for assessing the skin sensitization potential of most types of substances (ICCVAM 1999). United States and international regulatory agencies subsequently accepted the LLNA as a valid alternative test method for ACD testing. The LLNA provides several advantages compared to guinea pig test methods, including elimination of potential pain and distress, use of fewer animals, less time to perform, and availability of dose-response information. The LLNA is now used around the world.

In 2007, the U.S. Consumer Product Safety Commission (CPSC) asked ICCVAM and the National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM) to evaluate several new versions and applications of the LLNA, including use of the LLNA for determining skin sensitization potency categories. CPSC based the nomination on their interest in assessing the usefulness and limitations of the LLNA for identifying chemicals and products likely to be strong human skin sensitizers. ICCVAM assigned the nomination a high priority after considering favorable comments from the public and ICCVAM's Scientific Advisory Committee on Alternative Toxicological Methods (SACATM). As part of the NICEATM-ICCVAM collaboration with the European Centre for Validation of Alternative Methods (ECVAM) and the Japanese Center for the Validation of Alternative Methods (JaCVAM), scientists from these centers served as liaisons on the ICCVAM interagency Immunotoxicity Working Group (IWG). A detailed timeline of the LLNA potency evaluation is included with this report (**Appendix A**).

This test method evaluation report provides ICCVAM's recommendations regarding the usefulness and limitations of the LLNA for potency categorization of chemicals causing ACD in humans. The database of substances used to evaluate the accuracy of the LLNA for correctly determining skin sensitization potency categories is discussed and summarized.

ICCVAM solicited and considered public comments and stakeholder involvement throughout the evaluation process. ICCVAM considered the SACATM comments, the report of an independent international scientific peer review panel (Panel), and all public comments before finalizing the ICCVAM test method recommendations for use of the LLNA for determining skin sensitization potency categories. The recommendations and the background review document, which is provided here as **Appendix C**, are incorporated in this ICCVAM test method evaluation report. As required by the ICCVAM Authorization Act of 2000 (Public Law 106-545, 42 United States Code 285*l*-3), ICCVAM will forward this report and its recommendations to U.S. Federal agencies for consideration and acceptance decisions, where appropriate. Federal agencies must respond to ICCVAM within 180 days after receiving the ICCVAM test method recommendations. ICCVAM recommendations

[3] http://www.bls.gov/IIF

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are available to the public on the NICEATM-ICCVAM website,[4] and agency responses will also be made available on the website as they are received.

We gratefully acknowledge the many individuals who contributed to the preparation, review, and revision of this report. We especially recognize the Panel members for their thoughtful evaluations and generous contributions of time and effort. Special thanks are extended to Dr. Michael Luster for serving as the Panel Chair and to Dr. Michael Olson, Dr. Michael Woolhiser, and Kim Headrick for their service as Evaluation Group Chairs during the March 4-6, 2008, Panel meeting. We thank the interagency IWG for assuring a meaningful and comprehensive review. We especially thank Dr. Joanna Matheson (CPSC) and Dr. Abigail Jacobs (U.S. Food and Drug Administration Center for Drug Evaluation and Research) for serving as co-chairs of the interagency IWG. We also acknowledge Integrated Laboratory Systems, Inc., the NICEATM support contractor, for providing excellent scientific and operational support, including Dr. David Allen, Thomas Burns, Michael Paris, Dr. Eleni Salicru, Frank Stack, and Dr. Judy Strickland. Finally, we thank Drs. Silvia Casati and Alexandre Angers, and Dr. Hajime Kojima, the interagency IWG liaisons from ECVAM and JaCVAM, respectively, for their participation and contributions.

This ICCVAM evaluation of the LLNA for determining potency categories of skin-sensitizing chemicals is expected to assist regulatory agencies in determining when it may or may not be appropriate to use LLNA results for potency categorization and to facilitate regulatory agency decisions on the acceptability of the LLNA for this purpose. Appropriate use of the LLNA by industry is expected to significantly reduce and refine animal use required for ACD testing, while continuing to support the protection of human health.

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[4] http://iccvam.niehs.nih.gov

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Executive Summary

The Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) recently evaluated the use of the murine (mouse) local lymph node assay (LLNA) as a stand-alone test method to determine skin sensitization potency categories. The LLNA is used to identify chemicals and products that may cause allergic contact dermatitis (ACD), an allergic skin reaction characterized by redness, swelling, and itching. This test method evaluation report provides ICCVAM's recommendations regarding the usefulness and limitations of the LLNA for potency categorization of chemicals causing ACD in humans as well as recommendations for future studies. Also included in this report are a detailed timeline of the LLNA potency evaluation (**Appendix A**) and the final background review document (BRD) describing the validation status of the LLNA for this proposed usage (**Appendix C**).

Following a nomination by the U.S. Consumer Product Safety Commission (CPSC) to assess the validation status of the LLNA as a stand-alone test method for potency determinations for classification purposes, the National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM) and the ICCVAM interagency Immunotoxicity Working Group (IWG) prepared a draft BRD, and ICCVAM prepared draft test method recommendations. The CPSC, under the Federal Hazardous Substances Act, currently requires hazard labeling of only products that are considered to be strong skin sensitizers, based on a weight-of-evidence approach that considers frequency of responses in exposed human populations, severity of responses, and the dose at which allergic reactions occur (15 U.S.C. 1261). Criteria for test results from animal studies that could be used to identify potential strong human skin sensitizers would be helpful for the purposes of hazard identification for CPSC and other agencies with an interest in identifying strong skin sensitizers. Accordingly, ICCVAM evaluated the extent that LLNA results could be used to correctly predict strong versus other than strong human skin sensitizers as detailed in the BRD.

The draft BRD and draft ICCVAM test method recommendations were provided to an independent international scientific peer review panel (Panel) and the public for their consideration. The Panel met in public session on March 4-6, 2008, to discuss its review of the draft BRD and to provide conclusions and recommendations regarding the validation status of the LLNA as a stand-alone test method to determine skin sensitization potency categories. The Panel also reviewed how well the information in the draft BRD supported ICCVAM's draft test method recommendations. The Panel agreed with ICCVAM that the LLNA should not be used as a stand-alone test method for categorizing skin sensitizers based on potency but that it can be used as part of a weight-of-evidence evaluation for this purpose. The Panel recommended that NICEATM perform additional analyses using alternative human reference values that might be more appropriate for evaluating the use of the LLNA for skin sensitization potency determinations.

NICEATM performed these analyses for the final BRD, which is included as **Appendix C**, and ICCVAM finalized the test method recommendations. In finalizing this test method evaluation report and the BRD, ICCVAM considered (1) the conclusions and recommendations of the Panel, (2) comments from ICCVAM's Scientific Advisory Committee on Alternative Toxicological Methods (SACATM), and (3) public comments.

ICCVAM Recommendations: Test Method Usefulness and Limitations

ICCVAM concludes that the LLNA can be used to categorize substances as strong sensitizers (Globally Harmonized System of Classification and Labelling of Chemicals [GHS] Subcategory 1A) when the estimated concentration that produces a positive LLNA result (i.e., EC3) is ≤2%. However, because almost half (48% [13/27]) of the known strong human skin sensitizers have an EC3 > 2% or are negative in the LLNA, the LLNA cannot be considered a stand-alone assay to categorize skin

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sensitization potency. Additional information is required to categorize a substance as other than a strong sensitizer (GHS Subcategory 1B: "other" skin sensitizers) when the substance produces an LLNA EC3 > 2%. These recommendations are based on an accuracy analysis (see **Section 3.4**) that included 136 substances for which there were both LLNA and human data (i.e., 27 strong human skin sensitizers, 49 other than strong human skin sensitizers, and 60 human nonsensitizers).

ICCVAM Recommendations: Test Method Protocol

ICCVAM recommends use of the recently updated LLNA test method protocol (**Appendix B**), which includes improved dose selection procedures to guide selection of the highest dose that will aid in minimizing false negatives, and the procedures for calculating the EC3. The updated LLNA test method protocol provides for a 20% reduction in the number of animals required compared to the previously recommended LLNA protocol by reducing the number of required animals per group from five to four. Further, the collection of individual animal data and inclusion of both a concurrent vehicle and positive control are recommended for each study.

ICCVAM Recommendations: Future Studies

To further evaluate the usefulness and limitations of the LLNA for potency determinations, efforts should be made to identify additional high-quality human test data and experience for substances with comparative LLNA data. Emphasis should be placed on identifying substances that are classified as strong skin sensitizers based on a human threshold induction concentration of <500 $\mu g/cm^2$ to more adequately evaluate the LLNA EC3 value that will best distinguish strong from other than strong skin sensitizers. ICCVAM encourages the development, validation, and evaluation of integrated decision strategies that consider other types of relevant information such as quantitative structure-activity relationships, structural alerts, peptide reactivity, *in vitro* testing data, human data or experience, and related existing data from similar chemical entities.

Validation Status of the Use of the LLNA to Determine Skin Sensitization Potency Categories

The extent to which the LLNA correctly classifies strong versus other than strong human skin sensitizers was evaluated using a database of 136 substances with both LLNA and human data. The dose per skin area, which represents a defined incidence of a positive response among test subjects (i.e., 5%, DSA_{05} value) from the human maximization test or human repeat-insult patch test, was used as the human threshold response because it was viewed as analogous to the EC3 value, which is also a threshold positive response.

The 76 human sensitizers (among the 136 substances with LLNA and human data) were categorized as either "strong" or "other" sensitizers using the GHS criteria: $DSA_{05} \leq 500$ $\mu g/cm^2$ for strong sensitizers (GHS Subcategory 1A) and $DSA_{05} > 500$ $\mu g/cm^2$ for other sensitizers (GHS Subcategory 1B) (UN 2009). Of the 27 strong human sensitizers, 14 had LLNA EC3 ≤ 2%, 11 had EC3 > 2%, and two were negative in the LLNA. Forty-nine human sensitizers were other sensitizers: three with LLNA EC3 ≤ 2%, 35 with EC3 > 2%, and 11 with negative LLNA results. Of the 60 human nonsensitizers, 35 were sensitizers in the LLNA (four with LLNA EC3 ≤ 2%, 31 with EC3 > 2%), and 25 were nonsensitizers in the LLNA.

The correct classification, underclassification, and overclassification rates[5] of the LLNA versus human data were initially calculated using the GHS criteria of EC3 ≤ 2% for strong sensitizers and EC3 > 2% for other sensitizers. Based on this database, the LLNA correctly identified 52% (14/27) of

[5] The correct classification rate is the proportion of substances that are correctly assigned to a human potency category by the LLNA result. The underclassification rate is the proportion of substances that are incorrectly assigned to a less severe human potency category by the LLNA result, and the overclassification rate is the proportion of substances that are incorrectly assigned to a more severe human potency category by the LLNA result.

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the strong human sensitizers using EC3 ≤ 2%, but underclassified 48% (13/27) (see **Appendix C, Section 6.1.2**). Among the 21 substances that produced an EC3 ≤ 2%, 67% (14/21) were strong human skin sensitizers (GHS Subcategory 1A), but the remaining 33% (7/21) were either other human skin sensitizers (GHS Subcategory 1B, n = 3) or substances not classified as human skin sensitizers (n = 4).

Of the 13 strong human sensitizers that were not categorized as strong sensitizers using the GHS criterion of LLNA EC3 ≤ 2%, 77% (10/13) produced an LLNA EC3 value between 2% and 10%, one produced an LLNA EC3 of 30.9%, and two were negative in the LLNA. The 13 substances shared the following commonalities with regard to physicochemical characteristics:

- Twelve of 13 had molecular weights within a range of 100 (12/13 substances had molecular weights of 98.15 to 192.3).
- Eight of the 13 substances were liquids.
- All six of the substances for which peptide reactivity information was available had high (n = 5) or moderate (n = 1) peptide reactivity.

As noted above, most (77%) of the strong human sensitizers that were underclassified by the LLNA (10/13) had EC3 values between 2% and 10%. Use of LLNA EC3 ≤ 10% to classify substances as strong sensitizers correctly classified 89% (24/27) of the strong sensitizers compared with the 52% (14/27) of the strong sensitizers correctly classified using EC3 ≤ 2%. However, it also decreased the number of other than strong sensitizers classified correctly (31% [15/49] versus 71% [35/49]). The optimum EC3 value (3.8%) resulted in the highest correct classification rate for strong human sensitizers, other than strong human sensitizers, and nonsensitizers combined (55% [75/136]). The lowest underclassification rate was for strong and other than strong skin sensitizers (22% [17/76]).

ICCVAM Consideration of Independent Peer Review Panel Report and Other Comments

The ICCVAM evaluation process incorporates a high level of scientific peer review and transparency. The evaluation process for use of the LLNA as a stand-alone test method to determine skin sensitization potency categories included a public review meeting by an independent scientific peer review panel, multiple opportunities for public comments, and comments from SACATM. ICCVAM and the interagency IWG considered the Panel report, the SACATM comments, and all public comments before finalizing the ICCVAM test method evaluation report and BRD.

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1.0 Introduction

1.1 Background Information on the Murine Local Lymph Node Assay

The murine (mouse) local lymph node assay (LLNA) is an alternative skin sensitization test method that requires fewer animals and less time than the traditionally accepted guinea pig tests, the guinea pig maximization test and the Buehler test (EPA 2003; OECD 1992). It also avoids animal discomfort that can occur in the guinea pig tests when substances cause allergic contact dermatitis (ACD). The LLNA measures cell proliferation in the draining auricular lymph nodes of the mouse by analyzing incorporation of a radioactive marker into newly synthesized DNA. The LLNA was the first alternative test method evaluated and recommended by the U.S. Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM). International regulatory authorities have now recognized the LLNA as an acceptable alternative to guinea pig tests for most testing situations.

The use of the LLNA as a stand-alone test method to determine skin sensitization potency categories is one of several LLNA-related topics nominated by the U.S. Consumer Product Safety Commission (CPSC) for evaluation by ICCVAM together with the National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM).[6] This evaluation assessed the accuracy of the LLNA to correctly determine skin sensitization potency in humans.

1.2 ICCVAM, NICEATM, and Interagency Immunotoxicity Working Group

In accordance with the ICCVAM Authorization Act of 2000 (Public Law 106-545, 42 U.S.C. 285*l*-3), ICCVAM coordinates the technical evaluations of new, revised, and alternative test methods with regulatory applicability. NICEATM administers ICCVAM and provides scientific and operational support for ICCVAM's activities. After considering comments from the public and ICCVAM's advisory committee, the Scientific Advisory Committee on Alternative Toxicological Methods (SACATM), ICCVAM members unanimously agreed that an evaluation of the LLNA as a stand-alone test method to determine skin sensitization potency categories should have a high priority. A detailed timeline of this evaluation is provided in **Appendix A**.

ICCVAM established an interagency Immunotoxicity Working Group (IWG) to work with NICEATM to evaluate the use of the LLNA as a stand-alone test method to determine skin sensitization potency categories. The European Centre for the Validation of Alternative Methods (ECVAM) and the Japanese Center for the Validation of Alternative Methods (JaCVAM) designated liaison members to the interagency IWG.

A May 17, 2007, *Federal Register* (FR) notice (72 FR 27815)[7] requested data and information on these test methods and nominations of individuals to serve on an independent international scientific peer review panel (Panel). The request was also disseminated via the ICCVAM electronic mailing list and through direct requests to over 100 stakeholder organizations. In response to this request, a Panel of 19 experts representing eight countries was formed. The expertise of the Panel included alternative toxicity test methods, animal welfare, biostatistics, dermal toxicity, dermatology, human health risk assessment, immunotoxicology, pharmacology, regulatory toxicology, and occupational and environmental health.

1.3 LLNA Background Review Document

To facilitate peer review of the evaluation of the LLNA, the interagency IWG and NICEATM prepared a comprehensive draft background review document (BRD) that provided information and data from validation studies and the scientific literature. The final BRD is provided in **Appendix C**.

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[6] http://iccvam.niehs.nih.gov/methods/immunotox/llnadocs/CPSC_LLNA_nom.pdf
[7] http://iccvam.niehs.nih.gov/SuppDocs/FedDocs/FR/FR_E7_9544.pdf

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The draft BRD examined data derived from a database of over 500 substances tested in the LLNA. For each substance with comparative human reference data, skin sensitization potency was evaluated by comparing the LLNA EC3 value, the estimated concentration of a substance expected to produce a stimulation index (SI) of 3, the threshold value for a substance to be considered a sensitizer in the LLNA (Kimber et al. 2001), to the threshold concentration inducing a human response. On January 8, 2008, ICCVAM announced the availability of the draft BRD to the public.

1.4 Peer Review Panel

ICCVAM announced a March 4-6, 2008, public peer review panel (Panel) meeting to review the validation status of the LLNA as a stand-alone test method to determine skin sensitization potency categories (and other LLNA-related activities) (73 FR 1360).[8] All of the information provided to the Panel, including the draft BRD, ICCVAM draft test method recommendations, and all public comments received before the Panel meeting, were made publicly available via the NICEATM-ICCVAM website.[9]

The Panel evaluated (1) the extent to which the draft BRD addressed established validation and acceptance criteria and (2) the extent to which the draft BRD supported ICCVAM's draft proposed test method uses, recommended test method protocol, and proposed future studies. Interested stakeholders from the public were provided opportunities to comment at the Panel meeting. The Panel considered these comments as well as those submitted prior to the meeting before concluding their deliberations. As indicated in the Panel report (**Appendix D**), the Panel agreed with the ICCVAM draft recommendations that the LLNA should not be used as a stand-alone assay for categorizing skin sensitizers based on potency but that it could be used as part of a weight-of-evidence evaluation for this purpose. The Panel further recommended that NICEATM perform additional analyses using alternative human reference values that might be more appropriate for evaluating the use of the LLNA for skin sensitization potency determinations. On May 20, 2008, ICCVAM posted a report of the Panel's recommendations[10] (**Appendix D**) on the NICEATM-ICCVAM website for public review and comment (announced in 73 FR 29136).[11]

1.5 Scientific Advisory Committee on Alternative Toxicological Methods

The Scientific Advisory Committee on Alternative Toxicological Methods (SACATM) is a Federally chartered advisory committee that advises ICCVAM, NICEATM, and the Director of the NIEHS.[12] SACATM provides advice on priorities and activities related to the development, validation, scientific review, regulatory acceptance, implementation, and national and international harmonization of new, revised, and alternative toxicological test methods. The NIEHS Director appoints voting members to SACATM, which includes representatives from academia, state government, industry, and animal protection organizations.

ICCVAM provided SACATM with the draft BRD and draft test method recommendations, the Panel report, and all public comments for discussion at their meeting on June 18-19, 2008, where public stakeholders were given another opportunity to comment.

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[8] http://iccvam.niehs.nih.gov/SuppDocs/FedDocs/FR/FR_E7_25553.pdf
[9] http://iccvam.niehs.nih.gov
[10] http://iccvam.niehs.nih.gov/docs/immunotox_docs/LLNAPRPRept2008.pdf
[11] http://iccvam.niehs.nih.gov/SuppDocs/FedDocs/FR/FR-E8-11195.pdf
[12] http://ntp.niehs.nih.gov/index.cfm?objectid=720165EC-BDB7-CEBA-F517D1DEE4D7D129

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1.6 Final ICCVAM Test Method Recommendations and Final Background Review Document

ICCVAM and the interagency IWG considered the SACATM comments, the Panel report, and all public comments before finalizing ICCVAM test method recommendations for use of the LLNA as a stand-alone test method to determine skin sensitization potency categories. The recommendations (**Section 2.0**) and the final BRD (**Appendix C**) are incorporated in this ICCVAM test method evaluation report. As required by the ICCVAM Authorization Act of 2000, ICCVAM will forward this report and its recommendations to U.S. Federal agencies for consideration. Within 180 days after receiving ICCVAM test method recommendations, Federal agencies must respond to ICCVAM regarding their consideration and acceptance decisions, where appropriate. ICCVAM recommendations are available to the public on the NICEATM-ICCVAM website,[13] and agency responses will be made available as they are received.

[13] http://iccvam.niehs.nih.gov

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2.0 ICCVAM Recommendations: Usefulness and Limitations of the LLNA for Potency Categorization of Chemicals Causing Allergic Contact Dermatitis in Humans, Test Method Protocol, and Future Studies

ICCVAM has completed its evaluation of the validation status of the LLNA to classify substances into skin sensitization potency categories. NICEATM and ICCVAM prepared a comprehensive BRD that includes the data and information available to characterize the validity of this proposed use of the LLNA. The information included in the BRD (**Appendix C**) is based on a review of 136 substances with LLNA data and either (1) human maximization test (HMT) data (Kligman 1966; Kligman and Epstein 1975), (2) human repeat-insult patch test (HRIPT) data (Marzulli and Maibach 1974; Politano and Api 2008), or (3) other human data (for nonsensitizer status only). The database represents 76 human skin sensitizers and 60 human nonsensitizers, with 63 substances classified as skin sensitizers by both LLNA and human data.

The third revised edition of the Globally Harmonized System of Classification and Labelling of Chemicals (GHS) classifies skin sensitizers as Category 1 (UN 2009). Category 1 can be further subcategorized into 1A ("strong" skin sensitizers) and 1B ("other" skin sensitizers) based on results from human studies and/or animal studies (i.e., the LLNA and guinea pig tests). Under the GHS classification system, substances with positive responses in the HMT or HRIPT at induction thresholds $\leq 500\ \mu g/cm^2$ are classified as Subcategory 1A, and substances with positive responses at induction thresholds $>500\ \mu g/cm^2$ are classified as Subcategory 1B. The GHS also provides criteria for assigning these categories based on positive results in the LLNA using the EC3 value (i.e., the estimated concentration of a substance expected to produce an SI of 3, the threshold value for a substance to be considered a sensitizer in the LLNA) as the metric for relative potency (Kimber et al. 2001). Substances that produce an EC3 $\leq$ 2% are classified as Subcategory 1A, and substances with an EC3 > 2% are classified as Subcategory 1B (UN 2009). Nonsensitizers are not classified.

Most authorities do not currently regulate products based on skin sensitization potency, instead classifying them simply as "yes" or "no" for skin sensitization hazard. Under the Federal Hazardous Substances Act (15 U.S.C. 1261), CPSC currently requires hazard labeling of only those products considered to be strong skin sensitizers based on a weight-of-evidence approach that considers frequency of responses in exposed human populations, severity of responses, and the dose at which allergic reactions occur.[14] Criteria for test results from animal studies that could be used to identify potential strong human skin sensitizers would help in hazard identification for CPSC and other agencies with an interest in identifying strong skin sensitizers. Accordingly, ICCVAM evaluated the extent to which LLNA results could correctly predict strong versus other than strong human skin sensitizers.

2.1 ICCVAM Recommendations: Test Method Usefulness and Limitations

ICCVAM concludes that the LLNA, using the GHS classification criteria, can be used to categorize substances as strong sensitizers (GHS Subcategory 1A) when the estimated concentration that produces a positive LLNA result (i.e., EC3) is $\leq$2%. However, because almost half of the known strong human skin sensitizers have an EC3 > 2%, the LLNA cannot be considered a stand-alone assay to determine skin sensitization potency categories. Additional information is required to categorize a

[14] Substances that meet the CPSC's definition of strong sensitizer: (1) 4-phenylenediamine and products containing it; (2) powdered orris root and products containing it; (3) epoxy resins systems containing, in any concentration, ethylenediamine, diethylenetriamine, and diglycidyl ethers with molecular weight less than 200; (4) formaldehyde and products containing $\geq$1%; and (5) oil of bergamot and products containing $\geq$2% (16 C.F.R. 1500.13).

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substance as other than a strong sensitizer (GHS Subcategory 1B: "other" skin sensitizer) when the substance produces an LLNA EC3 > 2%.

These recommendations are based on an accuracy analysis (see **Section 3.4**) that included 136 substances for which there were both LLNA and human data (i.e., 27 strong human skin sensitizers, 49 other than strong human skin sensitizers, and 60 human nonsensitizers). Using the GHS criteria of LLNA EC3 ≤ 2% to classify substances as strong sensitizers and EC3 > 2% to classify substances as other than strong sensitizers, the overall correct prediction of human potency categories (i.e., strong sensitizers, other than strong sensitizers, and nonsensitizers) was 54% (74/136).

The LLNA EC3 ≤ 2% correctly identified 52% (14/27) of the strong human skin sensitizers. However, 48% (13/27) of strong human skin sensitizers were underclassified by the LLNA as either other than strong skin sensitizers (i.e., LLNA EC3 > 2%) or as nonsensitizers (i.e., negative in the LLNA). Among the 21 substances that produced an LLNA EC3 ≤ 2%, 67% (14/21) were correctly identified as strong sensitizers, but 33% (7/21) were incorrectly overclassified as strong skin sensitizers based on available human test data. Four of the seven substances were not classified as skin sensitizers (nonsensitizers) based on human test data.

Most substances with EC3 values between 2% and 10% should be considered to have the potential to be strong skin sensitizers unless there are data to support categorization as other than strong skin sensitizers. Of the strong human skin sensitizers in this database, 37% (10/27) produced EC3 values between 2% and 10%, which accounts for 76% (10/13) of the strong sensitizers that were underclassified by the LLNA. Therefore, it is likely that a considerable number of strong human skin sensitizers within the broader population of chemicals may produce EC3 values within this range.

By comparison, when the LLNA EC3 criterion for identifying strong skin sensitizers was increased to EC3 ≤ 10%, 89% (24/27) of the strong human skin sensitizers were correctly classified by the LLNA, and only 11% (3/27) were underclassified.

2.2 ICCVAM Recommendations: Test Method Protocol

ICCVAM recommends use of the recently updated LLNA test method protocol (**Appendix B**), which includes improved dose selection procedures to guide selection of the highest dose that will help minimize false negatives. The updated LLNA test method protocol provides for a 20% reduction in the required number of animals compared to the previously recommended LLNA protocol (ICCVAM 2001). The updated protocol reduces the number of required animals per group from five to four. It also recommends collection of individual animal data and inclusion of both a concurrent vehicle and a positive control in each study. These protocol modifications have resulted in an overall reduction of 20% in the number of animals used in a given test.

2.3 ICCVAM Recommendations: Future Studies

To further evaluate the usefulness and limitations of the LLNA for skin sensitization potency categorization, efforts should be made to identify additional high-quality human test data and human experience for substances with LLNA data for comparison. Emphasis should be placed on identifying substances that are classified as strong skin sensitizers based on a human threshold induction concentration of <500 $\mu g/cm^2$ to better evaluate the LLNA EC3 value that will best distinguish strong from other than strong human skin sensitizers. In order to develop a more accurate assessment of strong human skin sensitizers using LLNA results, especially for substances that produce an EC3 value between 2% and 10%, ICCVAM encourages the development, validation, and evaluation of integrated decision strategies that consider other types of relevant information such as quantitative structure-activity relationships, structural alerts, peptide reactivity, *in vitro* testing data, human test data or experience, and existing data from similar chemical entities.

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3.0 Validation Status for Use of the LLNA to Determine Skin Sensitization Potency Categories

The ICCVAM BRD (**Appendix C**) provides a comprehensive review of the validation status of the LLNA to determine skin sensitization potency categories. The BRD details the substances analyzed in the validation database, the accuracy and reliability of the LLNA for potency categorization, and all available data supporting its validity for the purpose of determining skin sensitization potency categories. This section summarizes the evaluation and validation status detailed in the BRD.

3.1 Test Method Description

The LLNA test method identifies potential skin sensitizers by quantifying lymphocyte proliferation in the draining auricular lymph nodes during the induction phase of skin sensitization. The magnitude of lymphocyte proliferation then correlates with the extent to which sensitization develops after topical exposure to the potential skin sensitizer. For the purposes of this analysis, relative potency in the LLNA is defined as the concentration of a fixed volume of a substance that is required for the induction phase of a skin sensitization reaction to occur. The more potent the substance the smaller the concentration needed.

3.1.1 General Test Method Procedures

The recently updated ICCVAM-recommended test method protocol for the LLNA describes the conduct of the assay in detail (**Appendix B**). A test substance-induced increase in lymphocyte proliferation in the draining lymph nodes of the ear, the site of application, is used to identify chemical sensitizers. Mice are injected with radiolabeled thymidine (or an analogue of thymidine), which is incorporated into the DNA of proliferating cells. The SI, the ratio of incorporated radioactivity in the auricular lymph nodes of treated versus control mice, is used to assess the sensitizing potential of the test substance. An SI ≥ 3 is used to classify a test substance as a skin-sensitizing agent. In the LLNA, a volume of 25 μL of the test substance is applied to each ear, and the estimated concentration expected to produce an SI of 3 (i.e., the EC3) is used as the metric for predicting skin-sensitization potency. Most recently, variations of the LLNA that do not employ radioactivity have also been evaluated and recommended by ICCVAM (ICCVAM 2010b, 2010a) and adopted as OECD test guidelines (OECD 2010b, 2010c). However, these nonradioactive LLNA methods have not been evaluated for skin sensitization potency determinations.

3.2 Validation Database

The validation database used to evaluate the LLNA's capacity to determine skin sensitization potency categories consists of 196 substances that have LLNA data with comparative guinea pig data, human data, or both. Data were obtained from published reports and unpublished data submitted to NICEATM in response to a *Federal Register* notice (72 FR 27815).[15] These 196 substances include 136 substances with comparative human data (76 sensitizers, 60 nonsensitizers), 116 substances with comparative guinea pig data (64 sensitizers, 52 nonsensitizers), and 56 substances with comparative human and guinea pig data (35 human sensitizers, 21 human nonsensitizers). Among the 136 substances with comparative human data and the 56 substances with comparative human and guinea pig data are 4-phenylenediamine and formaldehyde, two of the five substances that meet CPSC's definition of strong sensitizer (16 C.F.R. 1500.13).

Table 3-1 shows the chemical classes represented by the 196 substances tested in the LLNA with human and/or guinea pig skin sensitization data. Considering inorganics as one class, the 196 substances represent 30 chemical classes. Fifty-five substances are classified in more than one

[15] http://iccvam.niehs.nih.gov/SuppDocs/FedDocs/FR/FR_E7_9544.pdf

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chemical class. The classes with the highest number of substances are carboxylic acids (33 substances) and aldehydes (18 substances). In the entire NICEATM LLNA database of more than 600 substances (a sufficiently large representation for further analyses), 22 chemical classes are represented by at least five substances. Twenty of these classes have at least 60% of the LLNA results identified as positive (i.e., SI ≥ 3). These 20 classes are identified as those most likely to be associated with skin sensitization. In comparison, 19 of these 20 classes are also represented in the database of 196 substances included in this evaluation (i.e., the NICEATM LLNA potency database); only the class of macromolecular substances is not included. Further, all of the chemical classes previously found to contain common skin allergens (e.g., aldehydes, ketones, quinones, and acrylates) (Gerberick et al. 2004) are represented in this LLNA potency evaluation. **Annex III** of the BRD (**Appendix C**) provides the chemical classes to which each substance was assigned, information on the physicochemical properties (e.g., estimated log octanol-water partition coefficient), Chemical Abstracts Service Registry Number, and uses. Some substances were assigned to more than one chemical class, and some were not assigned to a specific chemical class.

Table 3-1 Chemical Classes Represented in the LLNA Potency Database

Chemical Class	No. of Substances[1]
Inorganic chemicals	**11**
Aluminum compounds	1
Chromium compounds	1
Elements	1
Gold compounds	1
Manganese compounds	1
Mercury compounds	1
Metals	5
Sulfur compounds	1
Zinc compounds	1
Organic chemicals	**185**
Alcohols	15
Aldehydes	18
Amides	5
Amines	16
Anhydrides	2
Azo compounds	5
Carbohydrates	6
Carboxylic acids	33
Cyanates	1
Esters	5

Chemical Class	No. of Substances[1]
Organic chemicals (continued)	**---**
Ethers	6
Formulations[2]	16
Heterocyclic compounds	15
Hydrocarbons, acyclic	5
Hydrocarbons, cyclic	12
Hydrocarbons, halogenated	1
Hydrocarbons, other	9
Ketones	3
Lactones	1
Lipids	15
Natural complex substances[2]	15
Nitriles	2
Nitro compounds	2
Onium compounds	1
Phenols	14
Polycyclic compounds	4
Quinones	1
Sulfur compounds	16
Ureas	2
Unknown[3]	**3**

Abbreviations: LLNA = murine local lymph node assay; No. = number.

Chemical classifications are based on the Medical Subject Headings classification for chemicals and drugs developed by the National Library of Medicine (http://www.nlm.nih.gov/mesh/meshhome.html).

[1] The total number of substances assigned to each chemical class does not equal the total number of substances evaluated because some substances were assigned to more than one chemical class and some substances were not assigned to a specific chemical class.

[2] Substances assigned to these classes were mixtures of two or more components. In some cases, another chemical class was also assigned based on the active ingredient (for formulations) or the principal component (for natural complex substances).

[3] The proprietary substances (fatty acid glutamate, fatty acid alcohol #1, and fatty acid alcohol #2) were not identified sufficiently for a chemical class to be assigned.

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3.3 Reference Test Method Data

The reference database for this evaluation consisted of (1) clinical studies that used the HMT or HRIPT or (2) other human information (for nonsensitizer status only). In the HMT and the HRIPT, potency information is determined from the no observed effect level (NOEL), the lowest observed effect level (LOEL), or the induction dose per skin area (DSA) that produces a positive response in 5% of the tested population (DSA_{05}). The third revised edition of the GHS classifies skin sensitizers as Category 1 (UN 2009) (see **Appendix E**). Category 1 substances are further subcategorized into 1A ("strong" skin sensitizers) or 1B ("other" skin sensitizers) based on results from human and/or animal studies (i.e., LLNA and guinea pig tests). Under the GHS classification system, substances with positive responses in the HMT or HRIPT at induction thresholds $\leq$500 $\mu g/cm^2$ are classified as Subcategory 1A, and substances with positive responses at >500 $\mu g/cm^2$ are classified as Subcategory 1B. The GHS criteria for using the LLNA to subcategorize sensitizers is based on the EC3 value: substances with EC3 $\leq$ 2% are classified as Subcategory 1A ("strong" skin sensitizers) and substances with EC3 > 2% are classified as Subcategory 1B ("other" skin sensitizers) (UN 2009). Nonsensitizers are not classified.

3.4 Test Method Accuracy

3.4.1 DSA_{05} and EC3 Values

The DSA_{05} value represents a defined incidence of a positive response among test subjects (i.e., 5%). It was used as the human threshold response because it corresponds best (compared with NOEL or LOEL values) to the EC3 value, which is also a threshold positive response. More than one LLNA test, often in different vehicles, was available for many of the substances in the validation database. Single EC3 and DSA_{05} values were established for each substance (see **Appendix C, Annex II-4**) before any analyses were conducted. Geometric mean EC3 and DSA_{05} values for each substance with multiple results were favored over the most potent EC3 and DSA_{05} values because the coefficient of determination, R^2, was higher for the geometric mean EC3 and DSA_{05} regression (0.448 versus 0.382; see **Appendix C, Section 6.1.1**). Geometric mean EC3 values were calculated regardless of vehicle because statistical analyses showed that vehicle had no impact on the relationship of LLNA EC3 and human DSA_{05} values for the substances tested (see **Appendix C, Annex IV**).

Forty-seven of the 98 substances with positive LLNA results had multiple EC3 values. The number of values for each substance ranged from 2 to 66. Individual EC3 values ranged from 0.0007% to 98.5%. Substances with a majority of negative LLNA test results were not assigned EC3 values. For example, nickel salts and streptomycin were each considered negative in the LLNA because most of the LLNA responses were negative (8/10 tests for nickel salts; 4/5 tests for streptomycin). Likewise, substances with multiple positive HMT or HRIPT responses were assigned geometric mean DSA_{05} values calculated from all the available DSA_{05} values (see **Appendix C, Annex II-4**). Thirty-two of the 76 substances with positive human results had multiple DSA_{05} values. The number of values ranged from 2 to 8. Individual DSA_{05} values ranged from 1.9 to 335545 $\mu g/cm^2$.

Table 3-2 shows the distribution of substances into the GHS potency categories using geometric mean LLNA EC3 values and geometric mean DSA_{05} values for substances with multiple results. The 76 human sensitizers include 27 strong sensitizers (14 with LLNA EC3 $\leq$ 2%, 11 with EC3 > 2%, and two with negative LLNA results) and 49 other than strong sensitizers (three with LLNA EC3 $\leq$ 2%, 35 with EC3 > 2%, and 11 with negative LLNA results). Of the 60 human nonsensitizers, 35 were LLNA sensitizers (four with LLNA EC3 $\leq$ 2%, 31 with EC3 > 2%) and 25 were LLNA nonsensitizers. **Figure 3-1** shows geometric mean LLNA EC3 values plotted against the geometric mean DSA_{05} values for the 63 LLNA and human sensitizers. Concordant LLNA and human nonsensitizers, LLNA false positives, and LLNA false negatives are shown on the edges of the graph.

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The GHS cutoffs, EC3 ≤ 2% and DSA_{05} ≤ 500 μg/cm^2, are marked to show the correspondence of the data with the GHS classification criteria for Subcategories 1A and 1B.

Table 3-2 Distribution of 136 Substances for Classification Rate Analyses[1]

LLNA + /Human +		LLNA + / Human -	LLNA - / Human +	LLNA - / Human -
Strong[2]	Other[3]			
25 (14 EC3 ≤ 2%; 11 EC3 > 2%)	38 (3 EC3 ≤ 2%; 35 EC3 > 2%)	35 (4 EC3 ≤ 2%; 31 EC3 > 2%)	13 (2 strong; 11 other)[2,3]	25

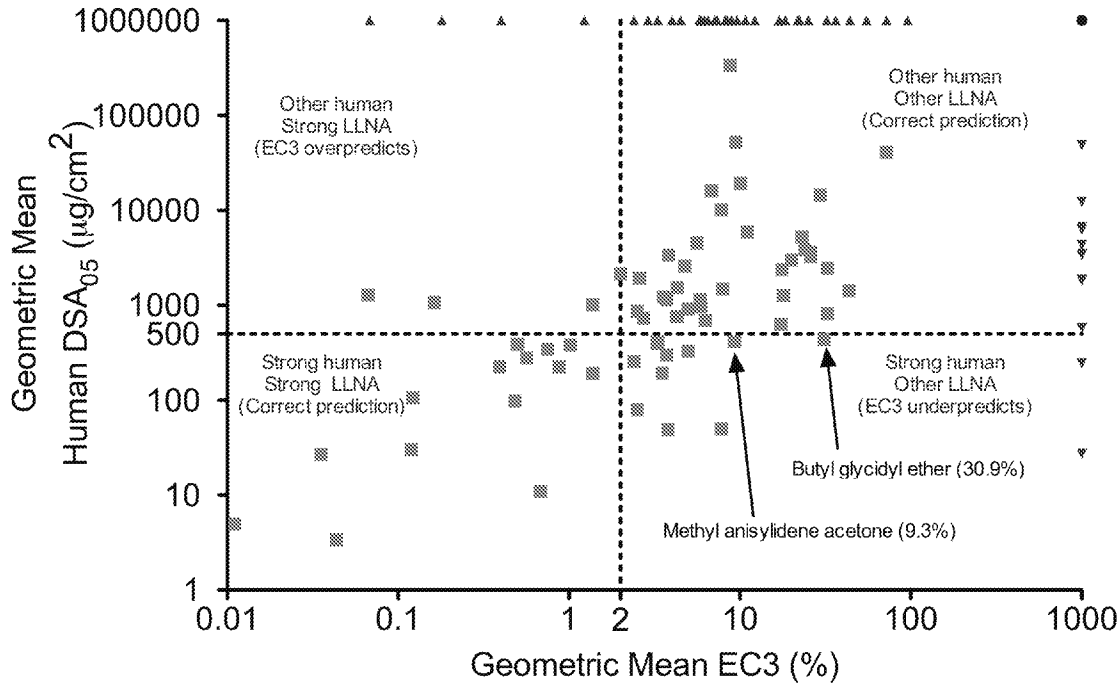
Abbreviations: DSA_{05} = induction dose per skin area, in μg/cm^2, in a human repeat-insult patch test or human maximization test that produces a positive response in 5% of the tested population; EC3 = estimated concentration of a substance expected to produce a stimulation index of 3, the threshold value for a substance to be considered a sensitizer in the LLNA; LLNA = murine local lymph node assay.

[1] Classification based on geometric mean EC3 and DSA_{05} values.

[2] Human sensitizers were classified as strong sensitizers if DSA_{05} ≤ 500 μg/cm^2.

[3] Human sensitizers were classified as other sensitizers if DSA_{05} > 500 μg/cm^2.

Figure 3-1 LLNA EC3 and Human Results by GHS Potency Category for 136 Substances



Legend: ▩ Human/LLNA sensitizers (n = 63); ▲ LLNA false positive (n = 35); ▼ LLNA false negative (n = 13); ● Concordant negative (n = 25).

Abbreviations: DSA_{05} = induction dose per skin area, in μg/cm^2, in a human repeat-insult patch test or human maximization test that produces a positive response in 5% of the tested population; EC3 = estimated concentration of a substance expected to produce a stimulation index of 3, the threshold value for a substance to be considered a sensitizer in the LLNA; GHS = Globally Harmonized System of Classification and Labelling of Chemicals (UN 2009); LLNA = murine local lymph node assay.

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3.4.2 LLNA Classification of Strong and Other Than Strong Sensitizers in Humans

The extent to which the LLNA correctly classifies strong and other than strong sensitizers in humans was evaluated using the criteria for human thresholds defined in the GHS (UN 2009). The correct classification, underclassification, and overclassification rates of the LLNA versus human data were initially calculated using the GHS criteria of EC3 ≤ 2% for strong sensitizers and EC3 > 2% for other sensitizers. As indicated in **Tables 3-3** and **3-4**, based on this database, the LLNA correctly identified 52% (14/27) of the strong human skin sensitizers using EC3 ≤ 2% but underclassified 48% (13/27). Among the 21 substances that produced an EC3 ≤ 2%, 67% (14/21) were strong human skin sensitizers (GHS Subcategory 1A), but the remaining 33% (7/21) were either other than strong human skin sensitizers (GHS Subcategory 1B: n = 3) or substances not classified as human skin sensitizers (n = 4).

As indicated in **Figure 3-1**, most of the strong human skin sensitizers that were underclassified by the LLNA (10/13) had EC3 values from 2% to 10%. Therefore, the classification rates for human skin sensitizer categories obtained using incremental EC3 cutoff values up to 10% were also evaluated (**Table 3-3**). From EC3 ≤ 2% to ≤4%, the increase in number of correctly classified strong sensitizers (14 to 21) was almost directly proportional to the decrease in the number of correctly classified other than strong sensitizers (35 to 29). The number of human nonsensitizers overclassified as strong sensitizers increased from four to seven when the LLNA EC3 cutoff value moved from ≤2% to ≤4%. With each additional increase of 2% in the LLNA EC3 cutoff value, the number of correctly classified strong sensitizers increased by one substance. Using LLNA EC3 ≤ 10% to classify substances as strong sensitizers correctly classified 89% (24/27) of the strong sensitizers compared to the 52% (14/27) of the strong sensitizers correctly classified using EC3 ≤ 2% (**Table 3-4**). However, the proportion of substances classified by the LLNA as strong sensitizers that actually are strong human skin sensitizers was higher for EC3 ≤ 2% than for EC3 ≤ 10%: 67% (14/21) versus 36% (24/67) (see **Table 3-3**).

Figure 3-2 shows the change in the correct classification and underclassification rates for the 27 strong human skin sensitizers over the entire range of LLNA EC3 cutoff values. The correct potency classification rate for strong human skin sensitizers increased, and the underclassification rate decreased as the EC3 value increased. The correct classification rate plateaued, however, because the two strong human skin sensitizers that yielded negative results in the LLNA were not correctly classified by any EC3 cutoff value.

Of the 13 strong human skin sensitizers that were underclassified by the GHS criterion of LLNA EC3 ≤ 2%, 11 were underclassified as other sensitizers and two were underclassified as nonsensitizers. The two strong human skin sensitizers that were classified by the LLNA as nonsensitizers also yielded sensitizer results in a few LLNA tests (2/10 for nickel salts and 1/5 for streptomycin). However, the GHS criterion of EC3 ≤ 2% would have underclassified these strong human sensitizers even if their positive results had been used in the analysis. The two positive nickel results were for nickel sulfate in dimethyl sulfoxide (EC3 = 4.8%) and nickel chloride in 30% ethanol (EC3 = 5.5%). The positive result for streptomycin yielded EC3 = 33% in dimethylformamide. Ten of the 11 remaining discordant substances had EC3 values less than 10%. The substance with EC3 > 10% was butyl glycidyl ether (EC3 = 30.9%). The physicochemical commonalities among these 13 strong human skin sensitizers include molecular weights within a range of 100 (12/13 substances had molecular weights of 98.15 to 192.3). Eight of the 13 substances were liquids; and all six of the substances for which peptide reactivity information was available had high (n = 5) or moderate (n = 1) peptide reactivity.

Table 3-3 Concordance of LLNA and Human Data for Strong Sensitizer, Other Sensitizer, and Nonsensitizer Categories for 136 Substances at Selected LLNA EC3 Values

		Strong Sensitizer	Other Sensitizer	Nonsensitizer	Total
		EC3 ≤ 2% (GHS)	**EC3 > 2% (GHS)**	**Negative LLNA**	
Human Data[1]	Strong Sensitizer	14	11	2	27
	Other Sensitizer	3	35	11	49
	Nonsensitizer	4	31	25	60
	Total	21	77	38	136
		EC3 ≤ 4%	**EC3 > 4%**	**Negative LLNA**	
Human Data[1]	Strong Sensitizer	21	4	2	27
	Other Sensitizer	9	29	11	49
	Nonsensitizer	7	28	25	60
	Total	37	61	38	136
		EC3 ≤ 6%	**EC3 > 6%**	**Negative LLNA**	
Human Data[1]	Strong Sensitizer	22	3	2	27
	Other Sensitizer	16	22	11	49
	Nonsensitizer	12	23	25	60
	Total	50	48	38	136
		EC3 ≤ 8%	**EC3 > 8%**	**Negative LLNA**	
Human Data[1]	Strong Sensitizer	23	2	2	27
	Other Sensitizer	20	18	11	49
	Nonsensitizer	16	19	25	60
	Total	59	39	38	136
		EC3 ≤ 10%	**EC3 > 10%**	**Negative LLNA**	
Human Data[1]	Strong Sensitizer	24	1	2	27
	Other Sensitizer	23	15	11	49
	Nonsensitizer	20	15	25	60
	Total	67	31	38	136

Abbreviations: EC3 = estimated concentration of a substance expected to produce a stimulation index of 3, the threshold value for a substance to be considered a sensitizer in the LLNA; GHS = Globally Harmonized System of Classification and Labelling of Chemicals (UN 2009); LLNA = murine local lymph node assay.

[1] Human sensitizer data were DSA_{05} values (induction dose per skin area, in $\mu g/cm^2$, that produced a positive response in 5% of the tested population in a human repeat-insult patch test or human maximization test). Sensitizers were classified as strong if $DSA_{05} \leq 500\ \mu g/cm^2$ and other if $DSA_{05} > 500\ \mu g/cm^2$.

Thirteen substances that had LLNA EC3 > 2% or were nonsensitizers in the LLNA were strong human skin sensitizers. Fourteen percent (11/77) of the substances with EC3 > 2% were strong human skin sensitizers ($DSA_{05} \leq 500\ \mu g/cm^2$). Five percent (2/38) of the substances that were negative in the LLNA were strong human skin sensitizers.

To determine the optimum EC3 value that could be used to identify strong and other than strong sensitizers, receiver-operator characteristic calculations (Fawcett 2006) were performed. The optimum EC3 value was defined as the value that resulted in the highest correct classification rate for strong human skin sensitizers, other human skin sensitizers, and nonsensitizers combined. The highest correct classification rate, 55% (75/136), occurred at both EC3 ≤ 3.8% and EC3 ≤ 3.5%. EC3 ≤ 3.8% was considered the optimum value based on the fact that it produced a lower underclassification rate for strong and other than strong skin sensitizers than EC3 ≤ 3.5%: 22% (17/76) versus 25% (19/76). These analyses are detailed in **Appendix C, Section 6.1.2**.

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Table 3-4 Classification Rates for the Prediction of Human Potency Categories by Selected LLNA EC3 Cutoff Values[1] for 136 Substances

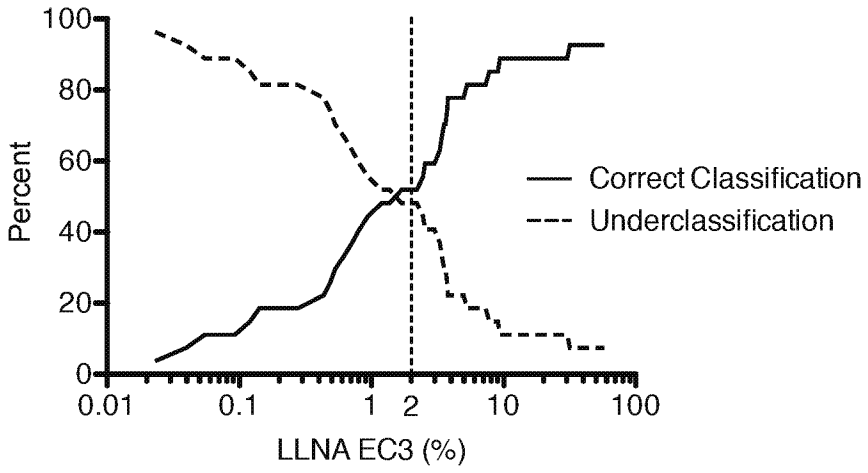
EC3 Cutoff for Strong versus Other Sensitizers	Strong Human Sensitizers ($DSA_{05} \leq 500$ µg/cm^2)		Other Human Sensitizers ($DSA_{05} > 500$ µg/cm^2)			Human Nonsensitizers		Overall Correct Potency Classification[2]
	Correct	Under	Over	Correct	Under	Correct	Over	
GHS Cutoff EC3 ≤ 2%	52 ± 19% (14/27)	48 ± 19% (13/27)	6 ± 7% (3/49)	71 ± 13% (35/49)	22 ± 12% (11/49)	42 ± 12% (25/60)	58 ± 12% (35/60)	54 ± 8% (74/136)
EC3 ≤ 4%	78 ± 16% (21/27)	22 ± 16% (6/27)	18 ± 11% (9/49)	59 ± 14% (29/49)	22 ± 12% (11/49)	42 ± 12% (25/60)	58 ± 12% (35/60)	54 ± 8% (74/136)
EC3 ≤ 6%	81 ± 15% (22/27)	19 ± 15% (5/27)	33 ± 13% (16/49)	45 ± 14% (22/49)	22 ± 12% (11/49)	42 ± 12% (25/60)	58 ± 12% (35/60)	50 ± 8% (68/136)
EC3 ≤ 8%	85 ± 13% (23/27)	15 ± 13% (4/27)	41 ± 14% (20/49)	37 ± 13% (18/49)	22 ± 12% (11/49)	42 ± 12% (25/60)	58 ± 12% (35/60)	48 ± 8% (65/136)
EC3 ≤ 10%	89 ± 12% (24/27)	11 ± 12% (3/27)	47 ± 14% (23/49)	31 ± 13% (15/49)	21 ± 12% (11/49)	42 ± 12% (25/60)	58 ± 12% (35/60)	47 ± 8% (64/136)

Abbreviations: DSA_{05} = induction dose per skin area, in µg/cm^2, in a human repeat-insult patch test or human maximization test that produces a positive response in 5% of the tested population; EC3 = estimated concentration of a substance expected to produce a stimulation index of 3, the threshold value for a substance to be considered a sensitizer in the LLNA; GHS = Globally Harmonized System of Classification and Labelling of Chemicals (UN 2009); LLNA = murine local lymph node assay.

[1] Classification rates are shown ±95% confidence limits.

[2] The overall correct classification rate includes the correct classification of strong human sensitizers, other than strong sensitizers, and nonsensitizers.

Figure 3-2 Classification Rates for the LLNA EC3 Prediction of 27 Strong Human Sensitizers



Abbreviations: EC3 = estimated concentration of a substance expected to produce a stimulation index of 3, the threshold value for a substance to be considered a sensitizer in the LLNA; LLNA = murine local lymph node assay.

Analysis was based on 27 substances identified as strong sensitizers in humans using the human maximization test and/or the human repeat-insult patch test because the induction dose per skin area that produced a positive response in 5% of the tested population was ≤500 µg/cm^2.

Fifty-six substances had LLNA, guinea pig (i.e., the guinea pig maximization test and/or the Buehler test), and human skin sensitization data. The overall correct classification rate of the LLNA, using EC3 ≤ 2% to classify substances as strong skin sensitizers and EC3 > 2% to classify substances as other than strong skin sensitizers, was similar to that of the guinea pig tests. The overall correct classification rate of human sensitizers and nonsensitizers was 61% (34/56) for the LLNA versus 59% (33/56) for the guinea pig tests. The LLNA correctly classified more strong sensitizers and other than strong sensitizers than did guinea pig tests; however, the LLNA correctly classified fewer nonsensitizers. The LLNA correctly classified 71% (10/14) of the strong human sensitizers versus 57% for the guinea pig tests and 67% (14/21) of the other human sensitizers versus 52% (11/21) for the guinea pig tests. The LLNA also correctly classified 48% (10/21) of the nonsensitizers versus 67% (14/21) for the guinea pig tests.

3.5 Test Method Reliability

3.5.1 Intra- and Interlaboratory Variability

Basketter and Cadby (2004) evaluated the intralaboratory variability associated with 29 individual EC3 values for isoeugenol. The EC3 values ranged from 0.5% to 2.6%. These data were used to support the "often-mentioned perspective that the biological variation associated with the estimation of EC3 values means that any particular EC3 value can be halved or doubled" (Basketter and Cadby 2004). Basketter et al. (2007) evaluated the interlaboratory reproducibility of EC3 data for 17 sensitizers tested in at least two laboratories using the same vehicle. The authors concluded that, although variability exists, it is less than an order of magnitude.

3.5.2 Influence of LLNA Vehicle

A number of analyses included in the BRD (**Appendix C**) highlight the potential impact of the LLNA vehicle on EC3 values and potency classification. Forty-five substances in the NICEATM LLNA database of over 600 substances had data from tests in multiple vehicles. Evaluation revealed that potency classifications differed for 18% (8/45) of these substances with the GHS classification system (e.g., the EC3 value would change from ≤2% to >2%, or vice versa). Nine percent (4/45) of these substances had EC3 values that varied by at least an order of magnitude depending on the vehicle used in the LLNA. Another 24% (11/45) of the substances were classified differently as either sensitizers or nonsensitizers depending on the vehicle. Additionally, there were instances in which LLNA results from the same vehicle produced discordant sensitizer and nonsensitizer outcomes (16% [7/45] of the substances).

Vehicle may be an important determinant of the EC3 value but perhaps not for every substance tested or for a particular group of substances. With respect to the accuracy analyses (see **Section 3.4**), two-way analyses of variance with chemical and vehicle as the factors indicated that two vehicles were responsible for a statistically significant effect of vehicle on the LLNA EC3 value, propylene glycol and Pluronic L92 (see **Appendix C, Annex IV**). Linear regression and Spearman correlation analyses (Steel and Torrie 1980) indicated that removing tests using these vehicles had no impact on the relationship of the EC3 value with human DSA_{05} values for the 63 substances that were sensitizers in the LLNA and in the HMT and/or HRIPT.

In the classification rate analyses (see **Section 3.4**), the variability of the LLNA EC3 values for sensitizers was similar to that of DSA_{05} values. For LLNA and human sensitizers, the coefficient of variation (CV) range for the LLNA EC3 values was 2% to 349%, and the CV range for the DSA_{05} values was 2% to 408%.

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3.6 Animal Welfare Considerations: Reduction, Refinement, and Replacement

The proposal for using the LLNA to determine potency does not impact its requirement for using animals or the number of animals that are required. However, this application could broaden the use of the LLNA protocol in place of guinea pig tests and thereby further reduce the number of guinea pigs being used to assess skin sensitization potential. The LLNA test method protocol requires a minimum of only four mice per treatment group, whereas currently recommended guinea pig tests require at least 10 guinea pigs per group for the Buehler test and at least five guinea pigs per group for the guinea pig maximization test. The LLNA is also a refinement compared with guinea pig tests because it avoids the pain and distress that occur in guinea pigs when substances cause allergic contact dermatitis.

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4.0 ICCVAM Consideration of Independent Peer Review Panel Report and Other Comments

The ICCVAM evaluation process incorporates a high level of scientific peer review and transparency. The evaluation of the use of the LLNA to determine skin sensitization potency included one public review meeting by an independent scientific peer review panel, comments from SACATM, and multiple opportunities for public comments (see **Sections 1.0** and **4.2**). ICCVAM and the interagency IWG considered the Panel report, the SACATM comments, and all public comments before finalizing the ICCVAM test method evaluation report and BRD. This section summarizes ICCVAM consideration of these reports and comments. The Panel report and public comments are provided in **Appendices D2** and **F2**, respectively.

4.1 ICCVAM Consideration of Independent Peer Review Panel Report

4.1.1 Comments on Draft ICCVAM Recommendations: Test Method Usefulness and Limitations

The Panel agreed with the ICCVAM draft recommendation made in January 2008 that the LLNA should not be considered as a stand-alone test method for determining skin sensitization potency but could instead be used as part of a weight-of-evidence evaluation (e.g., along with quantitative structure-activity relationship, peptide reactivity, human evidence). The Panel further stated that additional analyses suggested at the March 2008 Panel meeting might improve the correlation between the EC3 values and the human threshold values, thus providing more information on the usefulness of the LLNA for predicting skin sensitization potency categories. The Panel did note that the effect of vehicles should be recognized as a limitation in the data analyses and a likely source of within- and between-laboratory variability.

ICCVAM Response:
ICCVAM considered the Panel report and performed additional analyses to compare the EC3 values and alternative human threshold values. This exercise was reported in the final BRD (see **Appendix C, Section 6.1**). Based on these analyses, ICCVAM concluded that the LLNA could be used to categorize substances as strong sensitizers (GHS Subcategory 1A) when the estimated concentration that produces a positive LLNA result (i.e., EC3) is ≤2%. However, when the substance produces an LLNA EC3 > 2%, additional information is needed to categorize a substance as an other than strong sensitizer (GHS Subcategory 1B: "other" skin sensitizer) (see **Section 2.1**).

4.1.2 Comments on Draft ICCVAM Recommendations: Test Method Protocol

The Panel concurred with the ICCVAM draft recommendation made in January 2008 that the ICCVAM-recommended LLNA protocol should be used when generating data that might be considered for skin sensitization potency categorization. Furthermore, they supported the recommendation that data should always be collected from individual animals and not pooled. Some Panel members offered the opinion that pooled data (OECD 2002)[16] should also be considered acceptable. The Panel suggested that the calculation for the EC3 value be included as part of the LLNA protocol.

ICCVAM Response:
ICCVAM supports the Panel recommendation that the ICCVAM-recommended LLNA protocol should be used when generating data that might be considered for skin sensitization potency categorization. However, ICCVAM disagreed with the Panel minority with regard to the acceptability of pooled data. Rather, ICCVAM concluded that, if experiments are performed using the ICCVAM-

[16] Updated in 2010 (OECD 2010a).

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recommended LLNA protocol, the lymph nodes should be collected individually for each mouse. This is necessary in order to identify whether any of the individual animal responses are outliers. The capacity to identify outliers will help avoid false negative results for weaker sensitizers (i.e., substances that normally would produce an SI just above 3 might be incorrectly classified as negative due to a low outlier value).

The updated ICCVAM-recommended LLNA test method protocol, **Appendix B**, provides a detailed description of the LLNA and describes the calculation of the SI, which is used to determine the sensitizing potential of a test substance. Calculation of the EC3 value, which is the metric for predicting skin sensitization potency using the LLNA, is also included in this updated ICCVAM-recommended LLNA test method protocol.

4.1.3 Comments on Revised Draft ICCVAM Recommendations: Future Studies

The Panel agreed with the ICCVAM draft recommendation made in January 2008 that more data are needed to determine the optimal threshold to distinguish between weak and strong skin sensitizers in humans. However, the Panel discouraged conducting new animal studies unless it was likely that results from such studies would lead to an overall reduction in animal use. The Panel stated further that the LLNA could be used in conjunction with quantitative-structure activity relationship information, guinea pig assays, HMT/HRIPT, and quantitative data for elicitation and frequency of positive response in humans in a weight-of-evidence approach.

The Panel also suggested additional evaluations that might improve the correlation between LLNA and human data (e.g., dividing LOEL by a safety factor other than 10, using LOEL data only, or using NOEL data only). One Panel member suggested that using the DSA_{05} value was a better comparison to the EC3 value because the DSA_{05} represented a LOEL that was corrected to 5% incidence of an induction response. The Panel further stated that LLNA tests based on pooled or individual animal data should be evaluated independently to assess the impact of using pooled data on the accuracy for determining skin sensitization potency. The Panel recommended a statistical analysis to determine where an appropriate cutoff value between weak or strong sensitizers might be best defined for traditional LLNA data. For example, receiver-operator characteristic curves (Fawcett 2006) could be used to identify the optimum cutoff for determining the difference between weak and strong sensitizers. Finally, the Panel stated that the effect of different vehicles should be recognized as a limitation in the current data analysis, that this was a source of variability within and between laboratories, and that its impact should be considered in future analyses.

ICCVAM Response:
ICCVAM considered the Panel report and noted its positions regarding (1) the conduct of new animal studies; (2) the use of the LLNA in conjunction with other available information, data, and assay results in a weight-of-evidence approach; and (3) the conduct of additional evaluations that might improve the correlation between LLNA and human data. Accordingly, ICCVAM performed additional analyses to compare the EC3 values and the human threshold values (**Appendix C, Section 6.1**). Based on these analyses, ICCVAM concluded that the LLNA could be used to categorize substances as strong skin sensitizers (GHS Subcategory 1A) when the estimated concentration that produces a positive LLNA result (i.e., EC3) is ≤2%. However, additional information is required to categorize a substance as an other than strong sensitizer (GHS Subcategory 1B: "other" skin sensitizer) when the substance produces an LLNA EC3 > 2% (see **Section 2.0**).

4.2 ICCVAM Consideration of Public and SACATM Comments

The ICCVAM evaluation process provides numerous opportunities for stakeholder involvement, including submitting written public comments and providing oral comments at ICCVAM independent peer review panel meetings and SACATM meetings. **Table 4-1** lists the seven opportunities for

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public comment that were provided during the ICCVAM evaluation of the validation status of new versions and applications of the LLNA, which included assessing use of the LLNA to determine skin sensitization potency categories. The number of public comments received in response to each of the opportunities is also indicated. A total of 45 comments were submitted. Detailed comments received in response to or related to the *Federal Register* notices listed in **Table 4-1** are available on the NICEATM-ICCVAM website.[17] The following sections, delineated by *Federal Register* notice and public meeting, briefly discuss the public comments received.

Table 4-1 Opportunities for Public Comments

Opportunities for Public Comments	Date	Number of Public Comments
72 FR 27815: The Murine Local Lymph Node Assay: Request for Comments, Nominations of Scientific Experts, and Submission of Data	May 17, 2007	17
72 FR 52130: Draft Performance Standards for the Murine Local Lymph Node Assay: Request for Comments	September 12, 2007	4
73 FR 1360: Announcement of an Independent Scientific Peer Review Panel Meeting on the Murine Local Lymph Node Assay; Availability of Draft Background Review Documents; Request for Comments	January 8, 2008	7
Independent Scientific Peer Review Panel Meeting Assessing the Allergic Contact Dermatitis Potential of Chemicals and Products: Validation Status of New Versions and Applications of the Murine Local Lymph Node Assay	March 4-6, 2008	16
73 FR 25754: Meeting of the Scientific Advisory Committee on Alternative Toxicological Methods (SACATM)	May 7, 2008	1
73 FR 29136: Peer Review Panel Report on the Validation Status of New Versions and Applications of the Murine Local Lymph Node Assay (LLNA): A Test Method for Assessing the Allergic Contact Dermatitis Potential of Chemicals and Products: Notice of Availability and Request for Public Comments	May 20, 2008	0
SACATM Meeting, Radisson Hotel, RTP, NC	June 18-19, 2008	0

4.2.1 Public Comments in Response to 72 FR 27815 (May 17, 2007): The Murine Local Lymph Node Assay: Request for Comments, Nominations of Scientific Experts, and Submission of Data

NICEATM requested the following:

1. Public comments on the appropriateness and relative priority of evaluation of the validation status of the following:
    a. The LLNA as a stand-alone assay for determining potency (including severity) for the purpose of hazard classification
    b. The reduced LLNA approach (ESAC 2007; ICCVAM 2009; Kimber et al. 2006)
    c. Nonradioactive LLNA methods
    d. The use of the LLNA for testing mixtures, aqueous solutions, and metals
    e. The current applicability domain (i.e., the types of chemicals and substances for which the LLNA has been determined to be useful)

[17] http://ntp-apps.niehs.nih.gov/iccvampb/searchPubCom.cfm

ED_006472_00084346-00039

2. Nominations of expert scientists to consider as members of a possible independent scientific peer review panel
3. Submission of data for the LLNA and/or modified versions of the LLNA

NICEATM received 17 comments in response to this *Federal Register* notice. Six comments included additional data and information, while two others offered data and information upon request. Three commenters nominated four potential panelists for consideration. Three commenters suggested reference publications for consideration during the Panel evaluation. The nominees were included in the database of experts from which the Panel was selected. The data and suggested references were included in the ICCVAM draft review documents that were provided to the Panel for the March 2008 meeting.

Comment:
A commenter suggested rearranging the priority sequence of test method evaluation from most to least pressing: a, e, d, b, and c (see list above).

ICCVAM Response:
ICCVAM did not establish a relative priority for these activities because they were all considered to be high-priority activities. Accordingly, all LLNA-related activities described above were discussed at the March 2008 Panel meeting.

Comment:
One comment pertained to LLNA skin sensitization potency.

Acknowledging that the LLNA must be validated for determining skin sensitization potency for regulatory use, the commenter urged ICCVAM to take an abbreviated test validation approach. The commenter encouraged ICCVAM to spend its time and resources promoting the development and regulatory use of non-animal methods by engaging in integrated approaches to *in vitro* immunotoxicity.

ICCVAM Response:
Traditional regulatory test methods for skin sensitization (i.e., guinea pig maximization test, Buehler test, LLNA) have focused on "yes" or "no" determinations of skin sensitization hazard. In recent years, the LLNA has been proposed as an effective method for determining skin sensitization potency because of the dose-response information that is generated. ICCVAM evaluated the LLNA for potency use and concluded that the LLNA could be used to categorize substances as strong sensitizers (GHS Subcategory 1A) when the estimated concentration that produces a positive LLNA result (i.e., EC3) is $\leq 2\%$. However, additional information is required to categorize a substance as an other than strong sensitizer (GHS Subcategory 1B: "other" skin sensitizer) when the substance produces an LLNA EC3 $> 2\%$.

The proposal for using the LLNA for potency determinations does not impact its requirement for using animals or the number of animals that will be required. However, this application could broaden the use of the LLNA protocol in place of guinea pig tests and could thereby further reduce the number of guinea pigs that are being used to assess skin sensitization potential. ICCVAM acknowledges the desire to abbreviate the validation approach and is committed to performing test method validations in the most scientifically expeditious and efficient manner possible. However, ICCVAM is also committed to promoting human safety and, accordingly, is dedicated to ensuring the relevance and reliability of alternative test methods that reduce, refine, or replace animals used for such safety analyses. Further, ICCVAM is also committed to identifying *in vitro* models and integrated non-animal approaches for assessing ACD. ICCVAM is engaged with ECVAM and JaCVAM in the development of validation studies for such methods. Timely regulatory adoption of properly vetted and thoroughly validated test methods is the desired consequence and sought-after goal of these international validation organizations.

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4.2.2 Public Comments in Response to 72 FR 52130 (September 12, 2007): Draft Performance Standards for the Murine Local Lymph Node Assay: Request for Comments

NICEATM requested public comments on the September 2007 draft ICCVAM-recommended LLNA performance standards developed to facilitate evaluation of modified LLNA test method protocols. In response to this *Federal Register* notice, NICEATM received four comments.

None of the comments specifically addressed LLNA skin sensitization potency.

4.2.3 Public Comments in Response to 73 FR 1360 (January 8, 2008): Announcement of an Independent Scientific Peer Review Panel Meeting on the Murine Local Lymph Node Assay; Availability of Draft Background Review Documents; Request for Comments

NICEATM requested public comments on the January 2008 draft BRDs, draft ICCVAM test recommendations, draft test method protocols, and revised draft LLNA performance standards for an independent international scientific peer review panel meeting held March 4-6, 2008, to evaluate modifications and new applications for the LLNA. NICEATM received 23 comments in response to this FR notice; seven written comments were received in advance of the meeting, and 16 oral comments were offered at the Panel meeting.

Public Comments, Written

Five written comments were relevant to LLNA skin sensitization potency. One commenter submitted two separate comments.

Comment:

The commenter acknowledged the considerable detail and information that was involved in this evaluation but indicated that human data on skin sensitization thresholds has been given undue status as an accurate gold standard and delineated a number of issues that are problematic for the human no effect and lowest effect threshold data.

ICCVAM Response:

Uncertainties with the human data are acknowledged (e.g., variable human protocols and results) and discussed in the BRD (**Appendix C, Section 4.0**). An analysis of variability for human and LLNA skin sensitizers indicated that the CV range for DSA_{05} values (2%-408%) was similar to that for LLNA EC3 values (2%-349%) (see **Appendix C, Section 7.0**). Despite the limitations of the human data, a positive and statistically significant correlation between LLNA and human data exists. A linear regression analysis of geometric mean human DSA_{05} on the geometric mean LLNA EC3 yielded $R^2 = 0.448$ with a statistically significant slope ($p < 0.0001$) (see **Appendix C, Section 6.1.1**). As suggested by a Panel member, the analysis used the DSA_{05} for the human threshold as a better comparison to the EC3 value because it represents an LOEL corrected to 5% incidence of an induction response. As improvements are made in standardizing the predictive human tests, the results can be considered with more certainty.

Comment:

A second commenter indicated that the approach by ICCVAM to validate the LLNA for the prediction of strong and weak skin sensitizers poses a methodological challenge. The commenter noted that it is possible that available HMT and HRIPT data may lead to a false human skin sensitization potency categorization because it is often difficult to correctly interpret the total dose used in the human tests due to insufficient documentation of total area dosed or prior patient exposure. The commenter further indicated that the criteria used to select the LLNA data used in the analyses should also be more thoroughly discussed (e.g., LLNA protocols and solvents, geometric

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mean versus most conservative mean for substances with multiple studies, representation of substances in the LLNA database).

ICCVAM Response:
As noted above, uncertainties with the human data are acknowledged and discussed in the BRD (**Appendix C, Section 4.0**), and an analysis of variability for human and LLNA skin sensitizers indicated that the CV ranges for human and LLNA threshold values are similar (**Appendix C, Section 4.0**). Detail has been added on the calculations for the dose per unit area used in the human predictive tests, and the possibility of misclassification has been discussed. Such uncertainties did not prevent a statistically significant relationship of human DSA_{05} with the LLNA EC3 values ($R^2 = 0.448$ for the geometric mean linear regression; $p < 0.0001$ for slope) (see **Appendix C, Section 6.1.1**). The BRD also discusses numerous analyses performed by NICEATM to determine the optimal LLNA criteria for the current validation database. The geometric mean regression yielded $R^2 = 0.448$, and the most potent regression yielded $R^2 = 0.382$. The inclusion of LLNA results from different vehicles or from nonstandard protocols did not impact the relationship of the EC3 with the DSA_{05} values (see **Appendix C, Annex IV**).

Comment:
A third commenter observed the difficulties in comparing LLNA EC3 values to human data and to guinea pig data. The commenter also criticized the proposed classification categories for skin sensitization in the January 2008 draft BRD that use guinea pig tests for potency classification.

ICCVAM Response:
With regard to comparisons between LLNA EC3 values and human data, uncertainties with the human data are acknowledged (e.g., variable human protocols and results) and discussed in the BRD (**Appendix C, Section 4.0**). Such uncertainties did not prevent a statistically significant relationship of human DSA_{05} with the LLNA EC3 values ($R^2 = 0.448$ for the geometric mean linear regression; $p < 0.0001$ for slope) (see **Appendix C, Section 6.1.1**).

With regard to comparisons between LLNA EC3 values and guinea pig data, it is acknowledged in the BRD that the guinea pig tests are designed for hazard identification and are not well suited for potency estimations. However, the third revised edition of the GHS also includes criteria for sensitizer subcategories 1A ("strong" skin sensitizers) and 1B ("other" skin sensitizers) based on results from guinea pig tests (UN 2009). Since the January 2008 draft BRD, the analyses evaluating the accuracy of the LLNA to predict skin sensitization potency in guinea pigs have been removed from the evaluation. A comparison of the accuracy of the guinea pig outcomes to correctly classify human skin sensitization potency with the accuracy of the LLNA to correctly classify human skin sensitization potency has been retained (see **Appendix C, Section 6.2**). ICCVAM will continue to assess performance of new test methods against both the currently accepted test, as well as against existing human data and/or experience.

The proposed classification categories in the January 2008 draft BRD referred to by the commenter were finalized in the third revised edition of the GHS, which was recently adopted and published (UN 2009).

<u>Public Comments, Oral</u>
Two oral comments related to LLNA skin sensitization potency.

Comment:
One commenter stated that it might be difficult to split potency data into pooled and unpooled groups. This is because the majority of available data likely comes from pooled groups, and conclusions that individual animal data must be used were derived from analyses based primarily on pooled data from four animals.

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The commenter expressed concern about human threshold data being considered as the gold standard for the comparative analyses. However, the commenter considers the analyses adequate for recommending the LLNA as a part of a weight-of-evidence decision on human skin sensitization potency categorizations.

ICCVAM Response:
The BRD discusses numerous analyses performed by NICEATM to determine the representative LLNA EC3 value for the substances in the validation database (see **Appendix C, Annex IV**). Analyses separating LLNA data, based on the collection of either individual animal or pooled data, have not been performed and could be considered as part of ICCVAM's continuing efforts to assess test method performance.

Uncertainties with the human data are acknowledged (e.g., variable human protocols and results) and discussed in the BRD (see **Appendix C, Section 4.0**), but these uncertainties did not prevent a statistically significant relationship of human DSA_{05} with the LLNA EC3 values ($R^2 = 0.448$ for the geometric mean linear regression; $p < 0.0001$ for slope) (see **Appendix C, Section 6.1.1**).

Comment:
Another commenter noted that there has been much discussion about various ways of handling the potency data. The OECD expert task force on skin sensitization needs to see an analytical comparison of what is considered the most appropriate approach for evaluating the data. The question for categorization purposes is: what is the ideal testing modality for separating strong versus weak sensitizers for potency categorization? A regulator who must assign a categorization is going to be confronted with all available test data and must know which data should be given the greatest weight in their evaluation.

The commenter indicated that the OECD task force also reviewed the January 2008 draft BRD on potency determinations and sent a list of several questions to the Panel. One of the questions is whether the LLNA protocols can be refined (e.g., by selection of solvents or choice of other test parameters) to improve correlation. The commenter concluded by expressing hope that the additional analyses that the Panel has suggested will bring some clarity to the matter.

ICCVAM Response:
NICEATM considered numerous comments from the public and the Panel in finalizing the analyses related to LLNA skin sensitization potency. The BRD includes numerous analyses performed by NICEATM to determine the optimal LLNA criteria for the current validation database (see **Appendix C, Annex IV**). For the substances in the validation database, LLNA vehicle did not have a significant impact on the relationship of the LLNA EC3 value to the human DSA_{05} values. Additionally, the inclusion of LLNA results (20% [132/653] of the LLNA tests used) from nonstandard LLNA protocols that used different mouse strains, both sexes of mice, different dosing schedules, different durations between the last topical application and the injection of radioactive marker, and pretreatment with sodium lauryl sulfate did not have a significant impact on the relationship of the LLNA EC3 values to the human DSA_{05} values.

4.2.4 Public Comments in Response to 73 FR 25754 (May 7, 2008): Meeting of the Scientific Advisory Committee on Alternative Toxicological Methods (SACATM)

NICEATM announced the SACATM meeting and requested written and public oral comment on the agenda topics.

One public comment was received in response to this *Federal Register* notice, and it did not specifically address LLNA skin sensitization potency.

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4.2.5 Public Comments in Response to 73 FR 29136 (May 20, 2008): Peer Review Panel Report on the Validation Status of New Versions and Applications of the Murine Local Lymph Node Assay (LLNA): A Test Method for Assessing the Allergic Contact Dermatitis Potential of Chemicals and Products: Notice of Availability and Request for Public Comments

NICEATM requested written public comments on the *Peer Review Panel Report*.

No public comments were received in response to this *Federal Register* notice.

4.2.6 Public and SACATM Comments: SACATM Meeting on June 18-19, 2008

The June 18-19, 2008, SACATM meeting included a discussion of the ICCVAM review of the LLNA test method (see **Appendix F3**).

No public comments related specifically to the topic of LLNA skin sensitization potency were offered.

Regarding LLNA skin sensitization potency, one SACATM member noted that the use of the LLNA for potency determinations was unclear and asked if this was for a validation study.

ICCVAM Response:
In 2007, the CPSC expressed concern to ICCVAM that the LLNA was being proposed internationally for use in potency determinations for the purpose of classification even though the LLNA had not undergone formal validation for this purpose. Thus, CPSC requested that NICEATM-ICCVAM assess the validation status of the LLNA as a stand-alone assay for potency determinations (including severity) for classification purposes.

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5.0 References

Basketter DA, Cadby P. 2004. Reproducible prediction of contact allergenic potency using the local lymph node assay. Contact Dermatitis 50(1): 15-17.

Basketter DA, Gerberick F, Kimber I. 2007. The local lymph node assay and the assessment of relative potency: status of validation. Contact Dermatitis 57(2): 70-75.

Buehler EV. 1965. Delayed contact hypersensitivity in the guinea pig. Arch Dermatol 91: 171-177.

EPA. 2003. Health Effects Test Guidelines: OPPTS 870.2600 - Skin Sensitization. EPA 712-C-03-197. Washington, DC:U.S. Environmental Protection Agency. Available: http://www.epa.gov/opptsfrs/publications/OPPTS_Harmonized/870_Health_Effects_Test_Guidelines/Revised/870r-2600.pdf [accessed 17 September 2009].

ESAC. 2007. ESAC Statement on the Reduced Local Lymph Node Assay. Ispra, Italy:European Commission Directorate General, Joint Research Centre, Institute for Health and Consumer Protection, European Centre for the Validation of Alternative Methods. Available: http://ecvam.jrc.it/ft_doc/ESAC26_statement_rLLNA_20070525-1.pdf [accessed 6 July 2010].

Fawcett T. 2006. An introduction to ROC analysis. Pattern Recognition Letters 27(8): 861-874.

Gerberick GF, Ryan CA, Kern PS, Dearman RJ, Kimber I, Patlewicz GY, et al. 2004. A chemical dataset for evaluation of alternative approaches to skin-sensitization testing. Contact Dermatitis 50(5): 274-288.

Hutchings CV, Shum KW, Gawkrodger DJ. 2001. Occupational contact dermatitis has an appreciable impact on quality of life. Contact Dermatitis 45(1): 17-20.

ICCVAM. 1999. The Murine Local Lymph Node Assay: A Test Method for Assessing the Allergic Contact Dermatitis Potential of Chemicals/Compounds. The Results of an Independent Peer Review Evaluation Coordinated by the Interagency Coordinating Committee on the Validation of Alternative Methods and the National Toxicology Program (NTP) Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM). NIH Publication No. 99-4494. Research Triangle Park, NC:National Institute of Environmental Health Sciences. Available: http://iccvam.niehs.nih.gov/docs/immunotox_docs/llna/llnarep.pdf [accessed 21 October 2010].

ICCVAM. 2001. ICCVAM Immunotoxicology Working Group Recommended Protocol for the Murine Local Lymph Node Assay (LLNA): Testing of Chemicals for Contact Sensitizing (Allergic Contact Dermatitis [ACD]) Potential. Research Triangle Park, NC:National Institute of Environmental Health Sciences. Available: http://iccvam.niehs.nih.gov/docs/immunotox_docs/llna/LLNAProt.pdf [accessed 29 November 2010].

ICCVAM. 2009. ICCVAM Test Method Evaluation Report. The Reduced Murine Local Lymph Node Assay: An Alternative Test Method Using Fewer Animals to Assess the Allergic Contact Dermatitis Potential of Chemicals and Products. NIH Publication No. 09-6439. Research Triangle Park, NC:National Institute of Environmental Health Sciences. Available: http://iccvam.niehs.nih.gov/docs/immunotox_docs/LLNA-LD/TMER.pdf [accessed 21 October 2010].

ICCVAM. 2010a. ICCVAM Test Method Evaluation Report on the Murine Local Lymph Node Assay: BrdU-ELISA. A Nonradioactive Alternative Test Method to Assess the Allergic Contact Dermatitis Potential of Chemicals and Products. NIH Publication No. 10-7552. Research Triangle Park, NC:National Institute of Environmental Health Sciences. Available: http://iccvam.niehs.nih.gov/methods/immunotox/llna-ELISA/TMER.htm [accessed 21 October 2010].

ICCVAM. 2010b. ICCVAM Test Method Evaluation Report on the Murine Local Lymph Node Assay: DA. A Nonradioactive Alternative Test Method to Assess the Allergic Contact Dermatitis

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Potential of Chemicals and Products. NIH Publication No. 10-7551. Research Triangle Park, NC:National Institute of Environmental Health Sciences. Available: http://iccvam.niehs.nih.gov/methods/immunotox/llna-DA/TMER.htm [accessed 21 October 2010].

Kimber I, Basketter DA, Berthold K, Butler M, Garrigue JL, Lea L, et al. 2001. Skin sensitization testing in potency and risk assessment. Toxicol Sci 59(2): 198-208.

Kimber I, Dearman RJ, Betts CJ, Gerberick GF, Ryan CA, Kern PS, et al. 2006. The local lymph node assay and skin sensitization: a cut-down screen to reduce animal requirements? Contact Dermatitis 54(4): 181-185.

Kligman AM. 1966. The identification of contact allergens by human assay. 3. The maximization test: a procedure for screening and rating contact sensitizers. J Invest Dermatol 47(5): 393-409.

Kligman AM, Epstein W. 1975. Updating the maximization test for identifying contact allergens. Contact Dermatitis 1(4): 231-239.

Magnusson B, Kligman AM. 1970. Allergic Contact Dermatitis in the Guinea Pig. Springfield, IL:Charles C. Thomas.

Marzulli FN, Maibach HI. 1974. The use of graded concentrations in studying skin sensitizers: experimental contact sensitization in man. Food Cosmet Toxicol 12(2): 219-227.

OECD. 1992. Test No. 406. Skin Sensitisation [adopted 17 July 1992]. In: OECD Guidelines for the Testing of Chemicals, Section 4: Health Effects. Paris:OECD Publishing. Available: http://dx.doi.org/10.1787/9789264070660-en [accessed 30 July 2010].

OECD. 2002. Test No. 429. Skin Sensitisation: Local Lymph Node Assay [adopted 24 April 2002]. In: OECD Guidelines for the Testing of Chemicals, Section 4: Health Effects. Paris:OECD Publishing. Available: http://dx.doi.org/10.1787/9789264071100-en [accessed 30 July 2010].

OECD. 2010a. Test No. 429. Skin Sensitization: Local Lymph Node Assay [adopted 22 July 2010]. In: OECD Guidelines for the Testing of Chemicals, Section 4: Health Effects. Paris:OECD Publishing. Available: http://dx.doi.org/10.1787/9789264071100-en [accessed 6 August 2010].

OECD. 2010b. Test No. 442A. Skin Sensitization: Local Lymph Node Assay: DA [adopted 22 July 2010]. In: OECD Guidelines for the Testing of Chemicals, Section 4: Health Effects. Paris:OECD Publishing. Available: http://dx.doi.org/10.1787/9789264090972-en [accessed 6 August 2010].

OECD. 2010c. Test No. 442B. Skin Sensitization: Local Lymph Node Assay: BrdU-ELISA [adopted 22 July 2010]. In: OECD Guidelines for the Testing of Chemicals, Section 4: Health Effects. Paris:OECD Publishing. Available: http://dx.doi.org/10.1787/9789264090996-en [accessed 6 August 2010].

Politano VT, Api AM. 2008. The Research Institute for Fragrance Materials' human repeated insult patch test protocol. Regul Toxicol Pharmacol 52(1): 35-38.

Skoet R, Zachariae R, Agner T. 2003. Contact dermatitis and quality of life: a structured review of the literature. Br J Dermatol 149(3): 452-456.

Steel RGD, Torrie JH. 1980. Principles and Procedures of Statistics A Biometrical Approach. New York:McGraw Hill.

UN. 2009. Globally Harmonized System for Classification and Labelling of Chemicals. 3rd rev. ed. New York:United Nations. Available: http://www.unece.org/trans/danger/publi/ghs/ghs_rev03/03files_e.html [accessed 21 October 2010].

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Appendix A

Timeline for ICCVAM Evaluation on Usefulness and Limitations of the Murine Local Lymph Node Assay for Potency Categorization of Chemicals Causing Allergic Contact Dermatitis in Humans

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ICCVAM Evaluation Timeline

January 10, 2007	The Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) receives a nomination from the Consumer Product Safety Commission (CPSC) to review the validation status of new versions and applications of the murine local lymph node assay (LLNA), including use of the LLNA for determining skin sensitization potency categories.[1]
January 2007	The ICCVAM interagency Immunotoxicity Working Group (IWG) is re-established to work with the National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM) to carry out the LLNA evaluations.
January 24, 2007	ICCVAM endorses the CPSC-nominated LLNA review activities, including an evaluation on use of the LLNA for determining skin sensitization potency categories.
May 17, 2007	*Federal Register* notice (72 FR 27815) – The Murine Local Lymph Node Assay: Request for Comments, Nominations of Scientific Experts, and Submission of Data
June 12, 2007	The Scientific Advisory Committee on Alternative Toxicological Methods (SACATM) endorses with high priority the CPSC-nominated LLNA review activities, including an evaluation on use of the LLNA for determining skin sensitization potency categories.
January 8, 2008	*Federal Register* notice (73 FR 1360) – Announcement of an Independent Scientific Peer Review Panel Meeting on the Murine Local Lymph Node Assay; Availability of Draft Background Review Documents; Request for Comments
March 4–6, 2008	Independent Scientific Peer Review Panel holds a public meeting with opportunity for oral public comments at CPSC headquarters in Bethesda, MD, to discuss LLNA review activities, including an evaluation on use of the LLNA for determining skin sensitization potency categories. The Panel is charged with reviewing the current status of the LLNA for potency use and commenting on the extent to which the information in the draft LLNA background review document supports the draft ICCVAM recommendations.[2]
March 10–11, 2008	Organisation for Economic Co-operation and Development (OECD) Expert Meeting on Sensitization: Discussion of Globally Harmonized System of Classification and Labelling of Chemicals (GHS) hazard categories for skin sensitizers

[1] http://iccvam.niehs.nih.gov/methods/immunotox/llnadocs/CPSC_LLNA_nom.pdf
[2] http://iccvam.niehs.nih.gov/methods/immunotox/llna_PeerPanel08.htm

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May 20, 2008	*Federal Register* notice (73 FR 29136) – Peer Review Panel Report on the Validation Status of New Versions and Applications of the Murine Local Lymph Node Assay (LLNA): A Test Method for Assessing the Allergic Contact Dermatitis Potential of Chemicals and Products: Notice of Availability and Request for Public Comments
June 18–19, 2008	SACATM meeting: SACATM and public comments on the 2008 Panel report
July 2009	Publication of third revised edition of the GHS, which includes revised criteria for hazard classification and subcategories for skin sensitizers
July 2009 – August 2010	NICEATM performs additional analyses to evaluate the use of the LLNA for skin sensitization potency determinations based on comments from the independent scientific peer review panel, the public, and SACATM.
October 27, 2010	ICCVAM endorses the LLNA potency evaluation report, which includes ICCVAM's recommendations and the final background review document on the validity of the LLNA for determining skin sensitization potency categories.
Winter 2011 (published within two weeks after transmittal)	*Federal Register* notice announces availability of the ICCVAM evaluation report on the usefulness and limitations of the LLNA for potency categorization of chemicals causing allergic contact dermatitis in humans.

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Appendix B

Updated ICCVAM-Recommended Protocol: The Murine Local Lymph Node Assay: A Test Method for Assessing the Allergic Contact Dermatitis Potential of Chemicals and Products

Annex I
An Approach to Identification and Dissection of the Draining (Auricular) Lymph Nodes B-15

Annex II
An Example of How to Reduce the Number of Animals in the Concurrent Positive Control Group of the Murine Local Lymph Node Assay B-18

Annex III
Evaluating Local Irritation and Systemic Toxicity in the Murine Local Lymph Node Assay B-20

Annex IV
Procedures for Calculating the Estimated Concentration of a Substance Expected to Produce a Stimulation Index of 3 (EC3) in the Murine Local Lymph Node Assay B-22

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Preface

The murine local lymph node assay (LLNA) is a test method developed to assess whether a chemical may induce allergic contact dermatitis (ACD) in humans. In 1998, the LLNA was submitted to the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) for evaluation as a stand-alone alternative to the guinea pig sensitization tests accepted by U.S. regulatory agencies. In 1999, based on a comprehensive evaluation of the LLNA by an independent scientific peer review panel (Panel),[1] ICCVAM concluded that the LLNA is an acceptable alternative to the guinea pig test methods to assess the ACD hazard potential of most types of substances (Dean et al. 2001; ICCVAM 1999). The Panel also concluded that the LLNA offers animal welfare advantages compared to the traditional guinea pig test methods, in that it provides for animal use refinement (i.e., elimination of distress and pain) and reduces the total number of animals required.

An ICCVAM interagency Immunotoxicity Working Group (IWG) reviewed the 1999 Panel report (ICCVAM 1999) and developed recommendations applicable to the regulatory use of the LLNA. The interagency IWG then worked with the National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM) to produce a recommended test method protocol (ICCVAM 2001)[2] that would accurately reflect the ICCVAM and Panel recommendations. This current version of the ICCVAM-recommended test method protocol incorporates updates made in 2009, as described below, and an update made in 2010 to incorporate procedures for calculating the estimated concentration of a substance expected to produce a stimulation index of 3 (EC3), which is relevant to estimates of relative skin sensitization potency.

In March 2008 and April 2009, ICCVAM and NICEATM organized Panels to evaluate new versions and applications of the LLNA. The Panels provided conclusions and recommendations in their reports, many of which were applicable to the traditional LLNA test method protocol.[3] ICCVAM subsequently considered the Panels' conclusions and recommendations, as well as comments from the Scientific Advisory Committee on Alternative Toxicological Methods (SACATM) and the public, and updated the 2001 ICCVAM-recommended LLNA test method protocol (ICCVAM 2001). The updated LLNA test method protocol reduces animal numbers by 20% for each test by decreasing the minimum number of animals per dose group from five to four. A reduced LLNA procedure, which reduces animal numbers by 40% by using only one high-dose group for each test is also described. Further, improved guidance on dose selection and other procedures to improve assay accuracy and reproducibility are provided.

This updated ICCVAM-recommended LLNA test method protocol is based on evaluation of current experience and scientific data and is provided to Federal agencies for their consideration as a standardized test method protocol for generation of data for regulatory purposes. Prior to conducting an LLNA test to meet a regulatory requirement, the appropriate regulatory agency should be contacted for their current guidance on the conduct and interpretation of this assay. Users should be aware that the proposed test method protocol could be revised based on any additional optimization and/or validation studies that are conducted in the future. ICCVAM recommends that test method users consult the NICEATM-ICCVAM website (http://iccvam.niehs.nih.gov) to ensure use of the most current test method protocol. Additional information on the ICCVAM LLNA review process and deliberations of the Panel can also be found at the NICEATM-ICCVAM website (http://iccvam.niehs.nih.gov) or in the Panel reports (ICCVAM 2008, 2009b).

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[1] http://iccvam.niehs.nih.gov/docs/immunotox_docs/llna/llnarep.pdf
[2] http://iccvam.niehs.nih.gov/docs/immunotox_docs/llna/LLNAProt.pdf
[3] http://iccvam.niehs.nih.gov/docs/immunotox_docs/LLNAPRPRept2008.pdf
http://iccvam.niehs.nih.gov/docs/immunotox_docs/LLNAPRPRept2009.pdf

ED_006472_00084346-00053

We want to express our sincere appreciation to the ICCVAM interagency IWG for their careful deliberations and efforts in updating the LLNA test method protocol, and especially appreciate the efforts of the Working Group Co-chairs, Abigail Jacobs, Ph.D., from the U.S. Food and Drug Administration and Joanna Matheson, Ph.D., from the U.S. Consumer Product Safety Commission. We also want to acknowledge the outstanding support provided by NICEATM and the Integrated Laboratory Systems, Inc., support staff. Lastly, we appreciate the efforts of the Panel members for their diligent review, and the comments provided by SACATM and numerous stakeholders, including the public.

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Director, NICEATM
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1.0 General Principle of Detection of Skin Sensitization Using the Murine Local Lymph Node Assay

The basic principle underlying the murine local lymph node assay (LLNA) is that sensitizers induce proliferation of lymphocytes in the lymph nodes draining the site of test substance application. 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 cell proliferation as a function of *in vivo* radioisotope incorporation into the DNA of dividing lymphocytes. The LLNA assesses this proliferation in the draining lymph nodes proximal to the application site (see **Annex I**). This effect occurs as a dose response in which the proliferation in test groups is compared to that in the concurrent vehicle-treated control group. A concurrent positive control is added to each assay to provide an indication of appropriate assay performance.

2.0 Description of the Murine Local Lymph Node Assay

2.1 Sex and Strain of Animals

Young adult female mice (nulliparous and nonpregnant) of the CBA/Ca or CBA/J strain are recommended.[4] Females are used because most data in the existing database were generated using mice of this gender. At the start of the study, mice should be 8 to 12 weeks of age. All mice should be age matched (preferably within a one-week time frame). Weight variations between the mice should not exceed 20% of the mean weight.

2.2 Preparation of Animals

The temperature of the experimental animal room should be 22°C (±3°C) and the relative humidity 30% to 70% (although the aim is for 50%-60%). Lighting should be artificial, the sequence 12 hours light, 12 hours dark. Mice should be provided with an unlimited supply of standard laboratory mouse diets and drinking water. The mice should be quarantined/acclimatized for at least five days before the test (ILAR 1996). Mice should be allocated to small groups by a stratified randomization or other appropriate method before the start of the study unless adequate scientific rationale for housing mice individually is provided (ILAR 1996). Four animals per cage is the recommended housing arrangement. The mice should be uniquely identified before being placed in the study. The mice should not be identified via the ear (e.g., marking, clipping, or punching). Colored marks on the tail or other appropriate methods should be used. All mice should be examined (e.g., clinical signs, body weights, observation of excrement) before the start of the test to ensure good health and the absence of skin lesions.

2.3 Preparation of Doses

Solid test substances should be dissolved or suspended in appropriate solvents/vehicles and diluted, if appropriate, before the mice are dosed. Liquid test substances may be dosed directly (i.e., applied neat) or diluted before dosing. Insoluble substances, such as those generally seen in medical devices, should be subjected to an exaggerated extraction in an appropriate solvent to reveal all extractable constituents for testing before dosing. Fresh preparations of the test substance should be prepared daily unless stability over the test period is demonstrated.

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[4] Male mice or other strains of mice may be used if it is sufficiently demonstrated that these animals perform as well as female CBA mice in the LLNA.

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2.4 Test Conditions

2.4.1 Solvent/Vehicle

The selected solvent/vehicle should not interfere with or bias the test result and should be selected to maximize the test concentrations while producing a solution/suspension suitable for application of the test substance. Recommended solvents/vehicles are acetone: olive oil (4:1 by volume), *N,N*-dimethylformamide, methyl ethyl ketone, propylene glycol, and dimethyl sulfoxide. Others may be used (Kimber and Basketter 1992) if sufficient scientific rationale is provided. 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. Appropriate solubilizers (e.g., 1% Pluronic L92) should be incorporated, and wholly aqueous vehicles may need to be avoided. It may be necessary for regulatory purposes to test the substance in the clinically relevant solvent or product formulation.

2.4.2 Controls

Concurrent negative (solvent/vehicle) and positive controls should be included in each test to ensure that the test system is functioning properly and that the specific test is valid. In some circumstances (e.g., when using a solvent/vehicle not recommended in **Section 2.4.1**), it may be useful to include a naïve control. Aside from being treated with a solvent/vehicle, the mice in the negative control groups should be handled in an identical manner to the mice of the treatment groups.

Positive controls are used to demonstrate the appropriate performance of the assay by responding with adequate and reproducible sensitivity to a sensitizing substance for which the magnitude of the response is well characterized. Inclusion of a concurrent positive control is recommended because it demonstrates competency of the laboratory to successfully conduct each assay and allows for an assessment of intra- and interlaboratory reproducibility and comparability. The positive control should produce a positive LLNA response (i.e., a stimulation index [SI] ≥ 3). The positive control dose should be chosen such that the induction is reproducible but does not cause excessive skin irritation or systemic toxicity (i.e., SI > 20). Preferred positive control substances are 25% hexyl cinnamic aldehyde (HCA; Chemical Abstracts Service Registry Number [CASRN] 101-86-0) in acetone: olive oil (4:1 by volume) or 5% mercaptobenzothiazole (CASRN 149-30-4) in *N,N*-dimethylformamide. There may be circumstances where, given adequate justification, other positive control substances may be used.

Although the positive control substance should be tested in the same vehicle as the test substance, there may be certain regulatory situations where it is necessary to test the positive control substance in both a standard and a nonstandard vehicle (e.g., a clinically/chemically relevant formulation) to test for possible interactions. If the concurrent positive control substance is tested in a different vehicle than the test substance, then a separate vehicle control for the concurrent positive control should be included.

While a concurrent positive control group is recommended, there may be situations in which periodic testing (i.e., at intervals $\leq$6 months) of the positive control substance may be adequate for laboratories that conduct the LLNA regularly (i.e., at least once per month) and that have an established history and a documented proficiency for obtaining reproducible and accurate results with positive controls. Adequate proficiency with the LLNA can be successfully demonstrated by generating consistent positive results with the positive control in at least 10 independent tests conducted in less than one year.

A concurrent positive control group should be included whenever there is a procedural change to the LLNA (i.e., change in trained personnel, change in test method materials and/or reagents, change in test method equipment, change in source of test animals, etc.). Such changes should be documented in laboratory reports. Consideration should be given to the impact of these changes on the adequacy of

ED_006472_00084346-00056

the previously established historical database. It may be necessary to establish a new historical database to document consistency in the positive control results.

Investigators should be aware that the decision to include a positive control only periodically, instead of concurrently, will affect the adequacy and acceptability of negative study results generated without a concurrent positive control during the interval between each periodic positive control study. For example, if a false negative result is obtained in the periodic positive control study, all negative test substance results obtained in the interval between the last acceptable periodic positive control study and the unacceptable periodic positive control study may be questioned. Implications of these outcomes should be carefully considered when determining whether to include concurrent or periodic positive controls.

Consideration should also be given to using fewer animals in the concurrent positive control group when this is scientifically justified and if the laboratory demonstrates, based on laboratory-specific historical data,[5] that fewer mice can be used without substantially increasing the frequency with which studies will need to be repeated due to positive control failure. An example of how to reduce the number of mice in the concurrent positive control group is provided in **Annex II**.

Benchmark substances help demonstrate that the test method is functioning properly for detecting the skin sensitization potential of substances of a specific chemical class or a specific range of responses. Appropriate benchmark substances should have the following properties:

- Structural and functional similarity to the class of the substance being tested
- Known physicochemical characteristics
- Supporting data from the LLNA
- Supporting data on known effects in animal models and/or in humans

2.5 Methodology

A minimum of four animals per dose group is recommended, with at least three concentrations of the test substance, a concurrent negative control group treated only with the vehicle for the test substance, and a concurrent positive control. The processing of lymph nodes from individual mice allows for the identification of any individual animal responses that are outliers, in accordance with statistical tests such as Dixon's test (Dixon and Massey 1983). This will help to avoid false negative results for weaker sensitizers. That is, if an outlier is not identified and excluded, substances that normally would induce an SI just above 3 might be incorrectly classified as negative due to a low outlier value because the resulting mean SI may be less than 3. Individual animal measurements allow assessment of interanimal variability and a statistical comparison of the difference between test substance and vehicle control group measurements. Finally, reducing the number of mice in the positive control group is feasible only when individual animal data are collected.

Test substance treatment dose levels should be based on the recommendations given in Kimber and Basketter (1992) and in the ICCVAM Panel report (ICCVAM 1999). At least three consecutive doses are normally selected from an appropriate concentration series such as 100%, 50%, 25%, 10%, 5%, 2.5%, 1%, 0.5%, etc. Adequate scientific rationale should accompany the selection of the concentration series used. The maximum concentration tested should be the highest achievable level that does not cause excessive local irritation and/or overt systemic toxicity (**Annex III**). Efforts should be made to identify existing toxicological information (e.g., acute toxicity and dermal irritation) and structural and physicochemical information on the test material of interest (and/or structurally related test materials) that may aid in selecting the appropriate maximum test substance dose level. In the absence of such information, an initial prescreen test may be necessary (**Annex III**).

[5] A robust historical dataset should include at least 10 independent tests, conducted within a reasonable period of time (i.e., less than one year), with a minimum of four mice per negative and positive control groups.

ED_006472_00084346-00057

The LLNA experimental procedure is performed as follows:

Day 1. Identify and record the weight of each mouse and any clinical observations. Apply 25 μL/ear of the appropriate dilution of the test substance, the positive control, or the solvent/vehicle only, to the dorsum of both ears of each mouse.

Days 2 and 3. Repeat the application procedure as carried out on Day 1.

Days 4 and 5. No treatment.

Day 6. Record the weight of each mouse. Inject 250 μL of sterile phosphate-buffered saline (PBS) containing 20 μCi of tritiated (^{3}H)-methyl thymidine or 250 μL PBS containing 2 μCi of ^{125}I-iododeoxyuridine (^{125}IU) and 10^{-5} M fluorodeoxyuridine into each mouse via the tail vein (Kimber et al. 1995; Loveless et al. 1996). Five hours later, humanely kill each mouse and collect the draining (auricular) lymph nodes of both ears and place in PBS (one container per mouse). Both bilateral draining lymph nodes must be collected (see diagram and description of dissection in **Annex I**). To further monitor the local skin response, additional parameters such as scoring of ear erythema or ear thickness measurements (obtained either by using a thickness gauge, or ear punch weight determinations at necropsy) may be included in the study protocol.

Prepare a single-cell suspension of lymph node cells (LNC) excised bilaterally for each individual mouse. Prepare the single-cell suspension in PBS by either gentle mechanical separation through a 200-mesh stainless-steel gauze or another acceptable technique for generating a single-cell suspension. Wash LNC twice with an excess of PBS, and precipitate the DNA with 5% trichloroacetic acid (TCA) at 4°C for approximately 18 hours.

For the ^{3}H-methyl thymidine method, resuspend pellets in 1 mL TCA and transfer to 10 mL of scintillation fluid. Incorporation of ^{3}H-methyl thymidine is measured by β-scintillation counting as disintegrations per minute (dpm) for each mouse and expressed as dpm/mouse.

For the ^{125}IU method, transfer the 1 mL TCA pellet directly into gamma-counting tubes. Incorporation of ^{125}IU is determined by gamma counting and also expressed as dpm/mouse.

2.6 Reduced LLNA

This test method protocol includes a reduced LLNA (rLLNA) procedure that requires only the high-dose group. This reduction from three dose groups to one is the only difference between the multidose LLNA and the rLLNA (ESAC 2007; ICCVAM 2009a; Kimber et al. 2006). Like the multidose LLNA, the test substance concentration evaluated in the rLLNA should be the maximum concentration that does not induce overt systemic toxicity and/or excessive local skin irritation in the mouse. The rLLNA test method should be routinely used for the hazard identification of skin-sensitizing substances, except when a test article is expected to produce a positive result and dose-response information is needed. Because the rLLNA further decreases animal use by 40%, use of the multidose LLNA should be accompanied by appropriate scientific rationale.

2.7 Observations

Mice should be carefully observed at least once daily for any clinical signs, either of local irritation at the application site or of systemic toxicity (**Annex III**). Weighing mice before treatment and at the time of necropsy will help to assess systemic toxicity. Systematically record all observations and maintain records for each individual mouse. Animal monitoring plans should include criteria to promptly identify for euthanasia those mice exhibiting systemic toxicity, excessive irritation, or corrosion of skin (OECD 2000).

ED_006472_00084346-00058

3.0 Calculation of Results

Results for each treatment group are expressed as the mean SI. Each SI is the ratio of the mean dpm/mouse within each test-substance treatment group or the concurrent positive-control-treated group against the mean dpm/mouse for the solvent/vehicle-treated control group. The average SI for the solvent/vehicle-treated control group is then 1. The decision process regards a result as positive when SI $\geq$ 3.

The estimated concentration of a substance expected to produce an SI of 3 (i.e., the EC3) can also be calculated for positive LLNA results as an indicator of relative skin sensitization potency. The method for determining the EC3 is a simple linear interpolation of the points in the dose-response curve that lie immediately above and below SI = 3.

$$EC3 = c + \left[\frac{(3-d)}{(b-d)}\right] \times (a-c)$$

Coordinates:

(a = dose concentration immediately above SI = 3, b = SI immediately above 3)

(c = dose concentration immediately below SI = 3, d = SI immediately below 3)

When there are no data points that fall below SI = 3, a more complex log-linear extrapolation may be applied as described in Ryan et al. (2007) in which the two lowest test concentrations from the dose-response curve are used, provided the lowest SI value approaches the value of 3 and that a linear dose-response exists.

$$EC3_{ex} = 2^{\wedge}\left\{\log_2(c) + \frac{(3-d)}{(b-d)} \times \left[\log_2(a) - \log_2(c)\right]\right\}$$

Coordinates:

(a = dose concentration for next to lowest SI above 3, b = next to lowest SI above 3)

(c = dose concentration for lowest SI above 3, d = lowest SI above 3)

An example of how to calculate the EC3 using both the linear interpolation and the log-linear extrapolation approaches is provided in **Annex IV**.

In addition to an assessment of the magnitude of the SI, a statistical analysis for presence and degree of dose response may be conducted, which is possible only with the use of individual animals. Any statistical assessment should include an assessment of the dose-response relationship as well as suitably adjusted comparisons of test groups (e.g., pair-wise dosed group versus concurrent solvent/vehicle control comparisons). Statistical analyses may include, for instance, linear regression, Williams' test to assess dose-response trends, or Dunnett's test for pairwise comparisons. 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. In any case, the investigator may need to carry out SI calculations and statistical analyses with and without certain data points (outliers).

4.0 Evaluation and Interpretation of Results

In general, when the SI for any single treatment dose group is $\geq$3, the test substance is regarded as a skin sensitizer (Basketter et al. 1996; ICCVAM 1999; Kimber et al. 1994), and a test substance that does not meet this criterion is considered a nonsensitizer in the LLNA. However, the magnitude of the observed SI should not be the sole factor used to determine the biological significance of a skin sensitization response. Additional factors that could be considered include the outcomes of statistical analyses, the strength of the dose-response relationship, chemical toxicity, and solubility. For

ED_006472_00084346-00059

instance, a quantitative assessment may be performed by statistical analysis of individual mouse data and may provide a more complete evaluation of the test substance's ability to act as a sensitizer (see **Section 3.0**). Equivocal results (e.g., the SI does not reach 3, but it is near 3 and there is a positive dose-response relationship) should be clarified by performing statistical analyses and by considering structural relationship to known skin sensitizers, available toxicity information (including observations of excessive local skin irritation and/or systemic toxicity in the LLNA study), and consistency of the positive control and solvent/vehicle control responses.

Employing the optimized assay condition described previously, the mean SI value for the positive control group (25% HCA or 5% mercaptobenzothiazole) should be ≥3. If not, data derived from the experiment should not be considered for evaluation.

5.0 Data and Reporting

5.1 Data

Data should be summarized in tables showing the individual animal dpm values, the group mean dpm/mouse, the group's associated error term (e.g., standard deviation [SD], standard error of the mean [SEM]), and the mean SI value for each treated group, positive control group, and solvent/vehicle control group.

5.2 Test Report

The test report should contain the following information:

Test Substances and Control Substances:

- Identification data (e.g., CASRN, if available; source; purity; known impurities; lot number)
- Physical nature and physicochemical properties (e.g., volatility, stability, solubility, physicochemical properties relevant to the conduct of the study)
- Composition and relative percentages of components, if formulation

Solvent/Vehicle:

- Identification data (e.g., CASRN, if available; purity; concentration, where appropriate; volume used)
- Justification for choice of solvent/vehicle

Test Animals:

- Strain of mice used
- Source of mice, housing conditions, diet, etc.
- Number, age, and sex of mice
- Microbiological status of the mice, when known

Test Conditions:

- Details of test substance preparation and application
- Justification for dose selection (including results from prescreen test, if conducted)
- Vehicle and test substance concentrations used, and total amount of test substance applied
- Details of food and water quality (including diet type/source, water source)
- Detailed description of treatment and sampling schedules
- Methods for measurement of toxicity
- Criteria for considering studies as positive, negative, or equivocal

ED_006472_00084346-00060

- Details of any protocol deviations and an explanation of how the deviation affects the study design and results

Reliability Check:

- Summary of results of latest reliability check, including information on substance, concentration, and vehicle used
- Concurrent and/or historical positive and negative (solvent/vehicle) control data for testing laboratory
- Date and laboratory report for the most recent periodic positive control and a report detailing the historical positive control data for the laboratory justifying the basis for not conducting a concurrent positive control, if a concurrent positive control was not included

Results:

- Individual weights of mice at start of dosing and at scheduled kill, as well as mean and associated error term (e.g., SD, SEM) for each treatment group
- Time course of onset and signs of toxicity, including dermal irritation at site of administration, if any, for each animal
- Table of individual mouse dpm values and SI values for each treatment group
- Mean and associated error term (e.g., SD, SEM) for dpm/mouse for each treatment group and the results of outlier analysis for each treatment group
- Calculated SI and an appropriate measure of variability that takes into account the interanimal variability in both the test substance and control groups
- Calculated EC3 value (for positive LLNA results)
- Dose-response relationship
- Statistical analyses, where appropriate, and method applied

Discussion of the Results and Conclusion:

- A brief commentary on the results, the dose-response analysis, and the statistical analyses, where appropriate, with a conclusion as to whether the test substance should be considered a skin sensitizer

Quality Assurance Statement for GLP-Compliant Studies:

- This statement should indicate all inspections made during the study and the dates any results were reported to the Study Director. This statement should also confirm that the final report reflects the raw data.

6.0 References

Basketter DA, Gerberick GF, Kimber I, Loveless SE. 1996. The local lymph node assay: a viable alternative to currently accepted skin sensitization tests. Food Chem Toxicol 34(10): 985-997.

Basketter DA, Lea LJ, Dickens A, Briggs D, Pate I, Dearman RJ, et al. 1999. A comparison of statistical approaches to the derivation of EC3 values from local lymph node assay dose responses. J Appl Toxicol 19(4): 261-266.

Dean JH, Twerdok LE, Tice RR, Sailstad DM, Hattan DG, Stokes WS. 2001. ICCVAM evaluation of the murine local lymph node assay: II. Conclusions and recommendations of an independent scientific peer review panel. Reg Toxicol Pharmacol 34(3): 258-273.

Dixon WJ, Massey FJ. 1983. Introduction to Statistical Analysis. 4th ed. New York, NY:McGraw Hill.

ED_006472_00084346-00061

Ehling G, Hecht M, Heusener A, Huesler J, Gamer AO, Van Loveren H, et al. 2005. An European inter-laboratory validation of alternative endpoints of the murine local lymph node assay: 2nd round. Toxicology 212(1): 69-79.

EPA. 1998. Health Effects Test Guidelines: OPPTS 870.1200 - Acute Dermal Toxicity. EPA 712–C–98–192. Washington, DC:U.S. Environmental Protection Agency. Available: http://www.epa.gov/ocspp/pubs/frs/publications/Test_Guidelines/series870.htm [accessed 29 November 2010].

ESAC. 2007. ESAC Statement on the Reduced Local Lymph Node Assay. Ispra, Italy:European Commission Directorate General, Joint Research Centre, Institute for Health and Consumer Protection, European Centre for the Validation of Alternative Methods. Available: http://ecvam.jrc.it/ft_doc/ESAC26_statement_rLLNA_20070525-1.pdf [accessed 6 July 2010].

Hayes BB, Gerber PC, Griffey SS, Meade BJ. 1998. Contact hypersensitivity to dicyclohexylcarbodiimide and diisopropylcarbodiimide in female B6C3F1 mice. Drug Chem Toxicol 21(2): 195-206.

Hayes BB, Meade BJ. 1999. Contact sensitivity to selected acrylate compounds in B6C3F1 mice: Relative potency, cross reactivity, and comparison of test methods. Drug Chem Toxicol 22(3): 491-506.

Homey B, Von Schilling C, Blumel J, Schuppe HC, Ruzicka T, Ahr HJ, et al. 1998. An integrated model for the differentiation of chemical-induced allergic and irritant skin reactions. Toxicol Appl Pharmacol 153(1): 83-94.

ICCVAM. 1999. The Murine Local Lymph Node Assay: A Test Method for Assessing the Allergic Contact Dermatitis Potential of Chemicals/Compounds. The Results of an Independent Peer Review Evaluation Coordinated by the Interagency Coordinating Committee on the Validation of Alternative Methods and the National Toxicology Program (NTP) Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM). NIH Publication No. 99-4494. Research Triangle Park, NC:National Institute of Environmental Health Sciences. Available: http://iccvam.niehs.nih.gov/docs/immunotox_docs/llna/llnarep.pdf [accessed 21 October 2010].

ICCVAM. 2001. ICCVAM Immunotoxicology Working Group Recommended Protocol for the Murine Local Lymph Node Assay (LLNA): Testing of Chemicals for Contact Sensitizing (Allergic Contact Dermatitis [ACD]) Potential. Research Triangle Park, NC:National Institute of Environmental Health Sciences. Available: http://iccvam.niehs.nih.gov/docs/immunotox_docs/llna/LLNAProt.pdf [accessed 29 November 2010].

ICCVAM. 2008. Independent Scientific Peer Review Panel Report. Validation Status of New Versions and Applications of the Murine Local Lymph Node Assay: A Test Method for Assessing the Allergic Contact Dermatitis Potential of Chemicals and Products. Research Triangle Park, NC:National Institute of Environmental Health Sciences. Available: http://iccvam.niehs.nih.gov/docs/immunotox_docs/LLNAPRPRept2008.pdf [accessed 29 November 2010].

ICCVAM. 2009a. ICCVAM Test Method Evaluation Report. The Reduced Murine Local Lymph Node Assay: An Alternative Test Method Using Fewer Animals to Assess the Allergic Contact Dermatitis Potential of Chemicals and Products. NIH Publication No. 09-6439. Research Triangle Park, NC:National Institute of Environmental Health Sciences. Available: http://iccvam.niehs.nih.gov/docs/immunotox_docs/LLNA-LD/TMER.pdf [accessed 21 October 2010].

ICCVAM. 2009b. Independent Scientific Peer Review Panel Report: Updated Validation Status of New Versions and Applications of the Murine Local Lymph Node Assay: A Test Method for

ED_006472_00084346-00062

Assessing the Allergic Contact Dermatitis Potential of Chemicals and Products. Research Triangle Park, NC:National Institute of Environmental Health Sciences. Available: http://iccvam.niehs.nih.gov/docs/immunotox_docs/LLNAPRPRept2009.pdf [accessed 15 December 2010].

ICCVAM. 2009c. Report on the ICCVAM-NICEATM/ECVAM/JaCVAM Scientific Workshop on Acute Chemical Safety Testing: Advancing *In Vitro* Approaches and Humane Endpoints for Systemic Toxicity Evaluations. Research Triangle Park, NC:National Institute of Environmental Health Sciences. Available: http://iccvam.niehs.nih.gov/methods/acutetox/toxwksp-rpt.htm [accessed 8 July 2010].

ICCVAM. 2009d. Revised Draft Background Review Document: Non-radioactive Murine Local Lymph Node Assay: Flow Cytometry Test Method Protocol (LLNA: BrdU-FC). Research Triangle Park, NC:National Institute of Environmental Health Sciences. Available: iccvam.niehs.nih.gov/methods/immunotox/fcLLNA/recs.pdf [accessed 24 November 2010].

ILAR. 1996. Guide for the Care and Use of Animals. 7th ed. Washington, DC:National Academies Press.

Kimber I, Basketter DA. 1992. The murine local lymph node assay: a commentary on collaborative studies and new directions. Food Chem Toxicol 30(2): 165-169.

Kimber I, Dearman RJ, Betts CJ, Gerberick GF, Ryan CA, Kern PS, et al. 2006. The local lymph node assay and skin sensitization: a cut-down screen to reduce animal requirements? Contact Dermatitis 54(4): 181-185.

Kimber I, Dearman RJ, Scholes EW, Basketter DA. 1994. The local lymph node assay: developments and applications. Toxicology 93(1): 13-31.

Kimber I, Hilton J, Dearman RJ, Gerberick GF, Ryan CA, Basketter DA, et al. 1995. An international evaluation of the murine local lymph node assay and comparison of modified procedures. Toxicology 103(1): 63-73.

Loveless SE, Ladics GS, Gerberick GF, Ryan CA, Basketter DA, Scholes EW, et al. 1996. Further evaluation of the local lymph node assay in the final phase of an international collaborative trial. Toxicology 108(1-2): 141-152.

OECD. 2000. Guidance Document No. 19: Recognition, Assessment, and Use of Clinical Signs as Humane Endpoints for Experimental Animals Used in Safety Evaluation. Paris:OECD Publishing. Available: http://www.oecd.org/officialdocuments/displaydocumentpdf/?cote=env/jm/mono(2000)7&doclanguage=en [accessed 29 November 2010].

Patterson RM, Noga E, Germolec D. 2007. Lack of evidence for contact sensitization by Pfiesteria extract. Environ Health Perspect 115(7): 1023-1028.

Reeder MK, Broomhead YM, DiDonato L, DeGeorge GL. 2007. Use of an enhanced local lymph node assay to correctly classify irritants and false positive substances. Society of Toxicology Annual Meeting. Charlotte, NC.

Ryan CA, Chaney JG, Gerberick GF, Kern PS, Dearman RJ, Kimber I, et al. 2007. Extrapolating local lymph node assay EC3 values to estimate relative sensitizing potency. Cutan Ocul Toxicol 26(2): 135-145.

Tilney NL. 1971. Patterns of lymphatic drainage in the adult laboratory rat. J Anat 109: 369-383.

Vohr H, Jürgen AH. 2005. The local lymph node assay being too sensitive? Arch Toxicol 79(12): 721-728.

ED_006472_00084346-00063

Woolhiser MR, Hayes BB, Meade BJ. 1998. A combined murine local lymph node and irritancy assay to predict sensitization and irritancy potential of chemicals. Toxicol Meth 8(4): 245-256.

ED_006472_00084346-00064

Annex I:
An Approach to Identification and Dissection of the Draining (Auricular) Lymph Nodes

1.0 Background

Although minimal technical training of the murine local lymph node assay (LLNA) is required, extreme care must be taken to ensure appropriate and consistent dissection of the lymph nodes. It is recommended that technical proficiency in the identification and dissection of the lymph nodes draining the ear be achieved by practice on mice that have been (a) injected with a colored agent (dye) and/or (b) sensitized with a strong positive sensitizer. Brief descriptions of these practice dissections are provided below. Recognizing that nodes from vehicle-treated and naïve mice are smaller, laboratories performing the LLNA must also gain proficiency in the dissection of these nodes. It may be helpful for laboratories inexperienced in this procedure to request guidance from laboratories that have successfully performed the LLNA.

2.0 Training and Preparation for Node Identification

2.1 Identification of the Draining Node – Dye Treatment

Several methods can be used to provide color identification of the draining nodes. These techniques may be helpful for initial identification and should be performed to ensure proper isolation of the appropriate node. Examples of such treatments are listed below. It should be noted that other such protocols might be used effectively.

2.1.1 Evan's Blue Dye Treatment:

Inject approximately 0.1 mL of 2% Evan's Blue Dye (prepared in sterile saline) intradermally into the pinnae of an ear. Euthanize the mouse after several minutes and continue with the dissection as noted below.

2.1.2 Colloidal Carbon and Other Dye Treatments:

Colloidal carbon and India ink are examples of other dye treatments that may be used (Tilney 1971).

2.2 Identification of the Draining Node – Application of Strong Sensitizers

For node identification and training, a strong sensitizer is recommended. This agent should be applied in the standard acetone: olive oil vehicle (4:1 by volume). Suggested sensitizers for this training exercise include 0.1% oxazolone, 0.1% (weight per volume) 2,4-dinitrochlorobenzene, and 0.1% (by volume) dinitrofluorobenzene. After treating the ear with a strong sensitizer, the draining node will dramatically increase in size, thus aiding in identification and location of the node.

Using a procedure similar to that described in the test method protocol, apply the agent to the dorsum of both ears (25 μL/ear) for three consecutive days. On the fourth day, euthanize the mouse. Identification and dissection (described below) of the node should be performed in these sensitized animals before practice in nonsensitized or vehicle-treated mice, where the node is significantly smaller.

Please note: Due to the exacerbated response, the suggested sensitizers are not recommended as controls for assay performance. They should be used only for node identification and training.

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3.0 Dissection Approach

3.1 Lateral Dissection (Figure B-1)

Although lateral dissection is not the conventional approach used to obtain the nodes draining the ear, it may be helpful as a training procedure when used in combination with ventral dissection. Perform this procedure on both sides of the mouse. After euthanizing the mouse, place it in a lateral position. Wet the face and neck with 70% ethanol. Use scissors and forceps to make an initial cut from the neck area slightly below the ear. Carefully extend the incision toward the mouth and nose. Angle the tip of the scissors slightly upward during this procedure to prevent the damage of deeper tissue. Gently retract the glandular tissue in the area using the forceps. Using the masseter muscle, facial nerves, blood vessels, and the bifurcation of the jugular vein as landmarks, isolate and remove the draining node (**Figure B-1**). The draining auricular node will be positioned adjacent to the masseter muscle and proximal to and slightly above the jugular bifurcation.

3.2 Ventral Dissection (Figure B-2)

The most commonly used dissection approach is from the ventral surface of the mouse. This approach allows both right and left draining nodes to be obtained without repositioning the mouse. With the mouse ventrally exposed, wet the neck and abdomen with 70% ethanol. Use scissors and forceps to carefully make the first incision across the chest and between the arms. Make a second incision up the midline perpendicular to the initial cut, and then cut up to the chin area. Reflect the skin to expose the external jugular veins in the neck area. Take care to avoid salivary tissue at the midline and nodes associated with this tissue. The nodes draining the ear (auricular) are located distal to the masseter muscle, away from the midline, and near the bifurcation of the jugular veins.

4.0 Accuracy in Identification

The nodes can be distinguished from glandular and connective tissue in the area by the uniformity of the nodal surface and a shiny translucent appearance. Application of sensitizing agents (especially the strong sensitizers used in training) will cause enlargement of the node. If a dye is injected for training purposes, the node will take on the tint of the dye.

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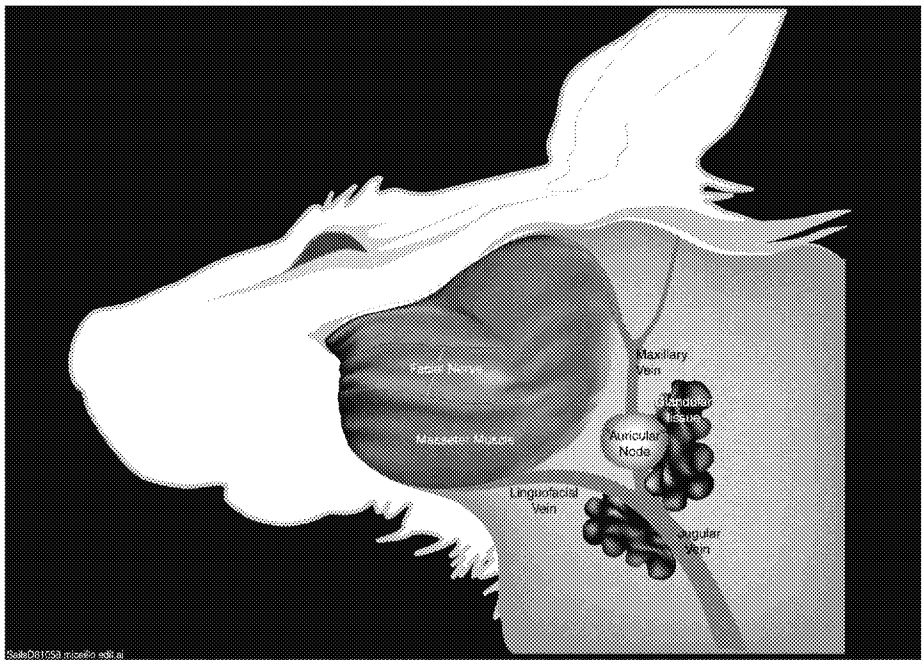
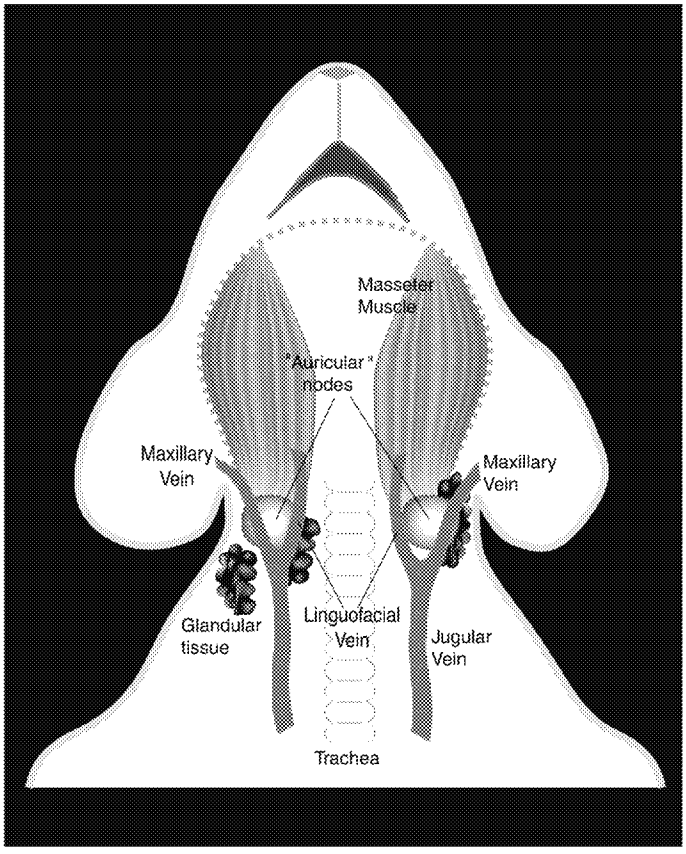
Figure B-1 Lateral Dissection



Credit: Dee Sailstad, U.S. EPA

Figure B-2 Ventral Dissection



Credit: Dee Sailstad, U.S. EPA

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Annex II:
An Example of How to Reduce the Number of Animals in the Concurrent Positive Control Group of the Murine Local Lymph Node Assay

As stated in the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) murine local lymph node assay (LLNA) test method protocol (**Section 2.4.2**), a concurrent positive control is recommended to ensure the appropriate performance of the assay. Appropriate performance is demonstrated when the test method responds with adequate and reproducible sensitivity to a sensitizing substance for which the magnitude of the response is well characterized. The number of mice in the concurrent positive control group may be reduced if the laboratory demonstrates, based on laboratory-specific historical data, that fewer mice can be used without compromising the integrity of the study (i.e., positive control results should always be positive compared to the vehicle control results). As illustrated in the example and accompanying explanation below, reducing the number of animals in the positive control group is feasible only when individual animal data are collected.

The stimulation index (SI) results for each positive control test can be used to generate mean SI values for every possible combination of SI values for as few as two animals. The mean SI values for every combination of numbers for each group size can then be used to calculate the failure rate of the positive control for each group size (i.e., the percentage of the combinations for which the mean SI < 3). **Table B-1** provides an example of positive control results from four tests in one laboratory of 30% hexyl cinnamic aldehyde (HCA) using six CBA/J mice per group. In these tests, with six animals, HCA produced "borderline" positive results (i.e., the mean SI values were marginally greater than 3). To determine whether the number of animals can be reduced, sample size reductions (i.e., N = 5, 4, 3, or 2) can be evaluated by taking all possible samples from the six values for each test given in **Table B-1**, which can occur in the following ways: N = 2 (15 samples), N = 3 (20 samples), N = 4 (15 samples), and N = 5 (6 samples).

Table B-1 Example of SI Results from Four Murine Local Lymph Node Assay Positive Control Studies with 30% HCA

Test	1	2	3	4
Animal 1	2.13	3.56	4.68	0.78
Animal 2	4.55	1.54	4.44	9.16
Animal 3	3.64	3.00	5.41	6.66
Animal 4	1.98	3.87	3.32	3.02
Animal 5	3.09	3.79	2.89	2.32
Animal 6	3.77	3.96	1.81	2.91
Mean SI	**3.19**	**3.29**	**3.76**	**4.14**

Abbreviations: HCA = hexyl cinnamic aldehyde; SI = stimulation index.

The failure rate of the positive control was then calculated using the SI results for each group of two, three, four, or five values to determine the likelihood of obtaining a mean SI < 3. The results for these four "borderline" HCA tests were then added to the results from an additional 12 robust positive control tests included in this laboratory's historical database to determine the overall likelihood of obtaining a mean SI < 3 for the positive control substance (**Table B-2**). The failure rate reflects the frequency with which a positive control test will fail, which would result in retesting the positive

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control and any concurrent test substances. Each laboratory is encouraged to determine the lowest number of animals to use in the positive control group based on the highest failure rate considered acceptable by the laboratory.

Table B-2 Example of Positive Control Failure Rate for 30% HCA Based on Data Collected in Single Laboratory

Number of Animals	HCA Test 1	HCA Test 2	HCA Test 3	HCA Test 4	Results from Other Tests[1]	Overall Likelihood of a Mean SI < 3
5	17% (1/6)	0% (0/6)	0% (0/6)	0% (0/6)	0% (0/72)	1% (1/96)
4	27% (4/15)	13% (2/15)	0% (0/15)	7% (1/15)	0% (0/180)	3% (7/240)
3	40% (8/20)	30% (6/20)	5% (1/20)	20% (4/20)	0% (0/240)	6% (19/320)
2	47% (7/15)	33% (5/15)	13% (2/15)	40% (6/15)	1% (1/180)	9% (21/240)

Abbreviations: HCA = hexyl cinnamic aldehyde; SI = stimulation index.

[1] These represent 12 positive control studies in the same laboratory where all mice in the positive control groups treated with 30% HCA produced an SI $\geq$ 3.

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Annex III: Evaluating Local Irritation and Systemic Toxicity in the Murine Local Lymph Node Assay

As noted in the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) murine local lymph node assay (LLNA) test method protocol, the maximum dose tested should be the maximum possible concentration that does not produce systemic toxicity and/or excessive local skin irritation after topical application in the mouse. In the absence of information to determine this concentration (e.g., acute toxicity and dermal irritation data, and/or structural and physicochemical information on the test material and/or structurally related test materials), a prescreen test should be performed using three dose levels of the test substance in order to define the appropriate dose to test in the LLNA. The maximum dose tested should be 100% of the test material for liquids or the maximum possible concentration for solids or suspensions.

The prescreen test is conducted under conditions identical to the main LLNA study, except there is no assessment of lymph node cell proliferation and fewer animals per dose group can be used. One or two animals per dose group are suggested. All mice are observed daily for any clinical signs of systemic toxicity or local irritation at the application site. For example, observations might occur before and after treatment on Days 1, 2, and 3. Body weights are recorded before testing and before termination (Day 6). Both ears of each mouse are observed for erythema and scored using **Table B-3**. Ear thickness measurements are taken using a thickness gauge (e.g., digital micrometer or Peacock Dial thickness gauge) on Day 1 (predose), Day 3 (approximately 48 hours after the first dose), and Day 6 (termination). Additionally, on Day 6, ear thickness can be determined by ear punch weight determinations, which should be performed after the animals are humanely killed.

Excessive local irritation is indicated by an erythema score ≥3 and/or an increase in ear thickness of ≥25% on any day of measurement (ICCVAM 2009d; Reeder et al. 2007). The highest dose selected for the main LLNA study will be the next lower dose in the prescreen concentration series that does not induce systemic toxicity and/or excessive local skin irritation.

Table B-3 Erythema Scores

Observation	Value
No erythema	0
Very slight erythema (barely perceptible)	1
Well-defined erythema	2
Moderate to severe erythema	3
Severe erythema (beet redness) to eschar (i.e., piece of dead tissue that is cast off from the surface of the skin) formation preventing grading of erythema	4

A statistically significant difference from control animals has also been used to delineate irritants from nonirritants in the LLNA (Ehling et al. 2005; Hayes et al. 1998; Hayes and Meade 1999; Homey et al. 1998; Patterson et al. 2007; Vohr and Jürgen 2005; Woolhiser et al. 1998). While these statistical differences often occur when the increase in ear thickness is less than 25%, they have not been associated specifically with excessive irritation (Ehling et al. 2005; Patterson et al. 2007; Vohr and Jürgen 2005; Woolhiser et al. 1998).

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Test guidelines for assessing acute systemic toxicity recommend a number of clinical observations for assessing systemic toxicity (EPA 1998). The following clinical observations, which are based on test guidelines and current practices (ICCVAM 2009c), may indicate systemic toxicity when used as part of an integrated assessment and, therefore, may indicate the maximum dose level to use in the main LLNA:

- Changes in nervous system function (e.g., piloerection, ataxia, tremors, and convulsions)
- Changes in behavior (e.g., aggressiveness, change in grooming activity, marked change in activity level)
- Changes in respiratory patterns (i.e., changes in frequency and intensity of breathing, such as dyspnea, gasping, and rales)
- Changes in food and water consumption
- Lethargy and/or unresponsiveness
- Any clinical signs of more than slight or momentary pain and distress
- Reduction in body weight >5% from Day 1 to Day 6
- Mortality

Moribund animals or animals obviously in pain or showing signs of severe and enduring distress should be humanely killed (OECD 2000).

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Annex IV: Procedures for Calculating the Estimated Concentration of a Substance Expected to Produce a Stimulation Index of 3 (EC3) in the Murine Local Lymph Node Assay

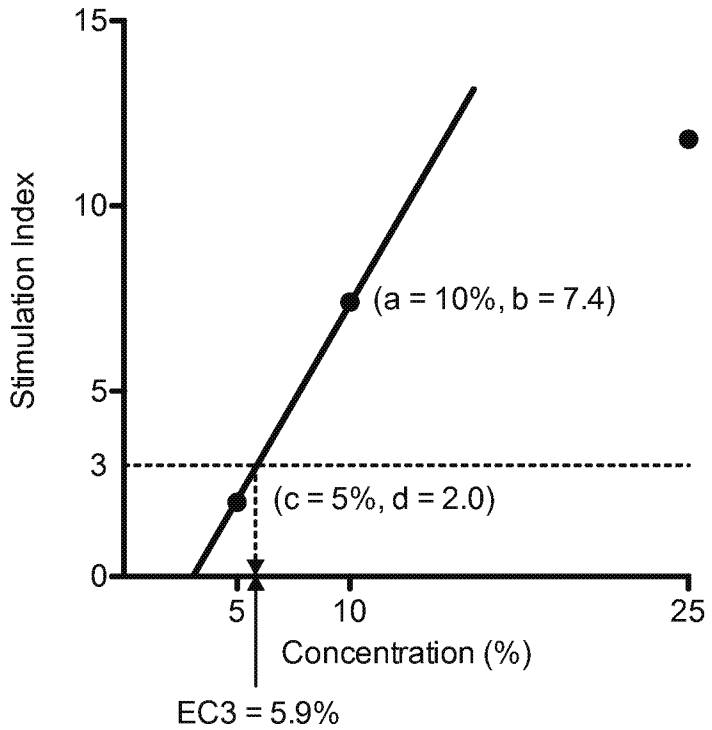
As mentioned in **Section 3.0** of the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) murine local lymph node assay (LLNA) test method protocol, the estimated concentration of a substance expected to produce a stimulation index (SI) of 3 (i.e., the EC3) is the metric for determining relative skin sensitization potency using the LLNA. The method for determining the EC3 is a simple linear interpolation of the points in the dose-response curve that lie immediately above and below SI = 3, the classification threshold for sensitizers in the LLNA. This method was chosen from an evaluation of a variety of statistical approaches to derive EC3 values from LLNA dose-response data (Basketter et al. 1999). An example of how to calculate the EC3 using linear interpolation is provided below using the LLNA data in **Table B-4**. **Figure B-3** illustrates this data.

Table B-4 LLNA Data Used for Calculating the EC3 by Linear Interpolation

Concentration	Mean SI
5%	2.0
10%	7.4
25%	11.8

Abbreviations: EC3 = estimated concentration of a substance expected to produce an SI of 3, the threshold value for a substance to be considered a sensitizer in the LLNA; LLNA = murine local lymph node assay; SI = stimulation index.

Figure B-3 Example of LLNA Data Above and Below Stimulation Index = 3



As shown in **Figure B-3**, the point in the dose-response curve lying immediately above SI = 3 corresponds to the concentration of 10% and the mean SI of 7.4. The point in the dose-response curve lying immediately below SI = 3 corresponds to the concentration of 5% and the mean SI of 2.0. Applying the following equation for linear interpolation to this data set results in EC3 = 5.9% as shown:

$$EC3 = c + \left[\frac{(3-d)}{(b-d)}\right] \times (a-c)$$

↓

Coordinates :

(a = 10% [dose concentration immediately above SI = 3], b = 7.4 [SI immediately above 3])

(c = 5% [dose concentration immediately below SI = 3], d = 2.0 [SI immediately below 3])

↓

$$EC3 = 5 + \left[\frac{(3-2)}{(7.4-2.0)}\right] \times (10-5) = 5.9\%$$

When there are no data points that fall below SI = 3, a more complex log-linear extrapolation may be applied as described in Ryan et al. (2007) in which the two lowest test concentrations from the dose-response curve are used, provided the lowest SI value approaches the value of 3 and that a linear dose-response exists. An example of how to calculate the EC3 using log-linear extrapolation is provided below using the LLNA data in **Table B-5** and **Figure B-4** provides a graphical illustration of this data.

Table B-5 LLNA Data Used for Calculating the EC3 by Log-linear Extrapolation

Concentration	Mean SI
2.5%	3.8
5%	5.8
10%	12.6

Note: The data used in this example was adapted from Ryan et al. (2007).

Abbreviations: EC3 = estimated concentration of a substance expected to produce an SI of 3, the threshold value for a substance to be considered a sensitizer in the LLNA; LLNA = murine local lymph node assay; SI = stimulation index.

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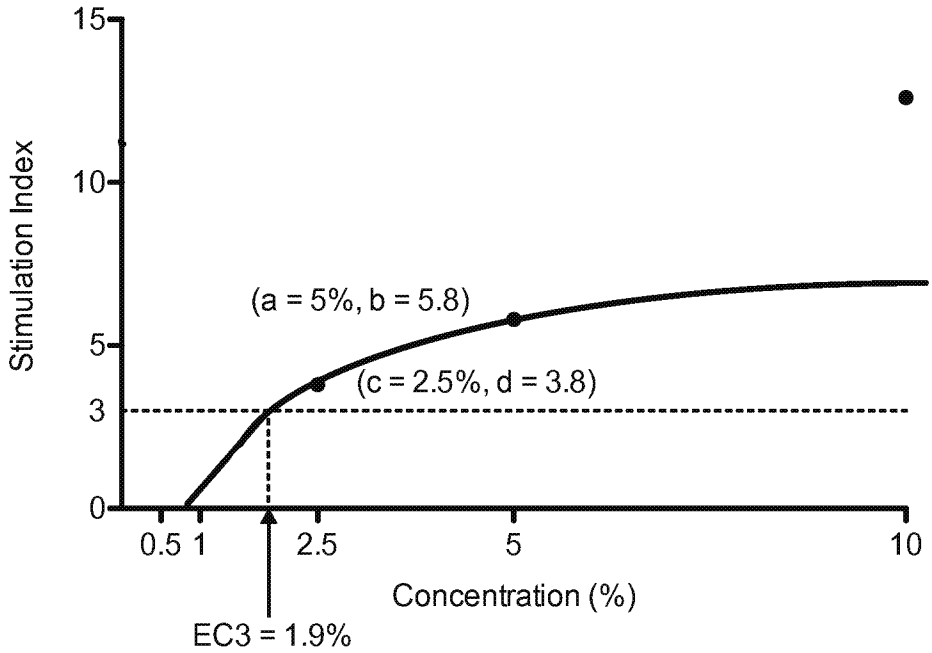
Figure B-4 Example of LLNA Data All Above Stimulation Index = 3



As shown in **Figure B-4**, all the points in the dose-response curve are above SI = 3. The two lowest SI values above 3 are 3.8 and 5.8 and their corresponding concentrations are 2.5% and 5%, respectively. Applying the following equation for log-linear extrapolation to this data set results in EC3 = 1.9% as shown:

$$EC3_{ex} = 2^{\wedge}\left\{\log_2(c) + \frac{(3-d)}{(b-d)} \times \left[\log_2(a) - \log_2(c)\right]\right\}$$

↓

Coordinates:

(a = 5% [dose concentration for next to lowest SI above 3], b = 5.8 [next to lowest SI above 3])

(c = 2.5% [dose concentration for lowest SI above 3], d = 3.8 [lowest SI above 3])

↓

$$EC3_{ex} = 2^{\wedge}\left\{\log_2(2.5) + \frac{(3-3.8)}{(5.8-3.8)} \times \left[\log_2(5) - \log_2(2.5)\right]\right\} = 1.9$$

Appendix C

Final Background Review Document:
Use of the Murine Local Lymph Node Assay for Potency Categorization of Chemicals Causing Allergic Contact Dermatitis in Humans

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Final Background Review Document: Use of the Murine Local Lymph Node Assay for Potency Categorization of Chemicals Causing Allergic Contact Dermatitis in Humans

Interagency Coordinating Committee on the Validation of Alternative Methods

National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods

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Table of Contents

List of Tables **C-8**

List of Figures **C-9**

List of Abbreviations and Acronyms **C-10**

Interagency Coordinating Committee on the Validation of Alternative Methods: Agency Representatives **C-12**

Acknowledgements **C-13**

Preface **C-16**

Executive Summary **C-18**

1.0 Introduction and Rationale for the Proposed Use of the Murine Local Lymph Node Assay for Potency Assessment **C-22**

1.1 Introduction C-22

1.1.1 Allergic Contact Dermatitis C-22

1.1.2 Historical Background C-22

1.1.3 Classification of Skin Sensitizers Based on Potency C-23

1.1.4 Use of the LLNA as a Stand-Alone Method for Potency Determinations C-26

1.2 Validation of the LLNA for Skin Sensitization Potential C-27

1.3 Selection of Citations for the BRD C-27

2.0 LLNA Test Method Protocol Components **C-28**

3.0 Substances Used for Validation of the LLNA for Potency Determinations **C-29**

4.0 Comparative *In Vivo* Reference Data **C-31**

4.1 Human Reference Data C-31

4.2 Guinea Pig Data C-32

4.3 Availability of Original Records for Human and Guinea Pig Data C-32

5.0 LLNA Data and Results **C-34**

5.1 Description of the LLNA Test Method Protocol Used to Generate Data C-34

5.2 Availability of Copies of Original LLNA Data Used to Evaluate Accuracy and Reliability C-34

5.3 Description of the Statistical Approach Used to Evaluate the Resulting Data C-34

5.4 Summary of Results C-34

5.5 Use of Coded Chemicals C-34

5.6 Lot-to-Lot Consistency of Test Substances C-35

5.7 Availability of Data for External Audit C-35

ED_006472_00084346-00079

6.0 Test Method Accuracy C-36

6.1 Usefulness of the LLNA in Predicting Skin Sensitization Potency in Humans C-36

6.1.1 Regression Analyses for LLNA EC3 versus Human Threshold Concentrations C-36

6.1.2 Correct, Underclassification, and Overclassification Rates for EC3 Value Predictions of Human Skin Sensitization Potency Categories C-40

6.1.3 Evaluation of Strong Sensitizers Underclassified by LLNA EC3 ≤ 2% C-48

6.2 Comparison of LLNA versus Guinea Pig Predictions of Human Skin Sensitization Potency C-49

7.0 Test Method Reliability C-54

7.1 Variability of LLNA EC3 Values Using the Same Vehicle C-54

7.2 Vehicle Effects on LLNA Results C-54

7.2.1 Published Studies Regarding Vehicle Effects on LLNA Results C-54

7.2.2 NICEATM Evaluation of Vehicle Effects on LLNA Results C-55

8.0 LLNA Data Quality C-64

8.1 Adherence to National and International GLP Guidelines C-64

8.2 Data Quality Audits C-64

8.3 Impact of Deviations from GLP Guidelines C-64

8.4 Availability of Laboratory Notebooks or Other Records C-64

9.0 Other Scientific Reports and Reviews C-65

9.1 Basketter, Gerberick, Kimber, and Colleagues C-65

9.1.1 Basketter et al. (2003) C-65

9.1.2 Kimber et al. (2003) C-65

9.1.3 Jowsey et al. (2006) C-66

9.1.4 Basketter et al. (2007a) C-66

9.1.5 Gerberick et al. (2007) C-66

9.1.6 Ryan et al. (2007) C-66

9.1.7 Loveless et al. (2010) C-67

9.2 McGarry (2007) C-68

9.3 Schlede et al. (2003) C-68

9.4 Zaghi and Maibach (2009) C-69

10.0 Animal Welfare Considerations C-70

10.1 Rationale for the Need to Use Animals C-70

10.2 Basis for Determining the Number of Animals Used C-70

10.3 Reduction Considerations C-70

11.0 Practical Considerations C-71

ED_006472_00084346-00080

12.0 References **C-72**

13.0 Glossary **C-78**

Annex I **LLNA/EC3 Validation – Submission from David Basketter, Frank Gerberick, and Ian Kimber** **C-83**

Annex II **Comparative LLNA, Guinea Pig, and Human Data Used in the Performance Evaluation** **C-121**

Annex III **Physicochemical Properties of Substances Evaluated** **C-263**

Annex IV **Analyses to Determine Representative EC3 Values** **C-307**

Annex V **Performance Characteristics for Use of LLNA EC3 Values to Predict Human Skin Sensitization Potency Categories** **C-321**

ED_006472_00084346-00081

List of Tables

Table C-1 GHS Classification Categories for Skin Sensitization C-25

Table C-2 Potency Categorization of Skin Sensitizers Based on LLNA EC3 Values C-26

Table C-3 Proposed Skin Sensitization Potency Categories Based on Guinea Pig Data C-26

Table C-4 Chemical Classes Represented in the LLNA Potency Database C-30

Table C-5 Distribution of 63 LLNA/Human Sensitizers by the Number of LLNA Studies Conducted and the Solvent Used C-37

Table C-6 Linear Regressions Obtained for LLNA EC3 Values versus Human Threshold Values C-39

Table C-7 Distribution of 136 Substances for Classsification Rate Analyses C-41

Table C-8 Concordance of LLNA and Human Data for Strong Sensitizer, Other Sensitizer, and Nonsensitizer Categories at Selected LLNA EC3 Values C-45

Table C-9 Correct, Underclassification, and Overclassification Rates for Prediction of Human Potency Categories by Selected LLNA EC3 Cutoff Values for 136 Substances C-46

Table C-10 Strong Human Sensitizers Underclassified by LLNA EC3 $\leq$ 2% C-49

Table C-11 Comparative Correct Classification, Underclassification, and Overclassification Rates When the GHS Criteria for Guinea Pig Tests and the LLNA EC3 Are Used to Determine Human Skin Sensitization Potency Category C-53

Table C-12 LLNA EC3 Values by Vehicle for 45 Substances with Positive Results (from the NICEATM LLNA Database) C-56

Table C-13 LLNA EC3 Values for 18 Skin Sensitizers Tested in Different Vehicles (from Jowsey et al. 2008a) C-58

Table C-14 LLNA EC3 Values for Seven Skin Sensitizers Tested in Different Vehicles (from McGarry 2007) C-60

ED_006472_00084346-00082

List of Figures

Figure C-1 Most Potent and Geometric Mean Regressions for LLNA EC3 Values versus Human DSA_{05} Values for 63 LLNA/Human Skin Sensitizers....C-38

Figure C-2 LLNA EC3 versus Human Results by GHS Potency Category for 136 Substances....C-42

Figure C-3 Classification Rates for the LLNA EC3 Prediction of Human Skin Sensitization Potency Categories for 63 Sensitizers....C-43

Figure C-4 Overall Correct, Underclassification, and Overclassification Rates for LLNA EC3 Prediction of Human Potency Category for 136 Substances....C-47

Figure C-5 Correct and Underclassification Rates for LLNA EC3 Prediction of 27 Strong Human Sensitizers....C-48

Figure C-6 Distribution of 28 Substances Classified as Sensitizers in Guinea Pig Tests, LLNA, and Human Tests for the Number of Guinea Pig Studies Conducted....C-50

Figure C-7 Representative Substances and Respective LLNA EC3 Values When Tested in Different Vehicles (from the NICEATM LLNA Database)....C-61

Figure C-8 Correlation of LLNA EC3 Values Between LLNA Tests with AOO and DMF or Acetone (from the NICEATM LLNA Database)....C-62

ED_006472_00084346-00083

List of Abbreviations and Acronyms

ACD	Allergic contact dermatitis
ACE	Acetone
AOO	Acetone: olive oil (4:1 by volume)
BRD	Background review document
BT	Buehler test
Conc.	Concentration tested
CPSC	U.S. Consumer Product Safety Commission
DEP	Diethyl phthalate
DMF	Dimethylformamide
DMSO	Dimethyl sulfoxide
2,4-DNCB	2,4-Dinitrochlorobenzene
dpm	Disintegrations per minute
DSA	Dose per skin area
DSA_{05}	Induction dose per skin area, in $\mu g/cm^2$, in a human repeat-insult patch test or human maximization test that produces a positive response in 5% of the tested population
EC3	Estimated concentration of a substance expected to produce a stimulation index of 3, the threshold value for a substance to be considered a sensitizer in the LLNA
ECETOC	European Centre for Ecotoxicology and Toxicology
ECPA	European Crop Protection Association
ECVAM	European Centre for the Validation of Alternative Methods
EPA	U.S. Environmental Protection Agency
EtOH	Ethanol
FDA	U.S. Food and Drug Administration
FHSA	Federal Hazardous Substances Act
FR	*Federal Register*
GCP	Good Clinical Practices
GHS	Globally Harmonized System of Classification and Labelling of Chemicals
GLP	Good Laboratory Practice
GPMT	Guinea pig maximization test
HCA	Hexyl cinnamic aldehyde
HMT	Human maximization test
HRIPT	Human repeat-insult patch test
ICCVAM	Interagency Coordinating Committee on the Validation of Alternative Methods
ICH	International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use
IPCS	International Programme on Chemical Safety
IWG	Immunotoxicity Working Group
ISO	International Organization for Standardization

ED_006472_00084346-00084

JaCVAM	Japanese Center for the Validation of Alternative Methods
K_{ow}	Estimated log octanol-water partition coefficient
LLNA	Murine local lymph node assay
LOEL	Lowest observed effect level
MEK	Methyl ethyl ketone
n	Number
NA	Not available
NC	Not calculated
NICEATM	National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods
NIEHS	National Institute of Environmental Health Sciences
NOEL	No observed effect level
NTP	National Toxicology Program
OECD	Organisation for Economic Co-operation and Development
OPPTS	Office of Prevention, Pesticides and Toxic Substances
PG	Propylene glycol
r	Correlation coefficient
R^2	Coefficient of determination
REACH	Regulation on Registration, Authorisation and Restriction of Chemicals
SACATM	Scientific Advisory Committee on Alternative Toxicological Methods
SI	Stimulation index
SLS	Sodium lauryl sulfate
TG	Test Guideline
WHO	World Health Organization

ED_006472_00084346-00085

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Preface

Allergic contact dermatitis (ACD) is an adverse health effect that frequently develops in workers and consumers following repeated exposed to skin sensitizing chemicals and products. ACD results in lost workdays[3] and can significantly diminish quality of life (Hutchings et al. 2001; Skoet et al. 2003). To minimize the occurrence of ACD, regulatory authorities require testing to identify substances that may cause skin sensitization. Sensitizing substances must be labeled with a description of the potential hazard and the precautions necessary for workers and consumers to avoid development of ACD.

In 1999, the U.S. Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) recommended the murine (mouse) local lymph node assay (LLNA) as a valid test method to assess the skin sensitization potential of most types of substances (Dean et al. 2001; Haneke et al. 2001; ICCVAM 1999; Sailstad et al. 2001). ICCVAM concluded that the LLNA provides several advantages compared to guinea pig test methods, including elimination of potential pain and distress, use of fewer animals, less time required to perform, and availability of dose-response information. United States and international regulatory authorities subsequently accepted the LLNA as an alternative test method for ACD testing. It is now used around the world.

In 2007, the U.S. Consumer Product Safety Commission (CPSC) asked ICCVAM and the National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM) to evaluate the scientific validity of the LLNA as a stand-alone assay for potency determinations for classification purposes. CPSC based the nomination on their interest in assessing the usefulness and limitations of the LLNA for identifying chemicals and products likely to be strong human sensitizers. ICCVAM assigned the nomination a high priority and established the ICCVAM interagency Immunotoxicity Working Group (IWG). The interagency IWG and NICEATM reviewed the current literature and evaluated available data to assess the application of the LLNA for this purpose.

The interagency IWG and NICEATM prepared a comprehensive draft background review document (BRD) that provided information, data, and analyses supporting the validation status of the LLNA for potency determinations for classification purposes. ICCVAM prepared draft test method recommendations, which included the usefulness and limitations, test method protocol, and future studies relevant to this application of the LLNA. Both documents were provided to an independent international scientific peer review panel (Panel) for their consideration at a public meeting on March 4–6, 2008.

A report of the Panel meeting was subsequently published on the NICEATM-ICCVAM website.[4] The Panel and ICCVAM concluded that the LLNA should not be used as a stand-alone assay for categorizing skin sensitizers based on potency but that it can be used as part of a weight-of-evidence evaluation for this purpose. The Panel recommended that NICEATM perform additional analyses using alternative human reference values that might be more appropriate for evaluating the accuracy of the LLNA for correctly determining skin sensitization potency categories. NICEATM performed these analyses for the final BRD.

ICCVAM considered the conclusions and recommendations of the Panel, along with comments from the public and the Scientific Advisory Committee on Alternative Toxicological Methods, and then finalized the BRD and test method recommendations. These will be forwarded to Federal agencies for their consideration and acceptance decisions, where appropriate.

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[3] http://www.bls.gov/IIF

[4] http://iccvam.niehs.nih.gov/methods/immunotox/llna_PeerPanel08.htm

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Executive Summary

Background

Allergic contact dermatitis (ACD) is an adverse health effect that frequently develops in workers and consumers following repeated exposed to skin sensitizing chemicals and products. ACD results in lost workdays[5] and can significantly diminish quality of life (Hutchings et al. 2001; Skoet et al. 2003). To minimize the occurrence of ACD, regulatory authorities require testing to identify substances that may cause skin sensitization. Sensitizing substances must be labeled with a description of the potential hazard and the precautions necessary for workers and consumers to avoid development of ACD.

In 1999, the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) recommended to U.S. Federal agencies that the murine local lymph node assay (LLNA) is a valid substitute for currently accepted guinea pig test methods to assess the ACD hazard potential of many, but not all, types of substances (Dean et al. 2001; Haneke et al. 2001; ICCVAM 1999; Sailstad et al. 2001). The recommendation was based on a comprehensive evaluation that included assessment of the LLNA's validation status by an independent international scientific peer review panel (Panel). The Panel and ICCVAM recommendations (ICCVAM 1999) are available at the National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM)-ICCVAM website.[6] The LLNA was subsequently incorporated into national and international test guidelines for the assessment of skin sensitization: U.S. Environmental Protection Agency Health Effects Test Guidelines on Skin Sensitization (EPA 2003), Organisation for Economic Co-operation and Development Test Guideline 429 (OECD 2002), and International Organization for Standardization (ISO) 10993-10: Tests for Irritation and Delayed-type Hypersensitivity (ISO 2002).[7]

In 2007, the U.S. Consumer Product Safety Commission formally requested that ICCVAM and NICEATM evaluate several activities related to the LLNA.[8] One of the nominated activities was an assessment of the validation status of the LLNA as a stand-alone assay for potency determinations for regulatory classification purposes. The information described in this background review document (BRD) was compiled by ICCVAM and NICEATM in response to this nomination. The BRD provides a comprehensive review of data and information regarding the usefulness and limitations of the LLNA as a stand-alone assay for determining potency (including severity) for the purpose of hazard classification.

Test Method Protocol

The LLNA test method identifies potential skin sensitizers by quantifying lymphocyte proliferation in the draining auricular lymph nodes during the induction phase of skin sensitization. The magnitude of lymphocyte proliferation then correlates with the extent to which sensitization develops after topical exposure to the potential skin sensitizer. For the purposes of this analysis, relative potency in the LLNA is defined as the concentration of a fixed volume of a substance that is required for the induction phase of a skin sensitization reaction to occur. The more potent the substance the smaller the quantity needed.

The recently updated ICCVAM-recommended test method protocol for the LLNA describes the conduct of the assay in detail (**Appendix B**). A test substance-induced increase in lymphocyte proliferation in the draining lymph nodes of the ear, the site of application, is used to identify chemical sensitizers. Mice are injected with radiolabeled thymidine (or an analogue of thymidine),

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[5] http://www.bls.gov/IIF
[6] http://iccvam.niehs.nih.gov/docs/immunotox_docs/llna/llnarep.pdf
[7] The OECD and ISO test guidelines were updated in 2010 (ISO 2010; OECD 2010a).
[8] http://iccvam.niehs.nih.gov/methods/immunotox/llnadocs/CPSC_LLNA_nom.pdf

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which is incorporated into the DNA of proliferating cells. The stimulation index (SI), the ratio of incorporated radioactivity in the auricular lymph nodes of treated versus control mice, is used to assess the sensitizing potential of the test substance. An SI of 3 or greater is used to classify a test substance as a skin-sensitizing agent. In the LLNA, a volume of 25 μL of the test substance is applied to each ear, and the estimated concentration expected to produce an SI of 3 (i.e., the EC3) is used as the metric for predicting skin-sensitization potency.

Validation Database

The information summarized in this BRD is based on a review of data from the LLNA. Data were obtained from published reports and unpublished data submitted to NICEATM in response to a *Federal Register* notice (72 FR 27815).[9] The information includes LLNA, guinea pig, and human data derived from a database of over 600 substances, 196 of which have LLNA data with comparative guinea pig and/or human data. These 196 substances include 136 substances with comparative human data (76 sensitizers, 60 nonsensitizers), 116 substances with comparative guinea pig data (64 sensitizers, 52 nonsensitizers), and 56 substances with comparative human and guinea pig data (35 sensitizers, 21 nonsensitizers).

The reference database for this evaluation consisted of human clinical studies, the human maximization test (HMT) and the human repeat-insult patch test (HRIPT) and, for nonsensitizers, other published reports. In the HMT and the HRIPT, potency information is determined from the no observed effect level (NOEL), the lowest observed effect level (LOEL), or the induction dose per skin area (DSA) that produces a positive response in 5% of the tested population (DSA_{05}). The third revised edition of the Globally Harmonized System of Classification and Labelling of Chemicals (GHS) classifies skin sensitizers as Category 1 (UN 2009). Category 1 substances are further subcategorized into Subcategory 1A ("strong" skin sensitizers) or Subcategory 1B ("other" skin sensitizers) based on results from human and/or animal studies (i.e., the LLNA and guinea pig tests). According to the GHS, substances with positive responses in the HMT or HRIPT at induction thresholds $\leq$500 μg/cm^2 are classified as Subcategory 1A, and substances with positive responses at >500 μg/cm^2 are classified as Subcategory 1B.

The GHS also includes criteria to use the LLNA to subcategorize sensitizers based on the EC3 value. Substances that produce an EC3 $\leq$ 2% are classified as Subcategory 1A (strong sensitizers), and substances with EC3 > 2% are classified as Subcategory 1B (other sensitizers) (UN 2009). Nonsensitizers are not classified.

Usefulness of the LLNA in Predicting Skin Sensitization Potency in Humans

The extent to which the LLNA correctly classifies strong versus other than strong human skin sensitizers was evaluated using the database of 136 substances for which both LLNA and human data were available. First, linear regression analyses using LLNA EC3 versus DSA_{05} values were conducted to establish a positive correlation, and to determine the optimum comparison based on either the most potent LLNA EC3 and DSA_{05} or the geometric mean LLNA EC3 and DSA_{05} for substances with multiple test results. Based on the higher R^2 value (0.448 versus 0.382) achieved when geometric means of multiply tested substances were used, this approach was carried forward in the performance analyses.

The correct, under- and overclassification rates of the LLNA versus human data for these 136 substances were initially calculated using the GHS criteria: EC3 $\leq$ 2% to classify substances as strong sensitizers and EC3 > 2% to classify substances as other sensitizers. The LLNA correctly identified 52% (14/27) of the strong human sensitizers using EC3 $\leq$ 2% but underclassified 48% (13/27). Among the 21 substances that produced an EC3 $\leq$ 2%, 67% (14/21) were strong human skin sensitizers (GHS Subcategory 1A), but the remaining 33% (7/21) were either other human skin

[9] http://iccvam.niehs.nih.gov/SuppDocs/FedDocs/FR/FR_E7_9544.pdf

sensitizers (GHS Subcategory 1B, n = 3) or substances not classified as human skin sensitizers (n = 4).

Of the 13 strong human sensitizers that were not categorized as strong sensitizers using the LLNA EC3 ≤ 2%, 11 produced an EC3 > 2% (10/11 had an EC3 between 2% and 10% and one produced an EC3 of 30.9%), and two were negative in the LLNA. The commonalities among these 13 substances with regard to physicochemical characteristics include molecular weights within a range of 100 g/mole (12/13 substances had molecular weights of 98.15 to 192.3 g/mole); 8/13 substances were liquids; and, of the six substances for which peptide reactivity information was available, all had high (n = 5) or moderate (n = 1) peptide reactivity.

As noted above, most (77% [10/13]) of the strong human sensitizers that are underclassified by the LLNA produced EC3 values from 2% to 10%. Using LLNA EC3 ≤ 10% to classify substances as strong sensitizers correctly classified 89% (24/27) of the strong sensitizers compared with the 52% (14/27) of the strong sensitizers correctly classified using EC3 ≤ 2%.

For the 56 substances that had LLNA, guinea pig (i.e., the guinea pig maximization test and/or the Buehler test), and human skin sensitization data, the overall correct classification rate produced by the LLNA, using EC3 ≤ 2% to classify substances as strong sensitizers and EC3 > 2% to classify substances as other sensitizers, was similar to that for the guinea pig tests. The overall correct classification rate of human sensitizers and nonsensitizers was 61% (34/56) for the LLNA versus 59% (33/56) for the guinea pig tests. The LLNA correctly classified more strong sensitizers and other sensitizers than did guinea pig tests; however, the LLNA correctly classified fewer nonsensitizers. The LLNA correctly classified 71% (10/14) of the strong human sensitizers versus 57% for the guinea pig tests, 67% (14/21) of the other human sensitizers versus 52% (11/21) for the guinea pig tests, and 48% (10/21) of the nonsensitizers versus 67% (14/21) for the guinea pig tests.

Test Method Reliability

Basketter and Cadby (2004) evaluated the intralaboratory variability associated with 29 individual EC3 concentrations for isoeugenol, which ranged from 0.5% to 2.6%. These data were used to support the "often-mentioned perspective that the biological variation associated with the estimation of EC3 values means that any particular EC3 can be halved or doubled" (Basketter and Cadby 2004). Basketter et al. (2007a) evaluated the interlaboratory reproducibility of EC3 data for 17 sensitizers tested in at least two laboratories using the same vehicle. The authors concluded that, although variability exists, it is less than an order of magnitude.

A number of analyses included in this BRD highlight the potential impact of the LLNA vehicle on EC3 values and potency classification. Forty-five substances in the NICEATM LLNA database had data from tests in multiple vehicles. Evaluation revealed that the vehicle-specific values for only 9% (4/45) of the substances varied by more than an order of magnitude. The GHS potency classifications differed by vehicle for 18% (8/45) of these substances. Another 24% (11/45) of the substances were classified differently as either sensitizers or nonsensitizers by the LLNA. Also, for 16% (7/45) of the substances, LLNA results from the same vehicle resulted in discordant sensitizer or nonsensitizer outcomes.

In a separate analysis, a correlation was calculated for EC3 values from two vehicles (dimethylformamide [DMF] and acetone) when compared to the EC3 values for the same substance obtained with acetone: olive oil (AOO; 4:1 by volume) as the vehicle. These data indicate that EC3 values for substances tested in acetone and AOO are similar, while EC3 values for substances tested in DMF are consistently lower than those obtained with AOO (i.e., the sensitizers are more potent in DMF than in AOO).

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While vehicle may be an important determinant of the calculated EC3 value, it had no impact on the relationship of the LLNA EC3 with DSA_{05} values for the 63 substances that were sensitizers in the LLNA and in the HMT and/or HRIPT (see **Annex IV**).

Animal Welfare Considerations

The proposal for using the LLNA for potency determinations does not impact its requirement for using animals or the number of animals that are required. However, this application could broaden the use of the LLNA protocol in place of guinea pig tests and thereby further reduce the number of guinea pigs that are being used to assess skin sensitization potential. The LLNA test method protocol requires a minimum of only four mice per treatment group, whereas currently recommended guinea pig tests require at least 10 guinea pigs per group for the Buehler test and at least 5 guinea pigs per group for the guinea pig maximization test. The LLNA is also a refinement compared with guinea pig tests because it avoids the pain and distress that occurs in guinea pig tests when substances cause ACD.

Test Method Transferability

No changes to the LLNA protocol are being proposed. Therefore, the transferability, training requirements, and time and cost considerations for the LLNA remain unchanged from the previous ICCVAM evaluations (ICCVAM 1999, 2010c).

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1.0 Introduction and Rationale for the Proposed Use of the Murine Local Lymph Node Assay for Potency Assessment

1.1 Introduction

1.1.1 Allergic Contact Dermatitis

Allergic contact dermatitis (ACD) is an adverse health effect that frequently develops in workers and consumers exposed to skin-sensitizing chemicals and products. ACD results in lost workdays[10] and can significantly diminish quality of life (Hutchings et al. 2001; Skoet et al. 2003). ACD develops in two phases: induction and elicitation. The induction phase occurs when a susceptible individual is exposed topically to a skin-sensitizing substance. During induction, the substance passes through the epidermis, where it forms a hapten complex with dermal proteins. The Langerhans cells, the resident antigen-presenting cells in the skin, process the hapten complex. The processed hapten complex then migrates to the draining lymph nodes. Antigen presentation to T-lymphocytes follows, which leads to the clonal expansion of these cells. At this point, the individual is sensitized to the substance (Basketter et al. 2003; Jowsey et al. 2006). Studies have shown that the magnitude of lymphocyte proliferation correlates with the extent to which skin sensitization develops (Kimber and Dearman 1991; Kimber and Dearman 1996).

The elicitation phase occurs when the individual is topically exposed to the same substance again. As in the induction phase, the substance penetrates the epidermis, is processed by the Langerhans cells, and is presented to circulating T-lymphocytes. The antigen-specific T-lymphocytes are then activated, which causes release of cytokines and other inflammatory mediators. This release produces a rapid dermal immune response that can lead to ACD (Basketter et al. 2003; ICCVAM 1999; Jowsey et al. 2006; Sailstad et al. 2001).

1.1.2 Historical Background

In 1999, the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) recommended the murine local lymph node assay (LLNA) as a valid substitute for currently accepted guinea pig test methods (i.e., the guinea pig maximization test [GPMT] and the Buehler test [BT]) to assess the ACD hazard potential of many, but not all, types of substances (Dean et al. 2001; Haneke et al. 2001; ICCVAM 1999; Sailstad et al. 2001). The recommendation was based on a comprehensive evaluation that included an assessment of the validation status of the LLNA by an independent scientific peer review panel (Panel). The Panel and ICCVAM recommendations (ICCVAM 1999) are available at the website of the National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM)-ICCVAM.[11]

ICCVAM recommended to U.S. Federal agencies that the LLNA should be considered for regulatory acceptance or other nonregulatory applications for assessing the ACD hazard potential of substances, while recognizing that some testing situations would still require the use of traditional guinea pig test methods (ICCVAM 1999; Sailstad et al. 2001). The LLNA was subsequently incorporated into national and international test guidelines for the assessment of skin sensitization:

- U.S. Environmental Protection Agency Health Effects Test Guidelines on Skin Sensitization (EPA 2003)
- Organisation for Economic Co-operation and Development Test Guideline 429 (OECD 2002)

[10] http://www.bls.gov/IIF
[11] http://iccvam.niehs.nih.gov/

ED_006472_00084346-00096

- International Organization for Standardization 10993-10: Tests for Irritation and Delayed-type Hypersensitivity (ISO 2002).[12]

On January 10, 2007, the U.S. Consumer Product Safety Commission (CPSC) formally requested that ICCVAM and NICEATM evaluate several activities related to the LLNA. One of the nominated activities was an assessment of the validation status of the LLNA as a stand-alone assay for determining the potency (including severity) of skin sensitizers for regulatory classification purposes. In response to this nomination, ICCVAM and NICEATM compiled a comprehensive draft background review document (BRD). ICCVAM and its interagency Immunotoxicity Working Group (IWG) evaluated the validation status of the LLNA as a stand-alone assay to determine the potency of skin sensitizers for regulatory classification purposes, and ICCVAM developed draft test method recommendations based on this initial evaluation.

An independent scientific peer review panel (Panel) reviewed the draft BRD in March 2008 to evaluate the extent to which the information in the draft BRD supported the draft test method recommendations. The Panel concluded that the LLNA should not be used as a stand-alone assay for categorizing skin sensitizers based on potency but that it could be used as part of a weight-of-evidence evaluation for this purpose. The Panel recommended that NICEATM perform additional analyses using alternative human reference values that might be more appropriate for evaluating the use of the LLNA for determining skin sensitization potency categories.

ICCVAM and the interagency IWG considered the conclusions and recommendations of the Panel, as well as comments from the public and the Scientific Advisory Committee on Alternative Toxicological Methods, in developing this final BRD. ICCVAM will provide the final BRD for consideration by U.S. regulatory agencies as part of the ICCVAM test method evaluation report.

1.1.3 Classification of Skin Sensitizers Based on Potency

Allergens are known to vary significantly in the potency with which they can induce skin sensitization. It has been suggested that skin-sensitizing chemicals vary as much as 10,000-fold in relative sensitization potency (Kimber et al. 2003). For the purposes of this BRD, *potency* is defined as a function of the concentration of a substance that is required for either induction or elicitation of skin sensitization. For induction, potency refers to the concentration of a substance needed to induce a skin sensitization response. The more potent the substance the smaller the quantity needed for induction. Likewise, for elicitation, potency refers to the concentration of a substance needed to elicit a response in a previously sensitized individual. The more potent the substance the smaller the quantity needed for elicitation (ECETOC 2003, 2008).

Interestingly, it has been widely reported that a defined test substance concentration does not necessarily result in a similar level of sensitization, or frequency of sensitization, every time. Actually, the key factor in the induction of skin sensitization is the dose of the substance per unit area of skin (Friedmann 1990; White et al. 1986). Kimber et al. (2008) discuss the evidence that in most typical situations it is dose per unit area that determines the effectiveness and extent of skin sensitization. Thus, it is recommended that sensitization thresholds obtained from animal and human data be expressed as the dose per unit area of skin (Boukhman and Maibach 2001).

The observed dose-response relationships associated with induction and elicitation allow thresholds for each phase to be determined (ECETOC 2003, 2008; Kimber et al. 2003). This includes thresholds for the applied dose of a substance below which (1) skin sensitization will not be induced in a naïve individual or (2) an elicitation reaction will not occur in a previously sensitized subject (Kimber et al. 1999). Although these thresholds are largely determined by the potency of a particular allergen, they

[12] The OECD and ISO test guidelines were updated in 2010 (ISO 2010; OECD 2010a).

ED_006472_00084346-00097

vary due to vehicle effects and the extent of dermal exposure (Lea et al. 1999; Marzulli and Maibach 1976). Additionally, it has been suggested that

- Induction thresholds for particular substances differ from the elicitation threshold for the same substance (i.e., in general, higher levels are needed for induction in a naïve individual than for elicitation in a previously sensitized individual) (Griem et al. 2003).
- Interindividual variability in thresholds for elicitation exists and is attributed largely to the extent to which individuals have been previously exposed (Basketter et al. 2003; ECETOC 2003, 2008; Kimber et al. 1999).

Most authorities do not currently regulate products based on skin sensitization potency, instead using simple "yes" or "no" designations of skin sensitization hazard. The CPSC, under the Federal Hazardous Substances Act,[13] currently requires hazard labeling of only those products that are considered to be strong skin sensitizers based on a weight-of-evidence approach that considers frequency of responses in exposed human populations, severity of responses, and the doses at which allergic reactions occur. The following substances meet the CPSC's definition of strong sensitizers:

- 4-Phenylenediamine and products containing it
- Powdered orris root and products containing it
- Epoxy resins systems containing, in any concentration, ethylenediamine, diethylenetriamine, and diglycidyl ethers with molecular weight less than 200
- Formaldehyde and products containing 1% or more of formaldehyde
- Oil of bergamot and products containing 2% or more of oil of bergamot

In December 2008, the third revised edition of the Globally Harmonized System of Classification and Labelling of Chemicals (GHS) was adopted; it was published in July 2009 (UN 2009). This edition of the GHS introduced two new subcategories for skin sensitizers:

- Subcategory 1A— strong skin sensitizers, for substances that occur frequently in humans and/or have high potency in animals
- Subcategory 1B— "other" skin sensitizers, for substances that show low frequency of occurrence in humans and/or a low to moderate potency in animals (see **Table C-1**)

Skin sensitizers are classified as Category 1 when the relevant regulatory authority does not require subcategorization or when data are insufficient for subcategorization. Nonsensitizers are not classified.

Kimber et al. (2003) proposed a four-level classification scheme for skin sensitization potency based on a log scale of EC3 values (see **Table C-2**). The Task Force on Contact Sensitization of the European Centre for Ecotoxicology and Toxicology also proposed a four-level classification scheme for assessing skin sensitization potency (see **Table C-3**) (ECETOC 2003). However, the evaluation in this BRD focuses on the usefulness of the LLNA as a stand-alone assay for determining skin sensitization potency based on the GHS classification scheme (see **Table C-1**). These other classification schemes are provided for reference only.

[13] Federal Hazardous Substances Act. 15 U.S.C. 1261, 16 C.F.R. 1500.

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Table C-1 GHS Classification Categories for Skin Sensitization

Category	Classification Criteria	LLNA EC3	Human Evidence	GPMT Response	BT Response
1 (Skin sensitizer)	Evidence that skin sensitization occurs in a substantial number of people OR Positive results from an appropriate animal test	NA	NA	NA	NA
1A (Strong skin sensitizer)	High frequency of occurrence in humans AND/OR High potency in animals May consider severity	≤2%	Positive response at ≤500 μg/cm^2 (HRIPT or HMT induction threshold[1])	EITHER: ≥30% responders at ≤0.1% intradermal induction dose OR ≥60% responders at >0.1% to ≤1% intradermal induction dose	EITHER: ≥15% responders at ≤0.2% topical induction dose OR ≥60% responders at >0.2% to ≤20% topical induction dose
1B (Other skin sensitizer)	Low to moderate frequency of occurrence in humans AND/OR Low to moderate potency in animals May consider severity	>2%	Positive response at >500 μg/cm^2 (HRIPT or HMT induction threshold[2])	EITHER: ≥30 to <60% responders at >0.1% to ≤1% intradermal induction dose OR ≥30% responders at >1% intradermal induction dose	EITHER: ≥15% to <60% responders at >0.2% to ≤20% topical induction dose OR ≥15% responders at >20% topical induction dose

Abbreviations: BT = Buehler test; EC3 = estimated concentration of a substance expected to produce a stimulation index of 3, which is the threshold value for a substance to be considered a sensitizer in the LLNA; GHS = Globally Harmonized System of Chemical Classification and Labelling (UN 2009); GPMT = guinea pig maximization test; HMT = human maximization test; HRIPT = human repeat-insult patch test; LLNA = murine local lymph node assay; NA = not applicable.

[1] Human evidence for strong skin sensitizers can also include (1) diagnostic patch test data where there is a relatively high and substantial incidence of reactions in a defined population in relation to relatively low exposure or (2) other epidemiological evidence where there is a relatively high and substantial incidence of allergic contact dermatitis in relation to relatively low exposure.

[2] Human evidence for other skin sensitizers can also include (1) diagnostic patch test data where there is a relatively low but substantial incidence of reactions in a defined population in relation to relatively high exposure or (2) other epidemiological evidence where there is a relatively low but substantial incidence of allergic contact dermatitis in relation to relatively high exposure.

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Table C-2 Potency Categorization of Skin Sensitizers Based on LLNA EC3 Values[1]

Potency Category	EC3 Value (%)
Extreme	<0.1
Strong	≥0.1 to <1
Moderate	≥1 to <10
Weak	≥10 to ≤100

Abbreviations: EC3 = estimated concentration of a substance expected to produce a stimulation index of 3, which is the threshold value for a substance to be considered a sensitizer in the LLNA; LLNA = murine local lymph node assay.

[1] Proposed by Kimber et al. (2003).

Table C-3 Proposed Skin Sensitization Potency Categories Based on Guinea Pig Data[1]

Induction Concentration (%)	GPMT Incidence (%)		BT Incidence (%)	
	30 to <60	≥60	15 to <60	≥60
<0.1	Strong	Extreme	Strong	Extreme
≥0.1 to <1	Moderate	Strong	Moderate	Strong
≥1 to <10	Weak	Moderate	Weak	Moderate
≥10 to ≤100	Weak	Weak	Weak	Weak

Abbreviations: BT = Buehler test; GPMT = guinea pig maximization test.

[1] Proposed by the ECETOC Task Force on Contact Sensitization (ECETOC 2003).

1.1.4 Use of the LLNA as a Stand-Alone Method for Potency Determinations

Traditional regulatory test methods for skin sensitization (i.e., GPMT, BT, LLNA) have focused on "yes" or "no" determinations of sensitization hazard. In recent years, the LLNA has been proposed as an effective method for determining skin sensitization potency because of the dose-response information that is generated. Originally suggested by Kimber and Basketter (1997), this concept was based on their characterization of the large difference in LLNA threshold response between 2,4-dinitrochlorobenzene (DNCB) and hexyl cinnamic aldehyde (HCA). A number of studies have been conducted in an attempt to support the use of the LLNA for this purpose (see **Section 9.0** for the review articles on this topic).

However, the LLNA had yet to be adequately validated for classifying skin sensitizers based on potency. Consequently, a number of workshops on skin sensitization reviewed the use of the LLNA to assess skin sensitization potency:

- CPSC Sensitizer Scientific Panel — July 2005 (Matheson 2006)
- World Health Organization International Programme on Chemical Safety (IPCS) International Workshop on Skin Sensitization in Chemical Risk Assessment — October 2006 (WHO/IPCS 2007)
- OECD Expert Group on Sensitization — February 2007 and March 2008

In each case, the participants concluded that the LLNA should be used in a weight-of-evidence approach to determine skin sensitization potency categories. The independent scientific peer review panel convened by ICCVAM in March 2008 was the first public independent peer review of the use of the LLNA as a stand-alone assay to assess the skin sensitization potency of test substances.

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1.2 Validation of the LLNA for Skin Sensitization Potential

The ICCVAM Authorization Act of 2000 (Sec. 4(c)) mandates that "[e]ach Federal Agency … shall ensure that any new or revised … test method … is determined to be valid for its proposed use prior to requiring, recommending, or encouraging [its use]" (Public Law 106-545. 42 U.S.C. 285*l*-3).

Validation is the process by which the reliability and relevance of a test method for a specific purpose are established. *Relevance* is the extent to which a test method will correctly predict or measure the biological effect of interest (ICCVAM 1997). *Reliability* is defined as the reproducibility of a test method within and among laboratories. Reliability should be assessed by testing a diverse set of substances that represent (1) the types of chemical and product classes expected to be tested and (2) the range of responses that needs to be identified. This validation process is intended to provide data and information to allow U.S. Federal agencies to develop guidance on the use of test methods in evaluating the potential of substances to cause skin sensitization.

The validation process begins with preparation of a BRD that provides a comprehensive review of a test method, including its mechanistic basis, proposed uses, data quality, and performance characteristics (i.e., relevance and reliability) (ICCVAM 1997). This BRD summarizes the available information on the use of the LLNA for potency categorization of chemicals causing ACD. It will also help to identify any additional studies that should be considered during future development and validation activities.

1.3 Selection of Citations for the BRD

The test method data summarized in this BRD are based on information obtained from the peer-reviewed scientific literature identified through online searches via PubMed and Scopus, through citations in publications, and in response to a *Federal Register* notice requesting LLNA, guinea pig, and/or human skin sensitization data and experience (72 FR 27815).[14] **Annex I** contains a document provided by Basketter et al. for consideration by ICCVAM and the European Centre for the Validation of Alternative Methods (ECVAM) during their evaluations of the LLNA for potency determinations. The NICEATM potency database includes 191 references relevant to this evaluation. Key words used in the online searches for this evaluation were ("LLNA" OR "Local Lymph Node" OR "Local lymph node" OR "local lymph node") AND "potency."

[14] http://iccvam.niehs.nih.gov/SuppDocs/FedDocs/FR/FR_E7_9544.pdf

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2.0 LLNA Test Method Protocol Components

The original ICCVAM-recommended LLNA test method protocol describes the conduct of the assay in detail (Dean et al. 2001; ICCVAM 2001). A test-substance-induced increase in lymphocyte proliferation in the draining lymph nodes of the ear, the site of test substance application, is used in the LLNA to identify chemical sensitizers. The test substance is first applied to the dorsum of the mice ears on three consecutive days. After 48 hours, mice are injected with radiolabeled compound (^{3}H-methyl thymidine or ^{125}I-iodeoxyuridine), which is incorporated into the DNA of proliferating cells. Five hours later, mice are euthanized and the auricular lymph nodes are harvested and processed so that the incorporated radioactivity can be measured. The stimulation index (SI), which is the ratio of incorporated radioactivity (measured as disintegrations per minute [dpm]) in the auricular lymph nodes of treated versus vehicle control mice, is used to assess the sensitizing potential of the test substance. Based on collecting lymph nodes from individual animals within each group, the formula for calculating the SI is:

$$SI = \frac{\textit{mean dpm from lymph nodes in substance - treated group}}{\textit{mean dpm from lymph nodes in vehicle - treated group}}$$

A test substance with an SI of 3 or greater is classified as a skin-sensitizing agent. The estimated concentration of a substance expected to produce an SI of 3 (i.e., the EC3) is the metric for determining skin sensitization potency using the LLNA. The method for determining the EC3 is a simple linear interpolation of the points in the dose-response curve that lie immediately above and below an SI of 3, the classification threshold for sensitizers in the LLNA. This method was chosen from an evaluation of a variety of statistical approaches to derive EC3 values from LLNA dose-response data (Basketter et al. 1999b). When there are no data points that fall below an SI value of 3, a more complex log-linear extrapolation may be applied as described in Ryan et al. (2007) in which the two lowest test concentrations from the dose-response curve are used, provided the lowest SI value approaches the value of 3 and that a linear dose-response exists.

The LLNA procedure recommended by ICCVAM (Dean et al. 2001; ICCVAM 2001) differs from the protocol described in OECD Test Guideline (TG) 429 (OECD 2002) in that the ICCVAM protocol requires a concurrent positive control and the collection and analysis of individual animal data rather than pooled animal data. The ICCVAM-recommended protocol and OECD TG 429 were recently updated to allow the use of four animals per dose group, rather than the minimum of five that was required previously, when individual animal data are collected (ICCVAM 2009; OECD 2010a). Most recently, ICCVAM evaluated and recommended variations of the LLNA that do not employ radioactivity (ICCVAM 2010b, 2010a). These were adopted as OECD test guidelines (OECD 2010c, 2010b). However, these nonradioactive LLNA methods have not been evaluated for potency determination.

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3.0 Substances Used for Validation of the LLNA for Potency Determinations

No new LLNA, guinea pig, or human skin sensitization studies were conducted for this evaluation. Rather, data from available studies were evaluated retrospectively. Data were obtained from 141 different sources, including published reports as well as unpublished data submitted to NICEATM in response to a *Federal Register* notice (72 FR 27815)[15] requesting LLNA, guinea pig, and human skin sensitization study data.

The information included in this BRD is derived from a database of over 600 substances, 196 of which have LLNA data with comparative guinea pig and/or human data. Among these 196 substances are 136 substances with comparative human data (76 sensitizers, 60 nonsensitizers), 116 substances with comparative guinea pig data (64 sensitizers, 52 nonsensitizers), and 56 substances with comparative human and guinea pig data (35 sensitizers, 21 nonsensitizers) (see **Annex II**). Two of the five substances that meet the CPSC's definition of strong sensitizers, 4-phenylenediamine and formaldehyde, are among the 136 substances with comparative human data and the 56 substances with comparative human and guinea pig data (see **Section 1.1.3**).

When available, chemical classes for each substance were retrieved from the National Library of Medicine Medical Subject Headings database.[16] If chemical classes were unavailable, they were assigned using a standard classification scheme based on the Medical Subject Headings classification system. A substance could be assigned to more than one chemical class; however, no substance was assigned to more than three classes. Chemical class information is presented only to provide an indication of the variety of structural elements that are present in the structures that were evaluated in this analysis. Classification of substances is not intended to indicate the impact of structure on biological activity with respect to sensitization potential.

Table C-4 shows the chemical classes represented by the 196 substances tested in the LLNA with human and/or guinea pig skin sensitization data. If *inorganic* is considered to be one class, the 196 substances represent 30 chemical classes. Fifty-five substances are classified in more than one chemical class. The classes with the highest number of substances are carboxylic acids (33 substances) and aldehydes (18 substances). In the entire NICEATM LLNA database of more than 600 substances, 22 chemical classes are represented by at least five substances, thereby providing a sufficiently large representation for further analyses. Twenty of those classes had at least 60% of the LLNA results identified as positive, and these 20 classes were identified as those most likely to be associated with skin sensitization. In comparison, 19 of these 20 classes were also represented in the database of 196 substances included in this evaluation (i.e., the LLNA potency database); only macromolecular substances were not included. Further, some of the chemical classes that have been previously identified as containing common skin allergens (aldehydes, ketones, quinones, acrylates) (Gerberick et al. 2004) were represented in this LLNA potency evaluation. **Annex III** provides the chemical class, information on the physicochemical properties (e.g., K_{ow} [estimated log octanol-water partition coefficient]), Chemical Abstracts Service Registry Number, and uses for each of the 196 substances. This information was obtained from the published reports, submitted data, or through online literature.

[15] http://iccvam.niehs.nih.gov/SuppDocs/FedDocs/FR/FR_E7_9544.pdf
[16] http://www.nlm.nih.gov/mesh/meshhome.html

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Table C-4 Chemical Classes[1] Represented in the LLNA Potency Database

Chemical Class	# of Substances[2]
Inorganic chemicals	**11**
Aluminum compounds	1
Chromium compounds	1
Elements	1
Gold compounds	1
Manganese compounds	1
Mercury compounds	1
Metals	5
Sulfur compounds	1
Zinc compounds	1
Organic chemicals	**185**
Alcohols	15
Aldehydes	18
Amides	5
Amines	16
Anhydrides	2
Azo compounds	5
Carbohydrates	6
Carboxylic acids	33
Cyanates	1
Esters	5

Chemical Class	# of Substances[2]
Organic chemicals (continued)	**---**
Ethers	6
Formulations[3]	16
Heterocyclic compounds	15
Hydrocarbons, acyclic	5
Hydrocarbons, cyclic	12
Hydrocarbons, halogenated	1
Hydrocarbons, other	9
Ketones	3
Lactones	1
Lipids	15
Natural complex substances[3]	15
Nitriles	2
Nitro compounds	2
Onium compounds	1
Phenols	14
Polycyclic compounds	4
Quinones	1
Sulfur compounds	16
Ureas	2
Unknown[4]	**3**

Abbreviations: LLNA = murine local lymph node assay.

[1] Chemical classifications are based on the Medical Subject Headings classification for chemicals and drugs, developed by the National Library of Medicine (http://www.nlm.nih.gov/mesh/meshhome.html).

[2] The total number of substances assigned to each chemical class does not equal the total number of substances evaluated because some substances were assigned to more than one chemical class, and some substances were not assigned to a specific chemical class.

[3] Substances assigned to these classes were mixtures of two or more components. In some cases, another chemical class was also assigned based on the active ingredient (for formulations) or the principal component (for natural complex substances).

[4] The proprietary substances (fatty acid glutamate, fatty acid alcohol #1, and fatty acid alcohol #2) were not identified sufficiently for a chemical class to be assigned.

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4.0 Comparative *In Vivo* Reference Data

4.1 Human Reference Data

The human reference data for this evaluation, which were obtained from 44 sources, were from human predictive skin sensitization tests (i.e., the human maximization test [HMT], the human repeat-insult patch test [HRIPT]) and, for nonsensitizers, other published reports. Protocols for the HMT include that of Kligman (1966) and Kligman and Epstein (1975). The HRIPT protocol is a modification of the Draize test (Marzulli and Maibach 1974; Politano and Api 2008). Both the HMT and HRIPT involve an induction phase of repeated applications of test substance to the skin for 24 (HRIPT) to 48 hours (HMT) with an occlusive dressing. This sensitization phase is followed by a rest period of 10 to 14 days and then a challenge phase that includes an additional application of test substance to the skin in an occlusive dressing for 24 (HRIPT) to 48 (HMT) hours. The application sites are then evaluated 24 (HRIPT), 48, and 72 hours after application; and the incidence and severity of a skin sensitization reaction are reported. The major differences between the HMT and HRIPT protocols are:

- The application of sodium lauryl sulfate (SLS) 24 hours before the induction test and one hour before the challenge test when testing nonirritating substances in the HMT
- The smaller number of subjects in the HMT (i.e., 25) versus approximately 100 for the HRIPT
- The length of patch application: five 48-hour patches for the HMT versus nine or ten 24-hour patches for the HRIPT

Human predictive test data were obtained from the published literature and from the Research Institute for Fragrance Materials (A. Api, personal communication). Skin sensitization potency in humans was identified as the threshold concentration inducing a sensitizing response in either the HMT or HRIPT. For the purposes of this evaluation, the threshold for induction of skin sensitization in humans was the induction dose per skin area in a HMT or HRIPT that produced a positive response in 5% of the tested population (i.e., the dose per skin area leading to a sensitization incidence of 5% [DSA_{05}]). The DSA_{05} value, which represents a defined low level of a positive response (i.e., 5%), was used as the human threshold response because it corresponds best (compared with the no observed effect levels [NOEL] or lowest observed effect level [LOEL]) to the LLNA EC3, which is also a threshold positive response. The DSA_{05} was used if it was reported in the literature, otherwise it was calculated from the LOEL and the incidence of a positive response in the study:

$$DSA_{05} = \frac{LOEL\ (\mu g/cm^2) \times 5\%}{incidence\ (\%)}$$

If the LOEL was not reported in $\mu g/cm^2$ skin area, it was calculated using the concentration of test substance applied, the weight or volume applied, and the size of the patch:

$$Dose\ applied = \frac{fractional\ concentration\ (\mu g\ substance/\mu g\ solution)\ \times\ weight\ applied\ (\mu g\ solution)}{patch\ size\ (cm^2)}$$

The volume applied is often reported in µL and must be multiplied by 1000 µg/µL to convert it to a weight. Substances in tests that resulted in only NOELs at the highest dose tested (i.e., no LOELs) were considered nonsensitizing.

The potency for each human skin sensitizer was determined, with DSA_{05} values as the metric for the positive responses, using the GHS criteria in **Table C-1**. Substances with $DSA_{05} \leq 500\ \mu g/cm^2$ were considered strong human skin sensitizers (GHS Subcategory 1A); and substances with $DSA_{05} > 500\ \mu g/cm^2$ were considered other human skin sensitizers (GHS Subcategory 1B). **Annex II-2** provides the available human data for each substance, which includes, where available,

the induction dose, vehicle, the NOEL and/or LOEL and DSA_{05} values for human sensitizers. Because they fail to elicit a positive response, nonsensitizers have no DSA_{05} values.

It is important to discuss some of the limitations associated with the human data, much of which come from older studies. First, the HMT and the HRIPT have differences in sensitivity. For instance, the HMT tends to give lower LOEL values than the HRIPT (Griem et al. 2003). Further, even when using the same human predictive test, the protocols often differ between laboratories in the application frequency, amount applied, and skin site used (Griem et al. 2003). In addition, the intraspecies variability of human susceptibility to skin sensitization (Friedmann 1990) may further confound the results from human predictive tests.

4.2 Guinea Pig Data

The guinea pig data for this evaluation were used not as reference data for the LLNA but as comparative data for usefulness in determining human skin sensitization potency categories. The guinea pig data, from 26 different sources, were obtained from the published literature or submitted reports and were generated using the currently accepted guinea pig test methods for skin sensitization (i.e., the GPMT and the BT). National and international test guidelines are available for these test methods (EPA 2003; OECD 1992).

Both test methods involve induction and elicitation phases. The GPMT requires intradermal injections, with and without Freund's complete adjuvant, followed by topical induction on Days 5 through 8. Induction concentrations should be systemically well tolerated but high enough to produce mild to moderate skin irritation. The challenge concentration, which is applied to the skin on Days 20 through 22, must be the highest nonirritating concentration.

The BT requires topical application of an induction concentration high enough to produce mild irritation on Days 0, 6 through 8, and 13 through 15. The challenge concentration, applied on Days 27 and 28, is the highest nonirritating concentration.

In both the GPMT and the BT, the challenge sites are evaluated 24 and 48 hours after removal of the challenge dose. The incidence and severity of skin sensitization reactions are reported. For the purposes of this evaluation, the potency for each guinea pig skin sensitizer (GHS Subcategory 1A—strong or GHS Subcategory 1B—other) is based on the percentage of responding guinea pigs and the associated induction concentration in accordance with the GHS criteria in **Table C-1**. Substances that produce positive responses in less than 30% of the test group for the GPMT and 15% of the test group for the BT are considered to be nonsensitizers in the guinea pig tests. **Annex II-3** provides the guinea pig test data for each substance, including, where available, the induction dose (intradermal for GPMT and topical for BT), the percentage of animals exhibiting a positive response, and the corresponding data source.

4.3 Availability of Original Records for Human and Guinea Pig Data

NICEATM was unable to obtain the original records and/or reports for the human and guinea pig reference data used in this evaluation. All animal data supporting the validity of a test method should be obtained and reported from studies conducted in accordance with Good Laboratory Practice (GLP) guidelines, which are internationally recognized principles designed to produce high-quality laboratory records (EPA 2006b, 2006a; FDA 2009; OECD 1998). Human studies should conform to Good Clinical Practice (GCP) guidelines (ICH 1996). GLP and GCP guidelines provide an internationally standardized procedure for study conduct, reporting requirements, archiving study data and records, and information about the test protocol in order to ensure the integrity, reliability, and accountability of a study.

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The extent to which the human or guinea pig studies complied with GLP or GCP guidelines, respectively, is based on the information provided in published and submitted reports. Information on GLP compliance was available for data from guinea pig studies submitted by E. Debruyne (Bayer CropScience SA) and P. Botham (European Crop Protection Association [ECPA]). None of the published references from which human or guinea pig data were obtained have GLP or GCP information specified.

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5.0 LLNA Data and Results

5.1 Description of the LLNA Test Method Protocol Used to Generate Data

The majority of studies included in this evaluation were reportedly conducted according to the original ICCVAM protocol (Dean et al. 2001; ICCVAM 1999) or following OECD TG 429 (OECD 2002). Where OECD TG 429 was the reference protocol, specifics on the number of animals per dose group tested, whether or not lymph nodes were pooled within dose groups, and/or whether a concurrent positive control was used were generally not available. In addition, in order to increase the LLNA database, NICEATM determined that data from nonstandard LLNA protocols could be used in the analyses without affecting the LLNA outcomes (see **Annex IV**). The nonstandard protocol deviations included use of a different strain of mouse, use of both sexes of mice, different dose schedule for topical application of test substance, different duration between the last topical application and the injection of radioactive marker, and pretreatment with SLS prior to topical application of the test substance.

5.2 Availability of Copies of Original LLNA Data Used to Evaluate Accuracy and Reliability

Copies of original data for the LLNA studies considered during the earlier ICCVAM evaluation (ICCVAM 1999) were made available to NICEATM for that evaluation. For the current evaluation, individual animal data for some of the LLNA studies submitted to NICEATM earlier were included; however, the original data for the vast majority of the LLNA studies used in this evaluation are not available. Individual animal data were submitted by P. Botham (ECPA), D. Germolec (NTP), H. Ott (Federal Institute for Occupational Safety and Health, Germany), and P. Ungeheuer (European Federation for Cosmetic Ingredients).

5.3 Description of the Statistical Approach Used to Evaluate the Resulting Data

Section 2.0 describes the derivation of the SI and the EC3. The EC3 (typically expressed as %) is the metric used to evaluate the capacity of the LLNA to predict skin sensitization potency.

To evaluate the correlation between EC3 values and human DSA_{05} values (expressed in $\mu g/cm^2$), EC3 values (in %) were converted to $\mu g/cm^2$ by multiplying by a factor of 250 (based on an exposed area of 1 cm^2 and a dosing volume of 25 μL in the LLNA) (Griem et al. 2003). For all other comparisons between LLNA and human or guinea pig test results, the EC3 was expressed in its traditional units (%).

5.4 Summary of Results

NICEATM obtained LLNA data for this evaluation from 95 sources. The data are provided in **Annex II-1**. Where available, the SI values for each concentration tested, the calculated EC3 values, and the corresponding data sources are provided. The information provided with the submitted data was used, but no additional attempt was made to identify the source or purity of the test substance.

5.5 Use of Coded Chemicals

Coding of substances to avoid potential scoring bias did not occur for any of the LLNA test substances evaluated by ICCVAM in the original evaluation (ICCVAM 1999) or for any of the more recently obtained studies used in the current evaluation.

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5.6 Lot-to-Lot Consistency of Test Substances

Ideally, a single lot of each substance is used during the validation of a test method. In situations where multiple lots of a substance must be used, lot-to-lot consistency must be evaluated to ensure the consistency of the substance evaluated over the course of the study. There was no available information in any of the reports included in this evaluation with which to assess lot-to-lot consistency.

5.7 Availability of Data for External Audit

The data for the LLNA test substances previously evaluated by ICCVAM (1999) were audited during that evaluation. Whether the other LLNA studies included in this evaluation are available for audit is unknown.

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6.0 Test Method Accuracy

This section evaluates the capacity of the LLNA to accurately predict skin sensitization potency in humans, based on data generated by the HMT and HRIPT for sensitizers. Other published data for nonsensitizers are included. The comparative capacity of the LLNA and guinea pig tests to predict skin sensitization potency in humans is also examined for substances tested in mice (LLNA), guinea pigs, and humans.

6.1 Usefulness of the LLNA in Predicting Skin Sensitization Potency in Humans

Two approaches were used to evaluate the capacity of the LLNA to predict skin sensitization potency in humans. In the first approach, for each substance classified as a sensitizer in both the LLNA and in humans, the LLNA EC3 (expressed in $\mu g/cm^2$ skin surface and not as a percent) was correlated against the human threshold response, the DSA_{05} (expressed in $\mu g/cm^2$).

In the second approach, using the same LLNA/human sensitizers as the first approach, the human sensitizers were classified as strong (GHS Subcategory 1A) or other sensitizers (GHS Subcategory 1B) based on the GHS decision criteria (strong sensitizers had $DSA_{05} \leq 500\ \mu g/cm^2$, and other sensitizers had $DSA_{05} > 500\ \mu g/cm^2$; see **Table C-1**). Classification rate analyses were then performed to determine the correct, overclassification, and underclassification rates of EC3 cutoffs, including that specified in the GHS, for classifying substances in the human skin sensitization potency categories (i.e., strong and other than strong).

In a variant of the second approach, substances that were classified in the LLNA as false positives (i.e., sensitizers in the LLNA but nonsensitizers in humans), false negatives (i.e., nonsensitizers in the LLNA but sensitizers in human tests), or nonsensitizers in both the LLNA and human tests were included. Then the correct classification rate as well as the under- and overclassification rates were recalculated for each skin sensitization category (strong sensitizer, other than strong sensitizer, nonsensitizer).

Data from more than one LLNA test were available for many of the substances in the NICEATM potency database, and some substances had more than one DSA_{05} value. Before conducting correlation/regression analyses, single EC3 and DSA_{05} values were established for each substance. The correlation/regression analyses used two different approaches for combining EC3 values or human DSA_{05} values where multiple values existed for individual substances: (1) most potent EC3 (i.e., the lowest) versus most potent DSA_{05} (i.e., the lowest) and (2) geometric mean EC3 versus geometric mean DSA_{05}. The regression with the highest coefficient of determination, R^2, was used to determine which approach to use for combining multiple values in the classification rate analyses. The impact of variability in the EC3 on skin sensitization potency categorization is discussed in **Section 7.0**.

6.1.1 Regression Analyses for LLNA EC3 versus Human Threshold Concentrations

The current NICEATM potency database includes 136 substances with both LLNA and human data. Sixty-three of the 136 are classified as skin sensitizers in both the LLNA and in the HMT and/or the HRIPT (see **Annex II**). Although there were 65 substances with positive LLNA and HMT/HRIPT responses, nickel salts and streptomycin were not considered to be LLNA sensitizers because the most prevalent LLNA responses were negative (8/10 tests for nickel salts and 4/5 tests for streptomycin). The distribution of the 63 LLNA sensitizers by the number of studies conducted and the solvent used is provided in **Table C-5**.

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Table C-5 Distribution of 63 LLNA/Human Sensitizers by the Number of LLNA Studies Conducted and the Solvent Used

Multiplicity of LLNA Studies for 63 Sensitizers					
1 Study	2 Studies	3 Studies	4 Studies	5 Studies	≥6 Studies
24 (38%)	12 (19%)	4 (6%)	5 (8%)	1 (2%)	17 (27%)
Number of Sensitizers Tested in Each Solvent[1]					
AOO	ACE	DMF	DMSO	EtOH-DEP	Other[2]
41 (65%)	9 (14%)	20 (32%)	11 (17%)	22 (35%)	49 (78%)

Abbreviations: ACE = acetone; AOO = acetone: olive oil (4:1 by volume); DMF = dimethylformamide; DMSO = dimethyl sulfoxide; EtOH-DEP = 1:3 or 3:1 ethanol: diethyl phthalate; LLNA = murine local lymph node assay.

[1] Numbers add up to more than 63 because 30 substances were tested in two or more solvents.

[2] Includes EtOH; DEP; methyl ethyl ketone; methyl ethyl ketone and olive oil; petrolatum; propylene glycol; Pluronic L92; hydroxypropyl cellulose in methanol; water; ACE and water; DMF and water; DMSO and water; unspecified solvents; and EtOH-DEP with additives such as tocopherol, Trolox C, butylated hydroxytoluene, and eugenol.

The analyses of the 63 LLNA/human sensitizers include both linear regressions and Spearman correlations (Steel and Torrie 1980) of the log-transformed LLNA EC3 values and human DSA_{05} values, both in units of $\mu g/cm^2$. **Annex IV** describes the analyses performed to evaluate a number of approaches to calculate the geometric mean EC3 in order to determine (1) the use of negative LLNA results for substances that also produced positive results (i.e., how to account for discordant negative results), (2) the use of vehicle-specific LLNA results for substances that had tests in multiple vehicles, and (3) the use of LLNA results from nonstandard protocols (**Section 5.1**). The preferred approach for calculating geometric mean EC3 values, presented in this section, ignores discordant negative results and vehicles (i.e., all EC3 values were pooled regardless of vehicle). It includes nonstandard protocols because these approaches had no impact on the EC3–DSA_{05} relationship (see **Annex IV**). Geometric mean DSA_{05} values were calculated using all available DSA_{05} values for each substance with multiple values.

Figure C-1 shows both the geometric mean and the most potent EC3–DSA_{05} regressions. Both regressions indicated a positive correlation between LLNA and human test results. The slopes for both regressions were significantly different from zero ($p < 0.001$). The geometric mean regression yielded $R^2 = 0.448$, and the most potent regression yielded $R^2 = 0.382$. The resulting regression equations are provided in **Table C-6** as regressions 1 (most potent) and 2 (geometric mean). Spearman correlations also indicated that the EC3–DSA_{05} relationship was statistically significant: $p < 0.0001$ for both correlations. The Spearman r (correlation coefficient) for the geometric mean regression was higher than that for the most potent regression ($r = 0.692$ versus 0.594). Because the geometric mean regressions produced a higher R^2 value than the most potent regression, and the Spearman r was also higher for the geometric mean regression, the geometric mean approach for calculating a single LLNA EC3 and DSA_{05} value for each substance was carried forward for the classification rate analyses in **Section 6.1.2** and for additional regressions that were performed using the NICEATM potency database (i.e., regressions 3 and 4).

Table C-6 compares the correlation results obtained using the NICEATM potency database (see **Annex II**):

- When LLNA EC3 data were correlated against HMT threshold data, HMT NOEL data only, or HMT LOEL/10 data only
- When LLNA EC3 data were correlated against HRIPT threshold data, HRIPT NOEL data only, or HRIPT LOEL/10 data only

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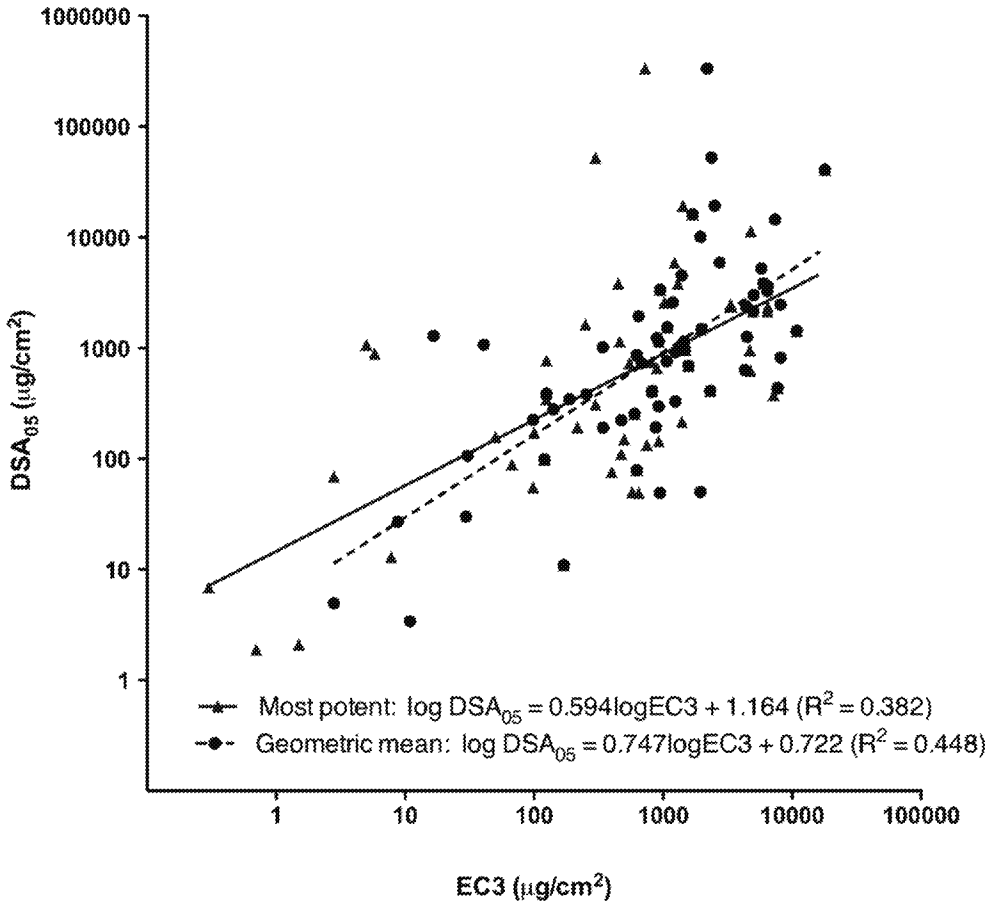
- For sensitizers tested in the LLNA using acetone: olive oil (AOO; 4:1 by volume), the most common solvent used, when correlated against human threshold data

For comparative purposes, **Table C-6** also provides linear regression data for LLNA EC3 values versus various sets of human threshold data published previously (Griem et al. 2003; Schneider and Akkan 2004) or submitted to NICEATM (Basketter et al. in **Annex I**). All of the sensitizers in these data sets are included in the NICEATM potency database (see **Annex II**).

Figure C-1 Most Potent and Geometric Mean Regressions for LLNA EC3 Values versus Human DSA_{05} Values for 63 LLNA/Human Skin Sensitizers



Abbreviations: DSA_{05} = induction dose per skin area, in µg/cm^2, in a human repeat-insult patch test or human maximization test that produces a positive response in 5% of the tested population; EC3 = estimated concentration of a substance expected to produce a stimulation index of 3, which is the threshold value for a substance to be considered a sensitizer in the LLNA; LLNA = murine local lymph node assay.

The solid line shows the regression line for the geometric mean LLNA EC3 versus the corresponding geometric mean human DSA_{05} (both in µg/cm^2) for 63 sensitizers. Geometric mean EC3 and DSA_{05} values were used for substances that had multiple values. The dashed line shows the regression line for the most potent LLNA EC3 versus the corresponding most potent human DSA_{05} for the same substances. The lowest EC3 and DSA_{05} values were used for substances that had multiple values. Human results were obtained from the human maximization test and/or human repeat-insult patch test.

Table C-6 Linear Regressions Obtained for LLNA EC3 Values versus Human Threshold Values

No.	Comparison	N	Regression Coefficient ($\mu g/cm^2$)[1]	Y-intercept[1]	R^2	p-value
1	NICEATM LLNA EC3 versus human DSA_{05} for sensitizers using most potent value	63	0.594 ± 0.097	1.164 ± 0.275	0.382	<0.0001
2	NICEATM LLNA EC3 versus human DSA_{05} for sensitizers using geometric mean values for multiply tested substances	63	0.747 ± 0.106	0.722 ± 0.322	0.448	<0.0001
3	NICEATM LLNA EC3 versus HMT DSA_{05}	36	0.579 ± 0.111	1.076 ± 0.344	0.441	<0.0001
4	NICEATM LLNA EC3 versus HRIPT DSA_{05}	42	0.832 ± 0.152	0.578 ± 0.455	0.427	<0.0001
5	Basketter et al. submission (see **Annex I**) reported EC3 data versus HMT/HRIPT NOEL, LOEL, and DSA_{05} values	66	0.896 ± 0.108	0.211 ± 0.335	0.519	<0.0001
6	Schneider and Akkan (2004) reported EC3 data versus HMT DSA_{05}	38	0.586 ± 0.115	0.936 ± 0.347	0.419	<0.0001
7	Schneider and Akkan (2004) reported EC3 data versus HRIPT DSA_{05}	24	0.765 ± 0.122	0.818 ± 0.355	0.641	<0.0001
8	Basketter et al. (2005) reported EC3 data versus HRIPT NOEL and LOEL data	25	1.121 ± 0.147	-0.533 ± 0.463	0.717	<0.0001
9	Griem et al. (2003) reported EC3 data versus HMT/HRIPT NOEL data	18	0.959 ± 0.129	0.111 ± 0.424	0.776	<0.0001
10	Griem et al. (2003) reported EC3 data versus HMT/HRIPT LOEL data	23	0.783 ± 0.123	0.682 ± 0.365	0.657	<0.0001
11	Griem et al. (2003) reported EC3 data versus HMT/HRIPT LOEL and NOEL data	41	0.854 ± 0.087	0.466 ± 0.271	0.711	<0.0001

Abbreviations: DSA_{05} = induction dose per skin area, in $\mu g/cm^2$, in a human repeat-insult patch test or human maximization test that produces a positive response in 5% of the tested population; EC3 = estimated concentration of a substance expected to produce a stimulation index of 3, which is the threshold value for a substance to be considered a sensitizer in the LLNA; HMT = human maximization test; HRIPT = human repeat-insult patch test; LLNA = murine local lymph node assay; LOEL = lowest observed effect level; N = number of studies included in analyses; No. = number of the analysis presented in the table; NOEL = no observed effect level; R^2 = coefficient of determination.

[1] Shown as parameter estimate with standard error.

Table C-6 shows that separating the HMT (regression 3; $R^2 = 0.441$) and HRIPT data (regression 4; $R^2 = 0.427$) did not increase the R^2 compared with the regression that combined HMT and HRIPT (regression 2) DSA_{05} values ($R^2 = 0.448$). For comparative purposes, linear regression data for LLNA EC3 values versus various sets of human threshold data published previously (Basketter et al. 2005; Griem et al. 2003; Schneider and Akkan 2004) and the Basketter et al. submission to NICEATM (see **Annex I)** are also provided in **Table C-6**. All of the sensitizers in these data sets are included in the NICEATM potency database (see **Annex II**).

As demonstrated in **Table C-6**, there are differences in R^2 values (which is a measure of the strength of the linear association between the LLNA EC3 and the DSA_{05}) among the various regressions. These differences presumably reflect differences in the number of substances with both LLNA EC3 and human skin sensitization threshold data; which human test is considered (HMT or HRIPT); whether NOEL, LOEL, and/or DSA_{05} values are used; and how data for substances tested multiple

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times are collapsed into a single value. For example, the R^2 value generated with the NICEATM potency database (n = 63) increased from 0.382 to 0.448 when geometric mean threshold values were used for multiply tested sensitizers (regression 2) instead of the most potent value (regression 1). The R^2 values generated from data in the Basketter et al. submission (see **Annex I**), Basketter et al. (2005), Schneider and Akkan (2004), and Griem et al. (2003) are generally higher than the R^2 values from the NICEATM potency database. There may be several reasons for this apparent discordance including the following facts:

- The Basketter et al. submission to NICEATM (see **Annex I**) (regression 5) and the NICEATM analysis (regression 2) used data from both the HMT and the HRIPT for a similar number of chemicals (66 versus 63, respectively), but the Basketter et al. submission used NOELs, LOELs, and DSA_{05} values while the NICEATM analysis used only DSA_{05} values. The NICEATM analysis combined HMT and/or HRIPT data when multiple results were available, but the Basketter et al. submission had only one HMT or HRIPT result for each substance. The parameter estimates (i.e., the regression coefficients and the y-intercepts) for the two regressions are close enough that the standard errors overlap.
- The NICEATM analyses (regressions 1 and 2) represent a larger set of substances (n = 63) than the published datasets (n = 18 to 41).
- Schneider and Akkan (2004) and the NICEATM analysis used DSA_{05} values. Schneider and Akkan (2004) performed separate regressions for the HMT (regression 6) and the HRIPT (regression 7). The HMT analysis (regression 6) was similar in the number of substances, regression coefficient, slope, and R^2 value to the NICEATM HMT analysis (regression 3). The HRIPT analysis (regression 7) was less similar to the NICEATM HRIPT analysis (regression 4), but the standard errors for the parameter estimates did overlap. The NICEATM HRIPT regression (4) contained 75% (18/24) more chemicals than the Schneider and Akkan regression (7).
- Basketter et al. (2005) (regression 8) used only the highest NOELs available (preferred) and LOELs (if NOELs were unavailable and sensitization incidence was <8%) from HRIPT data, while the NICEATM regression (regression 2) used DSA_{05} values from both the HMT and the HRIPT. NICEATM combined multiple HMT/HRIPT results for single substances using a geometric mean.
- Griem et al. (2003) (regressions 9-11) and the NICEATM analysis (regression 2) each included threshold doses from both HMT and HRIPT data. However, NICEATM used DSA_{05} values, while Griem et al. (2003) used NOELs (regression 9), LOELs (regression 10), or NOELs and LOELs combined (regression 11). Griem et al. considered incidences of positive responses below 10% to be LOELs. To derive LOELs for other incidences below 50%, uncertainty factors were applied: 10 for incidences between 25% and 50% and three for incidences between 10% and 25%.

6.1.2 Correct, Underclassification, and Overclassification Rates for EC3 Value Predictions of Human Skin Sensitization Potency Categories

In this analysis, the extent that the LLNA EC3 value correctly distinguished between strong and other sensitizers in humans was evaluated using the criteria for human thresholds (see **Table C-1**) recently published in the third revised edition of the GHS (UN 2009). The GHS criteria for human skin sensitization classifies sensitizers as strong (Subcategory 1A) if the positive response in an HMT or HRIPT test occurs at $\leq 500\ \mu g/cm^2$. The GHS criteria categorizes a sensitizer as other (Subcategory 1B) if the positive response in an HMT or HRIPT occurs at $>500\ \mu g/cm^2$. Substances that do not produce a positive response are not classified (i.e., nonsensitizers). Similarly, positive LLNA responses can be divided into Subcategory 1A or 1B with an EC3 $\leq$ 2% or >2%, respectively. Substances with negative LLNA responses are not classified (i.e., nonsensitizers).

Substances with multiple EC3 values were assigned a geometric mean EC3 value calculated from all of the available positive LLNA tests regardless of vehicle (see **Annex II-4**). Forty-seven of the 98 substances with positive LLNA results had multiple EC3 values; the number of values per substance ranged from 2 to 66. Individual EC3 values ranged from 0.0007% to 98.5%. If a majority of the LLNA tests for a substance were negative, however, it was not assigned an EC3 value. LLNA results for nickel salts and streptomycin were designated as negative because the most prevalent LLNA responses were negative (8/10 tests for nickel salts and 4/5 tests for streptomycin).

Substances with multiple DSA_{05} values were assigned a geometric mean DSA_{05} value calculated from all of the available DSA_{05} values (see **Annex II-4**). Thirty-two of the 76 substances with positive human results had multiple DSA_{05} values; the number of values per substance ranged from 2 to 8. Individual DSA_{05} values ranged from 1.9 $\mu g/cm^2$ to 335545 $\mu g/cm^2$. **Table C-7** shows the distribution of substances into the GHS potency categories using LLNA and human results.

Classification rates to determine the extent that the EC3 could predict strong and other human skin sensitizers were calculated from the results of receiver-operator characteristic calculations (Fawcett 2006), which provide sensitivity and 1-specificity results from the EC3 values divided into those associated with human $DSA_{05} \leq 500$ $\mu g/cm^2$ and those associated with $DSA_{05} > 500$ $\mu g/cm^2$. Two approaches were used to estimate the classification rates (correct, underclassification, and overclassification) for the EC3 classification of strong and other human skin sensitizers. In the first approach, the classification analysis considered only the 63 substances classified as sensitizers in both the LLNA and humans based on the HMT and/or HRIPT. In the second approach, the analysis took into consideration those substances that were LLNA false positives (35) and false negatives (13) against human skin sensitization data, as well as those classified as nonsensitizers in both the LLNA and in humans (25) (see **Table C-7**).

Table C-7 Distribution of 136 Substances for Classification Rate Analyses[1]

LLNA +/Human +		LLNA +/Human -	LLNA -/Human +	LLNA -/Human -
Strong[2]	**Other[3]**			
25 (14 EC3 ≤ 2%; 11 EC3 > 2%)	38 (3 EC3 ≤ 2%; 35 EC3 > 2%)	35 (4 EC3 ≤ 2%; 31 EC3 > 2%)	13 (2 strong; 11 other)[2,3]	25

Abbreviations: DSA_{05} = induction dose per skin area, in $\mu g/cm^2$, in a human repeat-insult patch test or human maximization test that produces a positive response in 5% of the tested population; EC3 = estimated concentration of a substance expected to produce a stimulation index of 3, which is the threshold value for a substance to be considered a sensitizer in the LLNA; LLNA = murine local lymph node assay.

[1] Classification based on geometric mean EC3 (regardless of vehicle and discordant negative results) and DSA_{05} values.

[2] Human skin sensitizers were classified as strong sensitizers if the geometric mean DSA_{05} from HMT and/or HRIPT was ≤ 500 $\mu g/cm^2$.

[3] Human skin sensitizers were classified as other sensitizers if the geometric mean DSA_{05} from HMT and/or HRIPT was > 500 $\mu g/cm^2$.

Figure C-2 shows geometric mean LLNA EC3 values plotted against the geometric mean DSA_{05} values for the 63 LLNA/human sensitizers. Also shown on the edges of the graph are concordant LLNA and human nonsensitizers (n = 25), LLNA false positives (n = 35), and LLNA false negatives (n = 13). The GHS cutoffs, 2% for EC3 and 500 $\mu g/cm^2$ for DSA_{05}, are marked to show the correspondence of the data with GHS Subcategories 1A and 1B.

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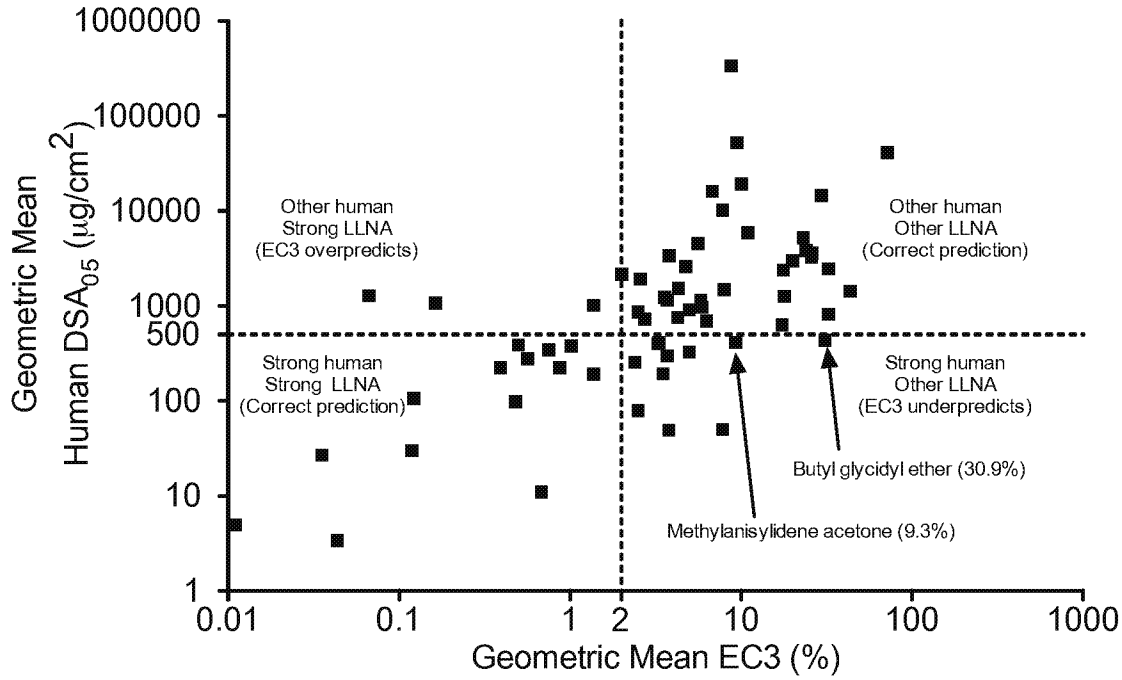
Figure C-2 LLNA EC3 versus Human Results by GHS Potency Category for 136 Substances



Legend:

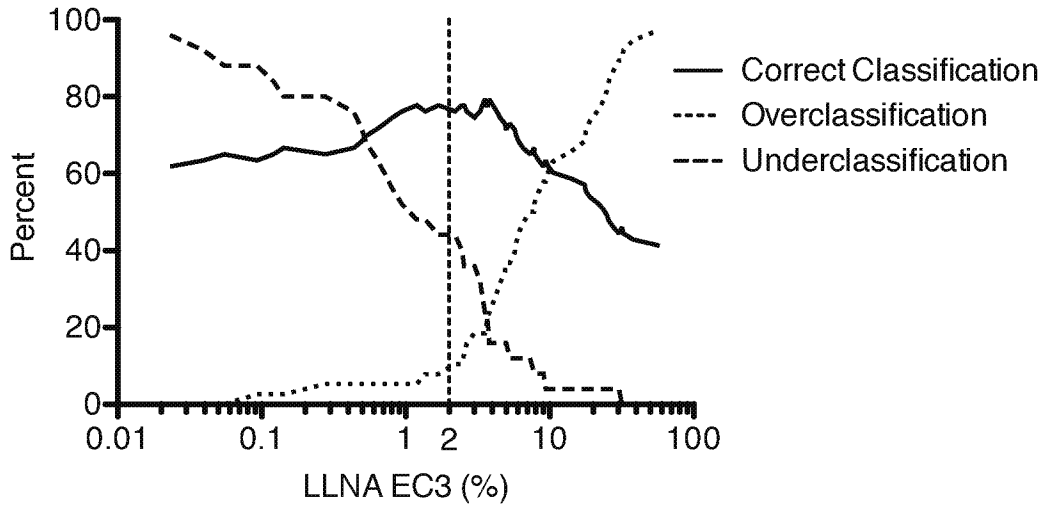
■ Human/LLNA sensitizers (63) ▼ LLNA false negative (13) ▲ LLNA false positive (35)
● Concordant negative (25)

Abbreviations: DSA_{05} = induction dose per skin area, in $\mu g/cm^2$, in a human repeat-insult patch test or human maximization test that produces a positive response in 5% of the tested population; EC3 = estimated concentration of a substance expected to produce a stimulation index of 3, which is the threshold value for a substance to be considered a sensitizer in the LLNA; GHS = Globally Harmonized System of Classification and Labelling of Chemicals (UN 2009); LLNA = murine local lymph node assay.

The relationship between the LLNA EC3 value and the correct human skin sensitization potency classification as well as the under- (i.e., EC3-classified strong human skin sensitizers as other than strong skin sensitizers) and overclassification (i.e., EC3-classified other than strong human sensitizers as strong sensitizers) rates for the 63 substances that were sensitizers in both the LLNA and in human tests are shown in **Figure C-3**. From EC3 ≤ 0.8% to ≤4.5%, the correct classification rate changes little, ranging from 75% (47/63) to 79% (50/63) (see **Annex V**). However, the under- and overclassification rates change remarkably, ranging from 56% (14/25) to 16% (4/25) and from 5% (2/38) to 32% (12/38), respectively. For these data, the optimal EC3 value was ≤3.79%. This EC3 produced the highest correct classification rate, 79% (50/63), with an underclassification rate of 16% (4/25) and an overclassification rate of 24% (9/38). Although EC3 ≤ 3.54% also produced a correct classification rate of 79% (50/63), it yielded a higher underclassification rate (24% [6/25]) than EC3 ≤ 3.79%. The correct classification rate using EC3 ≤ 2% to classify substances as strong skin sensitizers, as prescribed by the GHS, yielded a correct classification rate of 78% (49/63) with an underclassification rate of 44% (11/25) and an overclassification rate of 8% (3/38).

At an EC3 ≤ 9.38%, one strong human skin sensitizer was underclassified as an other than strong sensitizer, in comparison to 11 when using EC3 ≤ 2% to classify substances as strong sensitizers. This suggests that substances with EC3 values in the range of 2% to 10% should be considered as having the potential to cause strong human responses unless there is evidence that indicates otherwise. Also, of note, no strong human sensitizers were underclassified as other sensitizers with an EC3 ≤ 31.00% (see **Figures B-2** and **B-3**).

Figure C-3 Classification Rates for the LLNA EC3 Prediction of Human Skin Sensitization Potency Categories for 63 Sensitizers



Abbreviations: EC3 = estimated concentration of a substance expected to produce a stimulation index of 3, which is the threshold value for a substance to be considered a sensitizer in the LLNA; LLNA = murine local lymph node assay.

Analysis based on 63 substances identified as sensitizers both in the LLNA and in humans using the human maximization test and/or the human repeat-insult patch test. In humans, substances were classified as strong sensitizers (n = 25) if the induction dose (in $\mu g/cm^2$ skin surface) in a human repeat-insult patch test or human maximization test that produced a positive response in 5% of the tested population was ≤500 $\mu g/cm^2$. Those that produced values >500 $\mu g/cm^2$ were classified as other than strong human skin sensitizers (n = 38).

The second approach for the classification analysis included data for the 35 human nonsensitizers that produced false positive results in the LLNA, the 13 human sensitizers that were false negative in the LLNA, and the 25 substances that were concordant nonsensitizers in the LLNA and in humans (see **Table C-7**). This increased the number of substances with comparative LLNA and human data from 63 to 136. Thus, the overall correct classification rate for this analysis includes the correct classification rate for human nonsensitizers, as well as strong and other than strong human sensitizers. Likewise, the overall underclassification rate includes the underclassification of all categories that can be underclassified (strong and other than strong human sensitizers); and the overall overclassification rate includes all categories that can be overclassified (other than strong human sensitizers and nonsensitizers).

The correct, underclassification, and overclassification rates of the LLNA versus human data were initially calculated using EC3 ≤ 2%. As indicated in **Tables C-8** and **C-9**, based on the NICEATM potency database, the LLNA correctly identified 52% (14/27) of the strong human skin sensitizers using EC3 ≤ 2%, but 48% (13/27) were underclassified by the LLNA. Among the 21 substances that produced an EC3 ≤ 2%, 67% (14/21) were strong human skin sensitizers. The remaining 33% (7/21) were either other than strong human sensitizers (n = 3) or substances not classified as human skin sensitizers (nonsensitizers; n = 4).

As shown in **Figure C-2**, most of the strong human sensitizers that were underclassified by the LLNA occurred between EC3 ≤ 2% and ≤10%. With this in mind, the classification rates for human sensitization categories obtained using incremental EC3 cutoff values up to 10% were also evaluated (see **Table C-8**). From EC3 ≤ 2% to ≤4%, the increase in the number of correctly classified strong sensitizers (14 to 21) is almost directly proportional to the decrease in the number of correctly classified other than strong sensitizers (35 to 29). The number of human nonsensitizers overclassified as strong sensitizers increases from four to seven when the LLNA EC3 cutoff moves from ≤2% to ≤4%. With each further increase of 2% in the LLNA EC3 cutoff, the number of correctly classified strong sensitizers increases by one substance. Using LLNA EC3 ≤ 10% to classify substances as strong sensitizers correctly classifies 89% (24/27) of the strong sensitizers compared with the 52% (14/27) of the strong sensitizers correctly classified using EC3 ≤ 2% (see **Table C-9**). However, the proportion of substances classified by the LLNA as strong sensitizers that actually are strong human sensitizers is higher for EC3 ≤ 2% than for EC3 ≤ 10%, 67% (14/21) versus 36% (24/67) (see **Table C-8**).

Figure C-4 shows the change in the overall correct classification (for strong human and other than strong sensitizers and nonsensitizers combined), underclassification (for strong human sensitizers and other sensitizers combined), and overclassification (for human other sensitizers and nonsensitizers combined) rates for the entire range of EC3 cutoff values. From EC3 ≤ 0.8% to ≤4.5%, the overall correct potency classification rate changes little, ranging from 53% (72/136) to 55% (75/136) (see **Annex V**). However, the under- and overclassification rates change noticeably, ranging from 36% (27/76) to 22% (17/76) and 34% (37/109) to 43% (47/109), respectively. For this dataset, the optimal EC3 value was ≤3.79%. This EC3 value produced the highest correct classification rate, which was 55% (75/136), with an underclassification rate of 22% (17/76) and an overclassification rate of 40% (44/109). Although EC3 ≤ 3.54% also produced a correct classification rate of 55% (75/136), it yielded a higher underclassification rate (25% [19/76]) than EC3 ≤ 3.79%.

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Table C-8 Concordance of LLNA and Human Data for Strong Sensitizer, Other Sensitizer, and Nonsensitizer Categories at Selected LLNA EC3 Values

		Strong Sensitizer	Other Sensitizer	Nonsensitizer	Total
		EC3 ≤ 2% (GHS)	**EC3 > 2% (GHS)**	**Negative LLNA**	
Human Data[1]	**Strong Sensitizer**	14	11	2	27
	Other Sensitizer	3	35	11	49
	Nonsensitizer	4	31	25	60
	Total	21	77	38	136
		EC3 ≤ 4%	**EC3 > 4%**	**Negative LLNA**	
Human Data[1]	**Strong Sensitizer**	21	4	2	27
	Other Sensitizer	9	29	11	49
	Nonsensitizer	7	28	25	60
	Total	37	61	38	136
		EC3 ≤ 6%	**EC3 > 6%**	**Negative LLNA**	
Human Data[1]	**Strong Sensitizer**	22	3	2	27
	Other Sensitizer	16	22	11	49
	Nonsensitizer	12	23	25	60
	Total	50	48	38	136
		EC3 ≤ 8%	**EC3 > 8%**	**Negative LLNA**	
Human Data[1]	**Strong Sensitizer**	23	2	2	27
	Other Sensitizer	20	18	11	49
	Nonsensitizer	16	19	25	60
	Total	59	39	38	136
		EC3 ≤ 10%	**EC3 > 10%**	**Negative LLNA**	
Human Data[1]	**Strong Sensitizer**	24	1	2	27
	Other Sensitizer	23	15	11	49
	Nonsensitizer	20	15	25	60
	Total	67	31	38	136

Abbreviations: DSA_{05} = induction dose per skin area, in $\mu g/cm^2$, in a human repeat-insult patch test or human maximization test that produces a positive response in 5% of the tested population; EC3 = estimated concentration of a substance expected to produce a stimulation index of 3, which is the threshold value for a substance to be considered a sensitizer in the LLNA; GHS = Globally Harmonized System of Classification and Labelling of Chemicals (UN 2009); LLNA = murine local lymph node assay.

[1] Data for human sensitizers were DSA_{05} values (i.e., induction dose, in $\mu g/cm^2$ skin surface, in a human repeat-insult patch test or human maximization test that produced a positive response in 5% of the tested population). Sensitizers were classified as strong if $DSA_{05} \leq 500\ \mu g/cm^2$ and other if $DSA_{05} > 500\ \mu g/cm^2$ (UN 2009).

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Table C-9 Correct, Underclassification, and Overclassification Rates for Prediction of Human Potency Categories by Selected LLNA EC3 Cutoff Values[1] for 136 Substances

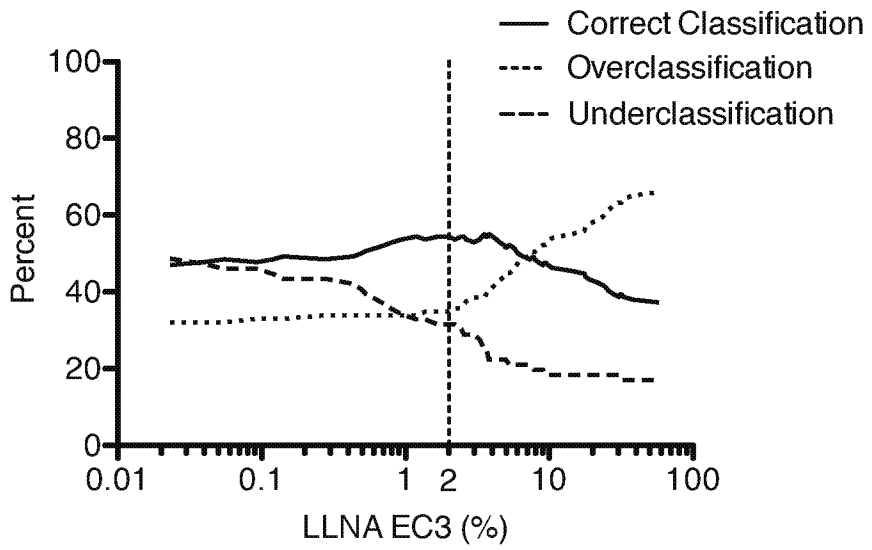
EC3 Cutoff for Strong versus Other Sensitizers	Strong Human Sensitizers ($DSA_{05} \leq 500$ µg/cm^2)		Other Human Sensitizers ($DSA_{05} > 500$ µg/cm^2)			Human Nonsensitizers		Overall Correct Potency Classification[2]
	Correct	Under	Over	Correct	Under	Correct	Over	
GHS Cutoff EC3 ≤ 2%	52 ± 19% (14/27)	48 ± 19% (13/27)	6 ± 7% (3/49)	71 ± 13% (35/49)	22 ± 12% (11/49)	42 ± 12% (25/60)	58 ± 12% (35/60)	54 ± 8% (74/136)
EC3 ≤ 4%	78 ± 16% (21/27)	22 ± 16% (6/27)	18 ± 11% (9/49)	59 ± 14% (29/49)	22 ± 12% (11/49)	42 ± 12% (25/60)	58 ± 12% (35/60)	54 ± 8% (74/136)
EC3 ≤ 6%	81 ± 15% (22/27)	19 ± 15% (5/27)	33 ± 13% (16/49)	45 ± 14% (22/49)	22 ± 12% (11/49)	42 ± 12% (25/60)	58 ± 12% (35/60)	50 ± 8% (68/136)
EC3 ≤ 8%	85 ± 13% (23/27)	15 ± 13% (4/27)	41 ± 14% (20/49)	37 ± 13% (18/49)	22 ± 12% (11/49)	42 ± 12% (25/60)	58 ± 12% (35/60)	48 ± 8% (65/136)
EC3 ≤ 10%	89 ± 12% (24/27)	11 ± 12% (3/27)	47 ± 14% (23/49)	31 ± 13% (15/49)	21 ± 12% (11/49)	42 ± 12% (25/60)	58 ± 12% (35/60)	47 ± 8% (64/136)

Abbreviations: DSA_{05} = induction dose per skin area, in µg/cm^2, in a human repeat-insult patch test or human maximization test that produces a positive response in 5% of the tested population; EC3 = estimated concentration of a substance expected to produce a stimulation index of 3, which is the threshold value for a substance to be considered a sensitizer in the LLNA; GHS = Globally Harmonized System of Classification and Labelling of Chemicals (UN 2009); LLNA = murine local lymph node assay.

[1] Classification rates are shown ±95% confidence limits.

[2] The overall correct classification rate includes the correct classifications of strong human sensitizers, other than strong sensitizers, and nonsensitizers.

Figure C-4 Overall Correct, Underclassification, and Overclassification Rates for LLNA EC3 Prediction of Human Potency Category for 136 Substances



Abbreviations: EC3 = estimated concentration of a substance expected to produce a stimulation index of 3, which is the threshold value for a substance to be considered a sensitizer in the LLNA; LLNA = murine local lymph node assay.

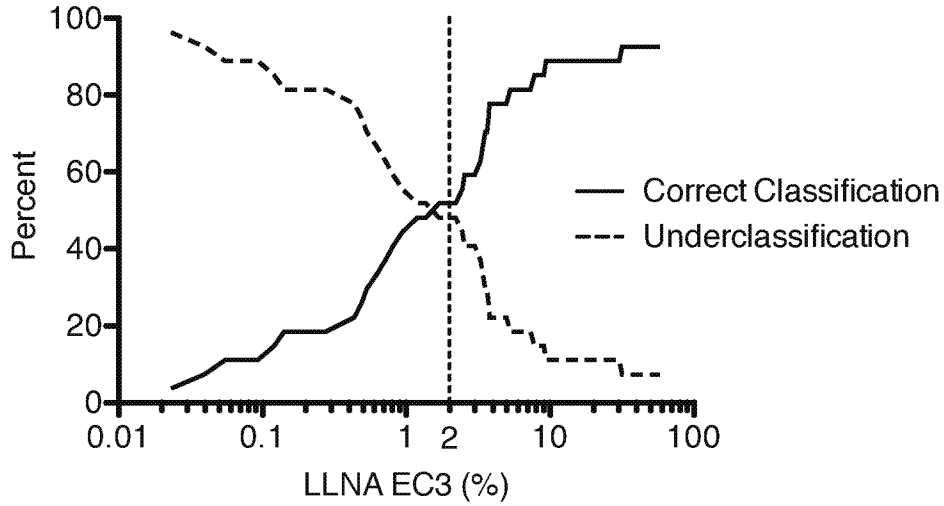
Analysis based on 136 substances: 63 sensitizers in both the LLNA and humans using the human maximization test and/or the human repeat-insult patch test (27 strong human sensitizers and 38 other than strong human sensitizers), 25 concordant nonsensitizers in the LLNA and in humans, 35 LLNA false positives, and 13 LLNA false negatives. In humans, sensitizers were classified as strong or other than strong sensitizers if the induction dose (in $\mu g/cm^2$ skin surface) in a human maximization test or a human repeat-insult patch test that produced a positive response in 5% of the tested population was $\leq 500\ \mu g/cm^2$. Sensitizers that produced a positive response of $>500\ \mu g/cm^2$ were classifed as other sensitizers (UN 2009).

The overall correct classification rate includes the correct classifications of strong human sensitizers, other than strong sensitizers, and nonsensitizers. The overall overclassification rate includes the overclassifications of other than strong human sensitizers and nonsensitizers, while the overall underclassification rate includes the underclassifications of strong human sensitizers and other than strong sensitizers.

Figure C-5 shows the change in the correct classification and underclassification rates for the 27 strong human sensitizers over the entire range of LLNA EC3 cutoff values. The correct potency classification rate for strong human sensitizers increases and the underclassification rate decreases as the EC3 increases. The correct classification rate plateaus, however, because the two strong human sensitizers that yielded negative results in the LLNA will not be correctly classified by any EC3 cutoff.

Fourteen percent (11/77) of the substances with EC3 > 2% are strong human sensitizers ($DSA_{05} \leq 500\ \mu g/cm^2$). In addition, 5% (2/38) of the substances that were negative in the LLNA were strong sensitizers.

Figure C-5 Correct and Underclassification Rates for LLNA EC3 Prediction of 27 Strong Human Sensitizers



Abbreviations: EC3 = estimated concentration of a substance expected to produce a stimulation index of 3, which is the threshold value for a substance to be considered a sensitizer in the LLNA; LLNA = murine local lymph node assay.

Analysis based on 27 substances identified as strong sensitizers in humans using the human maximization test and/or the human repeat-insult patch test based on the induction dose that produced a positive response in 5% of the tested population was ≤500 μg/cm^2.

6.1.3 Evaluation of Strong Sensitizers Underclassified by LLNA EC3 ≤ 2%

The thirteen strong human sensitizers that were underclassified by the LLNA EC3 ≤ 2% criterion are shown in **Table C-10** in the order of increasing EC3. The two substances that had predominantly negative LLNA results, nickel salts and streptomycin, would have been underclassified even if their positive results were used in the analysis. The two positive nickel results were for nickel sulfate in dimethyl sulfoxide (DMSO), EC3 = 4.8%, and nickel chloride in 30% ethanol, EC3 = 5.5% (see **Annex II-1**). The positive result for streptomycin yielded EC3 = 33% in dimethylformamide (DMF) (see **Annex II-1**). Ten of the 11 remaining discordant substances had EC3 values less than 10%. Butyl glycidyl ether was the only strong human sensitizer with LLNA EC3 > 10%. Using a criterion of LLNA EC3 ≤ 4% to classify substances as strong sensitizers would have correctly classified seven of the 13 discordant substances. Using LLNA EC3 ≤ 10% would have correctly classified 10 of the 13 discordant substances.

There are few commonalities among these 13 substances with regard to chemical class, physical form, molecular weight, peptide reactivity, and K_{ow} (see **Annex III** for physicochemical information):

- The 13 substances represent 10 chemical classes: aldehydes, amines, carbohydrates, carboxylic acids, ethers, heterocyclic compounds, inorganic chemicals, ketones, lipids, and organic sulfur compounds.
- Most (8/13) of the substances are liquids.
- The molecular weights of 12 of the 13 substances range from 98.15 (trans-2-hexenal) to 192.3 (delta-damascone). The exception is streptomycin (1457.39).
- Peptide reactivity information was available for only 6 of 13 substances. Five of the six substances (benzoisothiazolinone, benzylidene acetone, diethyl maleate, trans-2-hexenal, and methyl-2-nonynoate) had high peptide reactivity, and one substance (phenylacetaldehyde) had moderate peptide reactivity.
- K_{ow} ranged from -8.5 (streptomycin) to 4.16 (delta-damascone).

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Table C-10 Strong Human Sensitizers Underclassified by LLNA EC3 ≤ 2%[1]

Substance	LLNA EC3 (%/μg/cm²)	Human Results		
		DSA_{05} (μg/cm²)	Concentration Tested	Response Rate
2-Hexylidene cyclopentanone	2.40/600	255 (HRIPT)	NA	9.8% (5/51)
Methyl-2-nonynoate	2.50/625	79 (HRIPT)	NA	7.5% (5/67)
Diethylmaleate	3.27/818	400 (HMT, HRIPT)	4% 4%	100% (24/24) 7.5% (14/187)
Diethylenetriamine	3.30/825	411 (HMT)	10%	84% (21/25)
delta-Damascone	3.51/877	193 (HRIPT)	NA	13% (7/54)
Benzylidene acetone	3.70/925	299 (HMT, HRIPT)	2% 3%	48% (12/25) 9.7% (6/62)
trans-2-Hexenal	3.78/945	49 (HRIPT)	NA	24% (6/25)
Phenylacetaldehyde	4.99/1250	329 (HRIPT, HMT)	NA 2% 2% 2% 2%	13% (7/53) 44% (11/25) 16% (4/25) 52% (13/25) 8% (2/25)
Benzoisothiazolinone	7.79/1950	50 (HRIPT)	0.0725%	9% (5/58)
Methylanisylidene acetone	9.30/2325	412 (HMT)	8%	67% (16/24)
Butyl glycidyl ether	30.9/7725	437 (HMT)	10%	79% (19/24)
Nickel salts	Negative (8/10 tests)	27 (HMT)	1% 1% 10%	26% (6/323) 17% (4/24) 48% (12/25)
Streptomycin	Negative (4/5 tests)	245 (HMT)	0.1% 0.1% 5% 10% 10% 25%	4% (10/24) 13% (3/23) 50% (12/24) 65% (15/23) 100% (24/24) 80% (20/25)

Abbreviations: DSA_{05} = induction dose per skin area, in μg/cm², in an HRIPT or HMT that produces a positive response in 5% of the tested population; EC3 = estimated concentration of a substance expected to produce a stimulation index of 3, which is the threshold value for a substance to be considered a sensitizer in the LLNA; HMT = human maximization test; HRIPT = human repeat-insult patch test; LLNA = murine local lymph node assay; NA = not available.

[1] Shown in order of increasing LLNA EC3.

6.2 Comparison of LLNA versus Guinea Pig Predictions of Human Skin Sensitization Potency

Skin sensitization potency in guinea pigs from GPMT and BT results can also be used to classify substances as strong or other skin sensitizers (see **Section 1.1.3**). Thus, it was deemed useful to also evaluate the capacity of the guinea pig outcomes to agree with the human skin sensitization potency classification (see **Table C-1**) and to compare the guinea pig performance with the LLNA performance. Due to the categorical nature of the data collected in the guinea pig tests (i.e., using

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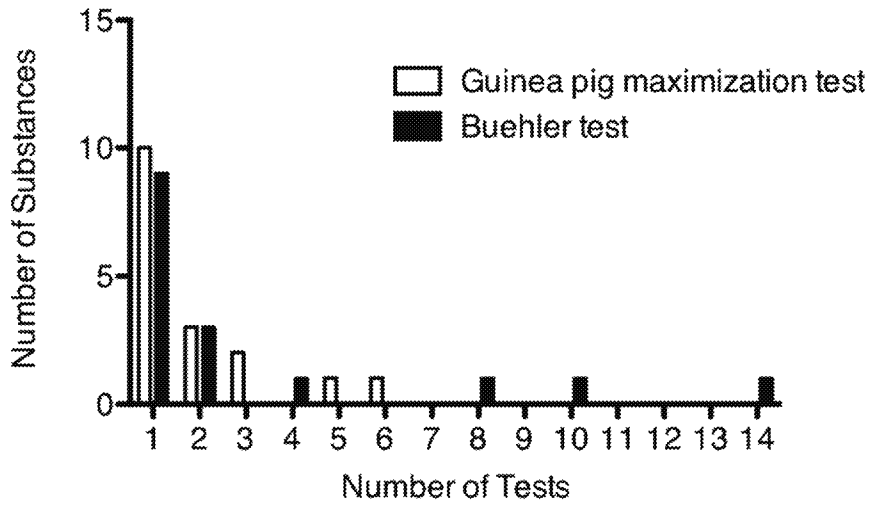
incidence of sensitized animals with particular test substance concentrations), a regression analysis with human DSA_{05} values could not be conducted. However, substances tested in the GPMT or the BT could be assigned a potency classification (strong, other, or nonsensitizer) based on the GHS decision criteria in **Table C-1**. The capacity of the guinea pig outcomes to correctly classify human skin sensitization potency was evaluated and compared with the capacity of the LLNA to correctly classify human skin sensitization potency using a classification rate analysis.

The current NICEATM potency database of 196 substances includes 116 substances with guinea pig test data and 56 substances with both human and guinea pig data (see **Annex III**). Twenty-eight substances are classified as sensitizers in the LLNA, guinea pig tests, and human tests. **Figure C-6** shows the frequency distribution of the 28 sensitizers and the number of guinea pig studies conducted. The number of GPMT results per substance ranged from one to six, and the number of BT results per substance ranged from one to 14. Sixteen substances had GPMT results, and 17 substances had BT results. Ten of the 28 sensitizers had both GPMT and BT results.

Figure C-6 Distribution of 28 Substances Classified as Sensitizers in Guinea Pig Tests, LLNA, and Human Tests for the Number of Guinea Pig Studies Conducted[1]



Abbreviations: LLNA = murine local lymph node assay.

[1] Analysis based on 28 substances that tested as sensitizers in the guinea pig tests (i.e., guinea pig maximization test and Buehler test), LLNA, and in humans (human maximization test and human repeat-insult patch test).

Results from each guinea pig test were assigned to strong (GHS Subcategory 1A), other (GHS Subcategory 1B), or nonsensitizer (not classified) categories based on the decision criteria in **Table C-1**. The most prevalent outcome was used to categorize the guinea pig test results for substances with multiple tests. In this approach, test results from either GPMT or BT tests (i.e., as per the decision criteria in **Table C-1**) were considered together when assigning an overall classification category according to **Table C-1**. For example, there were six GPMTs and one BT for benzocaine. Three of the GPMT results were classified as other sensitizers (GHS Subcategory 1B), and three yielded nonsensitizer results (i.e., not classified). The BT result was classified as other skin sensitizer (GHS Subcategory 1B). Thus, based on the four GHS Subcategory 1B tests versus the three not classified tests, benzocaine was classified as GHS Subcategory 1B (i.e., other skin sensitizer). If a substance had an equal number of tests classified in two or more categories, the most potent result was used to represent the guinea pig potency classification for the substance.

ED_006472_00084346-00124

Next, the correct classification rate as well as the under- and overclassification rates for guinea pig determinations of human potency category were calculated. Strong skin sensitizer (GHS Subcategory 1A), other skin sensitizer (GHS Subcategory 1B), and nonsensitizer (not classified) categories were calculated. The correct classification rate, underclassification rate, and overclassification rate for LLNA determinations of human potency category for the same substances were also calculated for comparison. For substances that had more than one LLNA EC3 or guinea pig response, the geometric mean EC3 value and the most prevalent guinea pig classification category were used. Two approaches were used to estimate the classification rates for the guinea pig determination of strong and other human sensitizers. In the first approach, the classification analysis considered only the 28 substances classified as sensitizers in guinea pigs, based on GPMT and/or BT results; in the LLNA; and in humans, based on the HMT and/or HRIPT.

In the second approach, the analysis included additional guinea pig and human sensitizers that were negative in the LLNA (n = 4), substances that were guinea pig false positives (n = 7) and false negatives (n = 3) against human skin sensitization data, and those classified as nonsensitizers in guinea pigs and humans (n = 14). The 56 substances used for the second approach also included

- Substances that were negative in guinea pigs but positive in the LLNA and humans (n = 1)
- LLNA false positives (n = 11) and false negatives (n = 6) against human skin sensitization data
- Substances classified as nonsensitizers both in the LLNA and in humans (n = 10)

The classification rate results are provided in **Table C-11**. In the first analysis, which focused only on the 28 substances classified as sensitizers in guinea pigs (i.e., GPMT and/or BT), the LLNA, and humans, overclassification means that other sensitizers (GHS Subcategory 1B) are misclassified as strong sensitizers (GHS Subcategory 1A), while underclassification means that strong sensitizers (GHS Subcategory 1A) are misclassified as other sensitizers (GHS Subcategory 1B). Using the guinea pig tests to determine human potency category, the overall correct classification rate (i.e., correctly classified strong human sensitizers plus correctly classified other human sensitizers) was 64% (18/28). The guinea pig tests correctly classified 67% (8/12) of the strong human sensitizers and 63% (10/16) of the other human sensitizers. Guinea pig results underclassified 33% (4/12) of the strong human sensitizers and overclassified 37% (6/16) of the other human sensitizers.

The correct classification rate of the LLNA, using EC3 ≤ 2% to classify substances as strong sensitizers and EC3 > 2% to classify substances as other sensitizers, was higher than that for the guinea pig tests (**Table C-11**). The overall correct classification rate of human sensitizers by the LLNA was 82% (23/28). The LLNA correctly classified 83% (10/12) of the strong human sensitizers and 81% (13/16) of the other human sensitizers. The LLNA underclassified 17% (2/12) of the strong human sensitizers and overclassified 19% (3/16) of the other human sensitizers.

The second analysis of 56 substances included guinea pig and LLNA false negatives, false positives, and concordant negatives relative to human data. The overall correct classification rate included correctly classified strong human sensitizers (GHS Subcategory 1A), other sensitizers (GHS Subcategory 1B), and nonsensitizers (not classified). Using the guinea pig tests to determine human potency categories, the overall correct classification rate was 59% (33/56). The guinea pig tests correctly classified 57% (8/14) of the strong human sensitizers, 52% (11/21) of the other human sensitizers, and 67% (14/21) of the human nonsensitizers. Guinea pig results underclassified 43% (6/14) of the strong human sensitizers and 14% of the other human sensitizers. Guinea pig results overclassified 33% (7/21) of the other human sensitizers and 33% (7/21) of the human nonsensitizers.

The overall correct classification rate produced by the LLNA, using EC3 ≤ 2% to classify substances as strong sensitizers and EC3 > 2% to classify substances as other sensitizers, was similar to that for the guinea pig tests (**Table C-11**). The LLNA's overall correct classification rate of human sensitizers and nonsensitizers was 61% (34/56) versus 59% (33/56) for the guinea pig tests. The LLNA correctly

classified more strong sensitizers and other sensitizers than the guinea pig tests did but correctly classified fewer nonsensitizers. The LLNA correctly classified 71% (10/14) of the strong human sensitizers versus 57% (8/14) for the guinea pig tests, 67% (14/21) of the other human sensitizers versus 52% (11/21) for the guinea pig tests, and 48% (10/21) of the nonsensitizers versus 67% (14/21) for the guinea pig tests. Guinea pig tests underclassified 43% (6/14) of the strong human sensitizers and overclassified 33% (7/21) of the other human sensitizers and 33% (7/21) of the human nonsensitizers. The LLNA underclassified 29% (4/14) of the strong human sensitizers and overclassified 14% (3/21) of the other human sensitizers and 52% (11/21) of the human nonsensitizers.

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Table C-11 Comparative Correct Classification, Underclassification, and Overclassification Rates[1] When the GHS Criteria[2] for Guinea Pig Tests and the LLNA EC3 Are Used to Determine Human Skin Sensitization Potency Category

Comparison	Classification							
	Strong Sensitizer ($DSA_{05} \leq 500$ µg/cm^2)		Other Sensitizer ($DSA_{05} > 500$ µg/cm^2)			Nonsensitizer		Overall Correct Classification[3]
	Correct	Under	Over	Correct	Under	Correct	Over	
GPMT and/or BT determination of human potency for 28 guinea pig, LLNA, and human sensitizers	67 ± 27% (8/12)	33 ± 27% (4/12)	37 ± 24% (6/16)	63 ± 24% (10/16)	NA	NA	NA	64 ± 18% (18/28)
LLNA EC3 determination of human potency (EC3 ≤ 2% for strong, EC3 > 2% for other) for 28 sensitizers in guinea pigs, LLNA, and humans	83 ± 21% (10/12)	17 ± 21% (2/12)	19 ± 19% (3/16)	81 ± 19% (13/16)	NA	NA	NA	82 ± 14% (23/28)
GPMT and/or BT determination of human potency for 56 substances that include guinea pig and LLNA false positives, false negatives, and concordant negatives	57 ± 26% (8/14)	43 ± 26% (6/14)	33 ± 20% (7/21)	52 ± 21% (11/21)	14 ± 25% (3/21)	67 ± 20% (14/21)	33 ± 20% (7/21)	59 ± 13% (33/56)
LLNA EC3 determination of human potency (EC3 ≤ 2% for strong, EC3 > 2% for other) for 56 substances that include guinea pig and LLNA false positives, false negatives, and concordant negatives	71 ± 24% (10/14)	29 ± 24% (4/14)	14 ± 15% (3/21)	67 ± 20% (14/21)	19 ± 17% (4/21)	48 ± 21% (10/21)	52 ± 21% (11/21)	61 ± 13% (34/56)

Abbreviations: BT = Buehler test; DSA_{05} = induction dose per skin area, in µg/cm^2, in a human repeat-insult patch test or human maximization test that produces a positive response in 5% of the tested population; EC3 = estimated concentration of a substance expected to produce a stimulation index of 3, which is the threshold value for a substance to be considered a sensitizer in the LLNA; GPMT = guinea pig maximization test; LLNA = murine local lymph node assay; NA = not applicable because only substances that were sensitizers in humans, guinea pigs, and the LLNA were evaluated (i.e., other sensitizers can only be overclassified, and nonsensitizers were not evaluated).

[1] Classification rates are shown ±95% confidence limits.

[2] The criteria for distinguishing between strong and other sensitizers using the LLNA, GPMT, BT, and human tests are provided in **Table C-1**. For substances multiply tested in the GPMT and/or BT, the majority classification category was used. When an equal number of discordant classifications were recorded, the most severe classification category was used. Substances that were tested in the LLNA and the human repeat-insult patch test and/or human maximization test were represented by a geometric mean EC3 value and DSA_{05} values, respectively.

[3] The proportion of substances correctly assigned to each GHS category for skin sensitization potency (i.e., strong sensitizer, other sensitizer, and nonsensitizer, if applicable).

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7.0 Test Method Reliability

An assessment of test method reliability (intralaboratory repeatability and intra- and interlaboratory reproducibility) is an essential element of any performance evaluation of an alternative test method (ICCVAM 2003). *Repeatability* refers to the closeness of agreement between test results obtained within a single laboratory when the procedure is performed on the same substance under identical conditions within a given time period (ICCVAM 1997, 2003). *Intralaboratory reproducibility* refers to the extent to which qualified personnel within the same laboratory can replicate results using a specific test protocol at different times. *Interlaboratory reproducibility* refers to the extent to which different laboratories can replicate results using the same protocol and test substances. It indicates the extent to which a test method can be transferred successfully among laboratories.

7.1 Variability of LLNA EC3 Values Using the Same Vehicle

As described in **Section 6.0**, the use of the LLNA for skin sensitization potency assessments depends on determining an accurate EC3 value for sensitizers. Thus, not only does the LLNA need to reproducibly achieve the correct sensitizer versus nonsensitizer result, but it also needs to reproducibly assign the proper skin sensitization potency classification. An evaluation of the intralaboratory variability associated with 29 individual EC3 values for isoeugenol, which ranged from 0.5% to 2.6%, was considered by Basketter and Cadby (2004) to support the "often-mentioned perspective that the biological variation associated with the estimation of EC3 values means that any particular EC3 can be halved or doubled."

The Basketter et al. submission evaluated EC3 data for 17 sensitizers tested in at least two laboratories as a measure of interlaboratory reproducibility of the EC3 value (see **Annex I**). The authors conclude that although there is biological variation in the EC3 values among substances tested multiple times using the same vehicle this variation is less than an order of magnitude.

Jowsey et al. (2008) assessed the inherent variability of the LLNA by examining the reproducibility of EC3 values for 14 substances tested more than once in AOO. These data were obtained from an LLNA dataset published by Basketter et al. (2007a). The variability was measured by dividing the maximum observed EC3 value by the minimum observed EC3 value. The results ranged from 1 to 4. Based on this outcome, a factor of five-fold was assumed a reasonable estimate of how variable EC3 values might be for a substance tested in the same vehicle multiple times.

Table C-12 provides available EC3 values for 45 substances tested in multiple vehicles. These data were selected from the current NICEATM LLNA database of over 600 substances by identifying the vehicles used in at least five LLNA studies. Thirteen percent (6/45) of the substances have discordant sensitizer/nonsensitizer LLNA results in the same vehicle.

7.2 Vehicle Effects on LLNA Results

Many factors affect skin sensitization. Two important factors are (1) the ability of the test substance to traverse the stratum corneum and reach the viable epidermis and (2) the efficiency of Langerhans cell migration from the skin. Both of these factors are susceptible to vehicle effects; therefore, the vehicle chosen for LLNA testing can have an impact on results (Basketter et al. 2001; Lea et al. 1999; McGarry 2007; Wright et al. 2001). Such effects need to be considered when evaluating the reproducibility of the LLNA in assigning the proper skin sensitization potency category.

7.2.1 Published Studies Regarding Vehicle Effects on LLNA Results

Jowsey et al. (2008) evaluated the impact of vehicle on the relative potency of skin-sensitizing substances tested in the LLNA. The authors compared EC3 values for 18 substances tested in at least two of 15 different vehicles using data from previously published results and unpublished Unilever

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results (**Table C-13**). The most common vehicles were AOO, DMF, and DMSO. The substances tested in AOO had EC3 values that ranged from 0.005% to 36.5% (nearing four orders of magnitude) with similar outcomes observed for DMF and DMSO. When evaluating EC3 values for each substance when it was tested in a different vehicle, the resulting variability for 50% (9/18) of the substances was no greater than the variability observed when substances were tested in the same vehicle (i.e., five-fold). Dinitrobenzene sulfonate, 1,4-dihydroquinone, and nickel sulfate were not included in this evaluation because their lowest or highest EC3 values were reported as greater than or less than a particular value. The EC3 values for 33% (6/18) of the substances differed by a factor of at least 10 when the substances were tested in different vehicles. In most cases (83% [5/6]), higher EC3 values (lower potency) were observed mostly with aqueous vehicles and propylene glycol. When these data were applied to the GHS classification scheme (see **Table C-1**) (UN 2009), seven substances (39% [7/18]) would have been assigned to different skin sensitization potency categories depending on the vehicle used (see **Table C-13**). 1,4-Dihydroquinone could not be evaluated because the highest EC3 was >1. The authors conclude that the LLNA vehicle can have an impact on potency. Although the effect is likely small, there are exceptions, and this knowledge is necessary for risk assessment.

McGarry (2007) performed an analysis similar to that in **Tables C-12** and **C-13** using the four-category LLNA potency system proposed by Kimber et al. (2003) (**Table C-2**) to demonstrate that the vehicle used affects the EC3 value and the resulting skin sensitization potency classification of a substance (see **Table C-14**). Among seven substances for which data from tests in multiple vehicles were available, six substances (86%) would have been assigned to different skin sensitization potency categories (see **Table C-2**) depending on the vehicle used. When these data were applied to the GHS classification scheme (see **Table C-1**) (UN 2009), two substances (29% [2/7]) would have been assigned to different skin sensitization potency categories depending on the vehicle used (see **Table C-14**). The EC3 values among the different vehicles differed by less than 10-fold for all seven substances evaluated.

Wright et al. (2001) also investigated the influence of application vehicle on sensitizing potency, using the LLNA to examine four recognized human contact allergens: isoeugenol, cinnamic aldehyde, and two fragrance chemicals. The fragrance chemicals are 3-dimethylaminopropylamine (a sensitizing impurity of cocamidopropyl betaine, a surfactant used in shower gel) and dibromodicyanobutane (the sensitizing component of Euxyl K 400, a preservative used in cosmetics). The four chemicals were applied in each of seven different vehicles (AOO, DMF, MEK, DMF, PG, and 50:50 and 90:10 mixtures of ethanol and water). It was found that the vehicle in which a chemical is presented to the epidermis had a marked effect on sensitizing activity. EC3 values ranged from 0.9% to 4.9% for isoeugenol, from 0.5% to 1.7% for cinnamic aldehyde, from 1.7% to >10% for 3-dimethylaminopropylamine, and from 0.4% to 6.4% for dibromodicyanobutane. These authors confirm that the vehicle in which a chemical is encountered on the skin has an important influence on the relative skin-sensitizing potency of chemicals and may have a significant impact on the elicitation of ACD.

7.2.2 NICEATM Evaluation of Vehicle Effects on LLNA Results

The data in **Table C-12** indicate that the assigned skin sensitization potency classification, strong versus other than strong sensitizer, differed by vehicle for 18% (8/45) of these substances when using the GHS classification scheme (see **Table C-1**) (UN 2009). Only 9% (4/45) of the substances had EC3 values that varied by at least an order of magnitude depending upon the vehicle used in the LLNA. Another 24% (11/45) of the substances were classified differently as either sensitizers or nonsensitizers depending on the vehicle. This is almost two times the number of substances that had discordant sensitizer or nonsensitizer results in the same vehicle (n = 6).

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Table C-12 LLNA EC3 Values by Vehicle for 45 Substances with Positive Results (from the NICEATM LLNA Database)

Substance	LLNA Vehicle and Associated EC3 Values (%)											GP	Human
	AOO[1]	DMF[1]	MEK[1]	PG[1]	DMSO[1]	ACE	DEP	EtOH	EtOH/DEP (3:1)	EtOH/DEP (1:3)	L92		
2-Amino-6-chloro-4-nitrophenol	2.20				6.85							NA	NA
5-Amino-o-cresol	7.72				3.80							NA	NA
3-Aminophenol	**3.20**	**0.24**										**+**	**NA**
Amylcinnamic aldehyde	11.70[2]									7.60		+	-
Aniline	37.95[3]		25.79[2]									+	+
Atrazine						NC					35.96[2]	-	NA
Benzocaine	8.26[3]	3.37[3]				NC						+	+
Benzyl benzoate	17.00									NC		NA	-
Beryllium sulfate		0.68			NC							NA	+
Carvone	12.95[2]									7.81[3]		NA	+
(Chloro)methylisothia-zolinone	0.012[2]	0.008[2]	0.007	0.055[2]	0.008	0.005[2]						+	+
Cinnamic aldehyde	1.67[2]	0.56[2]	1.09	1.36	1.06[2]				0.42[2]			+	+
Citral	8.48[2]								4.94[2]	2.75[2]		+	+
Coumarin	NC	29.58[3]										NA	+
delta-Damascone	2.12[2]									9.60		NA	+
3,4-Dihydrocoumarin	5.60	3.25										+	+
1,4-Dihydroquinone	0.11[2]	0.21[2]	0.09[2]	NC								+	NA[4]
2,4-Dinitrobenzene sulfonic acid		**0.83**			**1.98**						**6.40**	**NA**	**NA**
2,4-DNCB	0.047[2]				0.015	0.012						+	+
Disperse blue 106		0.008			0.014[2]							NA	NA
Ethylenediamine	2.20	3.40				NC						+	+
Eugenol	11.31[2]					18.16	15.10[2]	10.70	5.30	7.53[2]		+	+
Formaldehyde	**0.82[2]**	**0.30[2]**		**2.80**	**0.30**	**0.60[2]**					**7.03[2]**	**+**	**+**
Geraniol	38.50[3]						11.80	5.60	25.8	15.25[2]		+	+
Glutaraldehyde	0.12[2]	0.02		1.50		0.08[2]						+	+
Glyoxal	1.40	0.60										NA	+
trans-2-Hexenal	5.50									2.60		NA	+

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Substance	LLNA Vehicle and Associated EC3 Values (%)											GP	Human
	AOO[1]	DMF[1]	MEK[1]	PG[1]	DMSO[1]	ACE	DEP	EtOH	EtOH/DEP (3:1)	EtOH/DEP (1:3)	L92		
HCA	**9.53[2]**					**1.21**					**10.14[2]**	+	-
Hydroxycitronellal	24.23[2]	18.85					19.70	26.40	22.20	19.30		+	+
2-Hydroxyethyl acrylate	**2.96[2]**	**1.56**										+	**NA**
Isoeugenol	**1.31[2]**	**1.45**	**0.96**	**2.50**	**0.92**							+	+
Lilial	17.72[2]						4.10	2.90	8.80	13.90		NA	+
Limonene	69.00						44.19[2]	10.00	22.00	38.00		+	-
Methylhydrocinnamal	17.36[2]	23.10										NA	+
Methyl methacrylate	90.00					60.00						+	NA
Methyl salicylate	NC[2]	25.00	11.50			NC						-	-
Nickel salts		NC			4.80[3]						NC	+	+
Oxazolone	0.002[2]					0.001						+	NA
Penicillin G		5.46[2]			26.78[2]							+	+
Potassium dichromate		0.33			0.09[2]						0.20[2]	+	+
Resorcinol	5.92[3]	NC										NA[5]	-[6]
Salicylic acid	NC					12.22						-	-
SLS		4.32[2]			2.78[2]						4.90	-	-
Tetraethylthiuram disulfide	**1.40**					**5.42**						**NA**	+
Zinc diethyldithiocarbamate	0.24					1.01						NA	NA

Abbreviations: + = sensitizer; - = nonsensitizer; ACE = acetone; AOO = acetone: olive oil (4:1 by volume); DEP = diethyl phthalate; DMF = dimethylformamide; DMSO = dimethyl sulfoxide; DNCB = dinitrochlorobenzene; EC3 = estimated concentration of a substance expected to produce a stimulation index of 3, which is the threshold value for a substance to be considered a sensitizer in the LLNA; EtOH = ethanol; GHS = Globally Harmonized System of Classification and Labelling of Chemicals (UN 2009); HCA = hexyl cinnamic aldehyde; L92 = 1% Pluronic L92; LLNA = murine local lymph node assay; MEK = methyl ethyl ketone; NA = not available; NC = not calculated because the stimulation index < 3.0; NICEATM = National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods; PG = propylene glycol; SLS = sodium lauryl sulfate; TG = test guideline.

Boldface text highlights substances for which a discordant sensitizer subcategory (using the GHS EC3 cutoff of 2%; see **Table C-2**) would be assigned depending on the vehicle used in the LLNA.

[1] Vehicles recommended in OECD TG 429, listed in order of preference (OECD 2002). OECD TG 429 also indicates that other vehicles may be used with sufficient scientific rationale.

[2] Value represents a geometric mean of n ≥ 2 EC3 values, or, for negative results, n ≥ 2 negative tests.

[3] Value represents a geometric mean of n ≥ 2 EC3 values. Additional tests in this vehicle were negative (i.e., stimulation index < 3).

[4] Although no human maximization test or human repeat-insult patch test data were reported, 1,4-dihydroquinone has been reported to be a sensitizer in humans (Basketter et al. 1999a).

[5] Although the specific guinea pig test method and exposure/incidence data were not reported, resorcinol has been reported to be a nonsensitizer in guinea pigs (Basketter et al. 1996).

[6] Resorcinol was negative in the human maximization test (Kligman 1966), but sensitization in humans has been reported (Basketter et al. 2007b).

ED_006472_00084346-00131

Table C-13 LLNA EC3 Values for 18 Skin Sensitizers Tested in Different Vehicles (from Jowsey et al. 2008a)

Substance	LLNA Vehicle and Associated EC3 Values (%)															GP	Human
	AOO[1]	DMF[1]	MEK[1]	PG[1]	DMSO[1]	ACE	APS	L92	H_2O	EtOH/ H_2O (9:1)	EtOH/ H_2O (1:1)	EtOH	EtOH/ DEP (3:1)	EtOH/ DEP (1:3)	DEP		
Dinitro-benzene sulfonate		**<1**			**2**			**6.4**	**16**							**NA**	**NA**
1,4-Dihydro-quinone	0.15[2]	0.21[2]	0.09[2]	>1	0.35[2]	0.75[2]	>1									+	NA[3]
4-Phenylene-diamine	**0.11[2]**						**2.5**									+	+
3-Dimethyl-amino-propyl-amine	**2.2**	**1.7**	**1.8**	**>10**	**2.76**					**4.1**	**7.1**					+	+
Cinnamic aldehyde	**2.3[2]**	**0.48**	**1.09**	**1.36**	**0.93**					**1.56**	**1.2**					+	+
Dibromo-cyano-butane	**1.3[2]**	**6.4**	**0.4**							**1**						+	+
Ethylene glycol dimeth-acrylate	36.5	32.4	28.3	15.5	34.4	20										-	+
Eugenol	10.1[2]											10.7	5.3	10.5	15.1	+	+
Formal-dehyde	**0.76[2]**	**0.33**				**0.7**		**4.2**	**14.2**							+	+
Geraniol												5.6	25.8	20.4	11.8	+	+
Imidazol-idinyl urea		27.8[2]			27.1											+	+
Glutaral-dehyde	0.07			1.5		0.1										+	+
Hydroxy-citronellal	27.8[2]											26.4	22.2	19.3	19.7	+	+

Substance	LLNA Vehicle and Associated EC3 Values (%)															GP	Human
	AOO[1]	DMF[1]	MEK[1]	PG[1]	DMSO[1]	ACE	APS	L92	H_2O	EtOH/ H_2O (9:1)	EtOH/ H_2O (1:1)	EtOH	EtOH/ DEP (3:1)	EtOH/ DEP (1:3)	DEP		
Isoeugenol	**1.5[2]**	**1.4**	**1**	**2.5**	**0.9**		**2.9**			**1.8**	**4.9**					+	+
Lilial	18.7											3	8.8	13.9	4.2	NA	+
CMI/MI	0.005	0.0075	0.0068	0.048	0.0075	0.0076										+	+
Nickel sulfate		>5			4.8			2.5								+	+
Potassium dichromate		0.032			0.089[2]			0.17								+	+

Abbreviations: + = sensitizer; - = nonsensitizer; ACE = acetone; AOO = acetone: olive oil (4:1 by volume); APS = acetone: physiological saline (6:94 by volume); DEP = diethyl phthalate; DMF = dimethylformamide; DMSO = dimethyl sulfoxide; EC3 = estimated concentration of a substance expected to produce a stimulation index of 3, which is the threshold value for a substance to be considered a sensitizer in the LLNA; EtOH = ethanol; GP = guinea pig test result; H_2O = water; HU = human results; L92 = 1% Pluronic L92; LLNA = murine local lymph node assay; CMI/MI = 5-chloro-2-methyl-4-isothiazolin-3-one / 2-methyl-4-isothiazolin-3-one; MEK = methyl ethyl ketone; NA = not available; OECD = Organisation for Economic Co-operation and Development; PG = propylene glycol; TG = test guideline.

Boldface text highlights substances for which discordant sensitizer subcategory (using the GHS EC3 cutoff of 2%; see **Table C-2**) would be assigned depending on the vehicle used in the LLNA.

[1] Vehicles recommended in OECD TG 429, listed in order of preference (OECD 2002). OECD TG 429 also indicates that other vehicles may be used with sufficient scientific rationale.

[2] Value represents the arithmetic mean of $n \geq 2$ EC3 values.

[3] Although no human maximization test or human repeat-insult patch test data were reported, 1,4-dihydroquinone has been reported to be a sensitizer in humans (Basketter et al. 1999a).

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Table C-14 LLNA EC3 Values for Seven Skin Sensitizers Tested in Different Vehicles (from McGarry 2007)

Substance	LLNA Vehicle and Associated EC3 Value (%)									GP	Human
	AOO[1]	DMF[1]	MEK[1]	PG[1]	DMSO[1]	ACE	L92	EtOH/H_2O (90:10)	EtOH/H_2O (50:50)		
Cinnamic aldehyde	1.70	0.50	1.10	1.40	0.90			1.60	1.20	+	+
1,4-Dihydroquinone	0.15	0.21	0.09		0.35	0.08				+	NA[2]
3-Dimethylpropylamine	**2.20**	**1.70**	**1.80**	**>10.00**	**3.20**			**4.10**	**7.10**	**NA**	**NA**
Isoeugenol	**1.00**	**1.40**	**1.00**	**2.50**	**0.90**			**1.80**	**4.90**	**+**	**+**
(Chloro)methylisothiazolinone/ Methylisothiazolinone	0.0049	0.0075	0.0068	0.0480	0.0075	0.0076				+	+
Nickel sulfate		>5.00			4.80		2.50			+	+
Potassium dichromate		0.0327			0.0500		0.1700			+	+

Abbreviations: + = sensitizer; ACE = acetone; AOO = acetone: olive oil (4:1 by volume); DMF = dimethylformamide; DMSO = dimethyl sulfoxide; EC3 = estimated concentration of a substance expected to produce a stimulation index of 3, which is the threshold value for a substance to be considered a sensitizer in the LLNA; EtOH/H_2O = ethanol/water; GHS = Globally Harmonized System of Classification and Labelling of Chemicals (UN 2009); L92 = 1% Pluronic L92; LLNA = murine local lymph node assay; MEK = methyl ethyl ketone; NA = not available; OECD = Organisation for Economic Co-operation and Development; PG = propylene glycol; TG = test guideline.

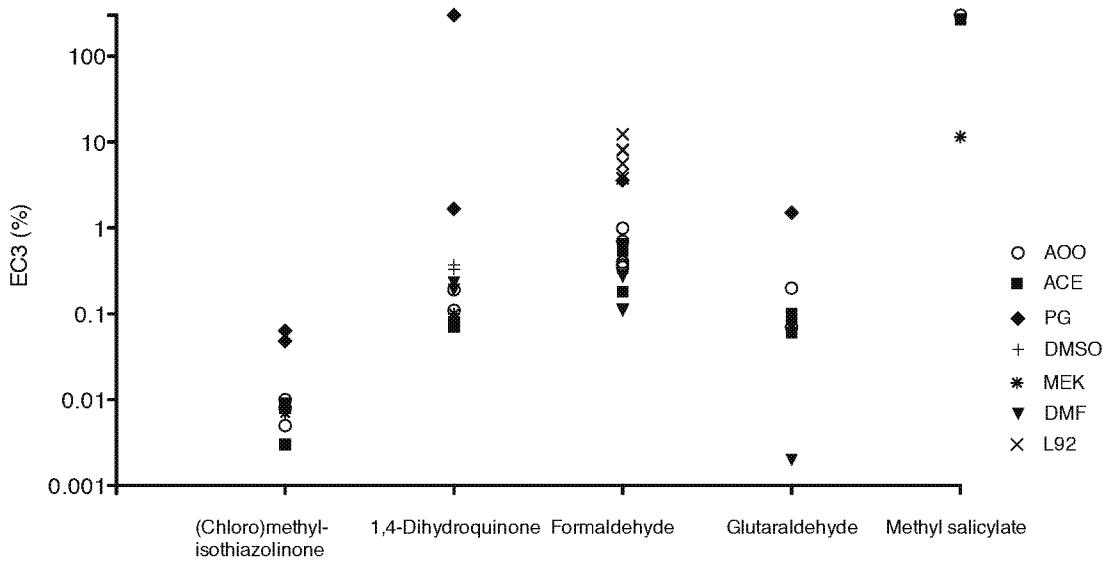
Boldface text highlights substances for which discordant classifications (using the GHS EC3 cutoff of 2%; see **Table C-2**) would be assigned depending on the vehicle used in the LLNA.

[1] Vehicles recommended in OECD TG 429, listed in order of preference (OECD 2002). OECD TG 429 also indicates that other vehicles may be used with sufficient scientific rationale.

[2] While no human maximization test or human repeat-insult patch test data were reported, 1,4-dihydroquinone has been reported to be a sensitizer in humans (Basketter et al. 1999a).

Figure C-7 further illustrates that the vehicle used has pronounced effects on the predicted skin sensitization potency when based on LLNA EC3 values. Five representative substances were selected from those listed in **Table C-12** based on available data from at least one LLNA test in multiple vehicles. These data demonstrate the potential impact of the vehicle on potency categorization when using the EC3 value. Greater than an order of magnitude difference can be seen for all five substances. This is in contrast to the conclusions of Jowsey et al. (2008) for multiple tests in different solvents (i.e., that EC3 values typically vary by no more than five-fold). Two substances in **Figure C-7** were either sensitizers or nonsensitizers in the LLNA, depending on the vehicle selected. One substance, 1,4-dihydroquinone, is sensitizing in guinea pigs and humans, although neither HMT nor HRIPT data were reported (Basketter et al. 1999a). The other substance (methyl salicylate) is nonsensitizing in guinea pigs and humans.

Figure C-7 Representative Substances and Respective LLNA EC3 Values When Tested in Different Vehicles (from the NICEATM LLNA Database)



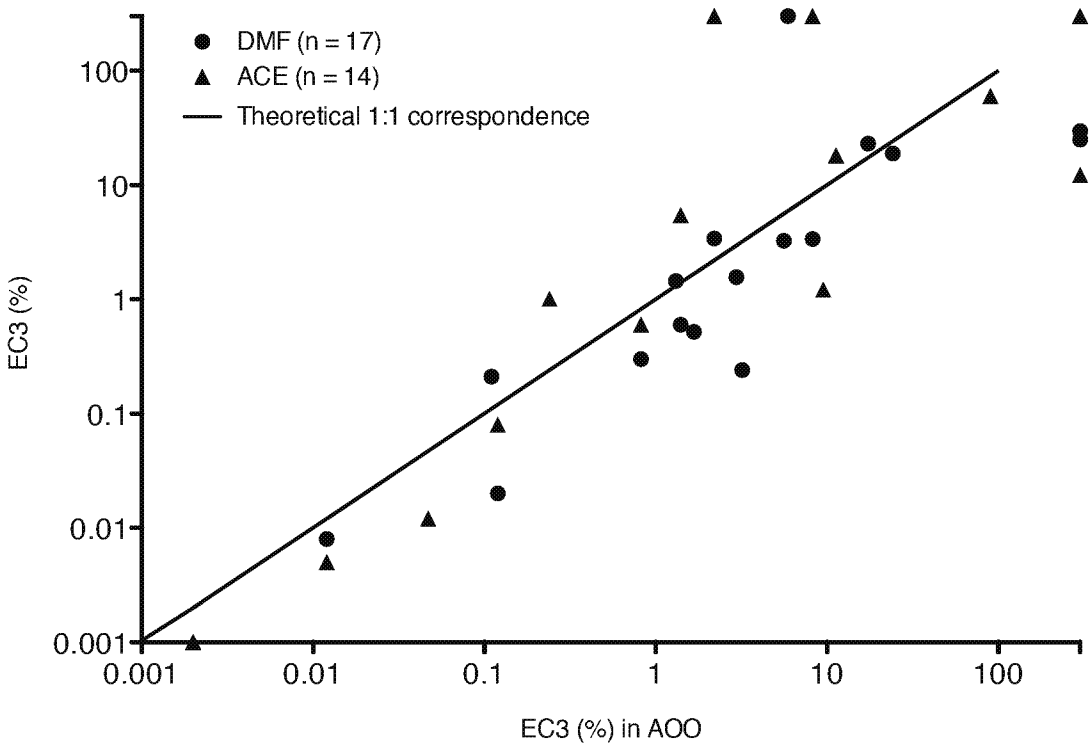
Abbreviations: ACE = acetone; AOO = acetone: olive oil (4:1 by volume); DMF = dimethylformamide; DMSO = dimethyl sulfoxide; EC3 = estimated concentration of a substance expected to produce a stimulation index of 3, which is the threshold value for a substance to be considered a sensitizer in the LLNA; L92 = 1% Pluronic L92; LLNA = murine local lymph node assay; MEK = methyl ethyl ketone; NICEATM = National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods; PG = propylene glycol.

Note: Values above 100 indicate studies where the substance was classified as a nonsensitizer.

As another analysis of vehicle effect on EC3 variability, a correlation was calculated for EC3 values from two vehicles (DMF and acetone) and compared to the EC3 values for the same substances obtained with AOO using the data in **Table C-12** (see **Figure C-8**). With AOO, a 1:1 correspondence would indicate that identical EC3 values had been obtained with the different vehicles. Substances that are nonsensitizers in either acetone or DMF are indicated as points that extend beyond 100% on the y-axis in **Figure C-8**. Substances that are nonsensitizers in AOO are indicated as points that extend beyond 100% on the x-axis. The figure suggests that EC3 values obtained with DMF and acetone are consistently lower than those obtained with AOO (i.e., the sensitizer is more potent when tested using DMF and acetone) because more points fall below the 1:1 correspondence line than above it. Spearman correlations of the log-transformed data show that the DMF ($r = 0.8743$; $p < 0.0001$) and acetone ($r = 0.8332$; $p = 0.0002$) results are significantly correlated with AOO. Negative results were arbitrarily set to EC3 = 110% so they could be used in the analysis.

Three substances were nonsensitizers when tested in acetone (i.e., benzocaine, ethylenediamine, and methyl salicylate). Methyl salicylate was also a nonsensitizer in AOO when tested at an even higher concentration. All three substances yielded sensitizer results when tested in DMF. Two of these three substances, benzocaine and ethylenediamine, were also sensitizers in guinea pigs and humans, while methyl salicylate was a nonsensitizer in both the guinea pig and human tests. Resorcinol was a nonsensitizer when tested in DMF but a sensitizer when tested in AOO (no tests in acetone were available). Resorcinol is a nonsensitizer in guinea pigs; however, the specific test protocol was not reported (Basketter et al. 1996). Although the HMT was negative (Kligman 1966), there is clinical evidence that resorcinol causes ACD in humans (Basketter et al. 2007b).

Figure C-8 Correlation of LLNA EC3 Values Between LLNA Tests with AOO and DMF or Acetone (from the NICEATM Database)



Abbreviations: ACE = acetone; AOO = acetone: olive oil (4:1 by volume); DMF = dimethylformamide; EC3 = estimated concentration of a substance expected to produce a stimulation index of 3, which is the threshold value for a substance to be considered a sensitizer in the LLNA; LLNA = murine local lymph node assay; NICEATM = National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods.

Note: Substances that are nonsensitizers in either acetone or DMF are indicated as points that extend beyond 100% on the y-axis, and substances that are nonsensitizers in AOO are indicated as points that extend beyond 100% on the x-axis.

While vehicle may be an important determinant of the EC3 value, it may not be important for every substance tested. With respect to the LLNA potency analyses in **Section 6.1**, two-way analyses of variance, with chemical and vehicle as the factors, indicated that two vehicles were responsible for a statistically significant effect of vehicle on the LLNA EC3, propylene glycol and Pluronic L92 (see **Annex IV**). Linear regression and Spearman correlation analyses indicated that removing tests using these vehicles had no impact on the relationship of the EC3 with human DSA_{05} values for the 63 substances that were sensitizers in the LLNA and in the HMT and/or HRIPT. When tests using propylene glycol and Pluronic L92 were excluded, the linear regression slope = 0.732 and

y-intercept = 0.773. Including the tests yielded slope = 0.747 and y-intercept = 0.722. In both cases, the slopes for the regressions had $p < 0.0001$ and the correlations yielded Spearman $r = 0.692$.

In the current potency evaluation of 196 substances, 58 LLNA sensitizers had more than one test (n = 2 to 66). The coefficients of variation (CV) for these sensitizers were calculated by combining results without regard to vehicle. When multiple vehicles were used, the CVs ranged from 0.4% to 349%. The CVs of the LLNA EC3 values for substances that were also sensitizers in humans ranged from 2% to 349%, while the CVs for the corresponding DSA_{05} values ranged from 2% to 408%.

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8.0 LLNA Data Quality

8.1 Adherence to National and International GLP Guidelines

From the available information, published papers, and data submissions, information on compliance with GLP guidelines was available only for data obtained from Gerberick et al. (2005), E. Debruyne (Bayer CropScience SA), and P. Botham (ECPA).

It was not feasible to formally assess the quality of the remaining LLNA data considered here. The published data on the LLNA were limited to tested concentrations and calculated SI and EC3 values. Auditing the reported values would require the original individual animal data for each LLNA experiment. Such data have not been obtained. Some of the studies were conducted according to GLP guidelines, which implies that an independent quality assurance audit was conducted. The impact of any deviations from GLP guidelines cannot be evaluated for the data reviewed here, because no data quality audits were obtained.

As noted in **Section 5.0,** the original records were not obtained for the studies included in the current evaluation. Data were available for several of the substances included in the initial ICCVAM (1999) evaluation, thus some of the raw data for these substances were available for review this time.

8.2 Data Quality Audits

Formal assessments of data quality, such as quality assurance audits, generally involve a systematic and critical comparison of the data provided in a study report to the laboratory records generated for a study.

Much of the data published by Gerberick et al. (2005) was obtained following GLP guidelines, or the studies were conducted in GLP-compliant facilities. Therefore, it was inferred that data audits had been conducted on the data (ICCVAM 1999).

A formal assessment of the quality of the remainder of the LLNA data included in this BRD was not feasible. The published data on the LLNA were limited to tested concentrations and calculated SI and EC3 values. Auditing the reported values would require obtaining the original individual animal data for each LLNA experiment. Such data were not obtained. However, some of the studies were conducted according to GLP guidelines, which implies that an independent quality assurance audit was conducted.

8.3 Impact of Deviations from GLP Guidelines

The impact of any deviations from GLP guidelines cannot be evaluated for the data reviewed in this BRD, because no information on data quality audits was obtained.

8.4 Availability of Laboratory Notebooks or Other Records

As noted in **Section 5.2,** the original records were not obtained for the studies included in this evaluation. Data were available for some of the substances included in the (ICCVAM 1999) evaluation and thus some of the raw data for these substances were available for review.

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9.0 Other Scientific Reports and Reviews

Several published studies have discussed the potential for using the LLNA to assess the relative skin sensitization potency of chemicals. The following section summarizes these reviews. Reviews by collaborating scientists are grouped together in **Section 9.1** and arranged by date. Reviews by other authors follow starting with **Section 9.2**.

9.1 Basketter, Gerberick, Kimber, and Colleagues

9.1.1 Basketter et al. (2003)

Basketter and colleagues discuss the usefulness of the LLNA for hazard identification and the test method's current regulatory status. The review also discusses the potential usefulness of the method to assess relative skin sensitization potency of chemicals and incorporation of the data into risk assessments.

The authors indicate that the use of the LLNA to assess potency has been extensively evaluated in recent years. They note the following factors to consider in the use of LLNA data for potency assessments:

- How the potency is estimated from the LLNA
- The robustness of the estimation
- The relevance of the estimation
- How the potency estimation is applied for risk assessment purposes.

The authors note that several studies have shown that the calculated EC3 values, as discussed in Basketter et al. (1999a), correlate well with human potency classifications (Basketter et al. 2000; Gerberick et al. 2001; Ryan et al. 2000).

The authors note that for the LLNA potency information to be useful, it should be capable of being incorporated into risk assessments. Various published proposals discuss incorporation of EC3 values into risk assessments (Basketter et al. 2001b; Gerberick et al. 2001; Robinson et al. 2000). They propose that combining various potential exposure conditions with calculated EC3 values would provide a way to incorporate the information into risk assessments (Basketter et al. 2002; Felter et al. 2002; Felter et al. 2003).

9.1.2 Kimber et al. (2003)

This review summarizes the efforts of the ECETOC Task Force (ECETOC 2003) that was charged with recommending approaches for the measurement of potency and defining thresholds for skin sensitization. The ECETOC Task Force focused primarily on categorization of sensitizers and identification of thresholds with respect to the induction phase of skin sensitization. Based on their deliberations, the task force concluded that the LLNA is the method of choice for prospective skin sensitization potency assessments. The task force proposed the following classification for skin sensitization potency based on EC3 values:

- Extreme: EC3 $< 0.1\%$
- Strong: $0.1\% \leq$ EC3 $< 1\%$
- Moderate: $1\% \leq$ EC3 $< 10\%$
- Weak: $10\% \leq$ EC3 $\leq 100\%$

Although the LLNA is preferred, the authors recognized that available data from guinea pig tests provide information of frequency and severity that could be used for potency assessments.

9.1.3 Jowsey et al. (2006)

Jowsey and colleagues discuss strategies for assessing skin sensitization without the use of animals. They also summarize the use of the LLNA for assessing the skin sensitization potential of chemicals. The authors note that the LLNA is useful for hazard characterization because it models all the events that occur during the process of skin sensitization and the extent to which skin sensitization will develop. That is, the magnitude of lymphocyte proliferation is an indicator of the extent of skin sensitization (Kimber and Dearman 1991). Based upon this observation, the authors proposed that using EC3 values derived from LLNA studies could be useful in assessing skin sensitization potency (Basketter et al. 2001b; Kimber and Basketter 1997). They also cite studies that demonstrate the accuracy and reliability of the EC3 value. They state that it consistently correlates with clinical estimates of human skin sensitization potency (Basketter et al. 2000; Dearman et al. 1998; Gerberick et al. 2001; Warbrick et al. 1999).

9.1.4 Basketter et al. (2007a)

This review provides an overview of the available data that the authors consider to be supportive of the validity of the LLNA for assessments of skin sensitization potency. The authors discuss the relevance of the LLNA EC3 value in evaluating human skin sensitization potency, the reliability of the EC3 value, and the interlaboratory transferability of the method based on EC3 values.

Most studies attempt to assign chemicals to various categories (e.g., nonsensitizers, weak sensitizers, strong sensitizers) based on predefined EC3 value cutoffs. While these studies tend to show good correlation between LLNA outcomes and human skin sensitization potential, more-recent studies have attempted to correlate experimental thresholds in humans (e.g., NOELs in HRIPTs) with the LLNA EC3 value. Although the outcomes depend on exposure conditions used in the patch tests, Basketter et al. conclude that the studies show a good relationship between EC3 values and the evaluated threshold levels (Basketter et al. 2005; Griem et al. 2003; Schneider and Akkan 2004).

The authors conclude that the EC3 value is a useful metric with which to predict the skin sensitization potential of chemicals in humans and that intra- and interlaboratory studies have shown that the EC3 value is reproducible within and among laboratories. The authors therefore propose that integration of the LLNA for potency identification in risk assessments would help to develop more accurate hazard identification and risk management strategies.

9.1.5 Gerberick et al. (2007)

In this review, the authors discuss the concept of using the LLNA to assess the skin sensitization potential of chemicals in humans. They cite several advantages of the LLNA (e.g., provides dose-response data, allows for quantification of threshold values) that make it amenable to potency determinations. They also cite several studies that have evaluated the accuracy and reliability of the EC3 value for assessing potency (Basketter and Cadby 2004; Dearman et al. 2001; Warbrick et al. 1999). These and other studies have reportedly demonstrated good correlation between LLNA potency estimates and human potency, as assessed by clinical studies and experience (Basketter et al. 2000; Gerberick et al. 2001).

Based on these findings, the authors conclude that the LLNA should be considered the preferred method for identifying human skin sensitization hazard and that it can provide important additional information regarding skin sensitization potency that facilitates scientifically sound risk assessments.

9.1.6 Ryan et al. (2007)

In this article, Ryan and colleagues review historical LLNA data from both published and unpublished sources and use the data to calculate and compare EC3 values using two different

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mathematical methods: linear interpolation and log-linear extrapolation. Usually the EC3 value is calculated by linear interpolation, which uses the dose and SI data points lying immediately above and below the SI value of 3 on the dose-response curve (see the following equation):

$$EC3 = c + \left[\frac{(3-d)}{(b-d)}\right] \times (a-c)$$

Coordinates:

(a = dose concentration immediately above SI = 3, b = SI immediately above 3)

(c = dose concentration immediately below SI = 3, d = SI immediately below 3)

In instances where all the test concentrations result in SI values that are greater than 3, a log-linear extrapolation is applied using the two SI values greater than 3 with the lowest of the SI values having the lowest percent concentration (see the following equation):

$$EC3_{ex} = 2^{\wedge}\left\{\log_2(c) + \frac{(3-d)}{(b-d)} \times \left[\log_2(a) - \log_2(c)\right]\right\}$$

Coordinates:

(a = dose concentration for next to lowest SI above 3, b = next to lowest SI above 3)

(c = dose concentration for lowest SI above 3, d = lowest SI above 3)

The authors evaluate 187 data sets with at least one SI value less than 3 and at least two SI values greater than 3. They use the same data sets to calculate the EC3 values using the linear interpolation and the log-linear extrapolation methods. Based on the resulting analyses, both methods of calculation are reliable and similar 88% of the time. When differences occur, the log-linear extrapolation tends to predict a stronger classification based on EC3 potency (i.e., extreme, strong, moderate, or weak). The authors also conclude from additional analyses that the quality of the dose-response curve determines the accuracy of the log-linear extrapolated EC3 values relative to the linear interpolated EC3 values. Thus, the authors indicate that using a log-linear extrapolation in instances where a linear interpolation is not possible could avoid the need for repeat animal testing with different test concentrations and may also allow for a potency classification.

9.1.7 Loveless et al. (2010)

This paper discusses how using potency information from LLNA EC3 values is applicable to classification, labeling, and risk assessment for skin sensitization hazard. The authors ask four main questions:

1. Could an EC3 value lower than 100% be defined and used as a threshold criterion for classification and labeling of skin-sensitizing substances?
2. Is there any reason to revise the recommendations of a previous ECETOC Task Force (see **Section 9.1.2**) (ECETOC 2003) regarding specific EC3 values used for subcategorization of substances based upon potency?
3. What recommendation could be made regarding classification and labeling of preparations under GHS?
4. How could LLNA data be integrated into risk assessment and provide a rationale for using concentration responses and corresponding no-effect concentrations?

The authors made the following overall conclusions to the four questions they posed.

1. The available data does not support using an EC3 value lower than 100% as the threshold for classification and labeling of a substance as a sensitizer because many chemicals with high EC3 values (>50%) are known to be human skin sensitizers.
2. After reviewing the potency categories for characterizing contact allergens recommended by a previous ECETOC Task Force (see **Section 9.1.2**) (ECETOC 2003), the use of the recommended four subcategories (i.e., extreme, strong, moderate, and weak) appears the most appropriate and scientifically based.
3. In order to classify preparations as Category 1 skin sensitizers under the current GHS regulation (UN 2009), the potency-related classification that applies to substances also applies to mixtures.
4. The authors recommend LLNA EC3 values for determination of a no-expected-sensitization induction level that represents the first step in a quantitative risk assessment.

9.2 McGarry (2007)

This review provides an overview of concerns that were raised upon implementation of the European chemicals legislation on the registration, evaluation, authorization, and restriction of chemicals (REACH). These concerns include that the LLNA is susceptible to vehicle effects (refer also to **Section 7.0**), it has not been validated for testing mixtures,[17] and it may result in a number of false positive responses when tested with skin irritants. The author states that these concerns have become heightened given the current requirements in the REACH legislation for skin sensitization testing, which specifies that the LLNA must be used for new *in vivo* testing for skin sensitization hazards, and only under "exceptional circumstances" can another method be used (EC 2006).

The intent of this review is to address these concerns from a European regulatory perspective and to discuss the potential utility of the LLNA to provide information on skin sensitization potency of substances. Evidence of vehicle effects, both on overall LLNA results (i.e., "yes" or "no" decisions) and on potency estimates (i.e., EC3 values), is described for several commonly used vehicles. Problems associated with testing mixtures and formulations (e.g., compatibility with traditional LLNA vehicles, alteration of the active substance's bioavailability by excipients) are also described. The author concludes with a discussion of the potential utility of the LLNA for estimating skin sensitization potency, while cautioning that the EC3 should not be considered a measure of absolute potency.

9.3 Schlede et al. (2003)

This article is the culmination of a 16-year collaboration among dermatologists, industry representatives, and regulators to assign potency rankings to chemicals with skin-sensitizing properties. Clinical and experimental data on humans and results of animal tests from the scientific literature were collected on 244 substances (i.e., technically produced chemicals as well as chemically defined single ingredients of natural products). Based primarily on "expert judgment" and in combination with reviews of the published literature, each substance was assigned to one of three defined categories:

- Significant contact allergen (Category A)
- Solid-based indication for contact allergenic effects (Category B)

[17] After the publication of McGarry (2007), ICCVAM recommended that the LLNA may be used to test any chemical or product, including pesticide formulations, metals, substances in aqueous solutions, and other products such as natural complex substances and dyes unless the chemical or product to be tested has properties that may interfere with the ability of the LLNA to detect skin-sensitizing substances (ICCVAM 2010c).

- Insignificant contact allergen or questionable contact allergenic effect (Category C).

Published data from human tests were obtained with the HMT or HRIPT, while the animal data were obtained with the GPMT, BT, and/or the LLNA. Most of the human experimental data correlate with sensitizing and sensitizing/nonsensitizing animal data. However, the authors state that published data on experimental human testing are limited in most cases to older studies with insufficient experimental design and/or limited documentation.

The authors conclude that results obtained with animal data are reliable and sensitive indicators for the determination of skin sensitization potential in humans.

9.4 Zaghi and Maibach (2009)

This paper compares the correlation between LLNA EC3 DSA and the HMT DSA_{05} as an indicator of allergic potency in humans as determined by the frequency of allergic reactions measured in patch test clinic populations. Eight compounds (nickel, cobalt chloride, neomycin, potassium dichromate, formaldehyde, p-phenylenediamine, benzocaine, and mercaptobenzothiazole) were evaluated. The compounds have LLNA, HMT, and patch test results from the North American Contact Dermatitis Group and the European Surveillance System on Contact Allergies. The authors quantitatively evaluated the role that other factors play in allergic reactions by subtracting the best potency correlation value from one. The data showed an inverse correlation for the weighted frequency of patch test positive responses and the LLNA or HMT. That is, as patch test positive responses decreased, LLNA DSA and HMT DSA_{05} increased. The correlation values for the LLNA and HMT with patch test clinic data were -0.56 and -0.71, respectively.

The authors suggest that there is a possible 20% error margin in the LLNA's capacity to predict potency. Further, because the best correlation value is only -0.71 (i.e., HMT correlation results), the authors suggest that other factors may play up to a 30% role in the determination of the frequency of an allergic reaction in the general population. The authors acknowledge that numerous variances in the collection of data (i.e., different laboratories, investigators, time) might have been limitations of the analyzed data set. Still, the authors conclude that while the LLNA and HMT might adequately predict allergic potency of a substance, a model that more accurately reflects human experience and takes into account environmental factors is needed.

ED_006472_00084346-00143

10.0 Animal Welfare Considerations

The proposal for using the LLNA for potency determinations does not affect its requirement for using animals or the number of animals that are required. These are defined in the ICCVAM-recommended LLNA protocol (ICCVAM 2009). However, this application could broaden the use of the LLNA protocol in place of guinea pig tests and thereby further reduce the number of guinea pigs being used to assess skin sensitization potential. The LLNA is also a refinement compared with guinea pig tests because it avoids the pain and distress that can occur in the guinea pig tests when substances cause ACD.

10.1 Rationale for the Need to Use Animals

There currently are no valid and accepted non-animal test methods to determine the ACD potential of substances and products, except for situations where human studies could be conducted ethically and where such studies would meet regulatory safety assessment requirements. Additionally, the most detailed information about the induction and regulation of immunological responses is available for mice (ICCVAM 1999).

10.2 Basis for Determining the Number of Animals Used

The number of animals used for the experimental, vehicle, and positive control groups, a minimum of four animals per group, is based on the number of animals specified in the ICCVAM-recommended LLNA protocol (ICCVAM 2009).

10.3 Reduction Considerations

Although a reduced version of the LLNA (i.e., use of only a negative control and a high-dose group) does not allow for the potency determination of a sensitizing chemical, the LLNA test method protocol (ICCVAM 2009) requires fewer mice per treatment group (a minimum of four animals per group) than either of the preferred guinea pig tests (a minimum of 10 animals/group for the Buehler test and 5 animals/group for the GPMT).

ED_006472_00084346-00144

11.0 Practical Considerations

The following issues must be taken into account when assessing the practicality of an alternative to an existing test method:

- Performance evaluations
- Assessments of the laboratory equipment and supplies needed to conduct the alternative test method
- Level of personnel training
- Labor costs
- Time required to complete the test method relative to the existing test method

The time, personnel cost, and effort required to conduct the proposed test method(s) must be considered to be reasonable when compared to the existing test method it is intended to replace. No such changes are being proposed for the LLNA protocol. Therefore, the transferability, training requirements, and time and cost considerations for using the LLNA for potency determinations remain unchanged from the previous ICCVAM evaluations (ICCVAM 1999, 2010c).

ED_006472_00084346-00145

12.0 References

ICCVAM Authorization Act of 2000. 2000. Public Law 106-545. 42 U.S.C. 2851-3.

Basketter DA, Blaikie L, Dearman RJ, Kimber I, Ryan CA, Gerberick GF, et al. 2000. Use of the local lymph node assay for the estimation of relative contact allergenic potency. Contact Dermatitis 42(6): 344-348.

Basketter DA, Cadby P. 2004. Reproducible prediction of contact allergenic potency using the local lymph node assay. Contact Dermatitis 50(1): 15-17.

Basketter DA, Clapp C, Jefferies D, Safford B, Ryan CA, Gerberick F, et al. 2005. Predictive identification of human skin sensitization thresholds. Contact Dermatitis 53(5): 260-267.

Basketter DA, Evans P, Gerberick GF, Kimber I. 2002. Factors affecting thresholds in allergic contact dermatitis: safety and regulatory considerations. Contact Dermatitis 47(1): 1-6.

Basketter DA, Gerberick F, Kimber I. 2007a. The local lymph node assay and the assessment of relative potency: status of validation. Contact Dermatitis 57(2): 70-75.

Basketter DA, Gerberick GF, Kimber I. 2001. Skin sensitisation, vehicle effects and the local lymph node assay. Food Chem Toxicol 39(6): 621-627.

Basketter DA, Gerberick GF, Kimber I. 2001b. Measurement of allergenic potency using the local lymph node assay. Trends Pharmacol Sci 22(6): 264-265.

Basketter DA, Gerberick GF, Kimber I, Loveless SE. 1996. The local lymph node assay: a viable alternative to currently accepted skin sensitization tests. Food Chem Toxicol 34(10): 985-997.

Basketter DA, Lea LJ, Cooper K, Stocks J, Dickens A, Pate I, et al. 1999a. Threshold for classification as a skin sensitizer in the local lymph node assay: a statistical evaluation. Food Chem Toxicol 37(12): 1167-1174.

Basketter DA, Lea LJ, Dickens A, Briggs D, Pate I, Dearman RJ, et al. 1999b. A comparison of statistical approaches to the derivation of EC3 values from local lymph node assay dose responses. J Appl Toxicol 19(4): 261-266.

Basketter DA, Sanders D, Jowsey IR. 2007b. The skin sensitization potential of resorcinol: Experience with the local lymph node assay. Contact Dermatitis 56(4): 196-200.

Basketter DA, Smith Pease CK, Patlewicz GY. 2003. Contact allergy: the local lymph node assay for the prediction of hazard and risk. Clin Exp Dermatol 28(2): 218-221.

Boukhman MP, Maibach HI. 2001. Thresholds in contact sensitization: immunologic mechanisms and experimental evidence in humans – an overview. Food Chem Toxicol 39(12): 1125-1134.

Dean JH, Twerdok LE, Tice RR, Sailstad DM, Hattan DG, Stokes WS. 2001. ICCVAM evaluation of the murine local lymph node assay: II. Conclusions and recommendations of an independent scientific peer review panel. Regul Toxicol Pharmacol 34(3): 258-273.

Dearman RJ, Hilton J, Evans P, Harvey P, Basketter DA, Kimber I. 1998. Temporal stability of local lymph node assay responses to hexyl cinnamic aldehyde. J Appl Toxicol 18(4): 281-284.

Dearman RJ, Wright ZM, Basketter DA, Ryan CA, Gerberick GF, Kimber I. 2001. The suitability of hexyl cinnamic aldehyde as a calibrant for the murine local lymph node assay. Contact Dermatitis 44(6): 357-361.

EC. 2006. Regulation (EC) No. 1907/2006 of the European Parliament and of the Council of 18 December 2006 concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH), establishing a European Chemicals Agency, amending Directive 1999/45/EC and

ED_006472_00084346-00146

repealing Council Regulation (EEC) No. 793/93 and Commission Regulation (EC) No. 1488/94 as well as Council Directive 76/769/EEC and Commission Directives 91/155/EEC, 93/67/EEC, 93/105/EC and 2000/21/EC. OJ L 396.

ECETOC. 2003. Technical Report No. 87. Contact Sensitization: Classification According to Potency. Brussels, Belgium:European Centre for Ecotoxicology and Toxicology of Chemicals. Available: for purchase at http://www.ecetoc.org/technical-reports [accessed 3 November 2010].

ECETOC. 2008. Document No. 46. Potency Values from the Local Lymph Node Assay: Application to Classification, Labelling and Risk Assessment. Brussels, Belgium:European Centre for Ecotoxicology and Toxicology of Chemicals. Available: for purchase at http://www.ecetoc.org/technical-documents [accessed 3 November 2010].

EPA. 2003. Health Effects Test Guidelines: OPPTS 870.2600 - Skin Sensitization. EPA 712-C-03-197. Washington, DC:U.S. Environmental Protection Agency. Available: http://www.epa.gov/opptsfrs/publications/OPPTS_Harmonized/870_Health_Effects_Test_Guidelines/Revised/870r-2600.pdf [accessed 17 September 2009].

EPA. 2006a. Good Laboratory Practice Standards. Federal Insecticide, Fungicide and Rodenticide Act. 40 CFR 160.

EPA. 2006b. Good Laboratory Practice Standards. Toxic Substances Control Act. 40 CFR 792.

Fawcett T. 2006. An introduction to ROC analysis. Pattern Recognition Letters 27(8): 861-874.

FDA. 2009. Good Laboratory Practice for Nonclinical Laboratory Studies. 21 CFR 58.

Felter SP, Robinson MK, Basketter DA, Gerberick GF. 2002. A review of the scientific basis for uncertainty factors for use in quantitative risk assessment for the induction of allergic contact dermatitis. Contact Dermatitis 47(5): 257-266.

Felter SP, Ryan CA, Basketter DA, Gilmour NJ, Gerberick GF. 2003. Application of the risk assessment paradigm to the induction of allergic contact dermatitis. Regul Toxicol Pharmacol 37(1): 1-10.

Friedmann PS. 1990. The immunology of allergic contact dermatitis: the DNCB story. In: Advances in Dermatology (Dahl MV, ed). Chicago:Yearbook Medical Publishers, 175-195.

Gerberick GF, Robinson MK, Ryan CA, Dearman RJ, Kimber I, Basketter DA, et al. 2001. Contact allergenic potency: correlation of human and local lymph node assay data. Am J Contact Dermat 12(3): 156-161.

Gerberick GF, Ryan CA, Dearman RJ, Kimber I. 2007. Local lymph node assay (LLNA) for detection of sensitization capacity of chemicals. Methods 41(1): 54-60.

Gerberick GF, Ryan CA, Kern PS, Dearman RJ, Kimber I, Patlewicz GY, et al. 2004. A chemical dataset for evaluation of alternative approaches to skin-sensitization testing. Contact Dermatitis 50(5): 274-288.

Gerberick GF, Ryan CA, Kern PS, Schlatter H, Dearman RJ, Kimber I, et al. 2005. Compilation of historical local lymph node data for evaluation of skin sensitization alternative methods. Dermatitis 16(4): 157-202.

Griem P, Goebel C, Scheffler H. 2003. Proposal for a risk assessment methodology for skin sensitization based on sensitization potency data. Regul Toxicol Pharmacol 38(3): 269-290.

Haneke KE, Tice RR, Carson BL, Margolin BH, Stokes WS. 2001. ICCVAM evaluation of the murine local lymph node assay: III. Data analyses completed by the National Toxicology Program

ED_006472_00084346-00147

Interagency Center for the Evaluation of Alternative Toxicological Methods. Regul Toxicol Pharmacol 34(3): 274-286.

Hutchings CV, Shum KW, Gawkrodger DJ. 2001. Occupational contact dermatitis has an appreciable impact on quality of life. Contact Dermatitis 45(1): 17-20.

ICCVAM. 1997. Validation and Regulatory Acceptance of Toxicological Test Methods: A Report of the *ad hoc* Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM). NIH Publication No. 97-3981. Research Triangle Park, NC:National Institute of Environmental Health Sciences. Available: http://iccvam.niehs.nih.gov/docs/about_docs/validate.pdf [accessed 23 December 2010].

ICCVAM. 1999. The Murine Local Lymph Node Assay: A Test Method for Assessing the Allergic Contact Dermatitis Potential of Chemicals/Compounds. The Results of an Independent Peer Review Evaluation Coordinated by the Interagency Coordinating Committee on the Validation of Alternative Methods and the National Toxicology Program (NTP) Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM). NIH Publication No. 99-4494. Research Triangle Park, NC:National Institute of Environmental Health Sciences. Available: http://iccvam.niehs.nih.gov/docs/immunotox_docs/llna/llnarep.pdf [accessed 21 October 2010].

ICCVAM. 2001. ICCVAM Immunotoxicology Working Group Recommended Protocol for the Murine Local Lymph Node Assay (LLNA): Testing of Chemicals for Contact Sensitizing (Allergic Contact Dermatitis [ACD]) Potential. Research Triangle Park, NC:National Institute of Environmental Health Sciences. Available: http://iccvam.niehs.nih.gov/docs/immunotox_docs/llna/LLNAProt.pdf [accessed 29 November 2010].

ICCVAM. 2003. ICCVAM Guidelines for the Nomination and Submission of New, Revised, and Alternative Test Methods. NIH Publication No. 03-4508. Research Triangle Park, NC:National Institute of Environmental Health Sciences. Available: http://iccvam.niehs.nih.gov/SuppDocs/SubGuidelines/SD_subg034508.pdf [accessed 4 November 2010].

ICCVAM. 2009. Recommended Performance Standards: Murine Local Lymph Node Assay. NIH Publication No. 09-7357. Research Triangle Park, NC:National Institute of Environmental Health Sciences. Available: http://iccvam.niehs.nih.gov/docs/immunotox_docs/llna-ps/LLNAPerfStds.pdf [accessed 8 July 2010].

ICCVAM. 2010a. ICCVAM Test Method Evaluation Report on the Murine Local Lymph Node Assay: BrdU-ELISA. A Nonradioactive Alternative Test Method to Assess the Allergic Contact Dermatitis Potential of Chemicals and Products. NIH Publication No. 10-7552. Research Triangle Park, NC:National Institute of Environmental Health Sciences. Available: http://iccvam.niehs.nih.gov/methods/immunotox/llna-ELISA/TMER.htm [accessed 21 October 2010].

ICCVAM. 2010b. ICCVAM Test Method Evaluation Report on the Murine Local Lymph Node Assay: DA. A Nonradioactive Alternative Test Method to Assess the Allergic Contact Dermatitis Potential of Chemicals and Products. NIH Publication No. 10-7551. Research Triangle Park, NC:National Institute of Environmental Health Sciences. Available: http://iccvam.niehs.nih.gov/methods/immunotox/llna-DA/TMER.htm [accessed 21 October 2010].

ICCVAM. 2010c. ICCVAM Test Method Evaluation Report on Using the Murine Local Lymph Node Assay for Testing Pesticide Formulations, Metals, Substances in Aqueous Solutions, and Other Products. NIH Publication No. 10-7512. Research Triangle Park, NC:National Institute of Environmental Health Sciences.

ED_006472_00084346-00148

ICH. 1996. Guidance for Industry: E6 Good Clinical Practice: Consolidated Guidance. Available: http://www.fda.gov/downloads/drugs/guidancecomplianceregulatoryinformation/guidances/ucm073122.pdf [accessed 1 November 2010].

ISO. 2002. Part 10: Tests for irritation and delayed-type hypersensitivity reaction. In: Biological Evaluation of Medical Devices, 2nd ed. Geneva:International Organization for Standardization. Available: Available for purchase at http://www.iso.org/iso/home.htm [accessed 4 November 2010].

ISO. 2010. Part 10: Tests for irritation and delayed-type hypersensitivity reaction. In: Biological Evaluation of Medical Devices 10993, 3rd ed. Geneva:International Organization for Standardization. Available: Available for purchase at http://www.iso.org/iso/home.htm [accessed 4 November 2010].

Jowsey IR, Basketter DA, Westmoreland C, Kimber I. 2006. A future approach to measuring relative skin sensitising potency: a proposal. J Appl Toxicol 26(4): 341-350.

Jowsey IR, Clapp CJ, Safford B, Gibbons BT, Basketter DA. 2008. The impact of vehicle on the relative potency of skin-sensitizing chemicals in the local lymph node assay. Cutan Ocul Toxicol 27(2): 67-75.

Kimber I, Basketter DA. 1997. Contact sensitization: a new approach to risk assessment. Hum Ecol Risk Assess 3(3): 385-395.

Kimber I, Basketter DA, Butler M, Gamer A, Garrigue JL, Gerberick GF, et al. 2003. Classification of contact allergens according to potency: proposals. Food Chem Toxicol 41(12): 1799-1809.

Kimber I, Dearman RJ. 1991. Investigation of lymph node cell proliferation as a possible immunological correlate of contact sensitizing potential. Food Chem Toxicol 29(2): 125-129.

Kimber I, Dearman RJ. 1996. Contact hypersensitivity: immunological mechanisms. In: Toxicology of Contact Hypersensitivity (Kimber I, Maurer T, eds). London:Taylor and Francis, 4-25.

Kimber I, Dearman RJ, Basketter DA, Ryan CA, Gerberick GF, McNamee PM, et al. 2008. Dose metrics in the acquisition of skin sensitization: Thresholds and importance of dose per unit area. Regul Toxicol Pharmacol 52(1): 39-45.

Kimber I, Gerberick GF, Basketter DA. 1999. Thresholds in contact sensitization: theoretical and practical considerations. Food Chem Toxicol 37(5): 553-560.

Kligman AM. 1966. The identification of contact allergens by human assay. 3. The maximization test: a procedure for screening and rating contact sensitizers. J Invest Dermatol 47(5): 393-409.

Kligman AM, Epstein W. 1975. Updating the maximization test for identifying contact allergens. Contact Dermatitis 1(4): 231-239.

Lea LJ, Warbrick EV, Dearman RJ, Kimber I, Basketter DA. 1999. The impact of vehicle on assessment of relative skin sensitization potency of 1,4-dihydroquinone in the local lymph node assay. Am J Contact Dermat 10(4): 213-218.

Loveless SE, Api AM, Crevel RWR, Debruyne E, Gamer A, Jowsey IR, et al. 2010. Potency values from the local lymph node assay: application to classification, labelling and risk assessment. Regul Toxicol Pharmacol 56(1): 54-66.

Marzulli FN, Maibach HI. 1974. The use of graded concentrations in studying skin sensitizers: experimental contact sensitization in man. Food Cosmet Toxicol 12(2): 219-227.

Marzulli FN, Maibach HI. 1976. Effects of vehicles and elicitation concentration in contact dermatitis testing. I. Experimental contact sensitization in humans. Contact Dermatitis 2(6): 325-329.

ED_006472_00084346-00149

Matheson JM. 2006. CPSC Staff Report on the Draft Proposed Revision of the FSHA "Strong Sensitizer" Supplemental Definition. Washington, DC:U.S. Consumer Product Safety Commission, 66.

McGarry HF. 2007. The murine local lymph node assay: regulatory and potency considerations under REACH. Toxicology 238(2-3): 71-89.

OECD. 1992. Test No. 406. Skin Sensitisation [adopted 17 July 1992]. In: OECD Guidelines for the Testing of Chemicals, Section 4: Health Effects. Paris:OECD Publishing. Available: http://dx.doi.org/10.1787/9789264070660-en [accessed 30 July 2010].

OECD. 1998. OECD Series on Good Laboratory Practice and Compliance Monitoring No. 1: OECD Principles on Good Laboratory Practice [as revised in 1997]. Paris:OECD Publishing. Available: http://www.oecd.org/document/63/0,3343,en_2649_34381_2346175_1_1_1_1,00.html [accessed 30 July 2010].

OECD. 2002. Test No. 429. Skin Sensitisation: Local Lymph Node Assay [adopted 24 April 2002]. In: OECD Guidelines for the Testing of Chemicals, Section 4: Health Effects. Paris:OECD Publishing. Available: http://dx.doi.org/10.1787/9789264071100-en [accessed 30 July 2010].

OECD. 2010a. Test No. 429. Skin Sensitization: Local Lymph Node Assay [adopted 22 July 2010]. In: OECD Guidelines for the Testing of Chemicals, Section 4: Health Effects. Paris:OECD Publishing. Available: http://dx.doi.org/10.1787/9789264071100-en [accessed 6 August 2010].

OECD. 2010b. Test No. 442A. Skin Sensitization: Local Lymph Node Assay: DA [adopted 22 July 2010]. In: OECD Guidelines for the Testing of Chemicals, Section 4: Health Effects. Paris:OECD Publishing. Available: http://dx.doi.org/10.1787/9789264090972-en [accessed 6 August 2010].

OECD. 2010c. Test No. 442B. Skin Sensitization: Local Lymph Node Assay: BrdU-ELISA [adopted 22 July 2010]. In: OECD Guidelines for the Testing of Chemicals, Section 4: Health Effects. Paris:OECD Publishing. Available: http://dx.doi.org/10.1787/9789264090996-en [accessed 6 August 2010].

Politano VT, Api AM. 2008. The Research Institute for Fragrance Materials' human repeated insult patch test protocol. Regul Toxicol Pharmacol 52(1): 35-38.

Robinson MK, Gerberick GF, Ryan CA, McNamee P, White IR, Basketter DA. 2000. The importance of exposure estimation in the assessment of skin sensitization risk. Contact Dermatitis 42(5): 251-259.

Ryan CA, Chaney JG, Gerberick GF, Kern PS, Dearman RJ, Kimber I, et al. 2007. Extrapolating local lymph node assay EC3 values to estimate relative sensitizing potency. Cutan Ocul Toxicol 26(2): 135-145.

Ryan CA, Gerberick GF, Cruse LW, Basketter DA, Lea L, Blaikie L, et al. 2000. Activity of human contact allergens in the murine local lymph node assay. Contact Dermatitis 43(2): 95-102.

Sailstad DM, Hattan D, Hill RN, Stokes WS. 2001. ICCVAM evaluation of the murine local lymph node assay: I. The ICCVAM review process. Regul Toxicol Pharmacol 34(3): 249-257.

Schlede E, Aberer W, Fuchs T, Gerner I, Lessmann H, Maurer T, et al. 2003. Chemical substances and contact allergy - 244 substances ranked according to allergenic potency. Toxicology 193(3): 219-259.

Schneider K, Akkan Z. 2004. Quantitative relationship between the local lymph node assay and human skin sensitization assays. Regul Toxicol Pharmacol 39(3): 245-255.

Skoet R, Zachariae R, Agner T. 2003. Contact dermatitis and quality of life: a structured review of the literature. Br J Dermatol 149(3): 452-456.

ED_006472_00084346-00150

Steel RGD, Torrie JH. 1980. Principles and Procedures of Statistics: A Biometrical Approach. New York:McGraw Hill.

UN. 2009. Globally Harmonized System of Classification and Labelling of Chemicals (GHS). 3rd rev. ed. New York:United Nations.

Warbrick EV, Dearman RJ, Lea LJ, Basketter DA, Kimber I. 1999. Local lymph node assay responses to paraphenylenediamine: intra- and inter-laboratory evaluations. J Appl Toxicol 19(4): 255-260.

White SI, Friedmann PS, Moss C, Simpson JM. 1986. The effect of altering area of application and dose per unit area on sensitization by DNCB. Br J Dermatol 115(6): 663-668.

WHO/IPCS. 2007. General Conclusions and Recommendations of an IPCS International Workshop on Skin Sensitization in Chemical Risk Assessment. IPCS International Workshop on Skin Sensitization in Chemical Risk Assessment. Berlin, Germany. Geneva:World Health Organization.

Wright ZM, Basketter DA, Blaikie L, Cooper KJ, Warbrick EV, Dearman RJ, et al. 2001. Vehicle effects on skin sensitizing potency of four chemicals: assessment using the local lymph node assay. Int J Cosmet Sci 23(2): 75-83.

Zaghi D, Maibach HI. 2009. The local lymph node assay compared with the human maximization test as an indicator of allergic potency in humans using patch test clinic populations. Cutan Ocul Toxicol 28(2): 61-64.

ED_006472_00084346-00151

13.0 Glossary

Accuracy*: (a) The closeness of agreement between a test method result and an accepted reference value. (b) The proportion of correct outcomes of a test method. It is a measure of test method performance and one aspect of *relevance*. The term is often used interchangeably with "concordance" (see also *two-by-two table*). Accuracy is highly dependent on the prevalence of positives in the population being examined.

Allergic contact dermatitis (ACD)*: A Type IV allergic reaction of the skin that results from skin contact with a skin sensitizer. Clinical signs of ACD include, but are not limited to, development of erythema (redness) and edema (swelling), blistering, and itching. Also referred to as skin sensitization.

Assay*: The experimental system used. Often used interchangeably with *test* and *test method*.

Classification rate: The correct classification rate is the proportion of substances that are correctly assigned to a human potency category by the LLNA (or guinea pig) result. The underclassification rate is the proportion of substances that are incorrectly assigned to a less severe human potency category by the LLNA (or guinea pig) result. The overclassification rate is the proportion of substances that are incorrectly assigned to a more severe human potency category by the LLNA (or guinea pig) result.

Coded substances: Substances labeled by code rather than name so that they can be tested and evaluated without knowledge of their identity or anticipation of test results. Coded substances are used to avoid intentional or unintentional bias when evaluating laboratory or test method performance.

Concordance*: The proportion of all substances tested that are correctly classified as positive or negative. It is a measure of test method performance and one aspect of *relevance*. The term is often used interchangeably with *accuracy* (see also *two-by-two table*). Concordance is highly dependent on the prevalence of positives in the population being examined.

EC3: the concentration of a substance estimated from the dose response curve to produce a three-fold increase in stimulation index, as compared to the concurrent vehicle control. The EC3 is the threshold value for a substance to be considered a sensitizer in the LLNA.

Essential test method component*: Structural, functional, and procedural elements of a test method that are used to develop the test method protocol. These components include unique characteristics of the test method, critical procedural details, and quality control measures. Adherence to essential test method components is necessary when the acceptability of a proposed test method is being evaluated based on performance standards derived from mechanistically and functionally similar validated test method. [Note: Previously referred to as *minimum procedural standards*]

False negative*: A substance incorrectly identified as negative by a test method.

False negative rate*: The proportion of all positive substances falsely identified by a test method as negative (see *two-by-two table*). It is one indicator of test method accuracy.

False positive*: A substance incorrectly identified as positive by a test method.

False positive rate*: The proportion of all negative substances that are falsely identified by a test method as positive (see *two-by-two table*). It is one indicator of test method accuracy.

Good Laboratory Practices (GLP)*: Regulations promulgated by the U.S. Food and Drug Administration and the U.S. Environmental Protection Agency, and principles and procedures adopted by the Organisation for Economic Co-operation and Development and Japanese authorities

* Definition used by ICCVAM (2003).

ED_006472_00084346-00152

that describe record keeping and quality assurance procedures for laboratory records that will be the basis for data submissions to national regulatory agencies.

Hazard*: The potential for an adverse health or ecological effect. A hazard potential results only if an exposure occurs that leads to the possibility of an adverse effect being manifested.

Human threshold response: In the evaluation included in this BRD, the threshold for induction of skin sensitization was considered to be the no observed effect level (NOEL, expressed as $\mu g/cm^2$) or, in the absence of negative data, the lowest observed effect level (LOEL, expressed as $\mu g/cm^2$), as described by Basketter et al. (2005).

Interlaboratory reproducibility*: A measure of whether different qualified laboratories using the same protocol and test substances can produce qualitatively and quantitatively similar results. Interlaboratory reproducibility is determined during the prevalidation and validation processes and indicates the extent to which a test method can be transferred successfully among laboratories.

Intralaboratory repeatability*: The closeness of agreement between test results obtained within a single laboratory when the procedure is performed on the same substance under identical conditions within a given time period.

Intralaboratory reproducibility*: The first stage of validation; a determination of whether qualified people within the same laboratory can successfully replicate results using a specific test protocol at different times.

Immunological: Relating to the immune system and immune responses.

In vivo: In the living organism. Refers to assays performed in multicellular organisms.

Murine local lymph node assay (LLNA): An *in vivo* test method used to assess the skin sensitization potential of a substance by measuring the proliferation of lymphocytes in the lymph nodes draining the ears (i.e., auricular lymph nodes) of mice, subsequent to topical exposure on the ear to the substance. The LLNA measures lymphocyte proliferation by quantifying the amount of ^{3}H-thymidine or ^{125}I-iododeoxyuridine incorporated into the cells of the draining lymph nodes.

Lymphocyte: A white blood cell found in the blood, lymph, and lymphoid tissues, which regulates and plays a role in acquired immunity.

Negative predictivity*: The proportion of correct negative responses among substances testing negative by a test method (see *two-by-two table*). It is one indicator of test method accuracy. Negative predictivity is a function of the sensitivity of the test method and the prevalence of negatives among the substances tested.

Nonsensitizer: A substance that does not cause skin sensitization following skin contact.

Performance*: The accuracy and reliability characteristics of a test method (see *accuracy*, *reliability*).

Positive control: A substance known to induce a positive response, which is used to demonstrate the sensitivity of the test method and to allow for an assessment of variability in the conduct of the assay over time. For most test methods, the positive control substance is tested concurrently with the test substance and the vehicle/solvent control. However, for some *in vivo* test methods, periodic studies using a positive control substance is considered adequate by the OECD.

Positive predictivity*: The proportion of correct positive responses among substances testing positive by a test method (see *two-by-two table*). It is one indicator of test method accuracy. Positive predictivity is a function of the sensitivity of the test method and the prevalence of positives among the substances tested.

* Definition used by ICCVAM (2003).

ED_006472_00084346-00153

Potency: For the purposes of this BRD, *potency* is defined as a function of the concentration of a substance that is required for either the induction or elicitation of a skin sensitization reaction. For induction, *potency* refers to the concentration of a substance needed to induce a skin sensitization response; the more potent the substance the smaller the quantity needed for induction. Likewise, for elicitation, potency refers to the concentration of a substance need to elicit a response in a previously sensitized individual; the more potent a substance, the smaller the quantity required for elicitation.

Prevalence*: The proportion of positives in the population of substances tested (see *two-by-two table*).

Protocol*: The precise, step-by-step description of a test, including the listing of all necessary reagents, criteria and procedures for the evaluation of the test data.

Quality assurance*: A management process by which adherence to laboratory testing standards, requirements, and record keeping procedures is assessed independently by individuals other than those performing the testing.

Reduction alternative*: A new or modified test method that reduces the number of animals required.

Reference test method*: The accepted *in vivo* test method used for regulatory purposes to evaluate the potential of a test substance to be hazardous to the species of interest.

Refinement alternative*: A new or modified test method that refines procedures to lessen or eliminate pain or distress in animals or enhances animal well-being.

Relevance*: The extent to which a test method correctly predicts or measures the biological effect of interest in humans or another species of interest. Relevance incorporates consideration of the *accuracy* or *concordance* of a test method.

Reliability*: A measure of the degree to which a test method can be performed reproducibly within and among laboratories over time. It is assessed by calculating intra- and interlaboratory reproducibility and intralaboratory repeatability.

Replacement alternative*: A new or modified test method that replaces animals with non-animal systems or replaces one animal species with a phylogenetically lower one (e.g., a mammal with an invertebrate).

Reproducibility*: The consistency of individual test results obtained in a single laboratory (intralaboratory reproducibility) or in different laboratories (interlaboratory reproducibility) using the same protocol and test substances (see intra- and interlaboratory reproducibility).

Sensitivity*: The proportion of all positive substances that are classified correctly as positive in a test method. It is a measure of test method accuracy (see *two-by-two table*).

Skin sensitizer: A substance that will lead to an allergic response following skin contact (UN 2009).

Specificity*: The proportion of all negative substances that are classified correctly as negative in a test method. It is a measure of test method accuracy (see *two-by-two table*).

Stimulation index (SI): A value calculated for the LLNA to assess the skin sensitization potential of a test substance. The value is calculated as the ratio of radioactivity incorporated into the auricular lymph nodes of a group of treated mice to the radioactivity incorporated into the corresponding lymph nodes of a group of vehicle control mice. For the LLNA, an SI equal to or greater than 3 classifies a substance as a potential skin sensitizer.

Test*: The experimental system used. Often used interchangeably with *test method* and *assay*.

Test method*: A process or procedure used to obtain information on the characteristics of a substance or agent. Toxicological test methods generate information regarding the ability of a

* Definition used by ICCVAM (2003).

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substance or agent to produce a specified biological effect under specified conditions. Often used interchangeably with *test* and *assay*. See also *validated test method* and *reference test.*

Transferability*: The ability of a test method or procedure to be accurately and reliably performed in different, competent laboratories.

Two-by-two table*: The two-by-two table can be used for calculating accuracy (concordance) ([a + d]/[a + b + c + d]), negative predictivity (d/[c + d]), positive predictivity (a/[a + b]), prevalence ([a + c]/[a + b + c + d]), sensitivity (a/[a + c]), specificity (d/[b + d]), false positive rate (b/[b + d]), and false negative rate (c/[a + c]).

		New Test Outcome		
		Positive	**Negative**	**Total**
Reference Test Outcome	**Positive**	a	c	a + c
	Negative	b	d	b + d
	Total	a + b	c + d	a + b + c + d

Validated test method*: An accepted test method for which validation studies have been completed to determine the relevance and reliability of this method for a specific proposed use.

Validation*: The process by which the reliability and relevance of a procedure are established for a specific purpose.

Vehicle control: An untreated sample containing all components of a test system, including the vehicle that is processed with the test substance-treated and other control samples to establish the baseline response for the samples treated with the test substance dissolved in the same vehicle.

Weight-of-evidence (process): The strengths and weaknesses of a collection of information are used as the basis for a conclusion that may not be evident from the individual data.

* Definition used by ICCVAM (2003).

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Annex I

LLNA/EC3 Validation - Submission

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LLNA/EC3 Validation

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Following the formal validation of the local lymph node assay (LLNA) as a method for hazard identification by ICCVAM and ECVAM (NIH, 1999; Gerberick et al, 2000; Balls and Hellsten, 2000; Dean et al, 2001), and it subsequent enshrinement in regulatory guidelines (OECD, 2002), considerable further evaluation and development of the LLNA has taken place. Most notably, this has been in the use of the LLNA to determine relative potency, so that potential skin sensitisers may be ranked and to provide a key input for skin sensitisation risk assessment. As a consequence, it has been proposed to perform a validation of the potency measurements provided by the LLNA. For this purpose the following questions are addressed in this dossier:

Q1: In those circumstances where an evaluation of skin sensitization potency is required for risk assessment purposes, do EC3 values derived from linear interpolation of LLNA dose response data provide an appropriate and reliable approach?

Q2: If yes, do EC3 values provide a suitable method for ranking of contact allergens according to skin sensitisation potency?

Q3: If yes, does ranking of potency based on LLNA-derived EC3 values correlate with available human data and clinical experience?

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Background

For the prediction of skin sensitisation potential, the local lymph node assay (LLNA) was proven several years ago to be a fully validated alternative to guinea pig tests. More recently, information from LLNA dose response analyses has been used to assess the relative potency of skin sensitising chemicals. These data are then deployed for risk assessment and risk management. EC3 measurements are reproducible in both intra- and inter-laboratory evaluations and are stable over time. It has been demonstrated also, by several independent groups, that EC3 values correlate closely with data on relative human skin sensitisation potency. In this dossier, the validity of these relative potency measurements are reviewed. It is concluded the LLNA conducted following the principles of OECD Guideline 429 does provide a valuable assessment of relative sensitising potency in the form of the EC3 value (estimated concentration of a chemical required to produce a 3-fold stimulation of draining lymph node cell proliferation compared with concurrent controls), and that all reasonable validation requirements have been addressed successfully. Consequently, the recommendation made here is that LLNA EC3 measurements should now be regarded as a validated method for the determination of the relative potency of skin sensitising chemicals.

Introduction

The LLNA has been formally validated and adopted into OECD guidelines. The internationally accepted method presented in Guideline 429 follows the standard protocol published 10 years earlier (Kimber and Basketter, 1992), but allows also for the use of a greater number of mice per group and pooling of nodes from individual animals. It also foresees the use of an alternative (radioactive) endpoint should it prove to be equally sensitive as the ^{3}HTdR employed in the standard assay. All the discussion that follows concerning the possibility of ranking potency in the LLNA draws on knowledge derived from LLNAs conducted according to OECD Guideline 429. In the few instances where this is not the case but it is felt that the information makes an important contribution, it has been clearly indicated with any limitations identified.

It is not appropriate here to review any aspect of the validation of the LLNA for basic "yes/no" hazard identification or to present a detailed protocol since this is now well established (NIH, 1999; Gerberick et al, 2000; Balls and Hellsten, 2000; Dean et al, 2001). However, it is worthwhile recalling why the classification threshold for this binary decision was set at a stimulation index (SI) value of 3. The SI itself simply represents the ratio of ^{3}HTdR counts in the test group compared to those in the concurrent vehicle treated control. In the earliest phase of assay development, it was judged that and SI of 3 was the point where a clear activation signal could be separated from the inherent biological noise. With greater experience and testing of greater numbers of chemicals, it became clearer that this value represented a good point of discrimination between sensitisers and irritants/non-sensitisers. Ultimately, a retrospective analysis of over a hundred chemicals confirmed that an SI of 3 was an appropriate, if slightly conservative, threshold (Basketter et al, 1999). It is worth noting that other workers, using a non-OECD compliant version of the LLNA (^{3}HTdR incorporation is measured *in vitro* in a manner very similar to the earliest published work on the LLNA in the late 1980s) have also found an SI of 3 a suitable threshold for the identification of skin sensitising chemicals (eg van Och et al, 2000; De Jong et al, 2002).

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Since the original validation of the LLNA, data on a considerable number of chemicals have been generated. Much of this work has been placed in the public domain via the peer reviewed literature (eg Gerberick et al, 2005; Basketter et al, 2007; Anderson et al, 2007). All of these publications have successfully used an SI value of 3 as a means of identifying skin sensitising chemicals. Currently, a further manuscript is being publication which adds approximately 100 further chemicals to the database and provides the corrections to the original Gerberick et al paper published at the end of 2005. The corrections are already available as are many of the new chemicals and so these are presented in Appendix 1. As this new data is still being compiled for publication, it has not been subjected to detailed analysis here.

Beyond these considerations however has emerged the question of whether and to what extent the quantitative output of the LLNA might also be used to provide some indication of the strength of a skin sensitiser. These thoughts were first fully encapsulated in a publication in 1997, where the concentration of the known potent allergen 2,4-dinitrochlorobenzne necessary to generate a LLNA threshold response was contrasted with that of the OECD weak positive control allergen, hexyl cinnamic aldehyde (Kimber and Basketter, 1997). The 160 fold difference in these concentrations was felt to be important and led to much further investigation, the culmination of which is encapsulated in the pages which follow. It is important to mention that this type of analysis is common in many other toxicology endpoints.

Data to support LLNA as a reliable and robust approach for skin sensitization dose response analysis

The protocol for the determination of the LLNA EC3 value is as follows. Essentially, the method represents a simple linear interpolation of the points in the dose response curve that lie immediately above and below the classification threshold, ie a stimulation index of 3. If the data points lying immediately above and below the SI value of 3 have the co-ordinates (a,b) and (c,d) respectively, then the EC3 value may be calculated using the equation: $EC3 = c+[(3-d)/(b-d)](a-c)$. This is represented graphically in Figure 1. Where this equation cannot be applied, then an approach to model a limited degree of extrapolation of LLNA dose response data can be deployed (Ryan et al, 2007).

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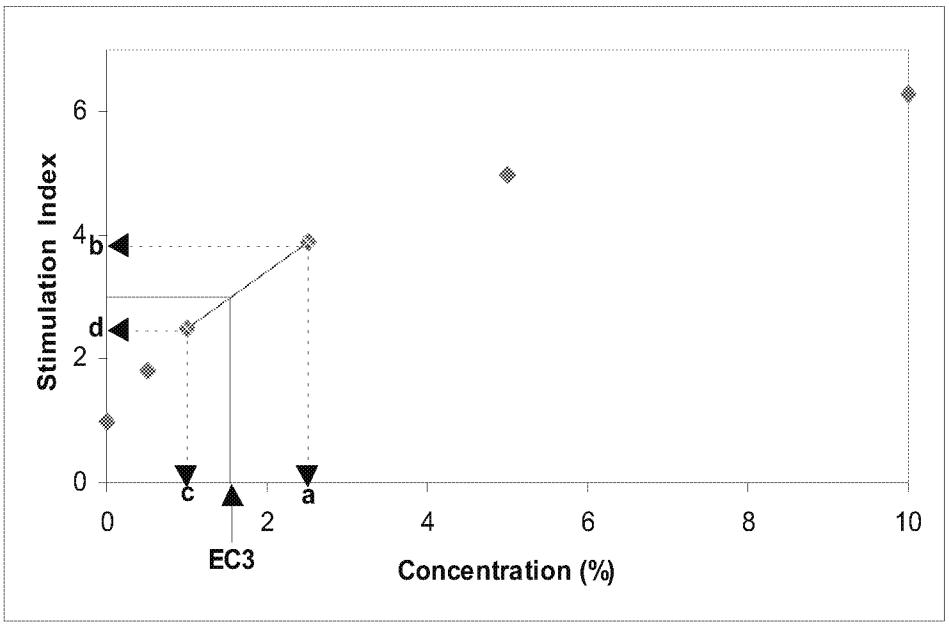

Figure 1. The calculation of the LLNA EC3 value by linear interpolation

The appropriateness of this simple approach compared to more complex methods was demonstrated several years ago (Basketter et al, 1999). Since that time, others have examined similar approaches, albeit with a non-OECD protocol, and have demonstrated that the outcome is the same as linear interpolation (van Och et al, 2000; De Jong et al, 2002). EC3 values for a large number of chemicals have now been published, much being collated in the seminal paper from 2005 on 211 substances, which also shows that these values span several (about 5) orders of magnitude (Gerberick et al, 2005). Subsequent to this, further EC3 values have been published (Betts et al, 2005; Anderson et al, 2007; Basketter et al, 2007b; Dearman et al, 2007; SCCP 2007). To date, the lowest value (most potent allergen) is benz(a)pyrene with an EC3 of 0.0009% and the highest value among sensitisers is 89% for aniline. The dataset comprises 42 non-sensitizers; 66 weak sensitizers; 69 moderate sensitizers; 21 strong sensitizers; and 13 extreme sensitizers if one used the categorization scheme proposed by ECETOC (Kimber et al, 2003).

It has also been noted also that the 211 chemicals reported with EC3 values in the 2005 publication span the full range of reactive chemistry associated with skin sensitisation (Roberts et al, 2006; Aptula et al, 2007). These workers have concluded that sensitisers fall into some 6 main categories with a modest number of special cases, all of which are populated by the >200 chemicals for which EC3 values have been derived. However, it is of course important also that a quantitative measure such as the LLNA EC3 value is robust and reproducible, within a laboratory, between laboratories and over time. These aspects are reviewed in the following paragraphs.

In the original validation of the LLNA, five laboratories used the assay with a set of sensitisers and non-sensitisers, and even with the technical variations which inevitably arose

in the detail of test conduct, came up with essentially identical threshold predictions on all the substances evaluated (Kimber et al, 1995; Loveless et al, 1996). It should be noted that this work was done before the final definition of the OECD protocol and also before the final definition of how to derive the EC3 value in 1999. On this foundation, the reliability (robustness) of the prediction of EC3 values has been further assessed within single laboratories. Data have been published that reveal that the OECD positive control, hexyl cinnamic aldehyde (HCA), a weak sensitiser, gives reproducible EC3 values over time in an individual laboratory (Dearman et al, 2001). This has also been shown for other weak allergens (Basketter et al, 2007a). The reproducibility of EC3 values has also been tested at the opposite end of the potency spectrum, for the very strong allergen, p-phenylenediamine (PPD) which was assessed in each of two laboratories (Warbrick et al, 1999). EC3 values were highly consistent over each of 4 monthly determinations in each laboratory. Lastly, the EC3 value for a moderate allergen, isoeugenol, was assessed in a single laboratory (Basketter and Cadby, 2004).

The outcome of these various assessments supplemented with a small amount of additional unpublished data for 17 chemicals of widely varying skin sensitisation potency has been collated in Table 1. What is of particular note here is that, whilst there is of course biological variation in the EC3 determination (eg isoeugenol, where 31 determinations give a mean and standard error EC3 value of 1.5% ± 0.1%), the values typically lie well within their order of magnitude banding. Putting this differently, the variation in EC3 value for any given chemical tested in the same vehicle is substantially less than an order of magnitude, whereas when a wide range of skin sensitisers are examined, then EC3 values for substances of different potency span several orders of magnitude. Of course, vehicles can, and do, have an impact on derived EC3 values (reviewed in Basketter et al, 2001). However, the extent of this variation is usually no greater than the variation in EC3 values found with repeated measurements in the same vehicle (Table 1). A manuscript presenting a statistical evaluation confirming this is being finalised for submission to a suitable journal.

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Table 1 Collation of EC3 data from repeat testing of 17 chemicals in multiple laboratories (data taken from Basketter et al, 2007a)

Substance	EC3 values (%)	Vehicle[1]	Mean EC3 (%) ± SE[2]
Bandrowski's base	0.04, 0.02	AOO	0.03
2,4-Dinitrochlorobenzene	0.04, 0.02, 0.05, 0.03, 0.03, 0.02, 0.06, 0.03, 0.06, 0.05, 0.05, 0.06, 0.05	AOO	0.04 ± 0.004
Potassium dichromate	0.05, 0.08, 0.14	DMSO	0.09 ± 0.046
p-Phenylenediamine	0.07, 0.12, 0.09, 0.08, 0.06, 0.14, 0.06, 0.18, 0.16, 0.13	AOO	0.11 ± 0.014
1,4-Hydroquinone	0.11, 0.19, 0.12	AOO	0.14 ± 0.04
Methyldibromoglutaronitrile	1.8, 0.9, 1.3	AOO	1.3 ± 0.45
Isoeugenol	1.7, 1.1, 1.4, 1.3, 1.3, 1.0, 1.4, 1.5, 2.9, 0.8, 1.3, 1.6, 2.8, 0.9, 1.0, 1.7, 1.2, 1.4, 0.8, 2.1, 2.3, 1.1, 1.2, 1.2, 0.7, 1.0, 2.3, 1.3, 2.0, 1.6, 1.3	AOO	1.5 ± 0.1
Cinnamal	3.1, 1.7, 2.7	AOO	2.3 ± 0.4
1-Bromopentadecane	5.2, 5.1	AOO	5.1 ± 0.02
L-Perillaldehyde	8.1, 7.8	AOO	8.0
Hexylcinnamal	6.6, 11.3, 10.6, 4.4, 11.5, 8.8, 7.6, 11.0, 7.0, 10.6, 11.9, 11.7, 10.9, 11.7, 12.2	AOO	9.9 ± 0.6
Eugenol	15.0, 4.9, 12.9, 7.5	AOO	10.1 ± 2.3
Abietic acid	14.7, 8.3, 10.6	AOO	11.3 ± 1.8
Penicillin G	16.7, 17.9, 30	DMSO	21.5 ± 4.3
Imidazolidinyl urea	23.9, 31.2	DMF	27.6
Hydroxycitronellal	33.0, 27.5, 23.0	AOO	27.8 ± 2.9
2-Ethylbutyraldehyde	60, 76	AOO	68

[1]AOO = acetone olive oil, 4:1, v/v; DMF = dimethyl formamide; DMSO = dimethylsulphoxide
[2]Numbers to no more than 2 significant figures; standard error not calculated if there were less than 3 data points.

Data to support that the LLNA EC3 is suitable for potency categorization and correlates with historical human data and clinical experience

The LLNA has been shown to be relevant as a model for the predictive identification chemicals with skin sensitization hazard. The protocol provides an objective measure of the crucial stage of the sensitisation process, the clonal expansion of lymphocytes that results from the application of a contact allergen by the appropriate route, epidermal application

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(Oort and Turk, 1965; Parrot and de Sousa, 1966). Both the route of administration and the immunological mechanisms involved are the same as those in man. The original validation of the LLNA contained a considerable number of known human sensitisers which were correctly identified by one or more of the contributing laboratories (NIH, 1999; Gerberick et al, 2000). This work was followed up with a specific study of a panel of known human contact allergens (n=11) which were correctly identified (Ryan et al, 2000). The quantitative element of the LLNA response was also noted some years ago (Kimber and Dearman, 1991). The method for the determination of the EC3 value having been fixed (see above), the relationship between LLNA EC3 values and human skin sensitisation potency was subsequently described.

Before reviewing this, two important points must be made: firstly, potency refers to the intrinsic property of a sensitising chemical, which is entirely independent from the frequency with which allergic contact dermatitis occurs in the general or a clinical population (since this depends heavily on exposure as well as potency); secondly, there is a paucity of data indicating the intrinsic potency of chemical skin sensitisers in humans, since this requires experimental studies of dubious ethics. Thus, the work that appears in the literature cannot offer the degree of certainty with regard to human/mouse correlations that would ideally be liked, and a degree of judgement is inevitable to help compensate for the relatively poor quality of the limited human data that are available. Hence, it has been important that many of the publications in this area have involved independent partners closely associated with the LLNA, including dermatologists, regulators and independent scientists (Hilton et al, 1998; Basketter et al, 1999, 2000, 2001, 2005; Gerberick et al, 2001; Griem, 2003; Schneider and Akkan, 2004).

The earlier potency comparisons referred to above tended only to assign human skin sensitisers into one of a number of categories (non, weak, moderate, strong, extreme) and to use the LLNA EC3 value to demonstrate that it was possible to assign the sensitising chemicals into these categories if certain cut-off limits were applied. Such an approach was strongly endorsed by industry groups (Kimber at al, 2001; 2003), by regulatory groups (Basketter et al, 2005) and most recently by the World Health Organisation (WHO, 2007). Although the outcome of this type of analysis could prove very useful, more interesting work was done by a number of groups who attempted to compare experimental thresholds in humans, typically a no effect level in a human repeated insult patch test (HRIPT) with the LLNA threshold, the EC3 value. Neither of these thresholds is of course absolute; they depend very much on the exposure conditions of the protocols. However, since each protocol is standardised, particularly the LLNA, then they represent a reasonable point of departure for such comparisons. Two groups have published such comparisons in 2003 and 2004. In one study, over 50 substances were assessed and a relationship between the LLNA and HRIPT thresholds shown (Schneider and Akkan, 2004). In a second study, a slightly different approach was chosen, but again a good relationship was demonstrated (Griem, 2003). Lastly, in a more recent analysis, a very critical approach was taken to selection of human data to try to ensure that only good quality HRIPT threshold information was used (Basketter et al, 2005). This restricted the analysis to just 25 substances, but again a good relationship between EC3 values and HRIPT thresholds was shown. In order to directly compare EC3 values, which are calculated as % concentration, to HRIPT thresholds, data from both test methods are expressed as dose per unit areas ($\mu g/cm^2$).

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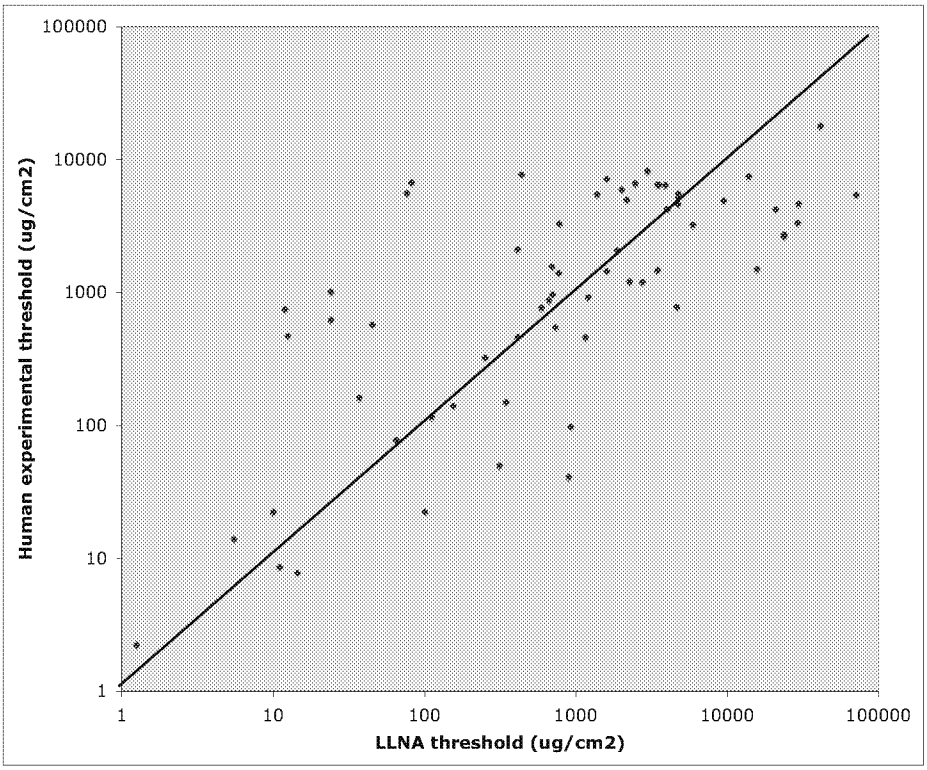
From these publications, it is possible to assemble all of the human intrinsic potency thresholds (ie the data from predictive human assays) and to compare them with LLNA EC3 values for the same chemicals. This is shown in figure 1.

Figure 1 Plot of human experimental thresholds v LLNA EC3 values



It is clear from this figure that there is a relationship between the two thresholds. The fact that the points fit well with the diagonal is also encouraging. It is our view that most of the variability in the dataset derives from the human studies. Within the publications reporting these data (Gerberick et al, 2001; Griem, 2003; Schneider and Akkan, 2004 and Basketter et al, 2005), several assumptions have had to be made. Furthermore, the human data were not produced to a well standardised protocol. Both of these factors are likely to contribute markedly to the spread of the human data.

It should be noted that the comparison of human and murine thresholds in Figure 1 comprises some 66 chemicals which cover a very wide spread of potency. The data underlying the figure is contained in Table 2. The threshold data for humans does not represent safe levels for occupational/consumer exposure.

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Table 2 Human experimental v LLNA EC3 thresholds

Substance	Human threshold (µg/cm^2)	Murine threshold (µg/cm^2)
MCI/MI	1.25[a]	2.25
Dinitrochlorobenzene	5.5[b]	14
Phenylenediamine	10[c]	22.5
Beryllium sulfate	11[b]	8.6
Phenylacetaldehyde	12[d,e]	750
Methylisothiazolinone	12.5[a]	475
Tetrachlorosalicylanilide	14.4[b]	7.8
Methyl-2-nonynoate	24[a]	625
Methyloctine carbonate	24[a]	625
trans-2-Hexenal	24[a]	1012
Formaldehyde	37[a]	162.5
Benzoisothiazolione	45[a]	575
Gold chloride	65[b]	78
Penicillin	76[b]	5606
Streptomycin	82[b]	6750
Glutaraldehyde	100[a]	22.5
Potassium dichromate	111[b]	116
Nickel sulfate	154[c]	140
Isoeugenol	250[a]	325
Cobalt sulfate	313[b]	50
Glyoxal	345[b]	150
Diethylenetriamine	411[b]	463
Methylanisylidene acetone	412[b]	2123
Butylglycidylether	437[b]	7725
Cinnamic aldehyde	591[a]	775
Thioglycerol	661[b]	878
Phenylpropionaldehyde	692[b]	1575
Oakmoss	700[a]	970
Ethylenediamine	732[b]	550
Dihydrocoumarin	769[b]	1402
Citral	775[a,f]	3300
Benzoyl peroxide	895[b]	41
Mercury bichloride	924[b]	98
Chlorpromazine	1150[b]	463
Benzylidene Acetone	1200[c]	925
Methylhydrocinnamal	1379[a]	5500
Diethylmaleate	1600[c]	1450
Ethyl acrylate	1600[c]	7175
Kanamycin	1874[b]	2075
Imidazolidnyl urea	2000[c]	5975
Pentachlorophenol	2155[b]	5000
Mercaptobenzothiazole	2269[b]	1214
Aniline	2463[b,g]	6658
Farnesol	2755[a]	1200
Hydroxycitronellal	2953[a,h]	8250
Anisyl alcohol	3448[a]	1475
Methylhexanedione	3448[c]	6500
Acetyl isovaleryl	3541[b]	6450
Geraniol	3875[a,i]	6475
Lyral HMPCC	4000[a]	4275
Tetramethylthiruamdisulphide	4610[b]	785
Benzyl cinnamate	4720[a]	4600
Cinnamic alcohol	4724[c,j]	5150

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Substance	Human threshold (μg/cm²)	Murine threshold (μg/cm²)
Cyclamen aldehyde	4724 [a]	5575
Eugenol	5905 [a]	3225
Phenyl benzoate	9448 [a]	4900
Linalool	13793 [a]	7500
Neomycin sulfate	15625 [b]	1500
Benzylbenzoate	20690 [a]	4250
Alpha Amyl cinnamic alcohol	23622 [d,k]	2650
Amylcinnamic aldehyde	23622 [a]	2750
Hexylcinnamic aldehyde	23622 [a]	2750
Benzocaine	29167 [b]	3338
Lilial	29525 [c,l]	4675
Pyridine	41051 [b]	17975
isoMethylionone	70866 [a]	5450

Abbreviations: DSA_{05} = dose per unit area of skin leading to a sensitization incidence of 5% calculated by linear interpolation from published HRIPT or HMT data; EC3 = estimated concentration of test substance necessary to produce an SI of 3; HMT = human maximization Test; HRIPT = Human repeated insult patch test; IFRA = International Fragrance Research Association; LLNA = murine local lymph node assay; LOEL = lowest observed effect level; NOEL = no observed effect level; SI = stimulation index.

[a] Value is a NOEL.
[b] Value is a DSA_{05} (Schneider and Akkan, 2004)
[c] Value is a LOEL.
[d] Source for this datum could not be verified.
[e] An HRIPT NOEL of 591 is reported in the IFRA std (see http://www.ifraorg.org/index).
[f] An HRIPT NOEL of 1400 is reported in the IFRA std (see http://www.ifraorg.org/index).
[g] A LOEL of 1379 is reported in Griem et al (2003).
[h] An HRIPT NOEL of 5000 is reported in the IFRA std (see http://www.ifraorg.org/index).
[i] An HRIPT NOEL of 11,811 is reported in the IFRA std (see http://www.ifraorg.org/index).
[j] An HRIPT NOEL of 3000 is reported in the IFRA std (see http://www.ifraorg.org/index).
[k] An HRIPT NOEL of 3543 is reported in the IFRA std (see http://www.ifraorg.org/index).
[l] An HRIPT NOEL of 4125 is reported in the IFRA std (see http://www.ifraorg.org/index).

Data to support the utility of the LLNA EC3 value (potency determination) in quantitative risk assessments for skin sensitization

For completeness, this section provides a succinct overview of how EC3 values might deliver value with respect to risk assessment and risk management. Two general possibilities have been considered. The first is placement of skin sensitising chemicals into one of a number of categories based on their potency (eg Gerberick et al, 2001; Kimber et al, 2003; Basketter et al, 2005). There are small differences between these various proposals, but all accept that skin sensitisers cover a very wide spectrum of relative potency and that strong and extreme allergens should be differentiated from moderate and weak allergens. It is known that the OECD is working on this concept and that the World Health Organisation convened an expert group which came to a similar, but as yet, unpublished, conclusion.

The second possibility is that the LLNA EC3 value can be used as a starting point for risk assessment (Kimber and Basketter, 1997). This option has been developed as fully as categorisation, but has the benefit of having also been implemented. The basic approach to the use of EC3 values in a quantitative risk assessment (QRA) has been outlined in a sequence of publications (Gerberick et al, 2001, Felter et al, 2002 and 2003). Use of the approach has been then detailed in several further publications (Basketter et al, 2003 and 2007; Zachiariae, 2003; Corea et al, 2006; Api et al, 2007; Jowsey et al, 2007; www.ifraorg.org, 2007).

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In principle, QRA for skin sensitisation follows the general principles of many toxicology endpoints: the determination of a no effect level in the animal model and then employment of a series of uncertainty factors to predict a safe exposure level for humans. The QRA approach as currently deployed identifies an acceptable daily exposure for specific skin sensitiser in a particular product use scenario. No doubt it could be modified to identify a general upper limit for daily exposure to a particular skin allergen, remembering always that this figure must be expressed in terms of dose per unit area. More detailed discussion of this topic can be found elsewhere (Kimber et al, 2007).

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Authors' response to the questions

Q1: In those circumstances where an evaluation of skin sensitization potency is required for risk assessment purposes, do EC3 values derived from linear interpolation of LLNA dose response provide an appropriate and reliable approach?

A1: It is the view of the authors of this document that LLNA EC3 values do provide and appropriate and reliable approach.

Q2: If yes, do EC3 values provide a suitable method for ranking of contact allergens according to skin sensitisation potency?

A2: It is the view of the authors of this document that EC3 values do permit a useful ranking of contact allergens according to skin sensitisation potency. Given that EC3 values span some 5 orders of magnitude, it is further noted that ranking into a similar number of categories should be possible.

Q3: If yes, does ranking of potency based on LLNA-derived EC3 values correlate with available human data and clinical experience?

A3: It is the view of the authors of this document that relative potency in the mouse correlates well with human data, always bearing in mind that the latter are available only in limited quantities and are not always of good quality.

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References

Anderson SE, Wells J, Fedorowicz A, Butterworth L, Meade B, Munson AE. Evaluation of the contact and respiratory sensitization potential of volatile organic compounds generated by simulated indoor air chemistry. Toxicol Sci 2007: 97, 355-363.

Api A M, Basketter D A, Cadby PA, Cano M-F, Ellis G, Gerberick G F, Griem P, McNamee P M, Ryan C A, Safford B. Dermal sensitization quantitative risk assessment (QRA) for fragrance ingredients. Regulatory Toxicology and Pharmacology, accepted.

Balls M,, Hellsten E., 2000 Statement on the validity of the local lymph node assay for skin sensitisation testing. ECVAM Joint Research Centre, European Commission, Ispra. Alternatives to Laboratory Animals 28, 366-367.

Basketter D A, Andersen K E, Lidén C, van Loveren H, Boman A, Kimber I, Alanko K, Berggren E. Evaluation of the skin sensitising potency of chemicals using existing methods and considerations of relevance for elicitation. Contact Dermatitis 2005: 52: 39 - 43.

Basketter D A, Blaikie L, Dearman R J, Kimber I, Ryan C A, Gerberick G F, Harvey P, Evans P, White I R, Rycroft R J G. Use of the local lymph node assay for the estimation of relative contact allergenic potency. Contact Dermatitis 2000: 42: 344 - 348.

Basketter D A, Cadby P. Reproducible prediction of contact allergenic potency using the local lymph node assay. Contact Dermatitis 2004: 50: 15 - 17.

Basketter D A, Clapp C J, Safford B J, Jowsey I R, McNamee P M, Ryan C A, Gerberick G F. Preservatives and skin sensitisation quantitative risk assessment: risk benefit considerations. Dermatitis 2007: accepted.

Basketter D A, Clapp C, Jefferies D, Safford R J, Ryan C A, Gerberick G F, Dearman R J, Kimber I. Predictive identification of human skin sensitisation thresholds. Contact Dermatitis 2005: 53: 260 - 267.

Basketter DA, Gerberick GF and Kimber I. (2007a) The local lymph node assay EC3 value: status of validation. Contact Dermatitis, in press.

Basketter D A, Gerberick G F, Kimber I, Loveless S E. The local lymph node assay - a viable alternative to currently accepted skin sensitization tests. Food and Chemical Toxicology 1996: 108: 141 – 152.

Basketter D A, Lea L, Cooper K, Dickens A, Briggs D, Pate I, Dearman R J, Kimber I. A comparison of statistical approaches to derivation of EC3 values from local lymph node assay dose responses. Journal of Applied Toxicology 1999b: 19: 261 - 266.

Basketter D A, Lea L, Cooper K, Dickens A, Stocks J, Pate I, Dearman R J, Kimber I. Thresholds for classification as a skin sensitiser in the local lymph node assay: a statistical evaluation. Food and Chemical Toxicology 1999a: 37: 1167 - 1174.

ED_006472_00084346-00171

Basketter D A, Roberts D W, Cronin M, Scholes E W. The value of the local lymph node assay in quantitative structure activity investigations. Contact Dermatitis 1992: 27: 137-142.

Basketter D A, Rodford R, Kimber I, Smith I, Wahlberg J E. Skin sensitization risk assessment: a comparative evaluation of 3 isothiazolinone biocides. Contact Dermatitis 1999c: 40:150-154.

Basketter D A, Sanders D and Jowsey I R. (2007b) The sensitisation potential of resorcinol: experience with the local lymph node assay. Contact Dermatitis, 56, 196 - 200.

Basketter D A, Wright Z M, Warbrick E V, Dearman R J, Kimber I, Ryan C A, Gerberick G F, White I R. Human potency predictions for aldehydes using the local lymph node assay. Contact Dermatitis 2001: 45: 89-94.

Betts C J, Dearman R J, Heylings J R, Kimber I, Basketter D A. Skin sensitization potency of methyl methacrylate in the local lymph node assay : comparisons with guinea-pig data and human experience. Contact Dermatitis 2006: 55: 140 - 147.

Betts C J, Dearman R J, Kimber I, Maibach H I. Potency and risk assessment of a skin-sensitizing disperse dye using the local lymph node assay. Contact Dermatitis 2005: 52: 268 - 272.

Corea N, Basketter DA, van Asten A, Marty J-P, Pons Guiraud A and Laverdet C (2006) Fragrance allergy: assessing the risk from fabric washing products. Contact Dermatitis, 55, 48 - 53.

De Jong W H, van Och F M, Den Hartopg Jager C F, Spiekstra S W, Slob W, Vandebriel R J, van Loveren H J. Ranking of allergenic potency of rubber chemicals in a modified local lymph node assay. Toxicological Sciences 2002: 66: 226 – 232.

Dean J.H., Twerdok L.E., Tice R.R., Sailstad D.M., Hattan D.G., Stokes W.S., 2001. ICCVAM evaluation of the murine local lymph node assay. II. Conclusions and recommendations of an independent scientific peer review panel. Regulatory Toxicology and Pharmacology 34, 258-273.

Dearman R J, Betts C, Farr C, McLaughlin J, Berdasco N, Wiench K and Kimber I. Comparative analysis of skin sensitisation potency of acrylates (methyl acrylate, ethyl acrylate, butyl acrylate and ethylhexyl acrylate) using the local lymph node assay. Contact Dermatitis, in press.

Dearman R J, Wright Z M, Basketter D A, Ryan C A, Gerberick G F, Kimber I. The suitability of hexyl cinnamic aldehyde as a calibrant for the using local lymph node assay. Contact Dermatitis 2001: 44: 357 - 361.

Felter S P, Robinson M K, Basketter D A, Gerberick G F. A review of the scientific basis for default uncertainty factors for use in quantitative risk assessment of the induction of allergic contact dermatitis. Contact Dermatitis 2002: 47: 257 - 266.

ED_006472_00084346-00172

Felter S P, Ryan C A, Basketter D A, Gerberick G F. Application of the risk assessment paradigm to the induction of allergic contact dermatitis. Regulatory Toxicol Pharmacol 2003: 37: 1 - 10.

Gerberick G F, Robinson M K, Felter S P, White I R, Basketter D A. Understanding fragrance allergy using an exposure-based risk assessment approach. Contact Dermatitis 2001b: 45: 333 - 340.

Gerberick G F, Robinson M K, Ryan C A, Dearman R J, Kimber I, Basketter D A, Wright Z, Marks J G. Contact allergenic potency: correlation of human and local lymph node assay data. American Journal of Contact Dermatitis 2001a: 12: 156 - 161.

Gerberick G F, Ryan C A, Kern P S, Schlatter H, Dearman R J, Kimber I, Patlewicz G, Basketter D A. Compilation of historical local lymph node assay data for the evaluation of skin sensitization alternatives. Dermatitis 2005: 16, 157 - 202.

Gerberick G F, Ryan C A, Kern P S, Schlatter H, Dearman R J, Kimber I, Patlewicz G, Basketter D A. Updating the compilation of historical local lymph node assay data for the evaluation of skin sensitization alternatives. Dermatitis 2007: MS in preparation.

Gerberick GF, Ryan CA, Kimber I, Dearman RJ, Lea, LJ and Basketter DA (2000) Local lymph node assay validation assessment for regulatory purposes. Am J Cont Derm, 11, 3-18.

Griem P, Goebel C, Scheffler H. Proposal for a risk assessment methodology for skin sensitization based on sensitization potency data. Regulatory Toxicology and Pharmacology 2003: 38: 269-290.

Hilton J, Dearman R J, Harvey P, Evans P, Basketter D, Kimber I. Estimation of the relative skin sensitizing potency using the local lymph node assay: a comparison of formaldehyde with glutaraldehyde. American Journal of Contact Dermatitis 1998: 9: 29-33.

www.ifraorg.org (International Fragrance Research Association (IFRA)) (2007) http://www.ifraorg.org/Enclosures/News/IFRA%20RIFM%20QRA%20Information%20booklet%20FINAL%20REVISED%202007%2006%2007.pdf
Jowsey IR, Kligman AM, White IR, Goossens A and Basketter DA. (2007) Evidence that two alkyl ester quaternary ammonium compounds (ester quats) lack substantial human skin sensitising potential. Dermatitis, 18, 32 - 39.

Kimber I, Basketter D A, Berthold K, Butler M, Garrigue J L, Lea L J, Newsome C, Roggeband R, Steiling W, Stropp G, Waterman S, Wiemann C. Skin sensitization testing in potency and risk assessment. Toxicological Sciences 2001: 59: 198-208.

Kimber I, Basketter D A. Contact sensitization: A new approach to risk assessment. Human and Ecological Risk Assessment 1997: 3: 385 - 395.

Kimber I, Basketter D A. The murine local lymph node assay; collaborative studies and new directions: A commentary. Food and Chemical Toxicology 1992: 30: 165 - 169.

ED_006472_00084346-00173

Kimber I, Dearman R J. Investigation of lymph node cell proliferation as a possible immunological correlate of contact sensitizing potential. Food and Chemical Toxicology 1991: 29: 125 – 129.

Kimber I, Basketter DA, Butler M, Gamer, A., Garrigue JL, Newsome C, Steiling W and Vohr H-W. (2003) Classification of allergens according to potency: proposals. Food Chem Toxicol, 41, 1799 - 1809.

Kimber I, Dearman R J, Basketter D A, Ryan C A, Gerberick G F, Lalko J and Api A M. (2007) Dose metrics in the acquisition of skin sensitization: thresholds and importance of dose per unit area. Regulatory Toxicology and Pharmacology, submitted.

Loveless S E, Ladics G S, Gerberick G F, Ryan C A, Basketter D A, Scholes E W, House R V, Hilton J, Dearman R J, Kimber I. Further evaluation of the local lymph node assay in the final phase of an international collaborative trial. Toxicology 1996: 108: 141 - 152.

NIH (1999). The murine local lymph node assay: a test method for assessing the allergic contact dermatitis potential of chemicals/compounds. NIH No. 99-4494.

Organisation for Economic Cooperation and Development. 2002. Guidelines for Testing of Chemicals No. 429. Skin sensitization: The Local Lymph Node Assay [http://www.oecd.org].

Oort J, Turk J L. A histological and autoradiographic study of lymph nodes during the development of contact sensitivity in guinea pigs. British Journal of Experimental Pathology 1965: 46: 147 - 154.

Parrot D M V, de Sousa M A B. Changes in the thymus-dependent areas of lymph nodes after immunological stimulation. Nature Lond. 1966: 212: 1316 - 1317.

Ryan C A, Chaney J G, Kern P S, Dearman R J, Kimber I, Basketter D A, Gerberick G F. Extrapolating local lymph node assay EC3 values to estimate relative sensitizing potency. Journal of Toxicology: Cutaneous and Ocular Toxicology 2007: accepted.

Ryan C A, Gerberick G F, Cruse L W, Basketter D A, Lea L, Blaikie L, Dearman R J, Warbrick EV, Kimber I. Activity of human contact allergens in the murine local lymph node assay. Contact Dermatitis 2000: 43: 95-102.

SCCP (Scientific Committee on Consumer Products in the European Union), 2007. http://ec.europa.eu/health/ph_risk/committees/04_sccp/docs/sccp_s_05.pdf

Schneider K, Akkan Z. Quantitative relationship between the local lymph node assay and human skin sensitization assays. Regulatory Toxicology and Pharmacology 2004: 39: 245-255.

van Och FM, Slob W, de Jong WH, Vandebriel RJ, van Loveren H. A quantitative method for assessing the sensitizing potency of low molecular weight chemicals using a local lymph node assay: employment of a regression method that includes determination of the uncertainty margins. Toxicology. 2000 146(1): 49-59.

ED_006472_00084346-00174

Warbrick E V, Dearman R J, Lea L J, Basketter D A, Kimber I. Local lymph node assay responses to paraphenylenediamine: intra- and inter-laboratory evaluations. Journal of Applied Toxicology 1999: 19: 255 - 260.

White J M L, Kullavanijaya P, Duangdeeden I, Zazzeroni R, Gilmour N J, Basketter D A, McFadden J P. p-Phenylenediamine allergy: The role of Bandrowski's base. Clinical and Experimental Allergy 2006: 36: 1289 - 1293.

Zachariae C, Rastogi S, Devantier C, Menné T, Johansen J D. Methyldibromo glutaronitrile: clinical experience and exposure-based risk assessment. Contact Dermatitis 2003: 48: 150-154.

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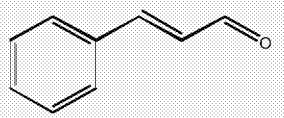
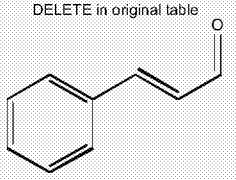
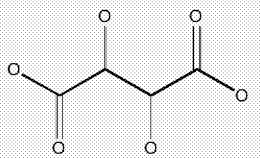
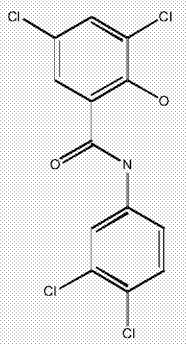
Appendix 1 Tabulation of corrections to Gerberick et al, 2005 database plus 31 additional chemicals

Table of Corrections

CHEMICAL STRUCTURE	CAS #	Vehicle[1]	LLNA %	LLNA %	LLNA %	LLNA %	LLNA %	LLNA %	LLNA SI	LLNA SI	LLNA SI	LLNA SI	LLNA SI	LLNA SI	LLNA EC3 %	Potency category	Ref.
 Cinnamic aldehyde	104-55-2	AOO	0.5	1.0	2.5	5.0	10.0		1.4	0.9	1.9	7.1	15.8		3.0	moderate	Basketter DA, Wright ZM, Warbrick EV, et al. Human potency predictions for aldehydes using the local lymph node assay. Contact Derm 2001; 45:89-94.
DELETE in original table  3-Phenyl propenal	14371-10-9	AOO	1.0	2.5	5.0	10.0	25.0		2.4	4.7	8.8	10.2	13.1		1.4	moderate	Patlewicz G, Wright ZM, Basketter DA, et al. Structure-activity relationships for selected fragrance allergens. Contact Derm 2002; 47:219-226.
 Tartaric acid	87-69-4	DMF	5	10	25				1.0	0.9	1.5					non-sensitizer	UL unpublished

Undec-10-enal	112-45-8	AOO	5.0	10.0	25.0	50.0	75.0		1.7	5.3	7.5	8.7	8.8		6.8	moderate	Patlewicz G, Wright ZM, Basketter DA, et al. Structure-activity relationships for selected fragrance allergens. Contact Derm 2002; 47:219-226.
 3, 3', 4', 5-Tetrachlorosalicylanilide	1154-59-2	Acetone	0.25	0.5	1.0				11.2	14.4	18.0				0.04 [3]	extreme	Basketter DA, Scholes EW, and Kimber I. The performance of the local lymph node assay with chemicals identified as contact allergens in the human maximization test. Fd Chem Tox 1994; 32:543-547.

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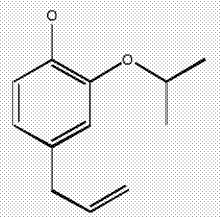
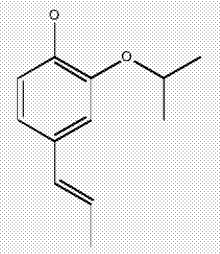
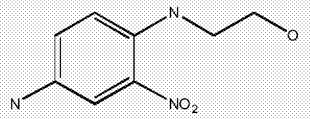
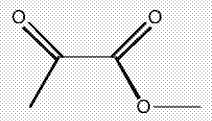
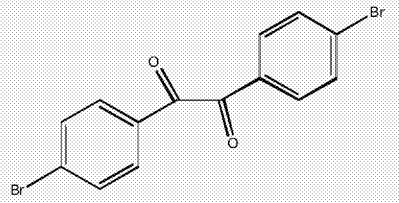
 Isopropyl eugenol	51474-90-9	AOO	12.0	29.0	59.0				1.8	1.8	2.2				NC	non-sensitizer	Bertrand F, Basketter DA, Roberts DW, and Lepoittevin J-P. Skin sensitization to eugenol and isoeugenol in mice: possible metabolic pathways involving ortho-quinone and quinone methide intermediates. Chem Res Toxicol 1997; 10:335-343.
 Isopropyl isoeugenol	186743-30-6	AOO	0.6	1.2	3.0				3	5.7	10.7				0.6 [3]	strong	Bertrand F, Basketter DA, Roberts DW, and Lepoittevin J-P. Skin sensitization to eugenol and isoeugenol in mice: possible metabolic pathways involving ortho-quinone and quinone methide intermediates. Chem Res Toxicol 1997; 10:335-343.
 2-(4-Amino-2-nitro-phenylamino)-ethanol (HC Red No3)	2871-01-4	AOO	0.1	0.25	0.5	1.0	2.5		0.5	1.2	1.9	1.8	3.3		2.2	moderate	Estrada E, Patlewicz G, Chamberlain M, et al.Computer-aided knowledge generation for understanding skin sensitization mechanisms: the TOPS-MODE approach. Chem Res Toxicol. 2003; 16:1226-1235

1-(2',3',4'5'-Tetramethylphenyl)-3-(4'-tertbutylphenyl) propane-1,3-dione	Not known	Acetone	10.0	20.0	40.0				1.6	1.2	1.6				NC	non-sensitizer	P&G unpublished
Resorcinol	108-46-3	AOO	1.0	2.5	5.0	10.0	25.0	50.0	1.8	2.3	2.6	6.3	10.1	12.5	5.5	moderate	Basketter DA., Sanders D., Jowsey IR., Contact Dermatitis 2007: 56: 196-200

ED_006472_00084346-00179

Table of new structures

CHEMICAL STRUCTURE	CAS #	Vehicle[1]	LLNA %	LLNA %	LLNA %	LLNA %	LLNA %	LLNA %	LLNA SI	LLNA SI	LLNA SI	LLNA SI	LLNA SI	LLNA SI	LLNA EC3 %	Potency category	Ref.
 Methyl pyruvate	600-22-6	AOO	1	2.5	5	10			1.2	3.1	4.7	8.0			2.4		UL unpublished
 4,4-Dibromobenzil	35578-47-3	AOO	5	10	25	50			1.5	1.6	3.6	5.7			20.5		UL unpublished

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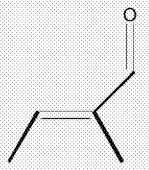
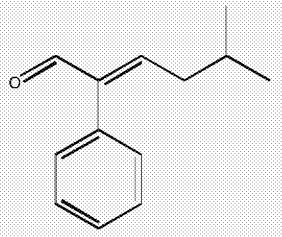
 Trans-2-methyl-2-butenal	497-03-0	AOO	10	25	50				1.5	1.0	2.8				?		UL unpublished
 5-Methyl-2-phenyl-2-hexenal	21834-92-4	AOO	0.5	1	2.5	5	10		1.0	1.3	0.5	3.8	17.7		4.4		UL unpublished
2,4-Hexadienal	142-83-6	AOO	0.5	1	2.5	5	10		0.9	1.5	2.2	4.2	14.8		3.5		UL unpublished

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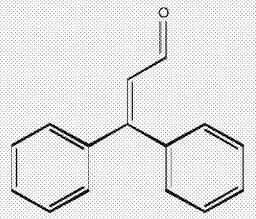
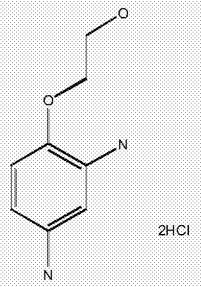
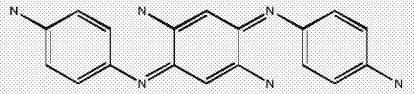
 ß-Phenylcinnamaldehyde	1210-39-5	AOO	0.1	0.25	0.5	1	2.5		2.0	2.3	1.9	5.9	10.6		0.6		UL unpublished
 2,4-Diaminophenoxyethanol HCl	66422-95-5	AOO	1	2.5	5	10	25		1.6	1.6	2.7	5.7	8.3		5.5		UL unpublished
 Bandrowski's base	20048-27-5	AOO	0.01	0.025	0.05	0.1	0.25		1.1	3.1	5.7	6.5	5.6		0.04		UL unpublished

ED_006472_00084346-00182

methylmethacrylate	80-62-6	AOO	10	30	50	75	100		1.4	1.5	1.5	2.1	3.6		90	weak	Betts CJ, Dearman RJ, Heylings JR, Kimber I and Basketter DA. Skin sensitization potency of methyl methacrylate in the local lymph node assay : comparisons with guinea pig data and human experience. Contact Derm 2006; 55: 140-127.
Butyl acrylate	141-32-2	AOO	1	2.5	5	10	25		0.7	1.3	1.4	2.5	8.7		11	weak	Dearman RJ et al. Comparative analysis of skin sensitisation potency of acrylates (methyl acrylate, ethyl acrylate, butyl acrylate and ethylhexyl acrylate) using the local lymph node assay. Submitted for publication

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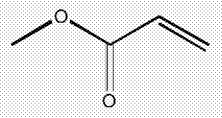
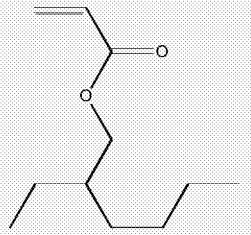
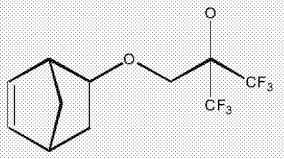
 Methyl acrylate	96-33-3	AOO	1	2.5	5	10	25		0.8	0.8	1.3	1.6	3.8		20	weak	Dearman RJ et al. Comparative analysis of skin sensitisation potency of acrylates (methyl acrylate, ethyl acrylate, butyl acrylate and ethylhexyl acrylate) using the local lymph node assay. Submitted for publication
 Ethylhexyl acrylate	103-11-7	AOO	0.5	1	2.5	5	10		1.1	1.2	0.9	1.2	3.1		10	weak	Dearman RJ et al. Comparative analysis of skin sensitisation potency of acrylates (methyl acrylate, ethyl acrylate, butyl acrylate and ethylhexyl acrylate) using the local lymph node assay. Submitted for publication
 2[(bicyclo[2.2.1]hept-5-ene-2-yloxy)methyl]-1,1,1,3,3,3-hexafluoro-2-propanol (norbornene fluoroalcohol)	305815-63-8	AOO	5	10	25	50	100		0.7	0.8	1.9	3.2	3.7				DeLorme MP, Ladics GS, Donner EM, Wagner VO, Finlay C, Frame SR, Everds NE, Loveless SE. Acute, subchronic and mutagenicity studies with norbornene fluoroalcohol. Drug Chem Toxicol 2005; 28: 379-395

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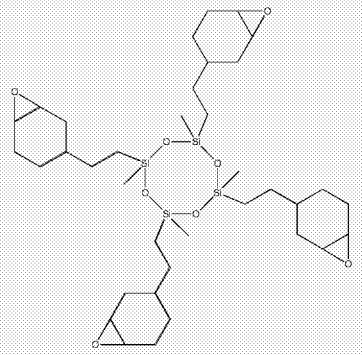
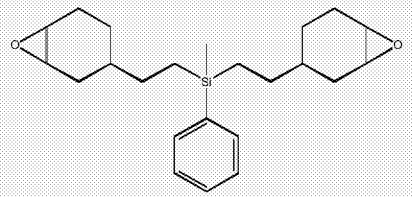
 3,4-epoxyclohexylethyl-cyclopolymethylsiloxane (Tet-sil)		AOO	50	100					1.2	1.2							Kostoryz EL, Zhu Q, Zhao H, Miller M and Eick JD. Assessment of the relative skin sensitization potency of siloranes and bis-GMA using the local lymph node assay and QSAR predicted potency. J Biomed Mat Res A 2006; 79: 684-688
 Bis-3,4-epoxycyclohexyl-ethyl-phenyl-methylsilane (Ph-Sil)		AOO	25	35	50				3.7	4.2	7.9						Kostoryz EL, Zhu Q, Zhao H, Miller M and Eick JD. Assessment of the relative skin sensitization potency of siloranes and bis-GMA using the local lymph node assay and QSAR predicted potency. J Biomed Mat Res A 2006; 79: 684-688

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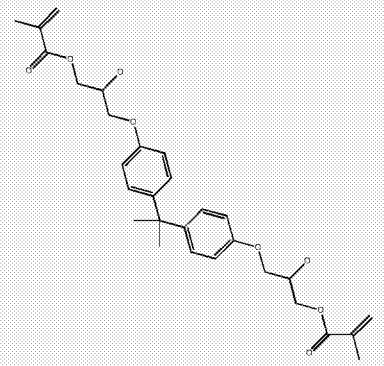
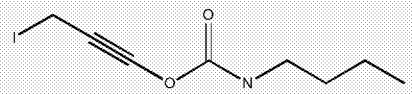
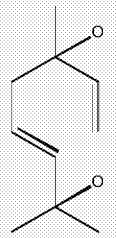
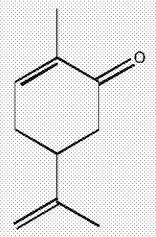
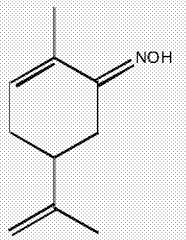
 2,2-bis-[4-(2-hydroxy-3 methacryloxypropoxy)phenyl)]-propane (Bis-GMA)	1565-94-2	AOO	35	75					2.0	5.9							Kostoryz EL, Zhu Q, Zhao H, Miller M and Eick JD. Assessment of the relative skin sensitization potency of siloranes and bis-GMA using the local lymph node assay and QSAR predicted potency. J Biomed Mat Res A 2006; 79: 684-688
 Iodopropynyl butylcarbamate	87977-28-4	AOO	0.1	1	5	10			0.7	3.4	4.2	12					Siebert J. The sensitizing potential of iodopropynyl butylcarbamate in the local lymph node assay. Contact Derm 2004; 51: 318-319

ED_006472_00084346-00186

Linalool aldehyde		AOO	1	5	15				1.2	2.0	4.2						Sköld M., Börje A., Harambasic E., Karlberg A.-T., Contact Allergens Formed on Air Exposure of Linalool. Identification and Quantification of Primary and Secondary Oxidation Products and the Effect on Skin Sensitization. Chem. Res. Toxicol. 2004, 17, 1697-1705
 Linalool alcohol		AOO	1	10	30				1.0	1.3	1.3						Sköld M., Börje A., Harambasic E., Karlberg A.-T., Contact Allergens Formed on Air Exposure of Linalool. Identification and Quantification of Primary and Secondary Oxidation Products and the Effect on Skin Sensitization. Chem. Res. Toxicol. 2004, 17, 1697-1705

 R-Carvone		AOO	6	12	20				1.3	2.6	6.2						Nilsson A.-M., Andresen Bergström M., Luthman K., Nilsson J.L.G., Karlberg A.-T. An a,b-unsaturated oxime identified as a strong contact allergen. Indications of antigen formation via several pathways. Food and Chem. Toxicol. 43 (2005) 1627-1636
 R-Carvoxime		AOO	0.1	1	5				2.1	3.7	8.1						Nilsson A.-M., Andresen Bergström M., Luthman K., Nilsson J.L.G., Karlberg A.-T. An a,b-unsaturated oxime identified as a strong contact allergen. Indications of antigen formation via several pathways. Food and Chem. Toxicol. 43 (2005) 1627-1636

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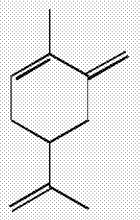
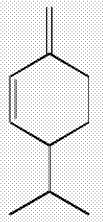
 (5R)-5-Isopropenyl-2-methyl-1-methylene-2-cyclohexene		AOO	0.5	5	15				0.94	1.9	6.6						Nilsson A.-M., Andresen Bergström M., Luthman K., Nilsson J.L.G., Karlberg A.-T. A Conjugated Diene Identified as a Prohapten: Contact Allergenic Activity and Chemica Reactivity of Proposed Epoxide Metabolites. Chem. Res. Toxicol. 2005, 18, 308-316.
 b- Phellandrene		AOO	1	10	20				1.1	4.8	23						Andresen Bergström M., Luthman K., Nilsson J.L.G., Karlberg A.-T. Conjugated Dienes as prohaptens in Contact Allergy: In Vivo and in Vitro Studies of Structure-Activity Relationships, Sensitizing Capacity, and Metabolic Activation. Chem. Res. Toxicol. 2006, 19, 760-769.

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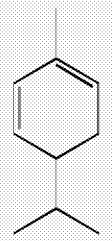
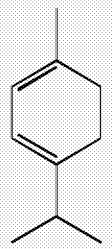
 a- Phellandrene		AOO	1	10	25				1.1	5	28						Andresen Bergström M., Luthman K., Nilsson J.L.G., Karlberg A.-T. Conjugated Dienes as prohaptens in Contact Allergy: In Vivo and in Vitro Studies of Structure-Activity Relationships, Sensitizing Capacity, and Metabolic Activation. Chem. Res. Toxicol. 2006, 19, 760-769
 a- Terpinene		AOO	1	5	10	15	25		1.1	1.5	3.4	8.9	23				Andresen Bergström M., Luthman K., Nilsson J.L.G., Karlberg A.-T. Conjugated Dienes as prohaptens in Contact Allergy: In Vivo and in Vitro Studies of Structure-Activity Relationships, Sensitizing Capacity, and Metabolic Activation. Chem. Res. Toxicol. 2006, 19, 760-769

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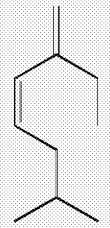
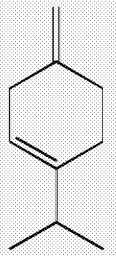
 (4Z)-2-Methyl-6-methyleneoct-4-ene		AOO	1	5	10	15	25		1.1	0.87	0.78	0.89	2.1				Andresen Bergström M., Luthman K., Nilsson J.L.G., Karlberg A.-T. Conjugated Dienes as prohaptens in Contact Allergy: In Vivo and in Vitro Studies of Structure-Activity Relationships, Sensitizing Capacity, and Metabolic Activation. Chem. Res. Toxicol. 2006, 19, 760-769
 b- Terpinene		AOO	1	10	25				1.4	1.3	2.1						Andresen Bergström M., Luthman K., Nilsson J.L.G., Karlberg A.-T. Conjugated Dienes as prohaptens in Contact Allergy: In Vivo and in Vitro Studies of Structure-Activity Relationships, Sensitizing Capacity, and Metabolic Activation. Chem. Res. Toxicol. 2006, 19, 760-769

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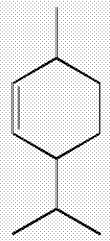
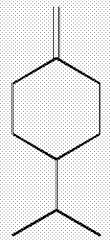
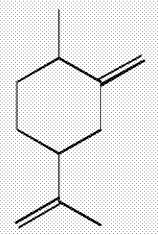
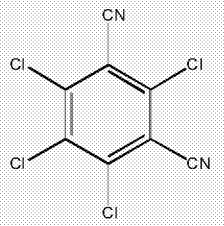
 (3S,6R)-3-isopropyl-6-methylcyclohexene		AOO	1	10	25				0.84	1.0	2.9						Andresen Bergström M., Luthman K., Nilsson J.L.G., Karlberg A.-T. Conjugated Dienes as prohaptens in Contact Allergy: In Vivo and in Vitro Studies of Structure-Activity Relationships, Sensitizing Capacity, and Metabolic Activation. Chem. Res. Toxicol. 2006, 19, 760-769
 4-Isopropyl-1-methylenecyclohexane		AOO	1	10	25				1.2	0.71	1.4						Andresen Bergström M., Luthman K., Nilsson J.L.G., Karlberg A.-T. Conjugated Dienes as prohaptens in Contact Allergy: In Vivo and in Vitro Studies of Structure-Activity Relationships, Sensitizing Capacity, and Metabolic Activation. Chem. Res. Toxicol. 2006, 19, 760-769

 (1R,4R)-4-Isopropenyl-1-methyl-2-methylenecyclohexane		AOO	1	5	10	15	25		1.3	1.8	1.2	2.3	2.9				Andresen Bergström M., Luthman K., Nilsson J.L.G., Karlberg A.-T. Conjugated Dienes as prohaptens in Contact Allergy: In Vivo and in Vitro Studies of Structure-Activity Relationships, Sensitizing Capacity, and Metabolic Activation. Chem. Res. Toxicol. 2006, 19, 760-769
 Chlorothalonil	1897-45-6	DMF	0.003	0.01	0.03	0.1	0.3		2.1	9.4	13.8	18.4	27.2				Boman A., Montelius J., Rissanen R.-L., Liden C. Sensitizing potential of chlorothalonil in the guinea pig and the mouse. Contact Dermatitis, 2000, 43, 273-279.

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Annex II

Comparative LLNA, Guinea Pig, and Human Data Used in the Performance Evaluation

Annex II-1
LLNA Data for 196 Substances Used for the Evaluation of Skin Sensitization Potency (Alphabetical Order) C-123

Annex II-2
Human Data for LLNA Potency Evaluation..... C-211

Annex II-3
Guinea Pig Data for LLNA Potency Evaluation C-233

Annex II-4
Summary LLNA, Human, and Guinea Pig Data Used in the Regression and Classification Rate Analyses C-253

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Annex II-1

LLNA Data for 196 Substances Used for the Evaluation of Skin Sensitization Potency (Alphabetical Order)

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ED_006472_00084346-00198

Chemical Name	LLNA Vehicle	Conc. (%)	SI	EC3 (%)	EC3 (µg/cm²)	Nonstd. LLNA Protocol	Mouse Strain	Mouse Source	LLNA Reference
Abietic acid	AOO	5.0 10.0 25.0	1.5 2 5.2	15.0	3750	N	CBA	NA	(Ashby et al. 1995)
Abietic acid	AOO	NA	NA	11.0	2750	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Basketter et al. 2003b)
Abietic acid	AOO	NA	NA	14.7	3675	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Basketter et al. 2007)
Abietic acid	AOO	NA	NA	8.3	2075	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Basketter et al. 2007)
Abietic acid	AOO	NA	NA	10.6	2650	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Basketter et al. 2007)
Acetyl isovaleryl[1]	AOO	25 50 100	2.9 6 14.3	25.8	6450	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Ryan et al. 2000)
AE F016382 00 TK71 A101	Pluronic L92	3.6 7.1 17.9 35.7	1.0 0.8 1.0 1.1	NC	NC	N	CBA/J	R. Janvier, Le Genest St Isle, France	(Debruyne 2007)
Aluminum chloride	Pet.	5 10 25	0.8 0.8 0.7	NC	NC	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Basketter et al. 1999b)
p-Aminobenzoic acid	AOO	0.5 1 2.5 5 10	1.2 1.2 1.1 1.6 1.4	NC	NC	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Loveless et al. 1996)

[1] The reference refers to this substance as 5-methyl-2,3-hexanedione.

ED_006472_00084346-00199

Chemical Name	LLNA Vehicle	Conc. (%)	SI	EC3 (%)	EC3 (µg/cm²)	Nonstd. LLNA Protocol	Mouse Strain	Mouse Source	LLNA Reference
3-Aminophenol	AOO	2.5 5.0 10.0	2.8 3.5 5.7	3.2	800	Y[2]	CBA/Ca	NA	(Basketter and Scholes 1992)
3-Aminophenol	DMF	NA	NA	0.2	60	N	NA	NA	(SCCP 2007)
Amylcinnamic aldehyde	NA	NA	NA	12.1	3013	NA	NA	NA	(Estrada et al. 2003)
Amylcinnamic aldehyde	AOO	NA	NA	13.5	3375	N	CBA	NA	(Basketter and Cadby 2004)
Amylcinnamic aldehyde	AOO	1.0 2.5 5.0 10.0 25.0	1.5 1.7 2.2 2.8 8.2	10.6	2650	N	NA	NA	(Patlewicz et al. 2001)[EC3] (Gerberick et al. 2005)[Dose-response data]
Amylcinnamic aldehyde	EtOH/DEP (1:3)	NA	NA	7.6	1900	N	NA	NA	(RIFM 2007)
Amylcinnamic aldehyde	AOO	NA	NA	11.2	2800	NA	NA	NA	(Smith and Hotchkiss 2001)
alpha-Amylcinnamyl alcohol	EtOH/DEP (1:3)	NA	NA	NC	NC	N	NA	NA	(RIFM 2007)
alpha-Amylcinnamyl alcohol	EtOH/DEP (1:3)	NA	NA	NC	NC	N	NA	NA	(RIFM 2007)
Aniline	AOO	NA	NA	37.0	9250	NA			(Griem et al. 2003) (Smith and Hotchkiss 2001)
Aniline	AOO	5.0 10.0 25.0 50.0 100.0	1.1 0.9 2.0 1.9 3.3	89	22250	N	CBA	NA	(Gerberick et al. 2005)

[2] Protocol used both sexes, and the test duration was 4 or 5 days.

ED_006472_00084346-00200

Chemical Name	LLNA Vehicle	Conc. (%)	SI	EC3 (%)	EC3 (µg/cm²)	Nonstd. LLNA Protocol	Mouse Strain	Mouse Source	LLNA Reference
Aniline	AOO	10 25 50	1.4 1.8 2.9	NC	NC	Y[3]	CBA/Ca	Animal Breeding Unit, Unilever Environmental Safety Laboratory	(Basketter et al. 1991) (Basketter and Scholes 1992)
Aniline	MEKOO	10 25 50	1.2 1.5 1.7	NC	NC	Y[4]	CBA/Ca	Animal Breeding Unit, Unilever Environmental Safety Laboratory	(Basketter et al. 1991)
Aniline	MEK	10 25 50	1.5 1.7 3	50.0	12500	Y[5]	CBA/Ca	Animal Breeding Unit, Unilever Environmental Safety Laboratory	(Basketter et al. 1991)
Aniline	AOO	10 25 50 100	1.9 4.4 3.6 1.7	16.6	4150	Y[6]	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Basketter et al. 1991)
Aniline	MEK	10 25 50 100	1.7 7.7 7.5 1.5	13.3	3325	Y[7]	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Basketter et al. 1991)
Anisyl alcohol	EtOH/DEP (1:3)	NA	NA	5.9	1475	N	NA	NA	(RIFM 2007)
A SC600	Pluronic L92	10 25 50 100	1.4 1.8 2.3 1.6	NC	NC	N	CBA/J	R. Janvier, Le Genest St Isle, France	(Debruyne 2007)

[3] Protocol used both sexes, and the test duration was 4 or 5 days.
[4] Protocol used both sexes, and the test duration was 5 days.
[5] Protocol used both sexes, and the test duration was 5 days.
[6] Protocol used both sexes, and the test duration was 5 days.
[7] Protocol used both sexes, and the test duration was 5 days.

ED_006472_00084346-00201

Chemical Name	LLNA Vehicle	Conc. (%)	SI	EC3 (%)	EC3 (µg/cm²)	Nonstd. LLNA Protocol	Mouse Strain	Mouse Source	LLNA Reference
Atrazine	ACE	10.0 20.0 30.0	1.3 1.4 0.8	NC	NC	Y[8]	B6C3F1	Taconic Farms	(NTP 1994)
Atrazine	Pluronic L92	12.5 25.0 50.0 75.0 100.0	1.8 2.8 3.6 7.1 7.3	31.3	7813	N	CBA/J	Jackson Laboratories, Bar Harbor, ME	(ECPA 2006c)
Atrazine	Pluronic L92	7.0 33.0 100.0	0.8 2.9 3.7	41.4	10344	N	CBA/J	Jackson Laboratories, Bar Harbor, ME	(ECPA 2006c)
Basil oil	EtOH/DEP (1:3)	2.5 5 10 25 50	3 3 8 17.6 25.2	6.2	1550	N	CBA/Ca	Harlan Interfauna UK, Shaw's Farm, Blackthorne, Bicester, Oxon, UK	(Lalko and Api 2006)
Benzalkonium chloride	ACE	0.5 1 2	9.0 11.1 7.6	0.1	17	Y[9]	CBA/J	Jackson Laboratories, Bar Harbor, ME	(Gerberick et al. 1992)
Benzocaine	AOO	NA	NA	37	9250	NA	NA	NA	(Griem et al. 2003) (Smith and Hotchkiss 2001)
Benzocaine	DMF	NA	NA	18	4500	NA	NA	NA	(Griem et al. 2003) (Smith and Hotchkiss 2001)
Benzocaine	AOO	1 5 25	1.3 1.8 2.9	NC	NC	N	CBA/Ca	B&K Universal, Sollentuna, Sweden	(Montelius et al. 1994)

[8] Mouse strain was not CBA.
[9] LLNA protocol modifications included daily treatment for 4, rather than 3, consecutive days and injection of ^{3}H-methyl thymidine on the fifth day.

ED_006472_00084346-00202

Chemical Name	LLNA Vehicle	Conc. (%)	SI	EC3 (%)	EC3 (µg/cm²)	Nonstd. LLNA Protocol	Mouse Strain	Mouse Source	LLNA Reference
Benzocaine	AOO	NA	NA	22	5500	Y[10]	BALB/c	National Institute of Public Health and the Environment Breeding Colony (RIVM), The Netherlands	(Van Och et al. 2000)
Benzocaine	AOO	5.0 10.0 20.0	4.5 7.2 7.6	3.1	775	Y[11]	CBA/Ca	NA	(Kimber et al. 1989)
Benzocaine	ACE	10 25 50	1.9 1.5 1.2	NC	NC	Y[12]	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Basketter et al. 1995)
Benzocaine	DMF	2.5 5 10	1.4 2.3 2.1	NC	NC	Y[13]	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Basketter et al. 1993)
Benzocaine	DMF	1 5 25	1.9 7.4 3	1.8	450	N	CBA/Ca	B&K Universal, Sollentuna, Sweden	(Basketter et al. 1995)
Benzocaine	DMF	1 5 12.5 25	1.7 3.1 2.4 1.4	4.7	1175	N	CBA/Ca	B&K Universal, Sollentuna, Sweden	(Basketter et al. 1995)
Benzocaine	DMF	1 5 12.5 25	1.4 1.4 2.2 1.5	NC	NC	N	CBA/Ca	B&K Universal, Sollentuna, Sweden	(Basketter et al. 1995)

[10] Mice were pretreated with SDS. Mouse strain was not CBA.
[11] LLNA study length was 3 days.
[12] Protocol used both sexes.
[13] Protocol used both sexes.

ED_006472_00084346-00203

Chemical Name	LLNA Vehicle	Conc. (%)	SI	EC3 (%)	EC3 (µg/cm²)	Nonstd. LLNA Protocol	Mouse Strain	Mouse Source	LLNA Reference
Benzocaine	DMF	1 5 12.5 25	1.6 1.5 2.4 1	NC	NC	N	CBA/Ca	B&K Universal, Sollentuna, Sweden	(Basketter et al. 1995)
Benzocaine	DMF	5 10 12.5 15 25	3.2 2.4 3.6 1.8 2.4	4.54[14]	1135	N	CBA/Ca	B&K Universal, Sollentuna, Sweden	(Basketter et al. 1995)
Benzoic acid	ACE	5 10 20	0.8 0.9 0.8	NC	NC	Y[15]	CBA/J	Jackson Laboratories, Bar Harbor, ME	(Gerberick et al. 1992)
Benzoisothiazolione	DMF	10 30 50	3.8 4.4 4.9	2.3	575	N	CBA	NA	(Ashby et al. 1995)
Benzoisothiazolione	NA	NA	NA	10.4	2600	N	CBA/Ca	NA	(Basketter et al. 1999d)
Benzoisothiazolione	DMF	3 10 30 50	1.56 1.22 2.79 4.53	32.4	8103	Y[16]	CBA/Ca	NA	(Botham et al. 1991)
Benzoisothiazolione	DMF	3 10 30 50	2.72 3.84 4.45 4.97	4.8	1188	Y[17]	CBA/Ca	NA	(Botham et al. 1991)

[14] Interpolation of the EC3 was linear (per Ryan et al. 2007) and used concentration = 0% and SI = 1 as the lowest point.
[15] LLNA protocol modifications included daily treatment for 4, rather than 3, consecutive days and injection of ^{3}H-methyl thymidine on the fifth day.
[16] Does not specify sex of mice used.
[17] Does not specify sex of mice used.

ED_006472_00084346-00204

Chemical Name	LLNA Vehicle	Conc. (%)	SI	EC3 (%)	EC3 (µg/cm²)	Nonstd. LLNA Protocol	Mouse Strain	Mouse Source	LLNA Reference
Benzoquinone	AOO	0.5 1 2.5	36.4 42.3 52.3	0.0099	2.5	Y[18]	CBA/Ca	NA	(Basketter and Scholes 1992)
Benzoyl peroxide	NA	NA	NA	0.30	75	NA	NA	NA	(Basketter and Kimber 2006)
Benzoyl peroxide	ACE	0.5 1 2.5 5 10	18.7 21 24.9 24.8 18.6	0.074	18	N	CBA/JHsd	Harlan Sprague-Dawley, Indianapolis, IN or Jackson Labs, Bar Harbor, ME	(Kimber et al. 1998)
Benzoyl peroxide	ACE	0.5 1 2.5 5 10	14.6 17.2 18.1 20.2 21.8	0.023	5.8	Y[19]	CBA/Ca	Harlan Seralab, Bicester, Oxfordshire, UK	(Kimber et al. 1998)
Benzoyl peroxide	ACE	0.5 1 2.5 5 10	23.4 22.8 21.8 22.5 16.1	0.056	14	Y[20]	CBA/Ca	Harlan Seralab, Bicester, Oxfordshire, UK	(Kimber et al. 1998)
Benzoyl peroxide	ACE	0.5 1 2.5 5 10	14.7 7.9 10.9 20.5 17.3	0.073	18	N	CBA/JHsd	Harlan Sprague-Dawley, Indianapolis, IN or Jackson Labs, Bar Harbor, ME	(Kimber et al. 1998)

[18] Protocol used both sexes, and the test duration was 4 or 5 days.
[19] Protocol used both sexes of mice.
[20] Protocol used both sexes of mice.

ED_006472_00084346-00205

Chemical Name	LLNA Vehicle	Conc. (%)	SI	EC3 (%)	EC3 (µg/cm²)	Nonstd. LLNA Protocol	Mouse Strain	Mouse Source	LLNA Reference
Benzoyl peroxide	ACE	0.5 1 2.5 5 10	24.4 22.1 33.7 31.4 26.5	0.043	11	N	CBA/JHsd	Harlan Sprague-Dawley, Indianapolis, IN or Jackson Labs, Bar Harbor, ME	(Kimber et al. 1998)
Benzyl alcohol	EtOH/DEP (1:3)	NA	NA	NC	NC	N	NA	NA	(RIFM 2007)
Benzylbenzoate	AOO	5 25	2.3 3.5	17.0	4250	NA	NA	NA	(Smith and Hotchkiss 2001)
Benzylbenzoate	EtOH/DEP (1:3)	NA	NA	NC	NC	N	NA	NA	(RIFM 2007)
Benzyl cinnamate	EtOH/DEP (1:3)	NA	NA	18.4	4600	N	NA	NA	(RIFM 2007)
Benzylidene acetone	AOO	10 25 50	8.5 13.6 12.8	3.7	925	N	CBA/J	Harlan Sprague-Dawley, Indianapolis, IN or Jackson Labs, Bar Harbor, ME	(Ryan et al. 2000)
Benzyl salicylate	EtOH/DEP (1:3)	NA	NA	2.9	725	N	NA	NA	(RIFM 2007)
Beryllium sulfate[21]	DMSO	0.25 1 4	1.03 1.17 1.28	NC	NC	Y[22]	BALB/c	Charles River, Germany	(Mandervelt et al. 1997)
Beryllium sulfate	DMF	2.5 5 10	8.4 7.1 9.4	0.68	170	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Basketter et al. 1994)
Bourgeonal	EtOH/DEP (1:3)	NA	NA	4.30	1075	N	NA	NA	(RIFM 2007)

[21] Data are for metal cation.
[22] Mouse strain was not CBA.

ED_006472_00084346-00206

Chemical Name	LLNA Vehicle	Conc. (%)	SI	EC3 (%)	EC3 (µg/cm^2)	Nonstd. LLNA Protocol	Mouse Strain	Mouse Source	LLNA Reference
1-Butanol	Water	5 10 20	1.6 1.2 1.4	NC	NC	N	CBA/J	Harlan Sprague-Dawley, Inc., Indianapolis, IN or Jackson Laboratories, Bar Harbor, ME	(Ryan et al. 2000)
Butyl acrylate	AOO	1 2.5 5 10 25	0.7 1.3 1.5 2.5 8.7	11	2750	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Dearman et al. 2007)
Butyl glycidyl ether	AOO	10 25 50	1.4 2.2 5.6	30.9	7725	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Basketter et al. 1994)
Carvone	AOO	6 12 20	1.3 2.6 6.2	12.9	3225	N	CBA/Ca	Harlan, Horst, Netherlands	(Nilsson et al. 2005)
Carvone	AOO	NA	NA	13	3250	N	NA	NA	(RIFM 2007)
Carvone	EtOH/DEP (1:3)	NA	NA	10.7	2675	N	NA	NA	(RIFM 2007)
Carvone	EtOH/DEP (1:3)	NA	NA	5.7	1425	N	NA	NA	(RIFM 2007)
Chloroamine T	NA	NA	NA	0.4	100	NA	NA	NA	(Basketter and Kimber 2006)
4-Chloroaniline	NA	NA	NA	6.5	1625	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Basketter et al. 2003b)
4-Chloroaniline	AOO	2.5 5 10	1.1 1.8 3.3	9	2250	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Scholes et al. 1992)
4-Chloroaniline	AOO	2.5 5 10	2.1 1.6 2.5	NC	NC	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Scholes et al. 1992)

ED_006472_00084346-00207

Chemical Name	LLNA Vehicle	Conc. (%)	SI	EC3 (%)	EC3 (µg/cm²)	Nonstd. LLNA Protocol	Mouse Strain	Mouse Source	LLNA Reference
4-Chloroaniline	AOO	2.5 5 10	1 1.5 1.8	NC	NC	N	CBA/Ca	Barriered Animal Breeding Unit, Alderley Park, UK	(Scholes et al. 1992)
4-Chloroaniline	AOO	2.5 5 10	1 1.5 1.8	NC	NC	Y[23]	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Basketter and Scholes 1992) (Scholes et al. 1992)
(Chloro)methylisothiazolinone	AOO	0.00375 0.0075 0.015 0.0375 0.075	1.3 2.6 7 10.9 14	0.0082	2.1	N	CBA	NA	(Ashby et al. 1995)
(Chloro)methylisothiazolinone	AOO	NA	NA	0.05	13	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Basketter et al. 2003a)
(Chloro)methylisothiazolinone	AOO	0.00075 0.0015 0.0075 0.015 0.0375	0.9 1.2 4.4 9.1 8.5	0.0049	1.2	N	CBA/Ca	Harlan Seralab, Oxon, UK	(Warbrick et al. 1999a)
(Chloro)methylisothiazolinone	AOO	0.0075 0.015 0.038 0.075 0.15	1.5 4.4 7.8 10.8 9.5	0.01	2.5	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Basketter et al. 1999c)
(Chloro)methylisothiazolinone	DMF	0.001 0.003 0.01 0.03 0.1	1.02 0.89 3.57 12.31 27.73	0.008	2.0	N	CBA/Ca	NA	(Botham et al. 1991)

[23] Protocol used both sexes, and the test duration was 4 or 5 days.

ED_006472_00084346-00208

Chemical Name	LLNA Vehicle	Conc. (%)	SI	EC3 (%)	EC3 (µg/cm^2)	Nonstd. LLNA Protocol	Mouse Strain	Mouse Source	LLNA Reference
(Chloro)methylisothiazolinone	DMF	0.0015 0.0075 0.015 0.0375 0.075	1.5 3 4.7 10.3 28	0.008	1.9	N	CBA/Ca	Harlan Seralab, Oxon, UK	(Warbrick et al. 1999a)
(Chloro)methylisothiazolinone	DMF	0.01 0.03 0.1	3.5 12.3 22.7	0.009	2.3	N	CBA	NA	(Ashby et al. 1995)
(Chloro)methylisothiazolinone	MEK	0.0015 0.0075 0.015 0.0375 0.075	0.9 3.3 8.4 14 17.6	0.007	1.7	N	CBA/Ca	Harlan Seralab, Oxon, UK	(Warbrick et al. 1999a)
(Chloro)methylisothiazolinone	DMSO	0.0015 0.0075 0.015 0.0375 0.075	1 3 9.5 6.4 10.3	0.008	1.9	N	CBA/Ca	Harlan Seralab, Oxon, UK	(Warbrick et al. 1999a)
(Chloro)methylisothiazolinone	PG	0.0015 0.0075 0.015 0.0375 0.075	2 0.8 2.1 2.3 4.7	0.048	12	N	CBA/Ca	Harlan Seralab, Oxon, UK	(Warbrick et al. 1999a)
(Chloro)methylisothiazolinone	PG	0.00375 0.0075 0.015 0.0375 0.075	0.8 0.8 0.8 1.5 3.7	0.063	16	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Basketter et al. 2003a)

ED_006472_00084346-00209

Chemical Name	LLNA Vehicle	Conc. (%)	SI	EC3 (%)	EC3 (µg/cm²)	Nonstd. LLNA Protocol	Mouse Strain	Mouse Source	LLNA Reference
(Chloro)methylisothiazolinone	ACE	0.005 0.05 0.1	8.1 27.8 48.2	0.003	0.69	Y[24]	CBA/J	Jackson Laboratories, Bar Harbor, ME	(Gerberick et al. 1992)
(Chloro)methylisothiazolinone	ACE	0.0015 0.0075 0.015 0.0375 0.075	1.2 2.9 9.3 17.7 23.5	0.008	1.9	N	CBA/Ca	Harlan Seralab, Oxon, UK	(Warbrick et al. 1999a)
Chlorpromazine	DMF	0.25 2.5 25	1.02 1.75 3.53	18.3	4575	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Basketter et al. 1994)
Chlorpromazine	DMF	10 25 50	11.8 13.7 8.9	1.85[25]	463[26]	N	CBA/Ca	NA	(Basketter et al. 1994)
Cinnamic aldehyde	AOO	NA	NA	1.4	350	NA	NA	NA	(Smith and Hotchkiss 2001)
Cinnamic aldehyde	AOO	5 10 25	12.5 18.4 15.4	2.00	500	Y[27]	CBA/Ca	NA	(Basketter and Scholes 1992)
Cinnamic aldehyde	AOO	1 2.5 5	4.8 7.4 9.8	0.53	133	Y[28]	CBA/Ca	NA	(Kimber et al. 1989)

[24] LLNA protocol modifications included daily treatment for 4, rather than 3, consecutive days and injection of ^{3}H-methyl thymidine on the fifth day.
[25] Interpolation of the EC3 was linear (per Ryan et al. 2007) and used concentration = 0% and SI = 1 as the lowest point.
[26] Schneider and Akkan (2004) report an EC3 of 463 µg/cm² from these data.
[27] Protocol used both sexes, and the test duration was 4 or 5 days.
[28] Does not specify sex of mice used.

Chemical Name	LLNA Vehicle	Conc. (%)	SI	EC3 (%)	EC3 (µg/cm^2)	Nonstd. LLNA Protocol	Mouse Strain	Mouse Source	LLNA Reference
Cinnamic aldehyde	AOO	0.5 1 2.5 5 10	1.4 0.9 1.9 7.1 15.8	3.1	775	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Basketter et al. 2001)
Cinnamic aldehyde	AOO	NA	NA	1.7	425	N	NA	NA	(Basketter et al. 2007)
Cinnamic aldehyde	AOO	NA	NA	2.7	675	N	NA	NA	(Basketter et al. 2007)
Cinnamic aldehyde	AOO	NA	NA	1.3	325	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Elahi et al. 2004)
Cinnamic aldehyde	AOO	NA	NA	2.2	550	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Elahi et al. 2004)
Cinnamic aldehyde	EtOH/DEP (3:1) + AO Mix	NA	NA	1.3	325	N	NA	NA	(RIFM 2007)
Cinnamic aldehyde	EtOH/DEP (3:1) + 0.1% TrlC	NA	NA	1.3	325	N	NA	NA	(RIFM 2007)
Cinnamic aldehyde	EtOH/DEP (3:1)	NA	NA	0.9	225	N	NA	NA	(RIFM 2007)
Cinnamic aldehyde	EtOH/DEP (3:1) + 0.1% Toc	NA	NA	1.1	275	N	NA	NA	(RIFM 2007)
Cinnamic aldehyde	DMSO	NA	NA	0.9	225	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Wright et al. 2001)
Cinnamic aldehyde	EtOH/DEP (3:1) + 2% Toc	NA	NA	0.8	200	N	NA	NA	(RIFM 2007)
Cinnamic aldehyde	EtOH/DEP (3:1) + 0.1% TrlC	NA	NA	0.7	175	N	NA	NA	(RIFM 2007)

ED_006472_00084346-00211

Chemical Name	LLNA Vehicle	Conc. (%)	SI	EC3 (%)	EC3 (µg/cm²)	Nonstd. LLNA Protocol	Mouse Strain	Mouse Source	LLNA Reference
Cinnamic aldehyde	EtOH/DEP (3:1) + AO Mix	NA	NA	0.7	175	N	NA	NA	(RIFM 2007)
Cinnamic aldehyde	EtOH/DEP (3:1) + 2% Toc	NA	NA	0.6	150	N	NA	NA	(RIFM 2007)
Cinnamic aldehyde	EtOH/DEP (3:1) + 0.1% Toc	NA	NA	0.2	50	N	NA	NA	(RIFM 2007)
Cinnamic aldehyde	EtOH/DEP (3:1)	NA	NA	0.2	50	N	NA	NA	(RIFM 2007)
Cinnamic aldehyde	AOO	1 2.5 5 10 25	2.2 3.9 4.6 7.6 5.4	1.7	426	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Wright et al. 2001)
Cinnamic aldehyde	DMF	1 5 25	4.3 9.8 12.8	0.7	171	N	CBA/Ca	B&K Universal, Sollentuna, Sweden	(Montelius et al. 1994)
Cinnamic aldehyde	DMF	0.25 0.5 1 2.5 5	1.5 3.1 4.5 8.3 8.6	0.5	116	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Wright et al. 2001)
Cinnamic aldehyde	MEK	1 2.5 5 10 25	2.8 6.2 8.5 14.6 13.2	1.1	272	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Wright et al. 2001)

ED_006472_00084346-00212

Chemical Name	LLNA Vehicle	Conc. (%)	SI	EC3 (%)	EC3 (µg/cm^2)	Nonstd. LLNA Protocol	Mouse Strain	Mouse Source	LLNA Reference
Cinnamic aldehyde	PG	1 2.5 5 10 25	2.1 5.8 8.2 16.3 17	1.4	341	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Wright et al. 2001)
Cinnamic aldehyde	DMSO	0.25 0.5 1 2.5 5	1.7 2.3 4.4 7.6 7.6	1.3	313	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Wright et al. 2001)
Cinnamic aldehyde	EtOH (10%)	1 2.5 5 10 25	2.7 3.5 4.8 5.2 5.8	1.6	391	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Wright et al. 2001)
Cinnamic aldehyde	EtOH (50%)	1 2.5 5 10 25	2.1 9.5 10.3 13.6 21.9	1.2	296	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Wright et al. 2001)
Cinnamyl alcohol	AOO	10 25 50 90.0	1.8 3.5 3.9 5.7	21	5250	N	NA	NA	(Gerberick et al. 2005)
Cinnamyl alcohol	AOO	NA	NA	19.1	4775	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Elahi et al. 2004)
Cinnamyl nitrile	EtOH/DEP (1:3)	NA	NA	NC	NC	N	NA	NA	(RIFM 2007)

ED_006472_00084346-00213

Chemical Name	LLNA Vehicle	Conc. (%)	SI	EC3 (%)	EC3 (µg/cm²)	Nonstd. LLNA Protocol	Mouse Strain	Mouse Source	LLNA Reference
C.I. Reactive Red 231	AOO	1 3 9 15	4.8 3.4 4.4 4.6	0.6	150	N	CBA	NA	(Haist et al. 2007)
C.I. Reactive Yellow 174	AOO	1 3 9 15	4.2 5.3 5.5 7.8	0.3	75	N	CBA	NA	(Haist et al. 2007)
Citral	EtOH/DEP (3:1) + 0.1% Toc	NA	NA	6.8	1700	N	NA	NA	(RIFM 2007)
Citral	AOO	5 10 20	2.1 5 9.3	6.6	1638	Y[29]	CBA/Ca	Animal Breeding Unit, Environmental Safety Laboratory, Unilever Research	(Basketter et al. 1991; Basketter and Scholes 1992)
Citral	AOO	5 10 20	0.9 2.2 6.2	12.0	3000	Y[30]	CBA/Ca	Harlan Olac, Ltd., Oxon, UK	(Basketter et al. 1991)
Citral	AOO	5 10 20	2.2 8.1 20.5	5.7	1419	Y[31]	CBA/Ca	Barriered Animal Breeding Unit, Alderley Park, UK	(Basketter et al. 1991)
Citral	AOO	5 10 20	0.9 2.4 4.7	12.6	3152	Y[32]	CBA/Ca	Harlan Olac, Ltd., Oxon, UK	(Basketter et al. 1991)
Citral[33]	AOO	5 10 25	2.9 6.4 12.9	5.1	1275	N	CBA	NA	(Ashby et al. 1995)

[29] Protocol used both sexes.
[30] Protocol used both sexes.
[31] Protocol used both sexes.
[32] Protocol used both sexes.
[33] Referred to as citral PQ in Ashby et al. (1995).

ED_006472_00084346-00214

Chemical Name	LLNA Vehicle	Conc. (%)	SI	EC3 (%)	EC3 (µg/cm²)	Nonstd. LLNA Protocol	Mouse Strain	Mouse Source	LLNA Reference
Citral	AOO	5 10 25	1.2 2.1 6.3	13	3250	Y[34]	NA	NA	(Gerberick et al. 2005)
Citral	EtOH/DEP (3:1)	NA	NA	5.3	1325	N	NA	NA	(RIFM 2007)
Citral	EtOH/DEP (1:3)	NA	NA	1.2	300	N	NA	NA	(RIFM 2007)
Citral	EtOH/DEP (1:3)	2.5 5 10 25 50	2.8 2.3 5.1 11.4 22.1	6.3	1575	N	CBA/Ca	Harlan Interfauna UK, Shaw's Farm, Blackthorne, Bicester, Oxon, UK	(Lalko and Api 2006)
Citral	EtOH/DEP (3:1)	NA	NA	4.6	1150	N	NA	NA	(RIFM 2007)
Citral	EtOH/DEP (3:1) + 0.1% TrlC	NA	NA	5.8	1450	N	NA	NA	(RIFM 2007)
Citral	EtOH/DEP (3:1) + AO Mix	NA	NA	4.6	1150	N	NA	NA	(RIFM 2007)
Citral	EtOH/DEP (3:1) + 0.1% TrlC	NA	NA	3.7	925	N	NA	NA	(RIFM 2007)
Citral	EtOH/DEP (3:1) + AO Mix	NA	NA	2.1	525	N	NA	NA	(RIFM 2007)
Citral	EtOH/DEP (3:1) + 0.1% Toc	NA	NA	1.5	375	N	NA	NA	(RIFM 2007)

[34] Protocol used a 4-day exposure period and both sexes.

ED_006472_00084346-00215

Chemical Name	LLNA Vehicle	Conc. (%)	SI	EC3 (%)	EC3 (µg/cm²)	Nonstd. LLNA Protocol	Mouse Strain	Mouse Source	LLNA Reference
Citronella oil	EtOH/DEP (1:3)	2.5 5 10 25 50	1.4 0.9 1.2 1.2 2.7	NC	NC	N	CBA/Ca	Harlan Interfauna UK, Shaw's Farm, Blackthorne, Bicester, Oxon, UK	(Lalko and Api 2006)
dl-Citronellol	EtOH/DEP (1:3)	NA	NA	43.5	10875	N	NA	NA	(RIFM 2007)
Clove oil (bud)	EtOH/DEP (1:3)	1.0 2.5 5.0 10.0 25.0	1.1 1.8 2.5 3.7 5.9	7.1	1775	N	NA	NA	(RIFM 2007)
Clove oil (leaf)	EtOH/DEP (1:3)	2.5 5 10 25 50	1.6 1.5 4.0 9.5 11.4	7.1	1775	N	CBA/Ca	Harlan Interfauna UK, Shaw's Farm, Blackthorne, Bicester, Oxon, UK	(Lalko and Api 2006)
Clove oil (stem)	EtOH/DEP (1:3)	1.0 2.5 5.0 10.0 25.0	1.6 1.7 2.2 4.2 8.9	7	1750	N	NA	NA	(RIFM 2007)
Cobalt (II) salts (cobalt chloride)	Water	0.5 1 2.5 5	2.08 3.51 3.77 7.21	0.8	200	Y[35]	CBA/N	Japan SLC Inc. Shizuoka, Japan	(Ikarashi et al. 1992)

[35] Test was terminated 24 hours after the last topical exposure.

ED_006472_00084346-00216

Chemical Name	LLNA Vehicle	Conc. (%)	SI	EC3 (%)	EC3 (µg/cm²)	Nonstd. LLNA Protocol	Mouse Strain	Mouse Source	LLNA Reference
Cobalt (II) salts (cobalt chloride)	DMSO	0.5 1 2.5	3.2 3.7 2.8	0.4	100	Y[36]	CBA/Ca	NA	(Basketter and Scholes 1992)
Cobalt (II) salts (cobalt chloride)	DMSO	1 2.5 5	1.5 1.6 2.7	NC	NC	Y	BALB/c	Charles River, Germany	(Mandervelt et al. 1997)
Copper (II) chloride	DMSO	1 2.5 5	8.1 13.8 13.6	0.4	100	Y[37]	CBA/Ca	NA	(Basketter and Scholes 1992; Basketter et al. 1999b)
Coumarin	AOO	5.0 10.0 25.0	2.7 2.9 2.3	NC	NC	N	NA	NA	(Gerberick et al. 2005)
Coumarin	DMF	10 25 50	0.9 2.05 3.2	45.7	11413	Y[38]	NA	Charles River, L'Arbresl, France	(Vocanson et al. 2006)
Coumarin	DMF	10 25 50	1.9 3.7 4	19.2	4792	Y[39]	NA	Charles River, L'Arbresl, France	(Vocanson et al. 2006)
Coumarin	DMF	NA	NA	NC	NC	N	NA	NA	(RIFM 2007)
Cyclamen aldehyde	AOO	1 2.5 10 25	1.4 1.34 1.84 3.26	22.3	5575	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Basketter et al. 2001)
Damascone	AOO	NA	NA	1.24	310	N	NA	NA	(RIFM 2007)
Damascone	AOO	NA	NA	1.22	305	N	NA	NA	(RIFM 2007)
t-alpha Damascone	AOO	NA	NA	3.30	825	N	NA	NA	(RIFM 2007)

[36] Protocol used both sexes, and the test duration was 4 or 5 days.
[37] Protocol used both sexes, and the test duration was 4 or 5 days.
[38] Protocol used CBA/J or Balb/c mice.
[39] Protocol used CBA/J or Balb/c mice.

ED_006472_00084346-00217

Chemical Name	LLNA Vehicle	Conc. (%)	SI	EC3 (%)	EC3 (µg/cm^2)	Nonstd. LLNA Protocol	Mouse Strain	Mouse Source	LLNA Reference
trans beta Damascone	AOO	NA	NA	2.40	600	N	NA	NA	(RIFM 2007)
D EC 25	Pluronic L92	0.5 1 2.5	0.56 0.63 0.59	NC	NC	N	CBA/J	R. Janvier, Le Genest St Isle, France	(Debruyne 2007)
delta Damascone	AOO	NA	NA	0.866	217	N	NA	NA	(RIFM 2007)
delta Damascone	AOO	NA	NA	5.19	1298	N	NA	NA	(RIFM 2007)
delta Damascone	EtOH/DEP (1:3)	NA	NA	9.6	2400	N	NA	NA	(RIFM 2007)
D EW 15	Pluronic L92	2.5 5 10 25	1.9 1.5 2.5 2.5	NC	NC	N	CBA/J	R. Janvier, Le Genest St Isle, France	(Debruyne 2007)
Dextran	AOO	NA	NA	NC	NC	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Basketter et al. 2003b)
1,2 Dibromo-2,4-dicyanobutane	AOO	0.5 1 2.5 5	1.4 3.4 3.5 5.4	0.9	225	N	NA	NA	(Gerberick et al. 2005)
1,2 Dibromo-2,4-dicyanobutane	AOO	NA	NA	1.3	325	N	NA	NA	(Basketter et al. 2007)
1,2 Dibromo-2,4-dicyanobutane	AOO	NA	NA	1.8	450	N	NA	NA	(Basketter et al. 2007)
1,2 Dibromo-2,4-dicyanobutane	AOO	NA	NA	5.2	1300	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Basketter et al. 2003b)
1,2 Dibromo-2,4-dicyanobutane	NA	NA	NA	2.0	500	N	CBA/Ca	B&K Universal AB, Sollentuna, Sweden	(Basketter et al. 2005)
1,2 Dibromo-2,4-dicyanobutane	NA	NA	NA	2.3	575	NA	NA	NA	(Estrada et al. 2003)
Diethylenetriamine	AOO	5 10	6.4 12.1	3.3	825	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Basketter et al. 1994)
Diethylmaleate	AOO	25 50 100	16.3 22.6 13.1	5.8	1450	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Ryan et al. 2000)

ED_006472_00084346-00218

Chemical Name	LLNA Vehicle	Conc. (%)	SI	EC3 (%)	EC3 (µg/cm²)	Nonstd. LLNA Protocol	Mouse Strain	Mouse Source	LLNA Reference
Diethylmaleate	AOO	NA	NA	2.1	525	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Basketter et al. 2003b)
Diethylmaleate	NA	1 2.5 5 10 25	2.1 3.3 3.5 7.5 16	2	500	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Basketter et al. 1999c)
Diethylmaleate	NA	NA	NA	4.7	1175	NA	NA	NA	(Estrada et al. 2003)
Diethyl phthalate	AOO	25 50 100	1.0 1.3 1.5	NC	NC	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Ryan et al. 2000)
Dihydrocoumarin	AOO	2.5 5 10	1.6 2.5 6.6	5.6	1400	N	CBA	NA	(Ashby et al. 1995)
Dihydrocoumarin	DMF	2.5 5 10	2.1 5.1 7	3.3	813	Y[40]	CBA/J or Balb/c	Charles River, L'Arbresl, France	(Vocanson et al. 2006)
1,4-Dihydroquinone	AOO	0.05 0.1 0.25 0.5 1.0	1.3 2.7 9.2 17.2 25.8	0.11	28	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Lea et al. 1999)
1,4-Dihydroquinone	AOO	0.05 0.1 0.25 0.5 1	1.3 1.2 4.3 11.2 12.1	0.19	48	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Lea et al. 1999)

[40] Protocol used CBA/J or Balb/c mice.

ED_006472_00084346-00219

Chemical Name	LLNA Vehicle	Conc. (%)	SI	EC3 (%)	EC3 (µg/cm²)	Nonstd. LLNA Protocol	Mouse Strain	Mouse Source	LLNA Reference
1,4-Dihydroquinone	AOO	0.1 0.25 0.50 0.1 2.5	2.8 5.8 13.7 15.2 13.1	0.11	28	Y[41]	CBA/Ca	Harlan Seralab, Bicester, Oxfordshire, UK	(Kimber et al. 1998)
1,4-Dihydroquinone	DMF	0.05 0.1 0.25 0.5 1.0	1.6 1.8 3.2 7.7 10.9	0.23	58	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Lea et al. 1999)
1,4-Dihydroquinone	DMF	0.05 0.1 0.25 0.5 1	0.8 1.8 3.7 7.3 8	0.19	48	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Lea et al. 1999)
1,4-Dihydroquinone	MEK	0.05 0.1 0.25 0.5 1	1.9 2.9 13.9 23 24.5	0.10	25	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Lea et al. 1999)
1,4-Dihydroquinone	MEK	0.05 0.1 0.25 0.5 1.0	2.2 3.6 14.0 19.8 30.9	0.08	20	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Lea et al. 1999)

[41] Protocol used both sexes of mice.

ED_006472_00084346-00220

Chemical Name	LLNA Vehicle	Conc. (%)	SI	EC3 (%)	EC3 (µg/cm²)	Nonstd. LLNA Protocol	Mouse Strain	Mouse Source	LLNA Reference
1,4-Dihydroquinone	PG	0.05 0.1 0.25 0.5 1.0	0.7 0.9 1.2 1.9 2.0	NC	NC	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Lea et al. 1999)
1,4-Dihydroquinone	AOO	0.1 0.25 0.50 0.1 2.5	2.5 5.8 6.0 8.4 12.2	0.12	30	Y[42]	CBA/Ca	Harlan Seralab, Bicester, Oxfordshire, UK	(Kimber et al. 1998)
1,4-Dihydroquinone	AOO	0.1 0.25 0.50 0.1 2.5	2.4 7.0 11.1 15.9 15.0	0.12	30	N	CBA/JHsd	Harlan Sprague-Dawley, Indianapolis, IN or Jackson Labs, Bar Harbor, ME	(Kimber et al. 1998)
1,4-Dihydroquinone	AOO	0.1 0.25 0.50 0.1 2.5	3.6 4.8 12.0 15.3 23.2	0.063	16	N	CBA/JHsd	Harlan Sprague-Dawley, Indianapolis, IN or Jackson Labs, Bar Harbor, ME	(Kimber et al. 1998)
1,4-Dihydroquinone	AOO	0.1 0.25 0.50 0.1 2.5	3.2 14.9 23.7 25.3 33.4	0.091	23	N	CBA/JHsd	Harlan Sprague Dawley, Indianapolis, IN or Jackson Labs, Bar Harbor, ME	(Kimber et al. 1998)
5,5-Dimethyl-3-methylenedihydro-2(3H)-furanone	AOO	2 4 8	3 7.4 9.2	1.8	450	N	CBA	NA	(Ashby et al. 1995)

[42] Protocol used both sexes of mice.

ED_006472_00084346-00221

Chemical Name	LLNA Vehicle	Conc. (%)	SI	EC3 (%)	EC3 (µg/cm²)	Nonstd. LLNA Protocol	Mouse Strain	Mouse Source	LLNA Reference
Dimethyl sulfoxide	AOO	25 50 100	2.7 2.3 3.9	72	18000	NA	NA	NA	(Estrada et al. 2003)
2,4-Dinitrochlorobenzene	NA	NA	NA	0.10	25	N	NA	NA	(Estrada et al. 2003)
2,4-Dinitrochlorobenzene	AOO	0.01 0.025 0.05 0.1 0.25	1.5 1.8 2.4 8.9 38.0	0.048	12	Y[43]	CBA/JHsd	Harlan Sprague Dawley, Inc., Frederick, MD	(Loveless et al. 1996)
2,4-Dinitrochlorobenzene	AOO	0.01 0.05 0.10	6.2 15.7 24.0	0.0058	1.5	Y[44]	CBA/Ca	NA	(Basketter and Scholes 1992)
2,4-Dinitrochlorobenzene	DMSO	0.01 0.025 0.05 0.1 0.25	2.4 4.2 7.3 14.7 14.7	0.015	3.8	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Betts et al. 2006)
2,4-Dinitrochlorobenzene	AOO	0.01 0.025 0.05 0.1 0.25	1.4 2.20 4.00 9.8 16.2	0.036	9.0	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Betts et al. 2006)
2,4-Dinitrochlorobenzene	AOO	0.1 0.25 0.5	5.9 19.9 36.7	0.083	21	Y[45]	CBA/Ca	Animal Breeding Unit, Alderley Park, UK	(Kimber et al. 1991)

[43] LLNA protocol modifications included daily treatment for 4, rather than 3, consecutive days and injection of ^{3}H-methyl thymidine on the fifth day.
[44] Protocol used both sexes, and the test duration was 4 or 5 days.
[45] Protocol did not specify sex, and the test duration was 4 days.

ED_006472_00084346-00222

Chemical Name	LLNA Vehicle	Conc. (%)	SI	EC3 (%)	EC3 (µg/cm²)	Nonstd. LLNA Protocol	Mouse Strain	Mouse Source	LLNA Reference
2,4-Dinitrochlorobenzene	AOO	0.1 0.25 0.5	6.2 15.7 24.0	0.073	18	Y[46]	CBA/Ca	Animal Breeding Unit, Unilever Environmental Safety Laboratory	(Kimber et al. 1991)
2,4-Dinitrochlorobenzene	AOO	0.1 0.25 0.5	10.3 29.7 49.6	0.071	18	Y[47]	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Kimber et al. 1991)
2,4-Dinitrochlorobenzene	AOO	0.1 0.25 0.5	4.7 15.8 26.8	0.087	22	Y[48]	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Kimber et al. 1991)
2,4-Dinitrochlorobenzene	AOO	0.01 0.025 0.05 0.10 0.25	2.0 2.3 5.3 10.5 35.5	0.027	6.8	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Kimber et al. 1995)
2,4-Dinitrochlorobenzene	AOO	0.01 0.025 0.05 0.10 0.25	0.8 1.8 3.3 8.7 40.9	0.046	12	N	CBA/J	Harlan Sprague Dawley Inc., Indianapolis, IN	(Kimber et al. 1995)
2,4-Dinitrochlorobenzene	AOO	0.01 0.025 0.05 0.10 0.25	1.1 1.4 2.5 4.6 11.5	0.062	16	Y[49]	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Kimber et al. 1995)

[46] Protocol did not specify sex, and the test duration was 4 days.
[47] Protocol did not specify sex, and the test duration was 4 days.
[48] Protocol did not specify sex, and the test duration was 4 days.
[49] LLNA protocol modifications included daily treatment for 4, rather than 3, consecutive days and injection of ^{3}H- methyl thymidine on the fifth day.

ED_006472_00084346-00223

Chemical Name	LLNA Vehicle	Conc. (%)	SI	EC3 (%)	EC3 (µg/cm²)	Nonstd. LLNA Protocol	Mouse Strain	Mouse Source	LLNA Reference
2,4-Dinitrochlorobenzene	AOO	0.01 0.025 0.05 0.1 0.25	0.8 1.2 1.7 3.1 22.5	0.094	24	N	CBA/J	Harlan Sprague Dawley Inc., Indianapolis, IN	(Kimber et al. 1995)
2,4-Dinitrochlorobenzene	AOO	0.01 0.025 0.05 0.1 0.25	1.3 1.5 2.1 7.7 43.9	0.057	14	Y[50]	CBA/J	Harlan Sprague Dawley Inc., Indianapolis, IN	(Kimber et al. 1995)
2,4-Dinitrochlorobenzene	AOO	0.01 0.025 0.05 0.1 0.25	1.5 1.9 3.1 6.5 25	0.05	13	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Loveless et al. 1996)
2,4-Dinitrochlorobenzene	AOO	0.01 0.025 0.05 0.1 0.25	1.2 0.9 2.9 4.5 13	0.06	15	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Loveless et al. 1996)
2,4-Dinitrochlorobenzene	AOO	0.01 0.025 0.05 0.1 0.25	2.5 2.9 3.2 7.1 25	0.033	8.3	Y[51]	CBA/JHsd	Harlan Sprague Dawley, Inc., Frederick, MD	(Loveless et al. 1996)

[50] LLNA protocol modifications included daily treatment for 4, rather than 3, consecutive days and injection of ^{3}H- methyl thymidine on the fifth day.
[51] LLNA protocol modifications included daily treatment for 4, rather than 3, consecutive days and injection of ^{3}H-methyl thymidine on the fifth day.

ED_006472_00084346-00224

Chemical Name	LLNA Vehicle	Conc. (%)	SI	EC3 (%)	EC3 (µg/cm²)	Nonstd. LLNA Protocol	Mouse Strain	Mouse Source	LLNA Reference
2,4-Dinitrochlorobenzene	AOO	0.01 0.025 0.05 0.10 0.25	1.17 1.12 1.93 1.95 7.10	0.131	33	Y[52]	BALB/c	Charles River Laboratories	(NTP 1997a)
2,4-Dinitrochlorobenzene	ACE	0.001 0.05 0.1 0.25 0.5	0.8 10.7 21.1 2.2 1.8	0.012	3.0	Y[53]	CBA/J	Jackson Laboratories, Bar Harbor, ME	(Gerberick et al. 1992)
2,4-Dinitrochlorobenzene	AOO	NA	NA	0.02	5.0	N	NA	NA	(Basketter et al. 2007)
2,4-Dinitrochlorobenzene	AOO	NA	NA	0.03	7.5	N	NA	NA	(Basketter et al. 2007)
2,4-Dinitrochlorobenzene	AOO	0.02 0.1 0.5	2 10.5 23	0.029	7.4	N	CBA/Ca	B&K Universal, Sollentuna, Sweden	(Montelius et al. 1994)
2,4-Dinitrochlorobenzene	AOO	NA	NA	0.044	11	Y[54]	Balb/c	National Institute of Public Health and the Environment Breeding Colony (RIVM), The Netherlands)	(Van Och et al. 2000)
2,4-Dinitrochlorobenzene	AOO	NA	NA	0.08	19	Y[55]	Balb/c	Harlan Olac Ltd., Bicester, Oxon, UK	(Basketter et al. 1997)
Dinocap EC	Pluronic L92	0.8 4 21	2 14.2 26.7	1.1	266	N	CBA/J	Jackson Laboratories, Bar Harbor, ME	(ECPA 2006c)

[52] Mouse strain was not CBA.
[53] LLNA protocol modifications included daily treatment for 4, rather than 3, consecutive days and injection of ^{3}H-methyl thymidine on the fifth day.
[54] Mice were pretreated with SDS. Mouse strain was not CBA.
[55] Mouse strain was not CBA.

ED_006472_00084346-00225

Chemical Name	LLNA Vehicle	Conc. (%)	SI	EC3 (%)	EC3 (µg/cm²)	Nonstd. LLNA Protocol	Mouse Strain	Mouse Source	LLNA Reference
Dinocap EC	Pluronic L92	0.8 4 21	2.23 25.77 14.38	0.9	226	N	CBA/Ca	NA	(ECPA 2007e)
Dinocap EC	Pluronic L92	0.8 4 20	1.3 11.5 15.6	1.3	333	N	CBA/J	R. Janvier, Le Genest St Isle, France	(ECPA 2007i)
Dinocap EC	Pluronic L92	0.8 4 10	1.3 4.08 10.94	2.8	689	N	CBA/JHsd	NA	(ECPA 2007g)
Dinocap EC	Pluronic L92	0.8 4 10	2.7 22.9 40.5	0.8	212	N	CBA/CaOla Hsd	NA	(ECPA 2006a)
Ethyl acrylate	AOO	NA	NA	28.7	7175	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Warbrick et al. 2001)
Ethyl acrylate	AOO	2.5 5 10 25 50	1.25 1.54 1.42 2.07 3.98	36.8	9200	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Dearman et al. 2007)
Ethylenediamine	AOO	0.5 1 2.5 5 10	1.6 1.9 3.3 6.1 17.4	2.20	550	Y[56]	NA	NA	(Kimber et al. 1998)
Ethylenediamine	ACE	1 5 10	1.1 1.1 2.2	NC	NC	Y[57]	CBA/J	Jackson Laboratories, Bar Harbor, ME	(Gerberick et al. 1992)
Ethylenediamine	DMF	10	6.8	3.4	850	NA	NA	NA	(Akkan et al. 2003)

[56] The LLNA protocol used both sexes of mice.
[57] LLNA protocol modifications included daily treatment for 4, rather than 3, consecutive days and injection of ^{3}H-methyl thymidine on the fifth day.

ED_006472_00084346-00226

Chemical Name	LLNA Vehicle	Conc. (%)	SI	EC3 (%)	EC3 (µg/cm²)	Nonstd. LLNA Protocol	Mouse Strain	Mouse Source	LLNA Reference
Ethylenediamine	ACE/Water (3:1)	0.1 0.25 0.5 1 2.5	0.8 1.7 1.1 1.2 1	NC	NC	Y[58]	NA	NA	(Kimber et al. 1998)
Ethylene glycol dimethacrylate	NA	NA	NA	35.0	8750	NA	NA	NA	(Basketter and Kimber 2001)
Ethylene glycol dimethacrylate	NA	NA	NA	36.5	9125	NA	NA	NA	(Estrada et al. 2003)
Ethylene glycol dimethacrylate	MEK	10 25 50	1.2 2.4 7	28.0	7000	NA	NA	NA	(Gerberick et al. 2005)
Ethyl vanillin	AOO	2.5 5 10 25 50	0.65 1.05 0.74 0.36 0.29	NC	NC	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Basketter et al. 2001)
Eugenol	AOO	2.5 5 10 25 50	1.6 1.5 2.4 5.5 16	11.9	2975	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Loveless et al. 1996)
Eugenol	AOO	2.5 5 10 25 50	1.1 1.7 1.8 9.1 12.4	8.9	2225	Y[59]	CBA/JHsd	Harlan Sprague Dawley, Inc., Frederick, MD	(Loveless et al. 1996)

[58] The LLNA protocol used both sexes of mice.
[59] LLNA protocol modifications included daily treatment for 4, rather than 3, consecutive days and injection of ^{3}H-methyl thymidine on the fifth day.

ED_006472_00084346-00227

Chemical Name	LLNA Vehicle	Conc. (%)	SI	EC3 (%)	EC3 (µg/cm²)	Nonstd. LLNA Protocol	Mouse Strain	Mouse Source	LLNA Reference
Eugenol	AOO	2.5 5 10 25 50	1.6 1.5 2.4 5.5 16	14.5	3625	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Loveless et al. 1996)
Eugenol	AOO	25 50 100	4.8 9.3 7.6	18.9	4737	Y[60]	CBA/Ca	Animal Breeding Unit, Unilever Environmental Safety Laboratory	(Basketter and Scholes 1992); (Kimber et al. 1991)
Eugenol	AOO	25 50 100	7.2 10.2 8.2	9.5	2368	Y[61]	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Kimber et al. 1991)
Eugenol	AOO	25 50	5.5 14.1	20.4	5109	Y[62]	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Kimber et al. 1991)
Eugenol	AOO	25 50 100	44.7 70.3 68.1	8.1	2021	Y[63]	CBA/Ca	Animal Breeding Unit, Alderley Park, UK	(Kimber et al. 1991)
Eugenol	AOO	25 50	1.2 4	40.9	10225	Y[64]	CBA/Ca	Barriered Animal Breeding Unit, Alderley Park, UK	(Kimber and Weisenberger 1991)
Eugenol	AOO	2.5 5 10 25 50	2 2.8 3.2 13 17	5.8	1450	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Loveless et al. 1996)

[60] Protocol did not specify sex, and the test duration was 4 days.
[61] Protocol did not specify sex, and the test duration was 4 days.
[62] Protocol did not specify sex, and the test duration was 4 days.
[63] Protocol did not specify sex, and the test duration was 4 days.
[64] Mice were exposed on flank by occluded patch prior to topical exposure on ears.

ED_006472_00084346-00228

Chemical Name	LLNA Vehicle	Conc. (%)	SI	EC3 (%)	EC3 (µg/cm²)	Nonstd. LLNA Protocol	Mouse Strain	Mouse Source	LLNA Reference
Eugenol	AOO	2.5 5 10 25 50	2.4 2.1 1.2 5.3 9.6	13.8	3450	Y[65]	CBA/JHsd	Harlan Sprague Dawley, Inc., Frederick, MD	(Loveless et al. 1996)
Eugenol	AOO	2.5 5 10 25 50	1.5 4.3 4.6 14 6.1	6.0	1500	N	CBA/JHsd	Harlan Sprague Dawley, Inc., Frederick, MD	(Loveless et al. 1996)
Eugenol	AOO	NA	NA	15.0	3750	N	NA	NA	(Basketter et al. 2007)
Eugenol	AOO	10 25 50	2.4 5.5 16.1	12.9	3225	N	CBA/Ca	NA	(Bertrand et al. 1997)
Eugenol	AOO	NA	NA	4.9	1225	N	NA	NA	(Basketter et al. 2007)
Eugenol	AOO	NA	NA	7.5	1875	N	NA	NA	(Basketter et al. 2007)
Eugenol	NA	NA	NA	13	3250	NA	NA	NA	(Basketter and Kimber 2001)
Eugenol	NA	NA	NA	11.6	2900	NA	NA	NA	(Kimber and Basketter 1997)
Eugenol	EtOH/DEP (1:3)	2.5 5 10 25 50	1.2 2.7 6 14.3 19.4	5.4	1350	N	CBA/Ca	Harlan Interfauna UK, Shaw's Farm, Blackthorne, Bicester, Oxon, UK	(Lalko and Api 2006)
Eugenol	ACE	25 50 75	5.4 10.6 10.5	18.2	4539	Y[66]	CBA/J	Jackson Laboratories, Bar Harbor, ME	(Gerberick et al. 1992)

[65] LLNA protocol modifications included daily treatment for 4, rather than 3, consecutive days and injection of ^{3}H-methyl thymidine on the fifth day.
[66] LLNA protocol modifications included daily treatment for 4, rather than 3, consecutive days and injection of ^{3}H-methyl thymidine on the fifth day.

ED_006472_00084346-00229

Chemical Name	LLNA Vehicle	Conc. (%)	SI	EC3 (%)	EC3 (µg/cm²)	Nonstd. LLNA Protocol	Mouse Strain	Mouse Source	LLNA Reference
Eugenol	EtOH/DEP (3:1)	NA	NA	5.3	1325	N	NA	NA	(Isola and Lalko 2001)
Eugenol	EtOH/DEP (1:3)	NA	NA	10.5	2625	N	NA	NA	(Isola and Lalko 2001)
Eugenol	EtOH	NA	NA	10.7	2675	N	NA	NA	(Isola and Lalko 2001)
Eugenol	DEP	NA	NA	15.1	3775	N	NA	NA	(Isola and Lalko 2001)
EXP 10810 A	Pluronic L92	10 25 50	6.4 8.4 9.2	2.1	527	N	CBA/J	R. Janvier, Le Genest St Isle, France	(Debruyne 2007)
EXP 11120 A	Pluronic L92	10 25 50 100	0.96 0.66 1.6 6.3	64.9	16223	N	CBA/J	R. Janvier, Le Genest St Isle, France	(Debruyne 2007)
FAR01042-00	Pluronic L92	10 25 50 100	1.4 2.1 1.4 2.5	NC	NC	N	CBA/J	R. Janvier, Le Genest St Isle, France	(Debruyne 2007)
FAR01060-00	Pluronic L92	10 25 50 100	0.4 0.8 1 3.6	88.5	22115	N	CBA/J	R. Janvier, Le Genest St Isle, France	(Debruyne 2007)
Farnesol	AOO	NA	NA	4.1	1025	N	NA	NA	(RIFM 2007)
Farnesol	AOO	NA	NA	5.5	1375	N	NA	NA	(RIFM 2007)
Fatty acid glutamate	NA	5 25 50 100	1.5 1.8 1.2 4.8	75.0	18750	N	NA	NA	(TNO 2006)

ED_006472_00084346-00230

Chemical Name	LLNA Vehicle	Conc. (%)	SI	EC3 (%)	EC3 (µg/cm²)	Nonstd. LLNA Protocol	Mouse Strain	Mouse Source	LLNA Reference
Fatty alcohol #1	NA	10 25 50	4.2 8.2 16.2	7.6	1899	N	NA	NA	(TNO 2006)
Fatty alcohol #2	NA	10 25 50	4 9.9 16	8.6	2140	N	NA	NA	(TNO 2006)
F & Fo WG 50 + 25	Pluronic L92	2.5 5 10 25	11.7 12.6 14.1 15.2	0.003	0.77	N	CBA/J	R. Janvier, Le Genest St Isle, France	(Debruyne 2007)
Formaldehyde	ACE	0.25 0.5 1 2.5 5	NC NC NC NC 4	0.54[67]	135	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Hilton et al. 1998)
Formaldehyde	ACE	0.093 0.185 0.37 0.925 1.85	1.1 2.3 2.3 3.9 4	0.61	153	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Hilton et al. 1998) [EC3]; (Gerberick et al. 2005) [Dose-response data]
Formaldehyde	ACE	NA	NA	0.65	163	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Basketter et al. 2005)
Formaldehyde	AOO	0.1 0.5 1 5 10	0.97 1.91 3.17 5.23 8.59	0.35	88	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Basketter et al. 2001)

[67] Hilton et al. (1998) report this EC3 as 0.18M.

ED_006472_00084346-00231

Chemical Name	LLNA Vehicle	Conc. (%)	SI	EC3 (%)	EC3 (µg/cm²)	Nonstd. LLNA Protocol	Mouse Strain	Mouse Source	LLNA Reference
Formaldehyde	AOO	5 10 25	9 10.6 11.9	0.37	93	Y[68]	CBA/Ca	Animal Breeding Unit, Alderley Park, UK	(Kimber et al. 1991)
Formaldehyde	AOO	NA	NA	0.40	100	NA	NA	NA	(Basketter and Kimber 2001)
Formaldehyde	AOO	0.093 0.185 0.37 0.925 1.85	1.1 2.3 2.3 3.9 4	0.70	175	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Hilton et al. 1998)
Formaldehyde	AOO	5 10 25	3.7 4 5.8	0.99	248	Y[69]	CBA/Ca	Animal Breeding Unit, Unilever Environmental Safety Laboratory	(Basketter and Scholes 1992; Kimber et al. 1991)
Formaldehyde	AOO	NA	NA	1.20	300	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Estrada et al. 2003)
Formaldehyde	DMF	1 10 20	6.7 13.2 17.7	0.27	67	N	CBA/Ca	Jackson Laboratories, Bar Harbor, ME	(Ryan et al. 2002)
Formaldehyde	DMF	0.25 0.5 1 2.5 5	NC NC NC NC >7	0.33[70]	83	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Hilton et al. 1998)
Formaldehyde	AOO	5 10 25	6.8 6.1 6.6	1.72	430	Y[71]	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Kimber et al. 1991)

[68] Protocol did not specify sex, and the test duration was 4 days.
[69] Protocol did not specify sex, and the test duration was 4 days.
[70] Hilton et al. (1998) report this EC3 as 0.11M.
[71] Protocol did not specify sex, and the test duration was 4 days.

ED_006472_00084346-00232

Chemical Name	LLNA Vehicle	Conc. (%)	SI	EC3 (%)	EC3 (µg/cm²)	Nonstd. LLNA Protocol	Mouse Strain	Mouse Source	LLNA Reference
Formaldehyde	AOO	5 10 25	4.6 4.7 4.2	2.78[72]	695	Y[73]	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Kimber et al. 1991)
Formaldehyde	DMSO	1 10 20	7.5 16 17.6	0.30[74]	74	N	CBA/Ca	Jackson Laboratories, Bar Harbor, ME	(Ryan et al. 2002)
Formaldehyde	Water	1 10 20	1.2 2.5 3.6	14.5	3636	N	CBA/Ca	Jackson Laboratories, Bar Harbor, ME	(Ryan et al. 2002)
Formaldehyde	PG	0.38 0.95 1.9 3.8 9.5	1.1 1.6 1.5 3.2 8.5	2.8	700	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Basketter et al. 2003a)
Formaldehyde	Pluronic L92	1 5 20	1.1 3.8 10.6	3.8	950	N	CBA/Ca	NA	(ECPA 2007d)
Formaldehyde	Pluronic L92	1 5 20	1.6 2.6 12	5.6	1400	N	CBA/J	Jackson Laboratories, Bar Harbor, ME	(ECPA 2006c)
Formaldehyde	Pluronic L92	1 5 20	0.99 2.16 6.15	8	2000	N	CBA/J	R. Janvier, Le Genest St Isle, France	(ECPA 2007i)
Formaldehyde	Pluronic L92	1 5 20	1.1 2.5 4.8	8.2	2050	N	CBA/JHsd	NA	(ECPA 2007j)

[72] Interpolation of the EC3 was linear (per Ryan et al. 2007) and used concentration = 0% and SI = 1 as the lowest point.
[73] Protocol did not specify sex, and the test duration was 4 days.
[74] Interpolation of the EC3 was linear (per Ryan et al. 2007) and used concentration = 0% and SI = 1 as the lowest point.

ED_006472_00084346-00233

Chemical Name	LLNA Vehicle	Conc. (%)	SI	EC3 (%)	EC3 (µg/cm²)	Nonstd. LLNA Protocol	Mouse Strain	Mouse Source	LLNA Reference
Formaldehyde	Pluronic L92	1 5 20	0.8 1.3 4.8	12.3	3075	N	CBA/CaOla Hsd	NA	(ECPA 2006b)
Fumaric acid	DMSO	5 10 25	1.3 2.3 1.4	NC	NC	N	CBA/CaOla Hsd	Harlan Winkelmann GmbH, D-33178 Borchen	(EFfCI 2006)
Fx + Me EW 69	Pluronic L92	5 10 25 50	0.83 1.55 2.95 8.55	25.2	6306	N	CBA/J	R. Janvier, Le Genest St Isle, France	(Debruyne 2007)
Geraniol	EtOH/DEP (3:1)	1 3 10 30 50	1 1 1.3 3.4 3.9	25.8	6450	Y[75]	CBA/Ca	Harlan Interfauna UK, Shaw's Farm, Blackthorne, Bicester, Oxon, UK	(Lalko et al. 2004)
Geraniol	EtOH/DEP (1:3)	NA	NA	20.4	5100	Y[76]	CBA/Ca	Harlan Interfauna UK, Shaw's Farm, Blackthorne, Bicester, Oxon, UK	(Lalko et al. 2004)
Geraniol	EtOH/DEP (1:3)	2.5 5 10 25 50	1.7 2.4 2.8 4.8 6	11.4	2850	N	CBA/Ca	Harlan Interfauna UK, Shaw's Farm, Blackthorne, Bicester, Oxon, UK	(Lalko and Api 2006)
Geraniol	AOO	NA	NA	57	14250	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon UK	(Griem et al. 2003)
Geraniol	AOO	12.5 25 50	0.9 1.2 2.6	NC	NC	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Basketter et al. 1994)

[75] Protocol used male mice.
[76] Protocol used male mice.

ED_006472_00084346-00234

Chemical Name	LLNA Vehicle	Conc. (%)	SI	EC3 (%)	EC3 (µg/cm²)	Nonstd. LLNA Protocol	Mouse Strain	Mouse Source	LLNA Reference
Geraniol	AOO	NA	NA	26	6500	N	NA	NA	(Roberts et al. 2007)
Geraniol	EtOH	NA	NA	5.6	1400	N	NA	NA	(Isola and Lalko 2001)
Geraniol	DEP	NA	NA	11.8	2950	N	NA	NA	(Isola and Lalko 2001)
Geranium oil	EtOH/DEP (1:3)	2.5 5 10 25 50	1.2 0.7 1.7 1.8 2.8	NC	NC	N	CBA/Ca	Harlan Interfauna UK, Shaw's Farm, Blackthorne, Bicester, Oxon, UK	(Lalko and Api 2006)
Glutaraldehyde	AOO	NA	NA	0.2	50	NA	NA	NA	(Basketter et al. 2000)
Glutaraldehyde	AOO	0.039 0.052 0.13 0.26 0.52	1.6 2.4 4.9 5.1 11.3	0.07	18	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Basketter et al. 2003a)
Glutaraldehyde	ACE	0.05 0.125 0.25 0.5 1.25	1.3 4.3 7.6 11.6 17.7	0.10	26	N	CBA/Ca	NA	(Gerberick et al. 2004)
Glutaraldehyde	ACE	NA	NA	0.09	23	NA	NA	NA	(Gerberick et al. 2001)
Glutaraldehyde	ACE	NA	NA	0.06	15	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Hilton et al. 1998)
Glutaraldehyde	DMF/Water	3.1 6.2 12.5	9.8 21.4 22.9	2.1	516	Y[77]	CBA/J	Jackson Laboratories, Bar Harbor, ME	(Gerberick et al. 1992)

[77] LLNA protocol modifications included daily treatment for 4, rather than 3, consecutive days and injection of ^{3}H-methyl thymidine on the fifth day.

ED_006472_00084346-00235

Chemical Name	LLNA Vehicle	Conc. (%)	SI	EC3 (%)	EC3 (µg/cm²)	Nonstd. LLNA Protocol	Mouse Strain	Mouse Source	LLNA Reference
Glutaraldehyde	DMF	0.25 0.5 1 2.5 5	>3 >3 >3 >3 >19	0.02	5.0	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Hilton et al. 1998)
Glutaraldehyde	PG	0.26 0.52 1.3 2.6	1 1.3 2.4 6.9	1.5	375	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Basketter et al. 2003a)
Glycerol	DMF	25 50 100	1.1 0.7 0.5	NC	NC	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon UK	(Ryan et al. 2000)
Glyceryl thioglycollate	AOO	10 25 50	8 14 31	4.7	1165	N	NA	NA	(TNO 2006)
Glyoxal	DMF	5 10 25	18.1 13.5 12.2	0.6	150	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Basketter et al. 1994)
Glyoxal	AOO	1 2.5 5 10 25	2.5 4.2 5.2 10.3 15.8	1.4	350	NA	NA	NA	(Patlewicz et al. 2002)[EC3]; (Gerberick et al. 2005) (Dose-response data)
Glyoxal	NA	NA	NA	0.5	125	NA	NA	NA	(Basketter and Kimber 2006)
Gold chloride	DMSO	5 10 25	21.8 10.9 17.2	0.48[78]	120	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Basketter et al. 1999b)

[78] Interpolation of the EC3 was linear (per Ryan et al. 2007) and used concentration = 0% and SI = 1 as the lowest point.

ED_006472_00084346-00236

Chemical Name	LLNA Vehicle	Conc. (%)	SI	EC3 (%)	EC3 (µg/cm²)	Nonstd. LLNA Protocol	Mouse Strain	Mouse Source	LLNA Reference
Hexane	AOO	25 50 100	0.8 0.8 2.2	NC	NC	NA	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Basketter et al. 1996)
trans-2-Hexenal	AOO	0.5 1 2.5 5.0 10.0	1.2 1.2 2.3 2.6 6.4	5.5	1375	NA	NA	NA	(Patlewicz et al. 2002)[EC3]; (Gerberick et al. 2005) [Dose-response data]
trans-2-Hexenal	EtOH/DEP (1:3)	NA	NA	2.6	650	N	NA	NA	(RIFM 2007)
Hexyl cinnamic aldehyde	AOO	2.5 5 10 25 50	2.2 3.2 7.1 13.9 17.6	4.4	1100	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Basketter et al. 1999c)
Hexyl cinnamic aldehyde	AOO	2.5 5 10 25 50	1.7 2.1 4.4 8.1 14.5	7.0	1750	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Dearman et al. 2001)
Hexyl cinnamic aldehyde	AOO	2.5 5 10 25 50	1.1 2.2 4.4 9.8 20.0	7.00	1750	Y[79]	CBA/JHsd	Harlan Sprague Dawley, Inc., Frederick, MD	(Loveless et al. 1996)

[79] LLNA protocol modifications included daily treatment for 4, rather than 3, consecutive days and injection of ^{3}H-methyl thymidine on the fifth day.

ED_006472_00084346-00237

Chemical Name	LLNA Vehicle	Conc. (%)	SI	EC3 (%)	EC3 (µg/cm²)	Nonstd. LLNA Protocol	Mouse Strain	Mouse Source	LLNA Reference
Hexyl cinnamic aldehyde	AOO	2.5 5 10 25 50	1.3 1.5 4.4 8.8 16.0	7.60	1900	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Loveless et al. 1996)
Hexyl cinnamic aldehyde	AOO	2.5 5 10 25 50	1.4 2.1 3.3 8.3 14.0	7.90	1975	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Loveless et al. 1996)
Hexyl cinnamic aldehyde	AOO	NA	NA	8	2000	NA	NA	NA	(Basketter and Kimber 2001)
Hexyl cinnamic aldehyde	AOO	2.5 5 10 25 50	1.3 1.3 4.2 8.8 17.0	8.10	2025	N	CBA/JHsd	Harlan Sprague Dawley, Inc., Frederick, MD	(Loveless et al. 1996)
Hexyl cinnamic aldehyde	AOO	2.5 5 10 25 50	1.3 1.1 2.5 10.0 17.0	8.40	2100	Y[80]	CBA/JHsd	Harlan Sprague Dawley, Inc., Frederick, MD	(Loveless et al. 1996)
Hexyl cinnamic aldehyde	AOO	2.5 5 10 25 50	1.4 2.1 3.3 8.4 14.0	8.8	2200	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Dearman et al. 2001)

[80] LLNA protocol modifications included daily treatment for 4, rather than 3, consecutive days and injection of ^{3}H-methyl thymidine on the fifth day.

ED_006472_00084346-00238

Chemical Name	LLNA Vehicle	Conc. (%)	SI	EC3 (%)	EC3 (µg/cm²)	Nonstd. LLNA Protocol	Mouse Strain	Mouse Source	LLNA Reference
Hexyl cinnamic aldehyde	AOO	10 25 50.0	3.2 6.0 10.0	9.40	2350	N	CBA	NA	(Ashby et al. 1995)
Hexyl cinnamic aldehyde	AOO	1 2.5 5 10 25	1.5 1.7 2.2 2.8 8.2	10.6	2650	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Dearman et al. 2001)
Hexyl cinnamic aldehyde	AOO	2.5 5 10 25 50	1.30 1.10 2.50 10.40 17.0	11	2750	Y[81]	CBA/Ca	Harlan Sprague-Dawley, Frederick, MD	(Dearman et al. 2001)
Hexyl cinnamic aldehyde	AOO	2.5 5 10 25 50	1.7 2.1 2.4 7.2 14.1	11.9	2975	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Dearman et al. 2001)
Hexyl cinnamic aldehyde	AOO	2.5 5 10 25 50	1 1.4 2 8.7 11.6	11.5	2875	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Basketter et al. 1999c)
Hexyl cinnamic aldehyde	AOO	5 10 25	1.6 2.5 6.8	11.70	2925	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Dearman et al. 2001)
Hexyl cinnamic aldehyde	AOO	5 10 25	1.4 2.7 5.3	11.70	2925	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Dearman et al. 2001)

[81] LLNA protocol modifications included daily treatment for 4, rather than 3, consecutive days and injection of ^{3}H-methyl thymidine on the fifth day.

ED_006472_00084346-00239

Chemical Name	LLNA Vehicle	Conc. (%)	SI	EC3 (%)	EC3 (µg/cm²)	Nonstd. LLNA Protocol	Mouse Strain	Mouse Source	LLNA Reference
Hexyl cinnamic aldehyde	AOO	NA	NA	12.02	3005	NA	NA	NA	(Patlewicz et al. 2001)
Hexyl cinnamic aldehyde	AOO	2.5 5 10 25 50	1.0 1.4 2.0 8.7 11.6	12.20	3050	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Dearman et al. 2001)
Hexyl cinnamic aldehyde	AOO	2.5 5 10 25 50	1.0 1.4 2.0 8.7 11.6	12.20	3050	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Dearman et al. 2001)
Hexyl cinnamic aldehyde	AOO	2.5 5 10 25 50	1.3 2.1 2.7 7.8 13.4	10.90	2725	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Dearman et al. 2001)
Hexyl cinnamic aldehyde	AOO	1 2.5 5 10 25	0.98 1 1.48 1.78 5.65	14.7	3682	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Basketter et al. 2001)
Hexyl cinnamic aldehyde	ACE	3 10 30	4.56 6.63 9.86	1.2	303	N	CBA/CaOla Hsd	Harlan Winkelmann GmbH, D-33178 Borchen	(Gamer et al. 2008)
Hexyl cinnamic aldehyde	Pluronic L92	3 10 30	1.2 4.6 18	6.7	1675	N	CBA/Ca	NA	(ECPA 2007a)
Hexyl cinnamic aldehyde	Pluronic L92	3 10 30	1.9 4.2 9.2	7	1750	N	CBA/J	R. Janvier, Le Genest St Isle, France	(ECPA 2007i)

ED_006472_00084346-00240

Chemical Name	LLNA Vehicle	Conc. (%)	SI	EC3 (%)	EC3 (µg/cm²)	Nonstd. LLNA Protocol	Mouse Strain	Mouse Source	LLNA Reference
Hexyl cinnamic aldehyde	Pluronic L92	3 10 30	1.9 2.2 10.3	12	3000	N	CBA/J	Jackson Laboratories, Bar Harbor, ME	(ECPA 2006c)
Hexyl cinnamic aldehyde	Pluronic L92	3 10 30	1.1 2.5 15.6	10.8	2700	N	CBA/JHsd	NA	(ECPA 2007j)
Hexyl cinnamic aldehyde	Pluronic L92	3 10 30	1.3 2.2 4.3	17.6	4400	N	CBA/CaHs dRcc(SPF)	RCC Ltd, Laboratory Animal Service, CH-4414 Füllinsdorf / Switzerland	(ECPA 2007b)
2-Hexylidene cyclopentanone	EtOH/DEP (1:3)	NA	NA	2.40	600	N	NA	NA	(RIFM 2007)
Hexyl salicylate	EtOH/DEP (1:3)	NA	NA	0.18	45	N	NA	NA	(RIFM 2007)
Hydrocortisone	NA	2.5 5 10	0.3 0.1 0.06	NC	NC	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Basketter et al. 1999a)
4-Hydroxybenzoic acid	DMSO	5 10 25	1.4 1.5 1.3	NC	NC	Y[82]	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Basketter and Scholes 1992); (Scholes et al. 1992)
4-Hydroxybenzoic acid	AOO	2.5 5 10	0.4 0.8 0.6	NC	NC	N	CBA/Ca	Barriered Animal Breeding Unit, Alderley Park. UK	(Scholes et al. 1992)
4-Hydroxybenzoic acid	AOO	5 10 25	0.9 1 0.9	NC	NC	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Scholes et al. 1992)
4-Hydroxybenzoic acid	AOO	5 10 25	1.4 1.5 1.3	NC	NC	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Scholes et al. 1992)

[82] Protocol used both sexes, and the test duration was 4 or 5 days.

ED_006472_00084346-00241

Chemical Name	LLNA Vehicle	Conc. (%)	SI	EC3 (%)	EC3 (µg/cm²)	Nonstd. LLNA Protocol	Mouse Strain	Mouse Source	LLNA Reference
Hydroxycitronellal	AOO	2.5 5 10 25 50	2.2 1 0.8 1.1 7.1	33.0	8250	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Basketter et al. 2001)
Hydroxycitronellal	AOO	NA	NA	20.0	5000	NA	NA	NA	(Basketter and Kimber 2001)
Hydroxycitronellal	AOO	NA	NA	25.0	6250	NA	NA	NA	(Estrada et al. 2003)
Hydroxycitronellal	AOO	25 50 100	3.6 5.9 8.5	21.0	5250	Y[83]	CBA/Ca	NA	(Basketter and Scholes 1992)
Hydroxycitronellal	AOO	10 25 50	1.7 3.2 6.7	23.0	5750	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Basketter et al. 1994)
Hydroxycitronellal	AOO	NA	NA	27.5	6875	N	NA	NA	(Basketter et al. 2007)
Hydroxycitronellal	AOO	NA	NA	22.4	5600	NA	NA	NA	(RIFM 2007)
Hydroxycitronellal	NA	NA	NA	25.3	6313	NA	NA	NA	(Patlewicz et al. 2002)
Hydroxycitronellal	DMF	1 5 25	1.3 2.1 3.4	18.8	4712	N	CBA/Ca	B&K Universal, Sollentuna, Sweden	(Montelius et al. 1994)
Hydroxycitronellal	EtOH/DEP (1:3)	NA	NA	19.3	4825	N	NA	NA	(Isola and Lalko 2001)
Hydroxycitronellal	DEP	NA	NA	19.7	4925	N	NA	NA	(Isola and Lalko 2001)
Hydroxycitronellal	EtOH/DEP (3:1)	NA	NA	22.2	5550	N	NA	NA	(Isola and Lalko 2001)
Hydroxycitronellal	EtOH	NA	NA	26.4	6600	N	NA	NA	(Isola and Lalko 2001)

[83] Protocol used both sexes, and the test duration was 4 or 5 days.

ED_006472_00084346-00242

Chemical Name	LLNA Vehicle	Conc. (%)	SI	EC3 (%)	EC3 (µg/cm²)	Nonstd. LLNA Protocol	Mouse Strain	Mouse Source	LLNA Reference
2-Hydroxyethyl acrylate	AOO	5 10 25	10.7 14.8 18.1	1.4	350	N	CBA/Ca	Barriered Animal Breeding Unit, Alderley Park, UK	(Scholes et al. 1992)
2-Hydroxyethyl acrylate	AOO	10 25	9 8.2	6.25	1563	Y[84]	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Basketter and Scholes 1992) (Scholes et al. 1992)
2-Hydroxyethyl acrylate	DMF	10 25 50	13.8 11 11.7	1.56[85]	390	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Scholes et al. 1992)
2-Hydroxypropyl methacrylate	AOO	10 25 50	1.1 1.2 1.3	NC	NC	Y[86]	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Basketter and Scholes 1992) (Scholes et al. 1992)
2-Hydroxypropyl methacrylate	AOO	10 25 50	0.8 1 0.9	NC	NC	N	CBA/Ca	Barriered Animal Breeding Unit, Alderley Park, UK	(Scholes et al. 1992)
2-Hydroxypropyl methacrylate	AOO	10 25 50	1 1.9 0.8	NC	NC	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Scholes et al. 1992)
2-Hydroxypropyl methacrylate	DMF	10 25 50	1.4 0.7 0.9	NC	NC	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Scholes et al. 1992)
Imidazolidinyl urea	DMF	10 25 50	1.7 3.1 5.5	24	6000	Y[87]	CBA/Ca	NA	(Basketter and Scholes 1992)
Isocyclemone E	EtOH/DEP (1:3)	NA	NA	25.14	6285	N	NA	NA	(RIFM 2007)

[84] Protocol used both sexes, and the test duration was 4 or 5 days.
[85] Interpolation of the EC3 was linear (per Ryan et al. 2007) and used concentration = 0% and SI = 1 as the lowest point.
[86] Protocol used both sexes, and the test duration was 4 or 5 days.
[87] Protocol used both sexes, and the test duration was 4 or 5 days.

ED_006472_00084346-00243

Chemical Name	LLNA Vehicle	Conc. (%)	SI	EC3 (%)	EC3 (µg/cm^2)	Nonstd. LLNA Protocol	Mouse Strain	Mouse Source	LLNA Reference
Isocyclocitral	EtOH/DEP (1:3)	NA	NA	7.35	1838	N	NA	NA	(RIFM 2007)
Isocyclogeraniol	EtOH/DEP (1:3)	NA	NA	NC	NC	N	NA	NA	(RIFM 2007)
Isoeugenol	AOO	0.5 1 5	0.9 6.3 31.0	0.5	125	N	CBA/Ca	NA	(Basketter and Cadby 2004)
Isoeugenol	AOO	0.5 1 5	1.5 2.5 29.8	0.6	150	N	CBA/Ca	NA	(Basketter and Cadby 2004)
Isoeugenol	AOO	0.5 1 5	1.6 4.3 24.4	0.6	150	N	CBA/Ca	NA	(Basketter and Cadby 2004)
Isoeugenol	AOO	0.5 1 5	1.8 2.9 23.2	0.6	150	N	CBA/Ca	NA	(Basketter and Cadby 2004)
Isoeugenol	AOO	0.5 1 5	2.3 1.6 23.6	0.6	150	N	CBA/Ca	NA	(Basketter and Cadby 2004)
Isoeugenol	AOO	NA	NA	0.6	150	N	NA	NA	(RIFM 2007)
Isoeugenol	AOO	NA	NA	0.6	150	N	NA	NA	(RIFM 2007)
Isoeugenol	AOO	NA	NA	0.7	175	N	NA	NA	(RIFM 2007)
Isoeugenol	AOO	0.5 1 5	1.2 4.2 18.4	0.7	175	N	CBA/Ca	NA	(Basketter and Cadby 2004)
Isoeugenol	AOO	NA	NA	0.7	175	N	NA	NA	(Basketter et al. 2007)
Isoeugenol	AOO	0.5 1 5	1.1 1.8 23.2	0.8	200	N	CBA/Ca	NA	(Basketter and Cadby 2004)

ED_006472_00084346-00244

Chemical Name	LLNA Vehicle	Conc. (%)	SI	EC3 (%)	EC3 (µg/cm²)	Nonstd. LLNA Protocol	Mouse Strain	Mouse Source	LLNA Reference
Isoeugenol	AOO	0.5 1 5	1.5 2.6 19.2	0.8	200	N	CBA/Ca	NA	(Basketter and Cadby 2004)
Isoeugenol	AOO	0.5 1 5	1.6 2.2 19.0	0.8	200	N	CBA/Ca	NA	(Basketter and Cadby 2004)
Isoeugenol	AOO	NA	NA	0.8	200	N	NA	NA	(RIFM 2007)
Isoeugenol	AOO	NA	NA	0.9	225	N	NA	NA	(Basketter et al. 2007)
Isoeugenol	AOO	0.5 1 5	0.7 2.3 13.8	1	250	N	CBA/Ca	NA	(Basketter and Cadby 2004)
Isoeugenol	AOO	0.5 1 5	1.3 2.2 13.1	1	250	N	CBA/Ca	NA	(Basketter and Cadby 2004)
Isoeugenol	AOO	0.5 1 5	0.8 1.6 14.1	1.1	275	N	CBA/Ca	NA	(Basketter and Cadby 2004)
Isoeugenol	AOO	0.5 1 5	1 1.1 12.4	1.2	300	N	NA	NA	(Gerberick et al. 2005)
Isoeugenol	AOO	NA	NA	1.2	300	N	NA	NA	(Basketter et al. 2007)
Isoeugenol	AOO	NA	NA	1.2	300	N	NA	NA	(Basketter et al. 2007)
Isoeugenol	AOO	NA	NA	1.2	300	N	NA	NA	(RIFM 2007)
Isoeugenol	AOO	NA	NA	1.3	325	N	NA	NA	(RIFM 2007)
Isoeugenol	AOO	2.5 5 10.0	9.9 17.0 29.5	1.3	319	Y[88]	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Kimber et al. 1991)

[88] Protocol did not specify sex, and the test duration was 4 days.

ED_006472_00084346-00245

Chemical Name	LLNA Vehicle	Conc. (%)	SI	EC3 (%)	EC3 (µg/cm²)	Nonstd. LLNA Protocol	Mouse Strain	Mouse Source	LLNA Reference
Isoeugenol	AOO	0.5 1 5	1.2 3.2 8.7	1.3	325	N	CBA/Ca	NA	(Basketter and Cadby 2004)
Isoeugenol	AOO	0.25 0.50 1 2.5 5	1.5 2.2 2.5 4.9 10.0	1.3	325	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Loveless et al. 1996)
Isoeugenol	AOO	NA	NA	1.3	325	N	NA	NA	(Basketter et al. 2007)
Isoeugenol	AOO	NA	NA	1.3	325	N	NA	NA	(Basketter et al. 2007)
Isoeugenol	AOO	0.5	1.1	1.3	332	N	CBA/Ca	NA	(Basketter and Cadby 2004)
Isoeugenol	AOO	2.5 5 10.0	7.8 13.1 14.6	1.3	334	Y[89]	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Kimber et al. 1991)
Isoeugenol	AOO	NA	NA	1.4	350	N	NA	NA	(Basketter et al. 2007)
Isoeugenol	AOO	0.5 1 5	1.6 1.6 14.7	1.4	357	N	CBA/Ca	NA	(Basketter and Cadby 2004)
Isoeugenol	AOO	2.5 5 10.0	7.5 13.1 25.3	1.4	358	Y[90]	CBA/Ca	Animal Breeding Unit, Unilever Environmental Safety Laboratory	(Basketter and Scholes 1992); (Kimber et al. 1991)
Isoeugenol	AOO	NA	NA	1.5	375	N	NA	NA	(RIFM 2007)
Isoeugenol	AOO	0.5 1 5	1.3 3.3 14.7	1.5	375	N	CBA/Ca	NA	(Basketter and Cadby 2004)

[89] Protocol did not specify sex, and the test duration was 4 days.
[90] Protocol did not specify sex, and the test duration was 4 days.

ED_006472_00084346-00246

Chemical Name	LLNA Vehicle	Conc. (%)	SI	EC3 (%)	EC3 (µg/cm²)	Nonstd. LLNA Protocol	Mouse Strain	Mouse Source	LLNA Reference
Isoeugenol	AOO	0.5 1 5	1.6 2.2 7.5	1.6	400	N	CBA/Ca	NA	(Basketter and Cadby 2004)
Isoeugenol	AOO	0.5 1 5	2.0 1.4 7.6	1.6	400	N	CBA/Ca	NA	(Basketter and Cadby 2004)
Isoeugenol	AOO	0.25 0.50 1 2.5 5	1.2 1.7 2.6 4.3 11.0	1.6	400	N	CBA/JHsd	Harlan Sprague Dawley, Inc., Frederick, MD	(Loveless et al. 1996)
Isoeugenol	AOO	NA	NA	1.7	425	N	NA	NA	(Basketter et al. 2007)
Isoeugenol	AOO	NA	NA	1.7	425	N	NA	NA	(Basketter et al. 2007)
Isoeugenol	AOO	0.5 1 5	1.0 1.3 7.5	1.8	450	N	CBA/Ca	NA	(Basketter and Cadby 2004)
Isoeugenol	AOO	0.5 1 5	1.2 1.4 19.3	1.8	450	N	CBA/Ca	NA	(Basketter and Cadby 2004)
Isoeugenol	AOO	0.25 0.50 1 2.5 5	2.9 1.7 2.3 3.8 6.8	1.8	450	Y[91]	CBA/JHsd	Harlan Sprague Dawley, Inc., Frederick, MD	(Loveless et al. 1996)
Isoeugenol	AOO	NA	NA	1.9	475	N	NA	NA	(RIFM 2007)
Isoeugenol	AOO	0.5 1 5	0.9 1.0 7.2	1.9	475	N	CBA/Ca	NA	(Basketter and Cadby 2004)

[91] LLNA protocol modifications included daily treatment for 4, rather than 3, consecutive days and injection of ^{3}H-methyl thymidine on the fifth day.

ED_006472_00084346-00247

Chemical Name	LLNA Vehicle	Conc. (%)	SI	EC3 (%)	EC3 (µg/cm²)	Nonstd. LLNA Protocol	Mouse Strain	Mouse Source	LLNA Reference
Isoeugenol	AOO	0.5 1 5	1.4 1.2 6.7	2	500	N	CBA/Ca	NA	(Basketter and Cadby 2004)
Isoeugenol	AOO	0.5 1 5	0.8 2.8 5.6	2.1	525	N	CBA/Ca	NA	(Basketter and Cadby 2004)
Isoeugenol	AOO	2.5 5 10.0	4.2 11.8 21.3	2.2	560	Y[92]	CBA/Ca	Animal Breeding Unit, Alderley Park, UK	(Kimber et al. 1991)
Isoeugenol	AOO	NA	NA	2.3	575	N	NA	NA	(Basketter et al. 2007)
Isoeugenol	AOO	NA	NA	2.3	575	N	NA	NA	(Basketter et al. 2007)
Isoeugenol	AOO	0.5 1 5	1.4 1.5 4.9	2.6	650	N	CBA/Ca	NA	(Basketter and Cadby 2004)
Isoeugenol	AOO	0.5 1 5	1.7 1.2 5.0	2.6	650	N	CBA/Ca	NA	(Basketter and Cadby 2004)
Isoeugenol	AOO	NA	NA	2.7	675	N	NA	NA	(Basketter et al. 2007)
Isoeugenol	AOO	NA	NA	2.8	700	N	NA	NA	(Basketter et al. 2007)
Isoeugenol	AOO	NA	NA	2.9	725	N	NA	NA	(Basketter et al. 2007)
Isoeugenol	AOO	0.25 0.50 1 2.5 5	0.7 0.7 0.9 2.1 7.2	3.1	775	Y[93]	CBA/JHsd	Harlan Sprague Dawley, Inc., Frederick, MD	(Loveless et al. 1996)

[92] Protocol did not specify sex, and the test duration was 4 days.
[93] LLNA protocol modifications included daily treatment for 4, rather than 3, consecutive days and injection of ^{3}H-methyl thymidine on the fifth day.

ED_006472_00084346-00248

Chemical Name	LLNA Vehicle	Conc. (%)	SI	EC3 (%)	EC3 (µg/cm^2)	Nonstd. LLNA Protocol	Mouse Strain	Mouse Source	LLNA Reference
Isoeugenol	AOO	0.25 0.50 1 2.5 5	1.0 1.3 2.1 2.3 4.1	3.3	825	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Loveless et al. 1996)
Isoeugenol	AOO	0.5 1 2.5 5 10	1.8 2.9 7.7 11.1 11.7	1.0	258	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Wright et al. 2001)
Isoeugenol	DMF	0.5 1 2.5 5 10	2.6 2.7 3.7 7.5 11.6	1.45	363	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Wright et al. 2001)
Isoeugenol	DMSO	0.5 1 2.5 5 10	1.9 3.2 7.4 20 17.1	0.9	231	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Wright et al. 2001)
Isoeugenol	EtOH (10%)	0.5 1 2.5 5 10	1.8 2 3.8 5.8 12.6	1.8	458	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Wright et al. 2001)
Isoeugenol	EtOH (50%)	0.5 1 2.5 5 10	1 1.2 2 3 5.4	5.0	1250	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Wright et al. 2001)

ED_006472_00084346-00249

Chemical Name	LLNA Vehicle	Conc. (%)	SI	EC3 (%)	EC3 (µg/cm²)	Nonstd. LLNA Protocol	Mouse Strain	Mouse Source	LLNA Reference
Isoeugenol	MEK	0.5 1 2.5 5 10	0.9 3.2 5 4.9 8.1	1.0	239	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Wright et al. 2001)
Isoeugenol	NA	NA	NA	1.40	350	NA	NA	NA	(Kimber and Basketter 1997)
Isoeugenol	NA	NA	NA	3.50	875	Y[94]	NA	NA	(Estrada et al. 2003)
Isoeugenol	PG	0.5 1 2.5 5 10	0.8 1.6 3 5.3 8.5	2.5	625	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Wright et al. 2001)
Isomethyl ionone	EtOH/DEP (1:3)	NA	NA	21.8	5450	N	NA	NA	(RIFM 2007)
Isopropanol	AOO	10 25 50	1.7 1.1 1.0	NC	NC	N	CBA	NA	(Basketter 1998)
Isopropyl myristate	AOO	25 50 100	2.1 3.3 3.4	44	11000	N	CBA/J	Harlan Sprague Dawley Inc., Indianapolis, IN	(Ryan et al. 2000)
Jasmine absolute (grandiflorum)	EtOH/DEP (1:3)	NA	NA	5.90	1475	N	NA	NA	(RIFM 2007)
Jasmine absolute (sambac)	EtOH/DEP (1:3)	NA	NA	36.40	9100	N	NA	NA	(RIFM 2007)
Kanamycin	AOO	5 10 25	2.2 0.8 1	NC	NC	NA	NA	NA	(Basketter et al. 1996)

[94] Protocol used both sexes of mice.

ED_006472_00084346-00250

Chemical Name	LLNA Vehicle	Conc. (%)	SI	EC3 (%)	EC3 (µg/cm^2)	Nonstd. LLNA Protocol	Mouse Strain	Mouse Source	LLNA Reference
Lead acetate	DMSO	2.5 5 10	0.7 0.8 1	NC	NC	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Basketter et al. 1999b)
Lemongrass oil	EtOH/DEP (1:3)	2.5 5 10 25 50	0.9 2.1 5.1 10.3 13.1	6.5	1625	N	CBA/Ca	Harlan Interfauna UK, Shaw's Farm, Blackthorne, Bicester, Oxon, UK	(Lalko and Api 2006)
Lilial	AOO	1 2.5 5 10 25	1.3 2.47 NA 2.02 3.71	18.7	4675	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Basketter et al. 2001)
Lilial	AOO	NA	NA	16.8	4200	N	NA	NA	(RIFM 2007)
Lilial	EtOH	NA	NA	2.9	725	N	NA	NA	(RIFM 2007)
Lilial	DEP	NA	NA	4.1	1025	N	NA	NA	(RIFM 2007)
Lilial	EtOH/DEP (1:3)	NA	NA	13.9	3475	N	NA	NA	(RIFM 2007)
Lilial	EtOH/DEP (3:1)	NA	NA	8.8	2200	N	NA	NA	(RIFM 2007)
d-Limonene	DEP	NA	NA	31	7750	N	NA	NA	(RIFM 2007)
d-Limonene	DEP	NA	NA	63	15750	N	NA	NA	(RIFM 2007)
d-Limonene	AOO	25 50 100	1.8 2.4 4.0	69	17250	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Gerberick et al. 2005) [EC3]; (Warbrick et al. 2001)[Dose-response data]
d-Limonene	EtOH	NA	NA	10	2500	N	NA	NA	(RIFM 2007)
d-Limonene	EtOH/DEP (3:1)	NA	NA	22	5500	N	NA	NA	(RIFM 2007)
d-Limonene	EtOH/DEP (1:3)	NA	NA	38	9500	N	NA	NA	(RIFM 2007)

ED_006472_00084346-00251

Chemical Name	LLNA Vehicle	Conc. (%)	SI	EC3 (%)	EC3 (µg/cm²)	Nonstd. LLNA Protocol	Mouse Strain	Mouse Source	LLNA Reference
Linalool	AOO	NA	NA	55	13750	N	NA	NA	(RIFM 2007)
Linoleic acid	AOO	10	1.5	14.1	3523	N	CBA/CaOla Hsd	Harlan Winkelmann GmbH, D-33178 Borchen	(EFfCI 2006)
Linolenic acid	AOO	10 25 50	3.1 9.3 10.3	9.9	2463	N	CBA/CaOla Hsd	Harlan Winkelmann GmbH, D-33178 Borchen	(EFfCI 2006)
Litsea cubeba oil	EtOH/DEP (1:3)	2.5 5 10 25 50	2 2.3 3.3 7.9 16	8.4	2100	N	CBA/Ca	Harlan Interfauna UK, Shaw's Farm, Blackthorne, Bicester, Oxon, UK	(Lalko and Api 2006)
Lyral HMPCC	AOO	1 2.5 5 10 25	0.6 0.7 0.6 1.3 4.9	17	4250	NA	NA	NA	(Patlewicz et al. 2002)[EC3] ; (Gerberick et al. 2005) [Dose-response data]
Lyral HMPCC	AOO	NA	NA	17.1	4275	N	NA	NA	(RIFM 2007)
Majantal	AOO	NA	NA	NC	NC	N	NA	NA	(RIFM 2007)
Maleic acid	DMSO	10 25 50	6.7 16.1 16.1	7.0	1743	N	CBA/CaOla Hsd	Harlan Winkelmann GmbH, D-33178 Borchen	(EFfCI 2006)
Manganese chloride	Petrolatum	5 10 25	1.10 0.60 1.00	NC	NC	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Basketter et al. 1999b)
Menthadiene-7-methyl formate	EtOH/DEP (1:3)	NA	NA	NC	NC	N	NA	NA	(RIFM 2007)
Menthadiene-7-methyl formate	EtOH/DEP (1:3)	NA	NA	NC	NC	N	NA	NA	(RIFM 2007)

ED_006472_00084346-00252

Chemical Name	LLNA Vehicle	Conc. (%)	SI	EC3 (%)	EC3 (µg/cm²)	Nonstd. LLNA Protocol	Mouse Strain	Mouse Source	LLNA Reference
2-Mercaptobenzothiazole	DMF	1 3 10	2.3 4.4 8.6	1.7	425	Y[95]	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Gerberick et al. 2005) [EC3]; (Basketter et al. 1993) [Dose-response data]
2-Mercaptobenzothiazole	DMF	10 25 50	4.5 4.6 5.5	5.7[96]	1428	Y[97]	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Basketter and Scholes 1992); (Scholes et al. 1992)
2-Mercaptobenzothiazole	DMF	10 25 50	5.2 9.1 4.8	6.0	1500	N	CBA/Ca	Barriered Animal Breeding Unit, Alderley Park, UK	(Scholes et al. 1992)
2-Mercaptobenzothiazole	DMF	10 25 50	9.8 9.5 8.9	2.3	570	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Scholes et al. 1992)
2-Mercaptobenzothiazole	DMF	10 25 50	10 10.8 8.1	2.2	555	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Scholes et al. 1992)
2-Mercaptobenzothiazole	DMF	1 5 25	3 9.9 17.1	1	250	N	CBA/Ca	B&K Universal, Sollentuna, Sweden	(Montelius et al. 1994)
Mercuric (II) chloride	AOO	5 10	19.9 11.8	0.4	98	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Basketter et al. 1994)
4-Methoxyacetophenone	AOO	10 25 50	1.3 1.0 1.0	NC	NC	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Gerberick et al. 2005) [EC3]; (Ryan et al. 2000) [Dose-response data]
Methoxy dicyclopentadiene carboxaldehyde	AOO	NA	NA	NC	NC	N	NA	NA	(RIFM 2007)
2-Methoxy-4-methylphenol	AOO	NA	NA	5.80	1450	N	NA	NA	(RIFM 2007)

[95] Protocol used both sexes of mice.
[96] Interpolation of the EC3 was linear (per Ryan et al. 2007) and used concentration = 0% and SI = 1 as the lowest point.
[97] Protocol used both sexes, and the test duration was 4 or 5 days.

ED_006472_00084346-00253

Chemical Name	LLNA Vehicle	Conc. (%)	SI	EC3 (%)	EC3 (µg/cm²)	Nonstd. LLNA Protocol	Mouse Strain	Mouse Source	LLNA Reference
4-Methylaminophenol sulfate	DMF	0.5 1 2.5	2.5 3.4 6.7	0.8	200	Y[98]	CBA/Ca	NA	(Gerberick et al. 2005) [EC3]; (Basketter and Scholes 1992) [Dose-response data]
Methylanisylidene acetone	AOO	10 25 50	3.5 10 26.1	9.3	2325	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Ryan et al. 2000)
alpha-Methyl cinnamic aldehyde	AOO	NA	NA	4.5	1125	N	NA	NA	(RIFM 2007)
6-Methylcoumarin	ACE	5 10 25	1.0 1.0 1.1	NC	NC	N	CBA	NA	(Ashby et al. 1995)
6-Methylcoumarin	ACE	5 10 25	1.2 0.9 0.8	NC	NC	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Scholes et al. 1991)
Methyl dodecanesulfonate	AOO	1 2.5 5	21.6 39.9 48.6	0.4	98	Y[99]	CBA/Ca	NA	(Gerberick et al. 2005) [EC3] (Basketter and Scholes 1992) [Dose-response data]
Methylhexanedione	AOO	25 50 100	2.9 6 14.3	26.0	6500	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Gerberick et al. 2005) [EC3]; (Ryan et al. 2000) [Dose-response data]
Methylhydrocinnamal	AOO	2.5 5 10 25 50	1.22 1.36 2.61 4.21 10.69	13.7	3425	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Basketter et al. 2001)
Methylhydrocinnamal	AOO	NA	NA	22.0	5500	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Griem et al. 2003)

[98] Protocol used both sexes, and the test duration was 4 or 5 days.
[99] Protocol used both sexes, and the test duration was 4 or 5 days.

ED_006472_00084346-00254

Chemical Name	LLNA Vehicle	Conc. (%)	SI	EC3 (%)	EC3 (µg/cm^2)	Nonstd. LLNA Protocol	Mouse Strain	Mouse Source	LLNA Reference
Methylhydrocinnamal	DMF	25 50 100	3.6 9 16.4	23.1	5775	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Ryan et al. 2000)
Methylhydrocinnamal	NA	NA	NA	14	3500	NA	NA	NA	(Basketter and Kimber 2001)
Methylisothiazolinone	AOO	0.25 0.5 1.0 2.5 5.0	1.5 1.5 1.8 3.8 2.5	1.9	475	NA	NA	NA	(Estrada et al. 2003) [EC3]; (Gerberick et al. 2005) [Dose-response data]
Methylisothiazolinone	AOO	0.049 0.099 0.197 0.493 0.985	1.5 1.5 1.8 3.8 2.5	0.4	100	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Basketter et al. 2003a)
Methyl methacrylate	ACE	10 30 50 75 100	1.5 2.3 2 4.4 7.3	60	15000	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Betts et al. 2006)
Methyl methacrylate	AOO	10 30 50 75 100	1.4 1.5 1.5 2.1 3.6	90	22500	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Betts et al. 2006)
Methyl 2-nonynoate	EtOH (80%)	5 10 20	10.4 17.7 24.4	2.5	625	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Ryan et al. 2000)
Methyl 2-octynoate	EtOH/DEP (1:3)	NA	NA	0.5	125	N	NA	NA	(RIFM 2007)

ED_006472_00084346-00255

Chemical Name	LLNA Vehicle	Conc. (%)	SI	EC3 (%)	EC3 (µg/cm²)	Nonstd. LLNA Protocol	Mouse Strain	Mouse Source	LLNA Reference
Methyl salicylate	DMF	5 10 25	2.3 2.5 3	25.0	6250	N	CBA/Ca	B&K Universal, Sollentuna, Sweden	(Montelius et al. 1994)
Methyl salicylate	MEK	5 10 25	2.5 2.5 7.5	11.5	2875	N	CBA/Ca	B&K Universal, Sollentuna, Sweden	(Montelius et al. 1994)
Methyl salicylate	AOO	1 2.5 5 10 20	1.2 1.5 1.2 1.8 2.9	NC	NC	Y[100]	CBA/J	Harlan Sprague Dawley Inc., Indianapolis, IN	(Kimber et al. 1995)
Methyl salicylate	ACE	1 2.5 5	0.8 0.8 0.8	NC	NC	Y[101]	CBA/J	NA	(Gerberick et al. 1992)
Methyl salicylate	AOO	1 2.5 5 10 20	1.1 1.4 1.4 1.4 2	NC	NC	Y[102]	CBA/Ca	Harlan Seralab, Bicester, Oxfordshire, UK	(Kimber et al. 1998)
Methyl salicylate	AOO	1 2.5 5 10 20	1.8 2 1.5 2.2 1.8	NC	NC	Y[103]	CBA/Ca	Harlan Seralab, Bicester, Oxfordshire, UK	(Kimber et al. 1998)

[100] LLNA protocol modifications included daily treatment for 4, rather than 3, consecutive days and injection of ^{3}H-methyl thymidine on the fifth day.
[101] LLNA protocol modifications included daily treatment for 4, rather than 3, consecutive days and injection of ^{3}H-methyl thymidine on the fifth day.
[102] The LLNA protocol used both sexes of mice.
[103] The LLNA protocol used both sexes of mice.

ED_006472_00084346-00256

Chemical Name	LLNA Vehicle	Conc. (%)	SI	EC3 (%)	EC3 (µg/cm^2)	Nonstd. LLNA Protocol	Mouse Strain	Mouse Source	LLNA Reference
Methyl salicylate	AOO	1 2.5 5 10 20	1 1.1 1.6 1.4 0.9	NC	NC	N	CBA/JHsd	Harlan Sprague-Dawley, Indianapolis, IN or Jackson Labs, Bar Harbor, ME	(Kimber et al. 1998)
Methyl salicylate	AOO	1 2.5 5 10 20	1.2 1.1 1.3 1.9 1.2	NC	NC	N	CBA/JHsd	Harlan Sprague-Dawley, Indianapolis, IN or Jackson Labs, Bar Harbor, ME	(Kimber et al. 1998)
Methyl salicylate	AOO	1 2.5 5 10 20	1.1 1.4 1.2 1.2 0.9	NC	NC	N	CBA/JHsd	Harlan Sprague-Dawley, Indianapolis, IN or Jackson Labs, Bar Harbor, ME	(Kimber et al. 1998)
NAVY 14 08 723	AOO	1 3 9 15	5.1 4.8 5.7 5.2	0.49	123	N	CBA	NA	(Haist et al. 2007)
Neomycin sulfate	EtOH (25%)	0.5 1 2	0.9 0.9 0.9	NC	NC	Y[104]	CBA/J	Jackson Laboratories, Bar Harbor, ME	(Gerberick et al. 1992)
Neomycin sulfate	DMSO	5 10 25	1 0.9 1	NC	NC	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Basketter et al. 1994)

[104] LLNA protocol modifications included daily treatment for 4, rather than 3, consecutive days and injection of ^{3}H-methyl thymidine on the fifth day.

ED_006472_00084346-00257

Chemical Name	LLNA Vehicle	Conc. (%)	SI	EC3 (%)	EC3 (µg/cm²)	Nonstd. LLNA Protocol	Mouse Strain	Mouse Source	LLNA Reference
Nickel (II) salts (nickel chloride)	DMF	0.25 0.9 1 2.5 5	2 2.4 1.6 1.6 2.2	NC	NC	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Ryan et al. 2002)
Nickel (II) salts (nickel sulfate)	DMSO	0.25 0.5 1 2.5 5	1.3 1.4 1.4 1.8 3.1	4.8	1202	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Ryan et al. 2002)
Nickel (II) salts (nickel chloride)	DMSO	1 2.5 5	1.5 2.2 2.4	NC	NC	Y[105]	CBA/Ca	NA	(Basketter and Scholes 1992)
Nickel (II) salts (nickel sulfate)	DMSO/ Water (9:1)	2.5 5	2.19 2.46	NC	NC	Y[106]	CBA/N	Japan SLC Inc., Shizuoka, Japan	(Ikarashi et al. 1992)
Nickel (II) salts (nickel sulfate)	EtOH (30%)	2.5 5 10	1.3 2.6 6.6	5.5	1375	Y[107]	CBA/J	Jackson Laboratories, Bar Harbor, ME	(Gerberick et al. 1992)
Nickel (II) salts (nickel sulfate)	Hydroxy-propyl cellulose in MeOH	2.5 5 10	0.8 1 2	NC	NC	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Scholes et al. 1992)
Nickel (II) salts (nickel sulfate)	Pluronic L92	0.25 0.5 1 2.5 5	2 2.4 2.8 3 2.3	NC	NC	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Ryan et al. 2002)

[105] Protocol used both sexes, and the test duration was 4 or 5 days.
[106] Test was terminated 24 hours after the last topical exposure.
[107] LLNA protocol modifications included daily treatment for 4, rather than 3, consecutive days and injection of ^{3}H-methyl thymidine on the fifth day.

ED_006472_00084346-00258

Chemical Name	LLNA Vehicle	Conc. (%)	SI	EC3 (%)	EC3 (µg/cm²)	Nonstd. LLNA Protocol	Mouse Strain	Mouse Source	LLNA Reference
Nickel (II) salts (nickel sulfate)	Hydroxyprop-yl cellulose in MeOH	2.5 5 10	1.4 1.2 1.2	NC	NC	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Scholes et al. 1992)
Nickel (II) salts (nickel sulfate)	Hydroxy-propyl cellulose in MeOH	2.5 5 10	0.6 0.7 0.5	NC	NC	N	CBA/Ca	Barriered Animal Breeding Unit, Alderley Park, UK	(Scholes et al. 1992)
Nickel (II) salts (nickel sulfate)	Hydroxy-propyl cellulose in MeOH	2.5 5 10	0.8 0.6 0.6	NC	NC	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Scholes et al. 1992)
Nickel (II) salts (nickel sulfate)	DMSO	0.5 1 2.5	1.1 1.5 1.5	NC	NC	Y[108]	CBA/Ca	NA	(Basketter and Scholes 1992)
Oakmoss	EtOH/DEP (1:3)	NA	NA	3.8	950	N	NA	NA	(RIFM 2007)
Octanoic acid	AOO	10 25 50	0.7 1 1.6	NC	NC	N	CBA	NA	(Basketter 1998)
1-Octen-3-yl acetate	EtOH/DEP (1:3)	NA	NA	NC	NC	N	NA	NA	(RIFM 2007)
Octinol	AOO	10 25 50	5.6 8.8 11.2	4.7	1187	N	CBA/CaOla Hsd	Harlan Winkelmann GmbH, D-33178 Borchen	(EFfCI 2006)
Oleic acid	NA	10 25 50	2.6 14.9 6.9	10.5	2622	N	CBA/CaOla Hsd	Harlan Winkelmann GmbH, D-33178 Borchen	(EFfCI 2006)
Oxalic acid	DMF	4 10 25	2.1 4.5 4.2	6.3	1563	N	CBA/Ca	B&K Universal, Sollentuna, Sweden	(Montelius et al. 1994)

[108] Protocol used both sexes, and the test duration was 4 or 5 days.

ED_006472_00084346-00259

Chemical Name	LLNA Vehicle	Conc. (%)	SI	EC3 (%)	EC3 (µg/cm²)	Nonstd. LLNA Protocol	Mouse Strain	Mouse Source	LLNA Reference
Oxalic acid	DMF	4 10 25	4.4 4.5 5	2.4	588	N	CBA/Ca	B&K Universal, Sollentuna, Sweden	(Montelius et al. 1994)
Oxazolone	ACE	0.0001 0.005 0.05	1.6 8.7 55.2	0.001	0.27	Y[109]	CBA/J	Jackson Laboratories, Bar Harbor, ME	(Gerberick et al. 1992)
Oxazolone	AOO	0.0025 0.005 0.01 0.025 0.5	3.8 6.2 7.7 15 23	0.0007	0.18	N	CBA/JHsd	Harlan Sprague Dawley, Inc., Frederick, MD	(Loveless et al. 1996)
Oxazolone	AOO	0.0025 0.005 0.01 0.025 0.5	3.9 4.8 6 12 13	0.0014	0.35	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Loveless et al. 1996)
Oxazolone	AOO	0.0025 0.005 0.01 0.025 0.05	3.4 4.4 4 5.9 8.9	0.002	0.50	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Loveless et al. 1996)
Oxazolone	AOO	0.0025 0.005 0.01 0.025 0.5	4 6.9 16 40 59	0.0025	0.63	Y[110]	CBA/JHsd	Harlan Sprague Dawley, Inc., Frederick, MD	(Loveless et al. 1996)

[109] LLNA protocol modifications included daily treatment for 4, rather than 3, consecutive days and injection of ^{3}H-methyl thymidine on the fifth day.
[110] LLNA protocol modifications included daily treatment for 4, rather than 3, consecutive days and injection of ^{3}H-methyl thymidine on the fifth day.

ED_006472_00084346-00260

Chemical Name	LLNA Vehicle	Conc. (%)	SI	EC3 (%)	EC3 (µg/cm²)	Nonstd. LLNA Protocol	Mouse Strain	Mouse Source	LLNA Reference
Oxazolone	AOO	0.0025 0.005 0.01 0.025 0.05	2.9 4.9 12 22 33	0.003	0.75	Y[111]	CBA/JHsd	Harlan Sprague Dawley, Inc., Frederick, MD	(Gerberick et al. 2005) [EC3]; (Loveless et al. 1996) [Dose-response data]
Oxazolone	AOO	0.1 0.25 0.5	19.4 24.2 32	0.0044	1.1	N	CBA/Ca	B&K Universal, Sollentuna, Sweden	(Montelius et al. 1994)
Oxazolone	Not specified	NA	NA	0.013	3.3	NA	NA	NA	(Estrada et al. 2003)
Oxyfluorfen EC	Pluronic L92	NA	NA	NC	NC	N	CBA/Ca	NA	(ECPA 2007f)
Oxyfluorfen EC	Pluronic L92	1 7 33	0.81 1.42 4.91	18.8	4700	N	CBA/J	R. Janvier, Le Genest St Isle, France	(ECPA 2007i)
Oxyfluorfen EC	Pluronic L92	1 7 33	1.13 1.49 3.14	30.8	7700	N	CBA/JHsd	NA	(ECPA 2007g)
Oxyfluorfen EC	Pluronic L92	1 7 33	1.2 1.2 5.4	18.1	4525	N	CBA/CaOla Hsd	NA	(ECPA 2007h)
Palmarosa oil	EtOH/DEP (1:3)	2.5 5 10 25 50	1.1 2.1 3.1 3.6 5	9.6	2400	N	CBA/Ca	Harlan Interfauna UK, Shaw's Farm, Blackthorne, Bicester, Oxon, UK	(Lalko and Api 2006)
Penicillin G	DMF	10 25 50	5.6 6.9 17	1.6	400	N	CBA/Ca	Barriered Animal Breeding Unit, Alderley Park, UK	(Scholes et al. 1992)

[111] LLNA protocol modifications included daily treatment for 4, rather than 3, consecutive days and injection of ^{3}H-methyl thymidine on the fifth day.

ED_006472_00084346-00261

Chemical Name	LLNA Vehicle	Conc. (%)	SI	EC3 (%)	EC3 (µg/cm²)	Nonstd. LLNA Protocol	Mouse Strain	Mouse Source	LLNA Reference
Penicillin G	DMF	5 10 25 50	1.1 2.7 6.3 6.5	11.7	2933	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Scholes et al. 1992)
Penicillin G	DMF	5 10 25	1 3.8 8.9	8.7	2165	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Scholes et al. 1992)
Penicillin G	DMSO	2.5 5.0 10.0 25.0 50.0	1.0 1.0 1.4 2.1 6.6	31.3	7825	N	CBA/Ca	Harlan Seralab, Bicester, Oxfordshire, UK	(Gerberick et al. 2005) [EC3]; (Kimber et al. 1998)
Penicillin G	DMSO	10 25 50	1.5 3.8 8.9	19.8	4946	Y[112]	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Basketter and Scholes 1992); (Scholes et al. 1992)
Penicillin G	DMSO	2.5 5.0 10.0 25.0 50.0	1.3 1.7 1.9 4.0 4.6	16.1	4025	N	CBA/Ca	Harlan Seralab, Bicester, Oxfordshire, UK	(Kimber et al. 1998)
Penicillin G	DMSO	2.5 5.0 10.0 25.0 50.0	0.8 0.7 0.8 1.3 3.4	46.4	11600	N	CBA/JHsd	Harlan Sprague-Dawley, Indianapolis, IN or Jackson Labs, Bar Harbor, ME	(Kimber et al. 1998)

[112] Protocol used both sexes, and the test duration was 4 or 5 days.

ED_006472_00084346-00262

Chemical Name	LLNA Vehicle	Conc. (%)	SI	EC3 (%)	EC3 (µg/cm²)	Nonstd. LLNA Protocol	Mouse Strain	Mouse Source	LLNA Reference
Penicillin G	DMSO	2.5 5.0 10.0 25.0 50.0	0.9 1.0 0.8 1.3 3.4	46.5	11625	N	CBA/JHsd	Harlan Sprague-Dawley, Indianapolis, IN or Jackson Labs, Bar Harbor, ME	(Kimber et al. 1998)
Penicillin G	DMSO	2.5 5.0 10.0 25.0 50.0	0.6 0.8 1.3 1.9 3.6	41.1	10275	N	CBA/JHsd	Harlan Sprague-Dawley, Indianapolis, IN or Jackson Labs, Bar Harbor, ME	(Kimber et al. 1998)
Penicillin G	DMSO	NA	NA	16.7	4175	NA	NA	NA	(Basketter et al. 2007)
Penicillin G	DMSO	NA	NA	17.9	4475	NA	NA	NA	(Basketter et al. 2007)
Pentachlorophenol	DMSO	10 25 50	2.1 3.5 5.4	20.0	5000	NA	NA	NA	(Gerberick et al. 2005) [EC3]; (Basketter et al. 1996) [Dose-response data]
Pentaerythritol triacrylate	ACE	0.005 0.01 0.05 0.1	1.19 0.92 1.68 2.43	NC	NC	Y[113]	BALB/c	Charles River, Laboratories	(NTP 1997b)
Perillaldehyde	AOO	0.5 1.0 2.5 5.0	1.2 1.1 0.9 4.3	8.1	2025	NA	NA	NA	(Gerberick et al. 2005)
Perillaldehyde	AOO	NA	NA	7.8	1950	NA	NA	NA	(Basketter et al. 2007)

[113] Mouse strain was not CBA.

ED_006472_00084346-00263

Chemical Name	LLNA Vehicle	Conc. (%)	SI	EC3 (%)	EC3 (µg/cm²)	Nonstd. LLNA Protocol	Mouse Strain	Mouse Source	LLNA Reference
Perillaldehyde	NA	NA	NA	7.95	1988	NA	NA	NA	(Estrada et al. 2003)
Peru balsam absolute	EtOH/DEP (1:3)	NA	NA	2.50	625	N	NA	NA	(RIFM 2007)
Phenyl benzoate	AOO	NA	NA	19.6	4900	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Basketter et al. 2005)
Phenyl benzoate	AOO	NA	NA	17.1	4263	NA	NA	NA	(Estrada et al. 2003)
Phenyl benzoate	AOO	1 2.5 5 10 25	2 6.4 9.3 8.7 11.1	1.2	300	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Basketter et al. 1999c)
Phenyl benzoate	AOO	5 10.0 25	2.3 2.1 3.5	20	5000	N	NA	NA	(Gerberick et al. 2005)
Phenylacetaldehyde	AOO	1 2.5 5 10.0 25	0.7 1.8 7.8 8.8 19	3.0	750	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Gerberick et al. 2005) [EC3]; (Basketter et al. 2001) [Dose-response data]
Phenylacetaldehyde	AOO	NA	NA	4.7	1175	NA	NA	NA	(Basketter et al. 2002)
Phenylacetaldehyde	AOO	25 50 100	15.5 23.8 24.1	8.8	2200	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Ryan et al. 2000)
4-Phenylenediamine	AOO	2.5 5 10	18.6 20 37.4	0.001	0.28	Y[114]	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Kimber et al. 1991)
4-Phenylenediamine	AOO	0.4 2	10.4 16.3	0.05	13	N	CBA/Ca	B&K Universal, Sollentuna, Sweden	(Montelius et al. 1994)

[114] Protocol did not specify sex, and the test duration was 4 days.

ED_006472_00084346-00264

Chemical Name	LLNA Vehicle	Conc. (%)	SI	EC3 (%)	EC3 (µg/cm²)	Nonstd. LLNA Protocol	Mouse Strain	Mouse Source	LLNA Reference
4-Phenylenediamine	AOO	0.05 0.1 0.25 0.5 1	2.6 4.7 10.3 15.5 14.2	0.06	15	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Warbrick et al. 1999b)
4-Phenylenediamine	AOO	0.05 0.1 0.25 0.5 1	2.2 4.2 13.73 20.77 25.28	0.07	18	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Warbrick et al. 1999b)
4-Phenylenediamine	AOO	NA	NA	0.08	20	N	CBA/Ca	NA	(Basketter et al. 2007)
4-Phenylenediamine	AOO	0.05 0.1 0.25 0.5 1	2 3.3 10.2 20.5 26.4	0.10	25	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Warbrick et al. 1999b)
4-Phenylenediamine	AOO	NA	NA	0.12	30	NA	NA	NA	(Basketter et al. 2007)
4-Phenylenediamine	AOO	0.01 0.025 0.05 0.1 0.25	0.9 1.5 1.3 1.9 7.1	0.13	33	Y[115]	CBA/Ca	RCC Basel, Itingen, Switzerland	(White et al. 2006)
4-Phenylenediamine	AOO	NA	NA	0.14	35	NA	NA	NA	(Basketter et al. 2007)

[115] Protocol used both sexes of mice.

ED_006472_00084346-00265

Chemical Name	LLNA Vehicle	Conc. (%)	SI	EC3 (%)	EC3 (µg/cm²)	Nonstd. LLNA Protocol	Mouse Strain	Mouse Source	LLNA Reference
4-Phenylenediamine	AOO	0.05 0.1 0.25 0.5 1	1.59 2.62 5.64 9.51 9.44	0.15	38	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Warbrick et al. 1999b)
4-Phenylenediamine	AOO	0.05 0.1 0.25 0.5 1	1.9 2.3 4 5.7 6.6	0.16	40	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Gerberick et al. 2005) [EC3]; (Warbrick et al. 1999b) [Dose-response data]
4-Phenylenediamine	AOO	NA	NA	0.18	45	NA	NA	NA	(Basketter et al. 2007)
4-Phenylenediamine	AOO	0.05 0.1 0.25 0.5 1	1.1 2.2 3.5 7.6 4.6	0.20	50	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Warbrick et al. 1999b)
4-Phenylenediamine	AOO	2.5 5 10	21 26 75.3	0.2	52	Y[116]	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Kimber et al. 1991)
4-Phenylenediamine	AOO	2.5 5 10	12.8 16.5 23.3	0.4	100	Y[117]	CBA/Ca	Animal Breeding Unit, Unilever Environmental Safety Laboratory	(Kimber et al. 1991); (Basketter and Scholes 1992)
4-Phenylenediamine	AOO	2.5 5	6.5 23.7	2.2	543	Y[118]	CBA/Ca	Animal Breeding Unit, Alderley Park, UK	(Kimber et al. 1991)
4-Phenylenediamine	NA	NA	NA	0.29	73	NA	NA	NA	(Estrada et al. 2003)

[116] Protocol did not specify sex, and the test duration was 4 days.
[117] Protocol did not specify sex, and the test duration was 4 days.
[118] Protocol did not specify sex, and the test duration was 4 days.

ED_006472_00084346-00266

Chemical Name	LLNA Vehicle	Conc. (%)	SI	EC3 (%)	EC3 (µg/cm²)	Nonstd. LLNA Protocol	Mouse Strain	Mouse Source	LLNA Reference
Phenylpropionaldehyde	AOO	NA	NA	6.3	1575	NA	NA	NA	(Schneider and Akkan 2004)
Phthalic anhydride	AOO	NA	NA	0.36	90	NA	NA	NA	(Basketter and Kimber 2006)
Potassium dichromate	DMF	0.025 0.05 0.1 0.25 0.5	2.9 4.3 9.1 15.1 22.6	0.33	83	N	CBA/J	Jackson Laboratories, Bar Harbor, ME	(Ryan et al. 2002)
Potassium dichromate	DMSO	0.25 0.5	8.8 10.1	0.01	2.8	Y[119]	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Kimber et al. 1991)
Potassium dichromate	DMSO	0.1 0.25 0.5	3.5 10.2 10.4	0.03	7.5	Y[120]	CBA/Ca	NA	(Basketter and Scholes 1992)
Potassium dichromate	DMSO	0.025 0.05 0.1 0.25 0.5	1.4 2.5 9.5 25.9 10.1	0.05	13	N	CBA/J	Jackson Laboratories, Bar Harbor, ME	(Ryan et al. 2002)
Potassium dichromate	DMSO	0.025 0.05 0.1 0.25 0.5	1.7 2.9 4.5 10.4 19.1	0.058	15	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Kimber et al. 1995)
Potassium dichromate	DMSO	0.1 0.25 0.5	7.9 22.6 33.6	0.07	18	Y[121]	CBA/Ca	Animal Breeding Unit, Alderley Park, UK	(Kimber et al. 1991)

[119] Protocol did not specify sex, and the test duration was 4 days.
[120] Protocol used both sexes, and the test duration was 4 or 5 days.
[121] Protocol did not specify sex, and the test duration was 4 days.

ED_006472_00084346-00267

Chemical Name	LLNA Vehicle	Conc. (%)	SI	EC3 (%)	EC3 (µg/cm²)	Nonstd. LLNA Protocol	Mouse Strain	Mouse Source	LLNA Reference
Potassium dichromate	DMSO	0.025 0.05 0.1 0.25 0.5	1.6 1.4 3.8 5.3 16.1	0.08	20	Y[122]	CBA/J	Harlan Sprague Dawley Inc., Indianapolis, IN	(Gerberick et al. 2005) [EC3] ; (Kimber et al. 1995) [Dose-response data]
Potassium dichromate	DMSO	0.025 0.05 0.1 0.25 0.5	1.9 1.7 2.2 5.9 13	0.122	31	N	CBA/J	Harlan Sprague Dawley Inc., Indianapolis, IN	(Kimber et al. 1995)
Potassium dichromate	DMSO	0.025 0.05 0.1 0.25 0.5	1.2 2.1 3.4 4.5 11.2	0.132	33	Y[123]	CBA/J	Harlan Sprague Dawley Inc., Indianapolis, IN	(Kimber et al. 1995)
Potassium dichromate	DMSO	0.025 0.05 0.1 0.25 0.5	1.1 1.3 2.3 5.1 13.1	0.15	38	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Kimber et al. 1995)
Potassium dichromate	DMSO	0.1 0.25 0.5	1.8 5.1 6.9	0.15	39	Y[124]	CBA/Ca	Animal Breeding Unit, Unilever Environmental Safety Laboratory	(Kimber et al. 1991)
Potassium dichromate	DMSO	0.1 0.25 0.5	2.0 4.1 5.4	0.17	43	Y[125]	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Kimber et al. 1991)

[122] LLNA protocol modifications included daily treatment for 4, rather than 3, consecutive days and injection of ^{3}H-methyl thymidine on the fifth day.
[123] LLNA protocol modifications included daily treatment for 4, rather than 3, consecutive days and injection of ^{3}H-methyl thymidine on the fifth day.
[124] Protocol did not specify sex, and the test duration was 4 days.
[125] Protocol did not specify sex, and the test duration was 4 days.

ED_006472_00084346-00268

Chemical Name	LLNA Vehicle	Conc. (%)	SI	EC3 (%)	EC3 (µg/cm²)	Nonstd. LLNA Protocol	Mouse Strain	Mouse Source	LLNA Reference
Potassium dichromate	DMSO	0.5 1 2.5 5	2.12 3.07 4.01 3.8	0.96	240	Y[126]	CBA/N	Japan SLC Inc., Shizuoka, Japan	(Ikarashi et al. 1992)
Potassium dichromate	NA	NA	NA	0.1	25	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Basketter et al. 1999c)
Potassium dichromate	Pluronic L92	0.02 0.1 0.5	2.4 2.9 7.9	0.11	28	N	CBA/J	Jackson Laboratories, Bar Harbor, ME	(ECPA 2006c)
Potassium dichromate	Pluronic L92	0.025 0.05 0.1 0.25 0.5	1.1 1.1 1.4 4.9 5.4	0.17	42	N	CBA/J	Jackson Laboratories, Bar Harbor, ME	(Ryan et al. 2002)
Potassium dichromate	Pluronic L92	0.02 0.1 0.5	1.4 1.8 7.8	0.18	45	N	CBA/JHsd	NA	(ECPA 2007j)
Potassium dichromate	Pluronic L92	0.02 0.1 0.5	1.06 1.04 5.55	0.3	75	N	CBA/J	R. Janvier, Le Genest St Isle, France	(ECPA 2007i)
Potassium dichromate	Pluronic L92	0.02 0.1 0.5	1.7 1.5 4.1	0.33	83	N	CBA/CaHs dRCC (SPF)	NA	(ECPA 2006d)
Produkt P-4G	AOO	1 3 9 15	2.4 2.5 1.9 2.5	NC	NC	N	CBA	NA	(Haist et al. 2007)
Propylene glycol	Water	50.0 100.0	1.2 1.6	NC	NC	N	CBA	NA	(Basketter 1998)

[126] Test was terminated 24 hours after the last topical exposure.

ED_006472_00084346-00269

Chemical Name	LLNA Vehicle	Conc. (%)	SI	EC3 (%)	EC3 (µg/cm²)	Nonstd. LLNA Protocol	Mouse Strain	Mouse Source	LLNA Reference
Propyl gallate	AOO	5 10 25	22.3 18.3 33.6	0.32	80	Y[127]	CBA/Ca	NA	(Basketter and Scholes 1992)
Propylidene phthalate	AOO	5 10.0 25	4.9 9.1 15.1	3.7	925	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Ryan et al. 2000)
Propylparaben	AOO	5 10.0 25	1.4 1.6 1.3	NC	NC	Y[128]	CBA/Ca	NA	(Basketter and Scholes 1992)
Pyridine	AOO	25 50 100	1.1 2.3 3.9	71.9	17975	NA	NA	NA	(Basketter et al. 1996)
Quinoxyfen/cyproconazole	Pluronic L92	7 33 100	2.09 10.66 20.3	9.8	2440	N	CBA/Ca	NA	(ECPA 2007g)
Quinoxyfen/cyproconazole	Pluronic L92	7 33 100	1.2 7.2 12.4	14.8	3700	N	CBA/J	R. Janvier, Le Genest St Isle, France	(ECPA 2007i)
Quinoxyfen/cyproconazole	Pluronic L92	12.5 25 50 75 100	2 2.3 8.6 15.8 30.1	27.8	6944	N	CBA/J	Jackson Laboratories, Bar Harbor, ME	(ECPA 2006c)
Quinoxyfen/cyproconazole	Pluronic L92	7 33 100	0.4 3.8 2.0	26.9	6721	N	CBA/J	Jackson Laboratories, Bar Harbor, ME	(ECPA 2006c)

[127] Protocol used both sexes, and the test duration was 4 or 5 days.
[128] Protocol used both sexes, and the test duration was 4 or 5 days.

ED_006472_00084346-00270

Chemical Name	LLNA Vehicle	Conc. (%)	SI	EC3 (%)	EC3 (µg/cm^2)	Nonstd. LLNA Protocol	Mouse Strain	Mouse Source	LLNA Reference
Quinoxyfen/cyproconazole	Pluronic L92	7 33 100	1.35 1.95 6.2	49.8	12438	N	CBA/JHsd	NA	(ECPA 2007g)
Quinoxyfen/cyproconazole	Pluronic L92	7 33 100	1.3 6.5 13.6	15.5	3875	N	CBA/CaOla Hsd	NA	(ECPA 2007g)
Quinoxyfen SC	Pluronic L92	7 33 100	1.1 1.7 0.8	NC	NC	N	CBA/J	Jackson Laboratories, Bar Harbor, ME	(ECPA 2006c)
Resorcinol	AOO	1 2.5 5 10 25	1.8 2.3 2.6 6.3 10.1	5.5	1385	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Basketter et al. 2007)
Resorcinol	AOO	1 5 10 25 50	0.7 2.2 5.2 8.4 10.4	6.3	1583	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Basketter et al. 2007)
Resorcinol	AOO	0.1 0.25 0.5 1 2.5	0.4 0.2 0.5 0.8 1	NC	NC	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Basketter et al. 2007)
Resorcinol	DMF	5 10 25	2.2 2.2 2.7	NC	NC	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Basketter et al. 1994)

ED_006472_00084346-00271

Chemical Name	LLNA Vehicle	Conc. (%)	SI	EC3 (%)	EC3 (µg/cm²)	Nonstd. LLNA Protocol	Mouse Strain	Mouse Source	LLNA Reference
Salicylic acid	ACE	1 10 20	0.9 1.8 7.2	12.2	3056	Y[129]	CBA/J	Jackson Laboratories, Bar Harbor, ME	(Gerberick et al. 1992)
Salicylic acid	AOO	5 10.0 25	0.8 1.5 2.5	NC	NC	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Basketter et al. 1994)
Sodium lauryl sulfate	DMF	1 2.5 5 10 20	2.7 4.2 4.6 8.9 8.6	1.5	375	Y[130]	CBA/JHsd	Harlan Sprague Dawley Inc., Frederick, MD	(Loveless et al. 1996)
Sodium lauryl sulfate	DMF	4 10 25	4.1 5.1 6.7	1.7	435	N	CBA/Ca	B&K Universal, Sollentuna, Sweden	(Montelius et al. 1994)
Sodium lauryl sulfate	DMF	5 10 25	4 5.1 7.6	2.7	665	N	CBA/Ca	B&K Universal, Sollentuna, Sweden	(Montelius et al. 1994)
Sodium lauryl sulfate	DMF	1 2.5 5 10 20	1.2 1.7 4.3 5.4 8	4.0	1000	N	CBA/JHsd	Harlan Sprague Dawley Inc., Frederick, MD	(Loveless et al. 1996)
Sodium lauryl sulfate	Pluronic L92	5 10 25	3.05 4.78 8.46	4.9	1225	N	CBA/CaOla Hsd	Harlan Winkelmann GmbH, D-33178 Borchen	(Gamer et al. 2008)

[129] LLNA protocol modifications included daily treatment for 4, rather than 3, consecutive days and injection of ^{3}H-methyl thymidine on the fifth day.
[130] LLNA protocol modifications included daily treatment for 4, rather than 3, consecutive days and injection of ^{3}H-methyl thymidine on the fifth day.

ED_006472_00084346-00272

Chemical Name	LLNA Vehicle	Conc. (%)	SI	EC3 (%)	EC3 (µg/cm²)	Nonstd. LLNA Protocol	Mouse Strain	Mouse Source	LLNA Reference
Sodium lauryl sulfate	DMF	1 2.5 5 10 20	1.5 2.3 3.8 4.1 5.3	4.4	1100	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Loveless et al. 1996)
Sodium lauryl sulfate	DMF	1 2.5 5 10 20	0.9 1.1 1.7 2.6 3.5	13.4	3350	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Loveless et al. 1996)
Sodium lauryl sulfate	DMF	1 2.5 5 10 20	1.6 2.1 2.8 1.6 3.6	17.1	4275	Y[131]	CBA/JHsd	Harlan Sprague Dawley Inc., Frederick, MD	(Loveless et al. 1996)
Sodium lauryl sulfate	DMSO	5 10 25	3.5 4 4.2	2.5	625	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Basketter et al. 1994)
Sodium lauryl sulfate	DMSO	5 10 25	3.2 4 4.2	3.1	773	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Gerberick et al. 2005) [EC3] (Basketter et al. 1996) [Dose-response data]
Spearmint oil	EtOH/DEP (1:3)	0.5 1 2.5 5 10	1.2 1.1 1.2 1.9 3.6	8.2	2050	N	CBA/Ca	Harlan Interfauna UK, Shaw's Farm, Blackthorne, Bicester, Oxon, UK	(Lalko and Api 2006)

[131] LLNA protocol modifications included daily treatment for 4, rather than 3, consecutive days and injection of ^{3}H-methyl thymidine on the fifth day.

ED_006472_00084346-00273

Chemical Name	LLNA Vehicle	Conc. (%)	SI	EC3 (%)	EC3 (µg/cm²)	Nonstd. LLNA Protocol	Mouse Strain	Mouse Source	LLNA Reference
Squalene	AOO	10 25 50	3.8 6.9 8.2	7.9	1975	N	CBA/CaOla Hsd	Harlan Winkelmann GmbH, D-33178 Borchen	(EFfCI 2006)
Streptomycin	DMF	2.5 5 10.0 25 50	1.4 1.6 2.1 2.9 3.2	33	8250	Y[132]	CBA/Ca	Harlan Seralab, Bicester, Oxfordshire, UK	(Kimber et al. 1998)
Streptomycin	DMF	2.5 5 10.0 25 50	1.2 1.4 1.3 2 1.9	NC	NC	N	CBA/JHsd	Harlan Sprague-Dawley, Indianapolis, IN or Jackson Labs, Bar Harbor, ME	(Kimber et al. 1998)
Streptomycin	DMF	2.5 5.0 10.0 25.0 50.0	1.3 1.2 1 1.2 1.3	NC	NC	Y[133]	CBA/Ca	Harlan Seralab, Bicester, Oxfordshire, UK	(Kimber et al. 1998)
Streptomycin	DMF	2.5 5.0 10.0 25.0 50.0	1.7 0.8 0.6 1.1 1.2	NC	NC	N	CBA/JHsd	Harlan Sprague-Dawley, Indianapolis, IN or Jackson Labs, Bar Harbor, ME	(Kimber et al. 1998)
Streptomycin	DMF	2.5 5.0 10.0 25.0 50.0	1 0.8 0.9 1.1 1.3	NC	NC	N	CBA/JHsd	Harlan Sprague-Dawley, Indianapolis, IN or Jackson Labs, Bar Harbor, ME	(Kimber et al. 1998)

[132] The LLNA protocol used both sexes of mice.
[133] The LLNA protocol used both sexes of mice.

ED_006472_00084346-00274

Chemical Name	LLNA Vehicle	Conc. (%)	SI	EC3 (%)	EC3 (μg/cm²)	Nonstd. LLNA Protocol	Mouse Strain	Mouse Source	LLNA Reference
Succinic acid	DMSO	5 10 25	1.2 1.2 1.3	NC	NC	N	CBA/CaOla Hsd	Harlan Winkelmann GmbH, D-33178 Borchen	(EFfCI 2006)
Sulfanilamide	DMF	10.0 25.0 50.0	1.0 1 0.9	NC	NC	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Basketter et al. 1994)
Sulfanilic acid	DMF	5 10 25	1.5 1.9 2.2	NC	NC	Y[134]	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Basketter et al. 1992)
Sulfanilic acid	DMF	5 10 25	1.1 1.2 1.3	NC	NC	Y[135]	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Basketter et al. 1992)
Sulfanilic acid	DMF	5 10 25	1.9 1.2 1.8	NC	NC	Y[136]	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Basketter et al. 1992)
Sulfanilic acid	DMSO	2.5 5 10	1.3 1.3 1.5	NC	NC	Y[137]	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Basketter et al. 1992)
Tartaric acid	DMF	5 10 25	1 0.9 1.5	NC	NC	N	NA	NA	(Gerberick et al. 2005)
Tea leaf absolute	DMF	NA	NA	NC	NC	N	NA	NA	(RIFM 2007)
Tetrachlorosalicylanilide	ACE	0.25 0.5 1	11.2 14.4 18	0.04	10	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Basketter et al. 1994)

[134] Protocol used both sexes.
[135] Protocol used both sexes.
[136] Protocol used both sexes.
[137] Protocol used both sexes, and the test duration was 4 days.

ED_006472_00084346-00275

Chemical Name	LLNA Vehicle	Conc. (%)	SI	EC3 (%)	EC3 (µg/cm²)	Nonstd. LLNA Protocol	Mouse Strain	Mouse Source	LLNA Reference
Tetrachlorosalicylanilide	ACE	0.1 0.25 0.5	16 27.8 40.5	0.03	7.8	N	CBA/Ca	Harlan Olac Ltd.	(Scholes et al. 1991)
Tetramethylthiruamdisulphide	AOO	2.5 5 10.0	2.4 2.9 5.1	5.2	1300	NA	NA	NA	(Basketter et al. 1996)
Tetramethylthiruamdisulphide	AOO	NA	NA	6.0	1500	NA	NA	NA	(Basketter and Kimber 2001)
Thioglycerol	DMF	10 25 50	6.7 10 10	3.6	895	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Basketter et al. 1994)
Toluene 2,4-diisocyanate	AOO	NA	NA	0.1	28	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Basketter et al. 2003b)
Treemoss	EtOH/DEP (1:3)	NA	NA	NC	NC	N	NA	NA	(RIFM 2007)
Trifluralin EC	Pluronic L92	7 33 100	5.96 30.04 75.24	5.8	1446	N	CBA/Ca	NA	(ECPA 2007c)
Trifluralin EC	Pluronic L92	7 33 100	1.9 8.7 25.7	11.2	2801	N	CBA/J	R. Janvier, Le Genest St Isle, France	(ECPA 2007i)
Trifluralin EC	Pluronic L92	7 33 100	3.1 26.3 61.5	7.0	1738	N	CBA/J	Jackson Laboratories, Bar Harbor, ME	(ECPA 2006c)
Trifluralin EC	Pluronic L92	7 33 100	1.03 6.98 16.12	15.6	3902	N	CBA/JHsd	NA	(ECPA 2007g)
Trifluralin EC	Pluronic L92	7 33 100	1.8 8.2 20.5	11.9	2969	N	CBA/CaOla Hsd	NA	(ECPA 2007k)

ED_006472_00084346-00276

Chemical Name	LLNA Vehicle	Conc. (%)	SI	EC3 (%)	EC3 (µg/cm²)	Nonstd. LLNA Protocol	Mouse Strain	Mouse Source	LLNA Reference
Trimellitic anhydride	NA	NA	NA	0.22	55	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Basketter et al. 2003b)
Tween 80	AOO	NA	NA	NC	NC	NA	NA	NA	(Basketter et al. 2000)
Undec-10-enal	AOO	5.0 10.0 25.0 50.0 75.0	1.7 5.3 7.5 8.7 8.8	6.8	1700	NA	NA	NA	(Patlewicz et al. 2002)
Undecylenic acid	AOO	10 25 50	2.5 3.3 4.4	19.4	4844	N	CBA/CaOla Hsd	Harlan Winkelmann GmbH, D-33178 Borchen	(EFfCI 2006)
Vanillin	AOO	2.5 5 10.0 25 50	0.9 1.4 1.5 1.2 1.4	NC	NC	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Basketter et al. 2001)
Xylene	AOO	NA	NA	95.8	23950	NA	NA	NA	(Estrada et al. 2003)
YELLOW E-JD 3442	AOO	1 3 9 15	1 0.8 0.9 0.9	NC	NC	N	CBA	NA	(Haist et al. 2007)
Ylang Ylang	EtOH/DEP (1:3)	NA	NA	6.80	1700	N	NA	NA	(RIFM 2007)
Zinc sulfate	DMSO	5 10 25	1.3 2 2.3	NC	NC	N	CBA/Ca	Harlan Olac Ltd., Bicester, Oxon, UK	(Basketter et al. 1999b)

Abbreviations: ACE = acetone; AO Mix = antioxidant mixture of 0.3% butylated hydroxytoluene/tocopherol/eugenol (0.1% each); AOO = acetone: olive oil (1:4 by volume); Conc. = concentration; DEP = diethylphthalate; DMF = dimethylformamide; DMSO = dimethylsulfoxide; EtOH = ethanol; EC3 = estimated concentration of a substance expected to produce a stimulation index of 3, the threshold value for a substance to be considered a sensitizer in the LLNA; LLNA = murine local lymph node assay; MEK = methyl ethyl ketone; N = no; NA = not available; NC = not calculated because SI < 3; Nonstd. = nonstandard; Pet. = petrolatum; PG = propylene glycol; SI = stimulation index; Toc = tocopherol; TrlC = Trolox C; UK = United Kingdom; Y = yes.

ED_006472_00084346-00277

References

Akkan Z, Kalberlah F, Oltmans J, Schneider K. 2003. Beurteilung der Wirkstärke hautsensibilisierender Chemikalien anhand des Local Lymph Node Assay (LLNA). Dortmund, Germany: Auftrag der Bundesanstalt für Arbeitsschutz und Arbeitsmedizin.

Ashby J, Basketter DA, Paton D, Kimber I. 1995. Structure activity relationships in skin sensitization using the murine local lymph node assay. Toxicology 103(3): 177-194.

Basketter D, Scholes EW, Kimber I, Botham PA, Hilton J, Miller K, et al. 1991. Interlaboratory evaluation of the local lymph node assay with 25 chemicals and comparison with guinea pig test data. Toxicology Methods 1(1): 30-43.

Basketter DA. 1998. Skin sensitization: risk assessment. Int J Cosmet Sci 20(3): 141-150.

Basketter DA, Blaikie L, Dearman RJ, Kimber I, Ryan CA, Gerberick GF, et al. 2000. Use of the local lymph node assay for the estimation of relative contact allergenic potency. Contact Dermatitis 42(6): 344-348.

Basketter DA, Cadby P. 2004. Reproducible prediction of contact allergenic potency using the local lymph node assay. Contact Dermatitis 50(1): 15-17.

Basketter DA, Clapp C, Jefferies D, Safford B, Ryan CA, Gerberick F, et al. 2005. Predictive identification of human skin sensitization thresholds. Contact Dermatitis 53(5): 260-267.

Basketter DA, Dearman RJ, Hilton J, Kimber I. 1997. Dinitrohalobenzenes: evaluation of relative skin sensitization potential using the local lymph node assay. Contact Dermatitis 36(2): 97-100.

Basketter DA, Evans P, Gerberick GF, Kimber I. 2002. Factors affecting thresholds in allergic contact dermatitis: safety and regulatory considerations. Contact Dermatitis 47(1): 1-6.

Basketter DA, Gerberick F, Kimber I. 2007. The local lymph node assay and the assessment of relative potency: status of validation. Contact Dermatitis 57(2): 70-75.

Basketter DA, Gerberick GF, Kimber I, Loveless SE. 1996. The local lymph node assay: a viable alternative to currently accepted skin sensitization tests. Food Chem Toxicol 34(10): 985-997.

Basketter DA, Gilmour NJ, Wright ZM, Walters T, Boman A, Liden C. 2003a. Biocides: characterization of the allergenic hazard of methylisothiazolinone. J Toxicol Cutaneous Ocul Toxicol 22(4): 187-199.

Basketter DA, Kimber I. 2001. Predictive testing in contact allergy: facts and future. Allergy 56(10): 937-943.

Basketter DA, Kimber I. 2006. Predictive tests for irritants and allergens and their use in quantitative risk assessment. In: Contact Dermatitis (Frosch P, Menné T, Lepoittevin J-P, eds). Heidelberg:Springer Verlag, 179-188.

ED_006472_00084346-00278

Basketter DA, Lea LJ, Cooper K, Stocks J, Dickens A, Pate I, et al. 1999a. Threshold for classification as a skin sensitizer in the local lymph node assay: a statistical evaluation. Food Chem Toxicol 37(12): 1167-1174.

Basketter DA, Lea LJ, Cooper KJ, Ryan CA, Gerberick GF, Dearman RJ, et al. 1999b. Identification of metal allergens in the local lymph node assay. Am J Contact Dermat 10(4): 207-212.

Basketter DA, Lea LJ, Dickens A, Briggs D, Pate I, Dearman RJ, et al. 1999c. A comparison of statistical approaches to the derivation of EC3 values from local lymph node assay dose responses. J Appl Toxicol 19(4): 261-266.

Basketter DA, Rodford R, Kimber I, Smith I, Wahlberg JE. 1999d. Skin sensitization risk assessment: a comparative evaluation of 3 isothiazolinone biocides. Contact Dermatitis 40(3): 150-154.

Basketter DA, Scholes EW. 1992. Comparison of the local lymph node assay with the guinea-pig maximization test for the detection of a range of contact allergens. Food Chem Toxicol 30(1): 65-69.

Basketter DA, Scholes EW, Cumberbatch M, Evans CD, Kimber I. 1992. Sulphanilic acid: divergent results in the guinea pig maximization test and the local lymph node assay. Contact Dermatitis 27(4): 209-213.

Basketter DA, Scholes EW, Kimber I. 1994. The performance of the local lymph node assay with chemicals identified as contact allergens in the human maximization test. Food Chem Toxicol 32(6): 543-547.

Basketter DA, Scholes EW, Wahlkvist H, Montelius J. 1995. An evaluation of the suitability of benzocaine as a positive control skin sensitizer. Contact Dermatitis 33(1): 28-32.

Basketter DA, Selbie E, Scholes EW, Lees D, Kimber I, Botham PA. 1993. Results with OECD recommended positive control sensitizers in the maximization, Buehler and local lymph node assays. Food Chem Toxicol 31(1): 63-67.

Basketter DA, Smith Pease CK, Patlewicz GY. 2003b. Contact allergy: the local lymph node assay for the prediction of hazard and risk. Clin Exp Dermatol 28(2): 218-221.

Basketter DA, Wright ZM, Warbrick EV, Dearman RJ, Kimber I, Ryan CA, et al. 2001. Human potency predictions for aldehydes using the local lymph node assay. Contact Dermatitis 45(2): 89-94.

Bertrand F, Basketter DA, Roberts DW, Lepoittevin JP. 1997. Skin sensitization to eugenol and isoeugenol in mice: possible metabolic pathways involving ortho-quinone and quinone methide intermediates. Chem Res Toxicol 10(3): 335-343.

Betts CJ, Dearman RJ, Heylings JR, Kimber I, Basketter DA. 2006. Skin sensitization potency of methyl methacrylate in the local lymph node assay: comparisons with guinea-pig data and human experience. Contact Dermatitis 55(3): 140-147.

Botham PA, Hilton J, Evans CD, Lees D, Hall TJ. 1991. Assessment of the relative skin sensitizing potency of 3 biocides using the murine local lymph node assay. Contact Dermatitis 25(3): 172-177.

ED_006472_00084346-00279

Dearman RJ, Betts CJ, Farr C, McLaughlin J, Berdasco N, Wiench K, et al. 2007. Comparative analysis of skin sensitization potency of acrylates (methyl acrylate, ethyl acrylate, butyl acrylate, and ethylhexyl acrylate) using the local lymph node assay. Contact Dermatitis 57(4): 242-247.

Dearman RJ, Wright ZM, Basketter DA, Ryan CA, Gerberick GF, Kimber I. 2001. The suitability of hexyl cinnamic aldehyde as a calibrant for the murine local lymph node assay. Contact Dermatitis 44(6): 357-361.

Debruyne E. 2007. Bayer Crop Science data submission to NICEATM.

ECPA. 2006a. Dinocap Emulsion Concentrate: Local Lymph Node Assay (LLNA) in Mice (Identification of Contact Allergens). RCC Study No. A85105. Report by CropLife International.

ECPA. 2006b. Formaldehyde Solution (36.5-38 % in H2O): Local Lymph Node Assay (LLNA) in Mice (Identification of Contact Allergens). RCC Study No. A83687. Report by CropLife International.

ECPA. 2006c. Oxyfluorfen EC, Atrazine SC, Quinoxyfen/Cyproconazole SC, Dinocap EC, Quinoxyfen SC and Trifluralin EC: Local Lymph Node Assay in CBA/J Mice. Report by Dow Chemical Company.

ECPA. 2006d. Potassium Dichromate: Local Lymph Node Assay (LLNA) in Mice (Identification of Contact Allergens). RCC Study Number A83665. Report by CropLife International.

ECPA. 2007a. Alpha-hexylcinnamaldehyde, 95+%: Murine Local Lymph Node Assay (LLNA) Positive Control Study. Project No. 58H0508/062114. Report by BASF.

ECPA. 2007b. Hexyl Cinnamaldehyde (HCA), Potassium Dichromate (PDC) and Formaldehyde (FRM) Evaluation of Potential Dermal Sensitization in the Local Lymph Node Assay in the Mouse. Study SA 06231. Report by Bayer.

ECPA. 2007c. Murine Local Lymph Node Assay: Summary Report. Trifluralin Emulsion Concentrate. Project No. 58H0513/062137. Report by BASF.

ECPA. 2007d. Murine Local Lymph Node Assay. Positive Control Study. Summary Report. Project No. 58H0509/062113. Report by BASF.

ECPA. 2007e. Murine Local Lymph Node Assay. Summary Report. Project No. 58H0511/062122. Report by BASF.

ECPA. 2007f. Murine Local Lymph Node Assay. Summary Report. Project No. 58H0512/062140. Report by BASF.

ECPA. 2007g. Oxyfluorfen Emulsion Concentrate, Quinoxyfen/Cyproconazole Suspension Concentrate, Dinocap Emulsion Concentrate, and Trifluralin Emulsion Concentrate: Local Lymph Node Assay (LLNA) in Mice. Study No. 20234. Study sponsor: CropLife International/ECPA; report by DuPont.

ECPA. 2007h. Oxyfluorfen Emulsion Concentrate: Local Lymph Node Assay (LLNA) in Mice (Identification of Contact Allergens). RCC Study Number A85094. Report by CropLife International.

ED_006472_00084346-00280

ECPA. 2007i. Oxyfluorfen, Quinoxyfen/Cyproconazole, Dinocap, Trifluralin Evaluation of Potential Dermal Sensitization in the Local Lymph Node Assay in the Mouse. Report by Bayer.

ECPA. 2007j. Pluronic L92® Surfactant: Local Lymph Node Assay (LLNA) in Mice. Study No. 20233. Study sponsor: CropLife International/ECPA; report by DuPont.

ECPA. 2007k. Trifluralin Emulsion Concentrate: Local Lymph Node Assay (LLNA) in Mice (Identification of Contact Allergens). RCC Study Number A85127. Report by CropLife International.

EFfCI. 2006. Comparative Experimental Study on the Skin Sensitising Potential of Selected Unsaturated Chemicals as Asssessed by the Murine Local Lymph Node Assay (LLNA) and the Guinea Pig Maximisation Test (GPMT): European Federation for Cosmetic Ingredients.

Elahi EN, Wright Z, Hinselwood D, Hotchkiss SAM, Basketter DA, Pease CKS. 2004. Protein binding and metabolism influence the relative skin sensitization potential of cinnamic compounds. Chem Res Toxicol 17(3): 301-310.

Estrada E, Patlewicz G, Chamberlain M, Basketter D, Larbey S. 2003. Computer-aided knowledge generation for understanding skin sensitization mechanisms: the TOPS-MODE approach. Chem Res Toxicol 16(10): 1226-1235.

Gamer AO, Nies E, Vohr HW. 2008. Local lymph node assay (LLNA): comparison of different protocols by testing skin-sensitizing epoxy resin system components. Regul Toxicol Pharmacol 52(3): 290-298.

Gerberick GF, House RV, Fletcher ER, Ryan CA. 1992. Examination of the local lymph node assay for use in contact sensitization risk assessment. Fundam Appl Toxicol 19(3): 438-445.

Gerberick GF, Robinson MK, Ryan CA, Dearman RJ, Kimber I, Basketter DA, et al. 2001. Contact allergenic potency: correlation of human and local lymph node assay data. Am J Contact Dermat 12(3): 156-161.

Gerberick GF, Ryan CA, Kern PS, Dearman RJ, Kimber I, Patlewicz GY, et al. 2004. A chemical dataset for evaluation of alternative approaches to skin-sensitization testing. Contact Dermatitis 50(5): 274-288.

Gerberick GF, Ryan CA, Kern PS, Schlatter H, Dearman RJ, Kimber I, et al. 2005. Compilation of historical local lymph node data for evaluation of skin sensitization alternative methods. Dermatitis 16(4): 157-202.

Griem P, Goebel C, Scheffler H. 2003. Proposal for a risk assessment methodology for skin sensitization based on sensitization potency data. Regul Toxicol Pharmacol 38(3): 269-290.

Haist I, Brummer D, Albrecht A. 2007. Untersuchung von Textilfarbstoffen im Local Lymph Node Assay (LLNA). Forschung Projekt 1877. Dortmund/Berlin/Dresden.

Hilton J, Dearman RJ, Harvey P, Evans P, Basketter DA, Kimber I. 1998. Estimation of relative skin sensitizing potency using the local lymph node assay: a comparison of formaldehyde with glutaraldehyde. Am J Contact Dermat 9(1): 29-33.

ED_006472_00084346-00281

Ikarashi Y, Ohno K, Tsuchiya T, Nakamura A. 1992. Differences of draining lymph node cell proliferation among mice, rats and guinea pigs following exposure to metal allergens. Toxicology 76(3): 283-292.

Isola D, Lalko J. 2001. Vehicle effects in the murine local lymph node assay. Int J Toxicol 20(6): 401.

Kimber I, Basketter DA. 1997. Contact sensitization: a new approach to risk assessment. Hum Ecol Risk Assess 3(3): 385-395.

Kimber I, Hilton J, Botham PA, Basketter DA, Scholes EW, Miller K, et al. 1991. The murine local lymph node assay: results of an inter-laboratory trial. Toxicol Lett 55(2): 203-213.

Kimber I, Hilton J, Dearman RJ, Gerberick GF, Ryan CA, Basketter DA, et al. 1998. Assessment of the skin sensitization potential of topical medicaments using the local lymph node assay: an interlaboratory evaluation. J Toxicol Environ Health A 53(7): 563-579.

Kimber I, Hilton J, Dearman RJ, Gerberick GF, Ryan CA, Basketter DA, et al. 1995. An international evaluation of the murine local lymph node assay and comparison of modified procedures. Toxicology 103(1): 63-73.

Kimber I, Hilton J, Weisenberger C. 1989. The murine local lymph node assay for identification of contact allergens: a preliminary evaluation of in situ measurement of lymphocyte proliferation. Contact Dermatitis 21(4): 215-220.

Kimber I, Weisenberger C. 1991. Anamnestic responses to contact allergens: application in the murine local lymph node assay. J Appl Toxicol 11(2): 129-133.

Lalko J, Api AM. 2006. Investigation of the dermal sensitization potential of various essential oils in the local lymph node assay. Food Chem Toxicol 44(5): 739-746.

Lalko J, Isola D, Api AM. 2004. Ethanol and diethyl phthalate: vehicle effects in the local lymph node assay. Int J Toxicol 23(3): 171-177.

Lea LJ, Warbrick EV, Dearman RJ, Kimber I, Basketter DA. 1999. The impact of vehicle on assessment of relative skin sensitization potency of 1,4-dihydroquinone in the local lymph node assay. Am J Contact Dermat 10(4): 213-218.

Loveless SE, Ladics GS, Gerberick GF, Ryan CA, Basketter DA, Scholes EW, et al. 1996. Further evaluation of the local lymph node assay in the final phase of an international collaborative trial. Toxicology 108(1-2): 141-152.

Mandervelt C, Clottens FL, Demedts M, Nemery B. 1997. Assessment of the sensitization potential of five metal salts in the murine local lymph node assay. Toxicology 120(1): 65-73.

Montelius J, Wahlkvist H, Boman A, Fernström P, Gråbergs L, Wahlberg JE. 1994. Experience with the murine local lymph node assay: inability to discriminate between allergens and irritants. Acta Derm Venereol 74(1): 22-27.

Nilsson AM, Bergström MA, Luthman K, Nilsson JLG, Karlberg AT. 2005. A conjugated diene identified as a prohapten: contact allergenic activity and chemical reactivity of proposed epoxide metabolites. Chem Res Toxicol 18(2): 308-316.

ED_006472_00084346-00282

NTP. 1994. Assessment of Contact Hypersensitivity to Atrazine in Female B6C3F1 Mice. Research Triangle Park, NC:National Institute of Environmental Health Sciences.

NTP. 1997a. Assessment of Contact Hypersensitivity to Dinitrochlorobenzene, Potassium Dichromate and Methyl Salicylate in BALB16 Female Mice. Research Triangle Park, NC:National Institute of Environmental Health Sciences.

NTP. 1997b. Assessment of Contact Hypersensitivity to Pentaerythritol Triacrylate in BALB16 Female Mice. Research Triangle Park, NC:National Institute of Environmental Health Sciences.

Patlewicz G, Basketter DA, Smith CK, Hotchkiss SAM, Roberts DW. 2001. Skin-sensitization structure-activity relationships for aldehydes. Contact Dermatitis 44(6): 331-336.

Patlewicz GY, Wright ZM, Basketter DA, Pease CK, Lepoittevin JP, Arnau EG. 2002. Structure-activity relationships for selected fragrance allergens. Contact Dermatitis 47(4): 219-226.

RIFM. 2007. Data submission to NICEATM. (NICEATM, ed). Woodcliff Lake, NJ.

Roberts DW, Patlewicz G, Kern PS, Gerberick F, Kimber I, Dearman RJ, et al. 2007. Mechanistic applicability domain classification of a local lymph node assay dataset for skin sensitization. Chem Res Toxicol 20(7): 1019-1030.

Ryan CA, Cruse LW, Skinner RA, Dearman RJ, Kimber I, Gerberick GF. 2002. Examination of a vehicle for use with water soluble materials in the murine local lymph node assay. Food Chem Toxicol 40(11): 1719-1725.

Ryan CA, Gerberick GF, Cruse LW, Basketter DA, Lea L, Blaikie L, et al. 2000. Activity of human contact allergens in the murine local lymph node assay. Contact Dermatitis 43(2): 95-102.

SCCP. 2007. Memorandum on hair dye substances and their skin sensitizing properties. Brussels, Belgium:European Commission. Available: http://ec.europa.eu/health/ph_risk/committees/04_sccp/docs/sccp_s_05.pdf [accessed 17 December 2010].

Schneider K, Akkan Z. 2004. Quantitative relationship between the local lymph node assay and human skin sensitization assays. Regul Toxicol Pharmacol 39(3): 245-255.

Scholes EW, Basketter DA, Lovell WW, Sarll AE, Pendington RU. 1991. The identification of photoallergic potential in the local lymph node assay. Photodermatol Photoimmunol Photomed 8(6): 249-254.

Scholes EW, Basketter DA, Sarll AE, Kimber I, Evans CD, Miller K, et al. 1992. The local lymph node assay: results of a final inter-laboratory validation under field conditions. J Appl Toxicol 12(3): 217-222.

Smith CK, Hotchkiss SAM. 2001. Allergic Contact Dermatitis. Chemical and Metabolic Mechanisms. London and New York:Taylor and Francis.

TNO. 2006. Limitations of the local lymph node assay (LLNA) as preferred test for skin sensitization: concerns about false positive and false negative test results. V 7217. Zeist, The Netherlands:CESIO (European Committee of Organic Surfactants and their Intermediates).

ED_006472_00084346-00283

Van Och FMM, Slob W, De Jong WH, Vandebriel RJ, Van Loveren H. 2000. A quantitative method for assessing the sensitizing potency of low molecular weight chemicals using a local lymph node assay: employment of a regression method that includes determination of the uncertainty margins. Toxicology 146(1): 49-59.

Vocanson M, Goujon C, Chabeau G, Castelain M, Valeyrie M, Floc'h F, et al. 2006. The skin allergenic properties of chemicals may depend on contaminants - evidence from studies on coumarin. Int Arch Allergy Immunol 140(3): 231-238.

Warbrick EV, Dearman RJ, Ashby J, Schmezer P, Kimber I. 2001. Preliminary assessment of the skin sensitizing activity of selected rodent carcinogens using the local lymph node assay. Toxicology 163(1): 63-69.

Warbrick EV, Dearman RJ, Basketter DA, Kimber I. 1999a. Influence of application vehicle on skin sensitization to methylchloroisothiazolinone/methylisothiazolinone: an analysis using the local lymph node assay. Contact Dermatitis 41(6): 325-329.

Warbrick EV, Dearman RJ, Lea LJ, Basketter DA, Kimber I. 1999b. Local lymph node assay responses to paraphenylenediamine: intra- and inter-laboratory evaluations. J Appl Toxicol 19(4): 255-260.

White JML, Kullavanijaya P, Duangdeeden I, Zazzeroni R, Gilmour NJ, Basketter DA, et al. 2006. p-Phenylenediamine allergy: the role of Bandrowski's base. Clin Exp Allergy 36(10): 1289-1293.

Wright ZM, Basketter DA, Blaikie L, Cooper KJ, Warbrick EV, Dearman RJ, et al. 2001. Vehicle effects on skin sensitizing potency of four chemicals: assessment using the local lymph node assay. Int J Cosmet Sci 23(2): 75-83.

ED_006472_00084346-00284

Annex II-2

Human Data for LLNA Potency Evaluation

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Test Substance	N	Vehicle	Conc (%)	Test Vol (µL)	Skin Area (cm^2)	Incid (%)	HMT		HRIPT		DSA_{05} ($\mu g/cm^2$)	Human Result	Reference
							NOEL ($\mu g/cm^2$)	LOEL ($\mu g/cm^2$)	NOEL ($\mu g/cm^2$)	LOEL ($\mu g/cm^2$)			
Acetyl isovaleryl[1]	25	Petrolatum	5	1000	14.5	8	NA	3448	NA	NA	2155	+	(Opdyke 1982)
Acetyl isovaleryl	29	Petrolatum	5	1000	14.5	3.5	NA	3448	NA	NA	4926		(Opdyke 1982)
Aluminum chloride	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	–	(Basketter et al. 1999b)
p-Aminobenzoic acid	23	Petrolatum	25	1000	14.5	0	17242	NA	NA	NA	NA	–	(Kligman 1966a)
Amylcinnamic aldehyde	25	1:3 EtOH/DEP	4.5	300	4.9	0	NA	NA	23622[2]	NA	NA	–	(RIFM 2007)
alpha-Amylcinnamyl alcohol	78	EtOH	8	200	5	1.3	NA	NA	NA	3200	12308	+	(Marzulli and Maibach 1980)
alpha-Amylcinnamyl alcohol	25	Petrolatum	8	1000	14.5	0	5517	NA	NA	NA	NA		(Opdyke 1974)
alpha-Amylcinnamyl alcohol	25	1:3 EtOH/DEP	4.5	300	4.9	0	NA	NA	3543[3]	NA	NA		(RIFM 2007)
Aniline	25	Petrolatum	20	1000	14.5	28	NA	13793	NA	NA	2463	+	(Kligman 1966a)
Anisyl alcohol	25	Petrolatum	NR	NR	NR	0	3448[4]	NA	NA	NA	NA	–	(RIFM 2007)
Basil oil	25	Petrolatum	4	1000	14.5	0	2759	NA	NA	NA	NA	–	(Lalko and Api 2006)
Benzalkonium chloride	24	Petrolatum	25	1000	14.5	0	17241	NA	NA	NA	NA	–	(Kligman 1966a)
Benzalkonium chloride	186	Petrolatum	5, then 1	500 mg	5[5]	0	NA	NA	5000	NA	NA		(Marzulli and Maibach 1976)
Benzocaine	22	Petrolatum	5	1000	14.5	4.5	NA	3448	NA	NA	3831	+	(Kligman 1966b)
Benzocaine	99	Water	20	500 mg	5	6	NA	NA	NA	20000	16667		(Marzulli and Maibach 1974)
Benzocaine	173	Water	10	500 mg	5	1.2	NA	NA	NA	10000	41667		(Marzulli and Maibach 1974)
Benzocaine	23	Petrolatum	25	1000	14.5	21.7	NA	17241	NA	NA	3973		(Kligman 1966a)
Benzocaine	92	Water	2	500 mg	5	0	NA	NA	2000	NA	NA		(Marzulli and Maibach 1974)

[1] Test substance is referred to as 5-methyl-2,3-hexanedione in the reference.
[2] No sensitization was observed in human studies. Doses reported reflect the highest concentration tested, not necessarily the highest achievable NOEL.
[3] No sensitization was observed in human studies. Doses reported reflect the highest concentration tested, not necessarily the highest achievable NOEL.
[4] No sensitization was observed in human studies. Doses reported reflect the highest concentration tested, not necessarily the highest achievable NOEL.
[5] Concentrations tested were 5% for first four applications and then 1% for last six applications. NOEL is for 5%.

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Test Substance	N	Vehicle	Conc (%)	Test Vol (µL)	Skin Area (cm^2)	Incid (%)	HMT		HRIPT		DSA_{05} (µg/cm^2)	Human Result	Reference
							NOEL (µg/cm^2)	LOEL (µg/cm^2)	NOEL (µg/cm^2)	LOEL (µg/cm^2)			
Benzoic acid	NA	NA	NA	NA	NA	0	NA	NA	NA	NA	NA	–	(Gad et al. 1986)
Benzoisothiazolione	54	Aqueous	0.036	NR	NR	0	NA	NA	45	NA	NA	+	(Basketter et al. 1999c)
Benzoisothiazolione	58	Aqueous	0.0725	NR	NR	9	NA	NA	NA	90	50		(Basketter et al. 1999c)
Benzoyl peroxide	69	PEG and 1% sulfur	10	250 mg	3.88	36	NA	NA	NA	6443	895	+	(Poole et al. 1970)
Benzoyl peroxide	25	Gel	5	300 mg	1	84	NA	15000	NA	NA	893		(Leyden and Kligman 1977)
Benzoyl peroxide	25	Gel	5	300 mg	1	68	NA	15000	NA	NA	1103		(Leyden and Kligman 1977)
Benzoyl peroxide	25	Gel	10	300 mg	1	84	NA	30000	NA	NA	1786		(Leyden and Kligman 1977)
Benzoyl peroxide	25	Gel	10	300 mg	1	68	NA	30000	NA	NA	2206		(Leyden and Kligman 1977)
Benzyl alcohol	NA	EtOH or EtOH/DEP	NA	NA	NA	0	NA	NA	5906	NA	NA	+	(RIFM 2007)
Benzyl alcohol	NA	NA	NA	NA	NA	0	6897	NA	NA	NA	NA		(RIFM 2007)
Benzyl alcohol	110	EtOH or EtOH/DEP	NA	NA	NA	0.91	NA	NA	NA	8858	48670		(RIFM 2007)
Benzylbenzoate	NA	NA	NA	NA	NA	0	20690[6]	NA	NA	NA	NA	–	(RIFM 2007)
Benzylbenzoate	NA	EtOH or EtOH/DEP	NA	NA	NA	0	NA	NA	59050	NA	NA		(RIFM 2007)
Benzyl cinnamate	NA	EtOH or EtOH/DEP	NA	NA	NA	0	NA	NA	4720[7]	NA	NA	–	(RIFM 2007)
Benzyl cinnamate	NA	NA	NA	NA	NA	0	5517	NA	NA	NA	NA		(RIFM 2007)
Benzylidene acetone	62	Petrolatum	3	200	5	9.7	NA	NA	NA	1200	619	+	(Marzulli and Maibach 1980)
Benzylidene acetone	25	Petrolatum	2	1000	14.5	48	NA	1379	NA	NA	144		(Opdyke 1973)
Benzyl salicylate	NA	EtOH or EtOH/DEP	NA	NA	NA	0	NA	NA	17717	NA	NA	–	(RIFM 2007)

[6] No sensitization was observed in human studies. Doses reported reflect the highest concentration tested, not necessarily the highest achievable NOEL.
[7] No sensitization was observed in human studies. Doses reported reflect the highest concentration tested, not necessarily the highest achievable NOEL.

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Test Substance	N	Vehicle	Conc (%)	Test Vol (µL)	Skin Area (cm^2)	Incid (%)	HMT		HRIPT		DSA_{05} ($\mu g/cm^2$)	Human Result	Reference
							NOEL ($\mu g/cm^2$)	LOEL ($\mu g/cm^2$)	NOEL ($\mu g/cm^2$)	LOEL ($\mu g/cm^2$)			
Benzyl salicylate	NA	NA	NA	NA	NA	0	20690	NA	NA	NA	NA		(RIFM 2007)
Beryllium sulfate[8]	22	Petrolatum	5	1000	14.5	82	NA	175	NA	NA	11	+	(Kligman 1966a)
Bourgeonal	26	NA	NA	NA	NA	23	NA	NA	NA	7087	1541	+	(RIFM 2007)
1-Butanol	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	–	(Ryan et al. 2000)
Butyl glycidyl ether	24	Petrolatum	10	1000	14.5	79	NA	6897	NA	NA	437	+	(Kligman 1966a)
Carvone	81	NA	NA	NA	NA	4.9	NA	NA	NA	18898	19284	+	(RIFM 2007)
(Chloro)methyl-isothiazolinone	200	Body lotion	0.0015	NA	NA	0	NA	NA	1.25	NA	NA		(Cardin et al. 1986)
(Chloro)methylisothia-zolinone	84	Shampoo	0.00125	300-500	3.5-3.6	1.2	NA	NA	NA	1.04	4.3		(Cardin et al. 1986)
(Chloro)methylisothia-zolinone	602	Conditioner, Fabric softener, Body lotion, Water	0.001	NA	NA	0	NA	NA	0.83	NA	NA		(Cardin et al. 1986)
(Chloro)methylisothia-zolinone	416	Soap, Shampoo, Conditioner	NR	NA	NA	0	NA	NA	0.42	NA	NA		(Cardin et al. 1986)
(Chloro)methylisothia-zolinone	103	Shampoo	NR	NA	NA	0	NA	NA	0.50	NA	NA	+	(Cardin et al. 1986)
(Chloro)methylisothia-zolinone	184	NA	0.00075	NA	NA	0	NA	NA	0.75	NA	NA		(Cardin et al. 1986)
(Chloro)methylisothia-zolinone	109	NA	0.005	NA	NA	0	NA	NA	2.5	NA	NA		(SCCNFP 2003)
(Chloro)methylisothia-zolinone	189	NA	0.0015	NA	NA	1.1	NA	NA	NA	1.34	6.1		(Cardin et al. 1986)
(Chloro)methylisothia-zolinone	45	Water	0.002	NA	NA	4.4	NA	NA	NA	1.67	1.9		(Cardin et al. 1986)
(Chloro)methylisothia-zolinone	116	NA	0.01	NA	NA	4.3	NA	NA	NA	5	5.8		(SCCNFP 2003)
(Chloro)methylisothia-zolinone	196	NA	0.015	NA	NA	3.6	NA	NA	NA	7.5	10.4		(SCCNFP 2003)
Chlorpromazine	24	Petrolatum	25	1000	14.5	75	NA	17241	NA	NA	1149	+	(Kligman 1966a)

[8] Data are for metal cation.

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Test Substance	N	Vehicle	Conc (%)	Test Vol (µL)	Skin Area (cm^2)	Incid (%)	HMT		HRIPT		DSA_{05} (µg/cm^2)	Human Result	Reference
							NOEL (µg/cm^2)	LOEL (µg/cm^2)	NOEL (µg/cm^2)	LOEL (µg/cm^2)			
Cinnamic aldehyde	NA	EtOH or EtOH/DEP	NA	NA	NA	0	NA	NA	591	NA	NA	+	(RIFM 2007)
Cinnamic aldehyde	55	EtOH	1	200	5	1.8	NA	NA	NA	400	1111		(Marzulli and Maibach 1980)
Cinnamic aldehyde	41	EtOH	1	200	5	12	NA	345	NA	NA	167		(Danneman et al. 1983)
Cinnamic aldehyde	41	EtOH or EtOH/DEP	NA	NA	NA	12	NA	NA	NA	775	322		(RIFM 2007)
Cinnamic aldehyde	53	Petrolatum	1	200	5	0	NA	NA	400	NA	NA		(Marzulli and Maibach 1980)
Cinnamic aldehyde	NA	EtOH	0.5	200	5	0	NA	NA	200	NA	NA		(Danneman et al. 1983)
Cinnamic aldehyde	25	Petrolatum	2	1000	14.5	44	NA	1379	NA	NA	157		(Opdyke 1979b)
Cinnamic aldehyde	25	Petrolatum	3	1000	14.5	12	NA	2069	NA	NA	862		(Opdyke 1979b)
Cinnamyl alcohol	150	70% EtOH	4	200	4	2.7	NA	NA	NA	2000	3704	+	(Jordan and King 1977)
Cinnamyl alcohol	54	Dimethyl phthalate	6	200	5	3.7	NA	NA	NA	2400	3243		(Steltenkamp et al. 1980)
Cinnamyl alcohol	25	70% EtOH	4	200	4	16	NA	2000	NA	NA	625		(Jordan and King 1977)
Cinnamyl alcohol	NA	EtOH or EtOH/DEP	NA	NA	NA	0	NA	NA	3000	NA	NA		(RIFM 2007)
Cinnamyl alcohol	109	EtOH or EtOH/DEP	NA	NA	NA	1.8	NA	NA	NA	4724	13122		(RIFM 2007)
Cinnamyl alcohol	25	Petrolatum	4	1000	14.5	0	2759	NA	NA	NA	NA		(Greif 1967)
Cinnamyl alcohol	25	Petrolatum	4	200	4	0	2000	NA	NA	NA	NA		(Jordan and King 1977)
Cinnamyl alcohol	150	Petrolatum	4	200	4	0	NA	NA	2000	NA	NA		(Jordan and King 1977)
Cinnamyl alcohol	200	Petrolatum	10	1000	14.5	20	NA	6897	NA	NA	1724		(Steltenkamp et al. 1980)
Cinnamyl alcohol	25	Hydrophilic ointment	10	1000	14.5	8	NA	6897	NA	NA	4310		(Steltenkamp et al. 1980)

ED_006472_00084346-00290

Test Substance	N	Vehicle	Conc (%)	Test Vol (µL)	Skin Area (cm^2)	Incid (%)	HMT		HRIPT		DSA_{05} ($\mu g/cm^2$)	Human Result	Reference
							NOEL ($\mu g/cm^2$)	LOEL ($\mu g/cm^2$)	NOEL ($\mu g/cm^2$)	LOEL ($\mu g/cm^2$)			
Cinnamyl nitrile	NA	NA	NA	NA	NA	0	NA	NA	1476	NA	NA	+	(RIFM 2007)
Cinnamyl nitrile	NA	EtOH or EtOH/DEP	NA	NA	NA	0	NA	NA	1063	NA	NA		(RIFM 2007)
Cinnamyl nitrile	NA	NA	NA	NA	NA	0	3448	NA	NA	NA	NA		(RIFM 2007)
Cinnamyl nitrile	38	EtOH or EtOH/DEP	NA	NA	NA	5.3	NA	NA	NA	1938	1828		(RIFM 2007)
Citral	84	EtOH	1	200	5	2.38	NA	NA	NA	400	840	+	(Steltenkamp et al. 1980)
Citral	56	Petrolatum	8	300	6.45	11	NA	NA	NA	3721	1691		(Opdyke 1979b)
Citral	24	Petrolatum	2	1000	14.5	8	NA	1379	NA	NA	862		(Opdyke 1979b)
Citral	NA	EtOH or EtOH/DEP	NA	NA	NA	0	NA	NA	1400	NA	NA		(RIFM 2007)
Citral	NA	EtOH or EtOH/DEP	8	NA	NA	62.5	NA	NA	NA	3876	310		(RIFM 2007)
Citral	82	EtOH	0.5	200	5	0	NA	NA	200	NA	NA		(Steltenkamp et al. 1980)
Citral	50	Petrolatum	4	300	6.45	11	NA	NA	NA	1861	1691		(Opdyke 1979b)
Citronella oil	25	Petrolatum	8	1000	14.5	0	5517	NA	NA	NA	NA	–	(Lalko and Api 2006)
dl-Citronellol	NA	EtOH or EtOH/DEP	NA	NA	NA	0	NA	NA	29528[9]	NA	NA	+	(RIFM 2007)
dl-Citronellol	NA	NA	NA	NA	NA	0	4138	NA	NA	NA	NA		(RIFM 2007)
dl-Citronellol	104	Petrolatum	10	200	5	0	NA	NA	4000	NA	NA		(RIFM 2007)
dl-Citronellol	25	Petrolatum	4	300	4	0	3000	NA	NA	NA	NA		(RIFM 2007)
dl-Citronellol	73	EtOH	10	200	5	14	NA	NA	NA	4000	1429		(RIFM 2007)
Clove Oil (bud)	25	Petrolatum	5	1000	14.5	0	3448	NA	NA	NA	NA	–	(Opdyke 1975a)
Clove oil (leaf)	25	Petrolatum	5	1000	14.5	0	3448	NA	NA	NA	NA		(Lalko and Api 2006)
Clove oil (stem)	25	Petrolatum	10	1000	14.5	0	6897	NA	NA	NA	NA		(Opdyke 1975b)

[9] No sensitization was observed in human studies. Doses reported reflect the highest concentration tested, not necessarily the highest achievable NOEL.

ED_006472_00084346-00291

Test Substance	N	Vehicle	Conc (%)	Test Vol (µL)	Skin Area (cm^2)	Incid (%)	HMT		HRIPT		DSA_{05} (µg/cm^2)	Human Result	Reference
							NOEL (µg/cm^2)	LOEL (µg/cm^2)	NOEL (µg/cm^2)	LOEL (µg/cm^2)			
Cobalt (II) salts[10]	25	Petrolatum	25	1000	14.5	40	NA	3620	NA	NA	453	+	(Kligman 1966a)
Cobalt (II) salts[11]	24	Petrolatum	10	1000	14.5	42	NA	1448	NA	NA	172		(Kligman 1966b)
Copper (II) chloride	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	–	(Basketter et al. 1999b)
Coumarin	NA	EtOH or EtOH/DEP	NA	NA	NA	0	NA	NA	3543	NA	NA	+	(RIFM 2007)
Coumarin	25	Petrolatum	8	1000	14.5	0	5517	NA	NA	NA	NA		(Marzulli and Maibach 1980)
Coumarin	42	EtOH or EtOH/DEP	NA	NA	NA	2.4	NA	NA	NA	8858	18454		(RIFM 2007)
Coumarin	104	Petrolatum	8	200	5	0	NA	NA	3200	NA	NA		(Marzulli and Maibach 1980)
Coumarin	73	EtOH	8	200	5	1.4	NA	NA	NA	3200	11429		(Marzulli and Maibach 1980)
Cyclamen aldehyde	64	NA	4	NA	NA	0	NA	NA	4724	NA	NA	–	(RIFM 2007)
Damascone	23	NA	NA	NA	NA	0	NA	NA	50	NA	NA	–	(RIFM 2007)
t-alpha Damascone	≥100	DEP	NA	NA	NA	0	NA	NA	250[12]	NA	NA	–	(RIFM 2007)
trans beta Damascone	54	Petrolatum	NA	NA	NA	0	NA	NA	310[13]	NA	NA	–	(RIFM 2007)
trans beta Damascone	100	DEP	NA	NA	NA	0	NA	NA	250	NA	NA		(RIFM 2007)
delta Damascone	54	EtOH or EtOH/DEP	NA	NA	NA	12.96	NA	NA	62	500	192.9	–	(RIFM 2007)
Diethylenetriamine	25	Petrolatum	10	1000	14.5	84	NA	6897	NA	NA	411	+	(Kligman 1966a)
Diethylmaleate	24	Petrolatum	4	300	4	100	NA	3000	NA	NA	150	+	(Marzulli and Maibach 1980)
Diethylmaleate	187	Petrolatum	4	200	5	7.5	NA	NA	NA	1600	1067		(Marzulli and Maibach 1980)
Diethyl phthalate	25	NR	10	1000	14.5	0	6896	NA	NA	NA	NA	–	(Greif 1967)
Dihydrocoumarin	62	Petrolatum	20	200	5	52	NA	NA	NA	8000	769	+	(Marzulli and Maibach 1980)

[10] Test substance was cobalt sulfate.
[11] Test substance was cobalt sulfate.
[12] No sensitization was observed in human studies. Doses reported reflect the highest concentration tested, not necessarily the highest achievable NOEL.
[13] No sensitization was observed in human studies. Doses reported reflect the highest concentration tested, not necessarily the highest achievable NOEL.

ED_006472_00084346-00292

Test Substance	N	Vehicle	Conc (%)	Test Vol (µL)	Skin Area (cm^2)	Incid (%)	HMT		HRIPT		DSA_{05} ($\mu g/cm^2$)	Human Result	Reference
							NOEL ($\mu g/cm^2$)	LOEL ($\mu g/cm^2$)	NOEL ($\mu g/cm^2$)	LOEL ($\mu g/cm^2$)			
Dihydrocoumarin	25	Petrolatum	20	300	4	100	NA	15000	NA	NA	750		(Marzulli and Maibach 1980)
Dimethyl sulfoxide	23	Petrolatum	75	1000	14.5	0	51724	NA	NA	NA	NA	–	(Kligman 1966a)
2,4-Dinitrochlorobenzene	165	Acetone	62.5 µg	100	7.1	8	NA	NA	NA	8.8	5.5	+	(Friedmann et al. 1983)
2,4-Dinitrochlorobenzene	22	Acetone	0.125	24	0.8	91	NA	NA	NA	38.2	2.1		(Rees et al. 1989)[14]
Ethyl acrylate	25	Petrolatum	4	300	4	40	NA	3000	NA	NA	375	+	(Marzulli and Maibach 1980)
Ethyl acrylate	78	EtOH	4	200	5	7.7	NA	NA	NA	1600	1039		(Marzulli and Maibach 1980)
Ethyl acrylate	70	Petrolatum	4	200	5	5.7	NA	NA	NA	1600	1404		(Marzulli and Maibach 1980)
Ethyl acrylate	28	Petrolatum	4	200	5	0	NA	NA	1600	NA	NA		(Marzulli and Maibach 1980)
Ethyl acrylate	27	Petrolatum	4	200	5	0	NA	NA	1600	NA	NA		(Marzulli and Maibach 1980)
Ethylenediamine	61	Petrolatum	3	200	5	8.2	NA	NA	NA	1200	732	+	(Marzulli and Maibach 1976)
Ethyl vanillin	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	–	(De Groot et al. 1994)
Eugenol	108	75% DEP/25% EtOH	5	300	4.9	0	NA	NA	5906	NA	NA	+	(RIFM 2007)
Eugenol	73	EtOH	8	200	5	2.7	NA	NA	NA	3200	5926		(Marzulli and Maibach 1980)
Eugenol	NA	EtOH	2.5	NA	NA	0	NA	NA	1938	NA	NA		(RIFM 2007)
Eugenol	25	Petrolatum	8	300	5	0	5517	NA	NA	NA	NA		(Marzulli and Maibach 1980)
Eugenol	104	Petrolatum	8	200	5	0	NA	NA	3200	NA	NA		(Marzulli and Maibach 1980)
Farnesol	NA	EtOH or EtOH/DEP	NA	NA	NA	0	NA	NA	2755	NA	NA	+	(RIFM 2007)
Farnesol	75	NA	NA	NA	NA	13	NA	6897	NA	NA	2593		(RIFM 2007)

[14] Nonstandard protocol. No repeated exposures.

ED_006472_00084346-00293

Test Substance	N	Vehicle	Conc (%)	Test Vol (µL)	Skin Area (cm^2)	Incid (%)	HMT		HRIPT		DSA_{05} ($\mu g/cm^2$)	Human Result	Reference
							NOEL ($\mu g/cm^2$)	LOEL ($\mu g/cm^2$)	NOEL ($\mu g/cm^2$)	LOEL ($\mu g/cm^2$)			
Formaldehyde	25	Petrolatum	1.85	1000	14.5	72	NA	NA	NA	1276	89	+	(Kligman 1966a)
Formaldehyde	89	Petrolatum	1	500 mg	5	4.5	NA	NA	NA	370	411		(Marzulli and Maibach 1974)
Formaldehyde	45	Petrolatum	0.1	500 mg	5	0	NA	NA	37	NA	NA		(Marzulli and Maibach 1974)
Geraniol	40	EtOH/DEP (75:25 w/w)	5	300	4.9	0	NA	NA	3061[15]	NA	NA	+	(Basketter et al. 2005)
Geraniol	25	Petrolatum	5	1000	14.5	80	NA	3448	NA	NA	216		(Malten et al. 1984)
Geraniol	73	EtOH	10	200	5	2.7	NA	NA	NA	4000	7407		(Marzulli and Maibach 1980)
Geraniol	NA	EtOH or EtOH/DEP	NA	NA	NA	0	NA	NA	11811	NA	NA		(RIFM 2007)
Geraniol	104	Petrolatum	10	200	5	0	NA	NA	4000	NA	NA		(Marzulli and Maibach 1980)
Geraniol	25	Petrolatum	6	1000	14.5	0	4138	NA	NA	NA	NA		(Marzulli and Maibach 1980)
Geranium oil	25	Petrolatum	10	1000	14.5	0	6897	NA	NA	NA	NA	–	(Lalko and Api 2006)
Glutaraldehyde	30	Petrolatum	5	500 mg	5	23.3	NA	NA	NA	5000	1073	+	(Marzulli and Maibach 1974)
Glutaraldehyde	102	Petrolatum	5	500 mg	5	0	NA	NA	100	NA	NA		(Marzulli and Maibach 1974)
Glycerol	NA	NA	NA	NA	NA	0	13793	NA	NA	NA	NA	–	(Gad et al. 1986)
Glyoxal	24	Petrolatum	10	1000	14.5	100	NA	6897	NA	NA	345	+	(Kligman 1966a)
Gold chloride	23	Petrolatum	2	1000	14.5	70	NA	1379	NA	NA	98.5	+	(Kligman 1966a)
Hexane	25	Petrolatum	100	1000	14.5	0	68966	NA	NA	NA	NA	–	(Kligman 1966a)
trans-2-Hexenal	NA	EtOH or EtOH/DEP	NA	NA	NA	0	NA	NA	24	NA	NA	+	(RIFM 2007)
trans-2-Hexenal	25	EtOH or EtOH/DEP	NA	NA	NA	24	NA	NA	NA	236	49.2		(RIFM 2007)

[15] Corrected NOEL to reflect RIFM HRIPT protocol.

ED_006472_00084346-00294

Test Substance	N	Vehicle	Conc (%)	Test Vol (µL)	Skin Area (cm^2)	Incid (%)	HMT		HRIPT		DSA_{05} ($\mu g/cm^2$)	Human Result	Reference
							NOEL ($\mu g/cm^2$)	LOEL ($\mu g/cm^2$)	NOEL ($\mu g/cm^2$)	LOEL ($\mu g/cm^2$)			
Hexyl cinnamic aldehyde	NA	EtOH or EtOH/DEP	NA	NA	NA	0	NA	NA	23662[16]	NA	NA	–	(RIFM 2007)
2-Hexylidene cyclopentanone	51	NA	NA	NA	NA	9.8	NA	NA	NA	500	255	+	(RIFM 2007)
Hexyl salicylate	NA	NA	NA	NA	NA	0	2069	NA	35433	NA	NA	–	(RIFM 2007)
Hydrocortisone	25	Petrolatum	25	1000	14.5	0	17242	NA	NA	NA	NA	–	(Kligman 1966a)
Hydroxycitronellal	26	Petrolatum	5	1000	14.5	0	3448	NA	NA	NA	NA	+	(Ford et al. 1988)
Hydroxycitronellal	104	Petrolatum	12	1000	14.5	16	NA	8276	NA	NA	2586		(Ford et al. 1988)
Hydroxycitronellal	66	EtOH/DEP (75:25 w/w)	5	300	4.9	2	NA	NA	NA	3061	7653		(Ford et al. 1988)
Hydroxycitronellal	38	EtOH	7.5	200	5	2.6	NA	NA	NA	3000	5769		(Steltenkamp et al. 1980)
Hydroxycitronellal	73	EtOH	20	200	5	19	NA	NA	NA	8000	2105		(Marzulli and Maibach 1980)
Hydroxycitronellal	150	Petrolatum	4	200	4	0.7	NA	NA	NA	2000	14286		(Jordan and King 1977)
Hydroxycitronellal	99	Petrolatum	20	200	5	1	NA	NA	NA	8000	40000		(Marzulli and Maibach 1980)
Hydroxycitronellal	25	Petrolatum	10	1000	14.5	8	NA	6897	NA	NA	4311		(Ford et al. 1988)
Hydroxycitronellal	81	EtOH or EtOH/DEP	NA	NA	NA	31	NA	NA	5000	5906	956		(RIFM 2007)
Hydroxycitronellal	65	EtOH/DEP (75:25 w/w)	2.5	300	4.9	0	NA	NA	1530	NA	NA		(Ford et al. 1988)
Hydroxycitronellal	25	Petrolatum	5	1000	14.5	0	3448	NA	NA	NA	NA		(Opdyke 1974)
Hydroxycitronellal	25	Petrolatum	12	1000	14.5	0	8276	NA	NA	NA	NA		(Opdyke 1974)
Imidazolidinyl urea	150	Aqueous soap solution	2	200	4	1.3	NA	NA	NA	1000	3846	+	(Jordan and King 1977)
Imidazolidinyl urea	25-30	Aqueous soap solution	2	1000	4	0	5000	NA	NA	NA	NA		(Jordan and King 1977)
Isocyclemone E	NA	NA	NA	NA	NA	0	NA	NA	47244	NA	NA	–	(RIFM 2007)

[16] No sensitization was observed in human studies. Doses reported reflect the highest concentration tested, not necessarily the highest achievable NOEL.

ED_006472_00084346-00295

Test Substance	N	Vehicle	Conc (%)	Test Vol (µL)	Skin Area (cm^2)	Incid (%)	HMT		HRIPT		DSA_{05} (µg/cm^2)	Human Result	Reference
							NOEL (µg/cm^2)	LOEL (µg/cm^2)	NOEL (µg/cm^2)	LOEL (µg/cm^2)			
Isocyclocitral[17]	NA	EtOH or EtOH/DEP	NA	NA	NA	0	NA	NA	7087	NA	NA	–	(RIFM 2007)
Isocyclocitral	NA	NA	NA	NA	NA	0	2759	NA	NA	NA	NA		(RIFM 2007)
Isocyclogeraniol	NA	EtOH or EtOH/DEP	NA	NA	NA	0	NA	NA	3898	NA	NA	+	(RIFM 2007)
Isocyclogeraniol	49	EtOH or EtOH/DEP	NA	NA	NA	4	NA	NA	NA	5000	6250		(RIFM 2007)
Isoeugenol	NA	EtOH	0.5	NA	NA	0	NA	NA	69	NA	NA	+	(RIFM 2007)
Isoeugenol	38	EtOH	1	NA	NA	5	NA	NA	NA	775	775		(RIFM 2007)
Isoeugenol	NA	EtOH	NA	NA	NA	0	NA	NA	250	NA	NA		(RIFM 2007)
Isoeugenol	73	EtOH	8	200	5	12	NA	NA	NA	3200	1333		(Marzulli and Maibach 1980)
Isomethyl ionone	NA	EtOH or EtOH/DEP	NA	NA	NA	0	NA	NA	70866[18]	NA	NA	–	(RIFM 2007)
Isopropanol	NA	NA	NA	NA	NA	0	6897	NA	NA	NA	NA	–	(Basketter et al. 1998)
Isopropyl myristate	25	NA	NA	NA	NA	0	13793	NA	NA	NA	NA	–	(Opdyke 1976b)
Jasmine absolute (grandiflorum)	75	EtOH or EtOH/DEP	NA	NA	NA	10.7	NA	NA	1475	2069	966.8	+	(RIFM 2007)
Jasmine absolute (sambac)	NA	EtOH or EtOH/DEP	NA	NA	NA	0	NA	NA	8858[19]	NA	NA	–	(RIFM 2007)
Kanamycin	24	Petrolatum	25	1000	14.5	46	NA	17241	NA	NA	1874	+	(Kligman 1966a)
Lead acetate	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	–	(Rycroft et al. 1993)
Lemongrass oil	25	Petrolatum	5	1000	14.5	0	3448	NA	NA	NA	NA	–	(RIFM 2007)

[17] No sensitization was observed in human studies. Doses reported reflect the highest concentration tested, not necessarily the highest achievable NOEL.
[18] No sensitization was observed in human studies. Doses reported reflect the highest concentration tested, not necessarily the highest achievable NOEL.
[19] No sensitization was observed in human studies. Doses reported reflect the highest concentration tested, not necessarily the highest achievable NOEL.

ED_006472_00084346-00296

Test Substance	N	Vehicle	Conc (%)	Test Vol (µL)	Skin Area (cm^2)	Incid (%)	HMT		HRIPT		DSA_{05} ($\mu g/cm^2$)	Human Result	Reference
							NOEL ($\mu g/cm^2$)	LOEL ($\mu g/cm^2$)	NOEL ($\mu g/cm^2$)	LOEL ($\mu g/cm^2$)			
Lilial	NA	DEP	5	NA	NA	0	NA	NA	3750	NA	NA	+	(RIFM 2007)
Lilial	106	75% DEP/ 25% EtOH	25	NA	NA	0	NA	NA	29527	NA	NA		(Cocchiara and Api 2005)
Lilial	NA	EtOH or EtOH/DEP	NA	NA	NA	0	NA	NA	4125	NA	NA		(RIFM 2007)
Lilial	225	EtOH or EtOH/DEP	NA	NA	NA	0.44	NA	NA	NA	29528	335545		(RIFM 2007)
d-Limonene	NA	EtOH or EtOH/DEP	NA	NA	NA	0	NA	NA	10000[20]	NA	NA	–	(RIFM 2007)
d-Limonene	NA	NA	NA	NA	NA	0	5517	NA	NA	NA	NA		(RIFM 2007)
Linalool	NA	NA	NA	NA	NA	0	13793 [21]	NA	NA	NA	NA	–	(RIFM 2007)
Linalool	25	NR	8	1000	14.5	0	5517	NA	NA	NA	NA		(Greif 1967)
Linalool	NA	EtOH or EtOH/DEP	NA	NA	NA	0	NA	NA	15000	NA	NA		(RIFM 2007)
Litsea cubeba oil	25	Petrolatum	8	1000	14.5	0	5517	NA	NA	NA	NA	–	(RIFM 2007)
Lyral HMPCC	NA	EtOH or EtOH/DEP	NA	NA	NA	0	NA	NA	4000	NA	NA	–	(RIFM 2007)
Lyral HMPCC	108	75% EtOH/25% DEP	15	300	NR	0	NA	NA	8264	NA	NA		(RIFM 2007)
Majantal	NA	EtOH or EtOH/DEP	NA	NA	NA	0	NA	NA	9900	NA	NA	–	(RIFM 2007)
Manganese chloride	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	-	(Nguyen and Allen 1979)
Menthadiene-7-methyl formate	79	NA	NA	NA	NA	10.13	690	NA	1063	6900	3405.7	+	(RIFM 2007)
2-Mercaptobenzothiazole	24	Petrolatum	25	1000	14.5	38	NA	17241	NA	NA	2269	+	(Kligman 1966a)
2-Mercaptobenzothiazole	24	Petrolatum	10	1000	14.5	21	NA	6987	NA	NA	1642		(Kligman 1966b)

[20] No sensitization was observed in human studies. Doses reported reflect the highest concentration tested, not necessarily the highest achievable NOEL.
[21] No sensitization was observed in human studies. Doses reported reflect the highest concentration tested, not necessarily the highest achievable NOEL.

ED_006472_00084346-00297

Test Substance	N	Vehicle	Conc (%)	Test Vol (µL)	Skin Area (cm^2)	Incid (%)	HMT		HRIPT		DSA_{05} ($\mu g/cm^2$)	Human Result	Reference
							NOEL ($\mu g/cm^2$)	LOEL ($\mu g/cm^2$)	NOEL ($\mu g/cm^2$)	LOEL ($\mu g/cm^2$)			
Mercuric (II) chloride[22]	24	Petrolatum	2	500 mg	5	8	NA	NA	NA	1478	924	+	(Marzulli and Maibach 1974)
Mercuric (II) chloride	25	Petrolatum	2	1000	14.5	92	NA	1019	NA	NA	55		(Kligman 1966a)
4-Methoxyacetophenone	25	Petrolatum	6	1000	14.5	0	4138	NA	NA	NA	NA	–	(Opdyke 1974)
Methoxy dicyclopentadiene carboxaldehyde[23]	NA	DEP	NA	NA	NA	0	NA	NA	5000	NA	NA	–	(RIFM 2007)
2-Methoxy-4-methylphenol	NA	EtOH or EtOH/DEP	NA	NA	NA	0	NA	NA	118[24]	NA	NA	–	(RIFM 2007)
Methylanisylidene acetone	24	Petrolatum	8	1000	14.5	67	NA	552	NA	NA	412	+	(Opdyke 1979a)
alpha-Methyl cinnamic aldehyde	>100	EtOH or EtOH/DEP	NA	NA	NA	0	NA	NA	3543[25]	NA	NA	–	(RIFM 2007)
alpha-Methyl cinnamic aldehyde	NA	NA	NA	NA	NA	0	5517	NA	NA	NA	NA		(RIFM 2007)
6-Methylcoumarin	25	Petrolatum	4	1000	14.5	0	2759	NA	NA	NA	NA	–	(Opdyke 1976a)
Methylhexanedione	25	Petrolatum	5	300	4	8	NA	3750	NA	NA	2344	+	(Opdyke 1982)
Methylhexanedione	29	Petrolatum	5	300	4	3.4	NA	3750	NA	NA	5515		(Opdyke 1982)
Methylhydrocinnamal	23	DEP	2	1000	14.5	0	1379	NA	NA	NA	NA	+	(RIFM 2007)
Methylhydrocinnamal	24	NA	20	1000	14.5	29	NA	13793	NA	NA	2378		(RIFM 2007)
Methylisothiazolinone	98	Water	0.03	200	4	0	NA	NA	15	NA	NA	+	(SCCNFP 2003)
Methylisothiazolinone	100	Water	0.02	200	4	0	NA	NA	10	NA	NA		(Basketter et al. 2007)
Methylisothiazolinone	97	NA	0.01	NA	NA	0	NA	NA	5	NA	NA		(SCCNFP 2003)
Methylisothiazolinone	NA	NA	0.04	NA	NA	0.9	NA	NA	NA	20	111		(SCCNFP 2003)
Methylisothiazolinone	NA	NA	0.05	NA	NA	0.5	NA	NA	NA	45	450		(SCCNFP 2003)
Methyl 2-nonynoate	NA	75% EtOH/ 25% DEP	NA	NA	NA	0	NA	NA	24	NA	NA	+	(RIFM 2007)
Methyl 2-nonynoate	67	75% EtOH/ 25% DEP	NA	NA	NA	7.5	NA	NA	NA	118	79		(RIFM 2007)

[22] LOEL and DSA_{05} expressed as amount of mercury (Hg) metal.
[23] No sensitization was observed in human studies. Doses reported reflect the highest concentration tested, not necessarily the highest achievable NOEL.
[24] No sensitization was observed in human studies. Doses reported reflect the highest concentration tested, not necessarily the highest achievable NOEL.
[25] No sensitization was observed in human studies. Doses reported reflect the highest concentration tested, not necessarily the highest achievable NOEL.

ED_006472_00084346-00298

Test Substance	N	Vehicle	Conc (%)	Test Vol (µL)	Skin Area (cm^2)	Incid (%)	HMT		HRIPT		DSA_{05} (µg/cm^2)	Human Result	Reference
							NOEL (µg/cm^2)	LOEL (µg/cm^2)	NOEL (µg/cm^2)	LOEL (µg/cm^2)			
Methyl 2-octynoate	NA	EtOH or EtOH/DEP	NA	NA	NA	0	NA	NA	118	NA	NA	+	(RIFM 2007)
Methyl 2-octynoate	41	EtOH or EtOH/DEP	NA	NA	NA	2.5	NA	NA	NA	194	388		(RIFM 2007)
Methyl salicylate	27	Petrolatum	NA	NA	NA	0	5517	NA	NA	NA	NA	–	(Opdyke 1979b)
Neomycin sulfate	186	Petrolatum	5	500,000 µg	14.5	1.6	NA	NA	NA	5000	15625	+	(Marzulli and Maibach 1974)
Neomycin sulfate	54	Petrolatum	0.5	500,000 µg	14.5	5.6	NA	NA	NA	500	446		(Marzulli and Maibach 1974)
Neomycin sulfate	23	Petrolatum	10	1000	14.5	17	NA	6897	NA	NA	2028		(Kligman 1966b)
Neomycin sulfate	24	Petrolatum	25	1000	14.5	21	NA	17241	NA	NA	4105		(Kligman 1966b)
Neomycin sulfate	25	Petrolatum	10	1000	14.5	4	NA	6897	NA	NA	8621		(Kligman 1966b)
Nickel (II) salts	23	Petrolatum	1	1000	14.5	26	NA	154	NA	NA	28	+	(Kligman 1966b)
Nickel (II) salts	24	Petrolatum	1	1000	14.5	17	NA	154	NA	NA	45		(Kligman 1966b)
Nickel (II) salts	25	Petrolatum	10	1000	14.5	48	NA	1540	NA	NA	16		(Kligman 1966a)
Oakmoss	NA	EtOH or EtOH/DEP	NA	NA	NA	0	NA	NA	700	NA	NA	+	(RIFM 2007)
Oakmoss	NA	NA	NA	NA	NA	0	1724	NA	NA	NA	NA		(RIFM 2007)
Oakmoss	95	EtOH or EtOH/DEP	NA	NA	NA	2.1	NA	NA	NA	1417	3374		(RIFM 2007)
Octanoic acid	NA	NA	NA	NA	NA	0	690	NA	NA	NA	NA	–	(Basketter et al. 1999a)
1-Octen-3-yl acetate	175	EtOH or EtOH/DEP	NA	NA	NA	5.14	NA	NA	3543	6900	6712.1	+	(RIFM 2007)
Palmarosa oil	25	Petrolatum	4	1000	14.5	0	5517	NA	NA	NA	NA	–	(RIFM 2007)
Penicillin G	24	Petrolatum	25	1000	14.5	67	NA	17241	NA	NA	1287	+	(Kligman 1966a)
Penicillin G	22	Petrolatum	0.2	1000	14.5	9.1	NA	138	NA	NA	76		(Kligman 1966b)
Penicillin G	25	Petrolatum	10	1000	14.5	36	NA	6897	NA	NA	958		(Kligman 1966b)
Penicillin G	23	Petrolatum	0.1	1000	14.5	0	69	NA	NA	NA	NA		(Kligman 1966b)
Penicillin G	25	Petrolatum	10	1000	14.5	20	NA	6897	NA	NA	1724		(Kligman 1966b)
Pentachlorophenol	25	Petrolatum	5	1000	14.5	8	NA	3448	NA	NA	2155	+	(Kligman 1966b)
Pentaerythritol triacrylate	9	Petrolatum	10%	360	4	78	NA	NA	NA	9000	576	+	(Nethercott 1978)

ED_006472_00084346-00299

Test Substance	N	Vehicle	Conc (%)	Test Vol (µL)	Skin Area (cm^2)	Incid (%)	HMT		HRIPT		DSA_{05} ($\mu g/cm^2$)	Human Result	Reference
							NOEL ($\mu g/cm^2$)	LOEL ($\mu g/cm^2$)	NOEL ($\mu g/cm^2$)	LOEL ($\mu g/cm^2$)			
Perillaldehyde	54	NA	NA	NA	NA	9.3	NA	NA	NA	2760	1484	+	(RIFM 2007)
Peru balsam absolute	25	NA	NA	NA	NA	32	5517	NA	950	5517	862	+	(RIFM 2007)
Phenyl benzoate	107	DEP	8	NA	NA	0.9	NA	NA	NA	9448	52489	+	(RIFM 2007)
Phenylacetaldehyde	110	75% DEP, 25% EtOH	0.5	NA	NA	0	NA	NA	591	NA	NA	+	(RIFM 2007)
Phenylacetaldehyde	53	EtOH or EtOH/DEP	NA	NA	NA	13	NA	NA	591	1181	454		(RIFM 2007)
Phenylacetaldehyde	25	Petrolatum	2	1000	14.5	44	NA	1379	NA	NA	157		(Opdyke 1979b)
Phenylacetaldehyde	25	Petrolatum	2	1000	14.5	16	NA	1379	NA	NA	431		(Opdyke 1979b)
Phenylacetaldehyde	23	Petrolatum	2	1000	14.5	52	NA	1379	NA	NA	133		(Opdyke 1979b)
Phenylacetaldehyde	25	Petrolatum	2	300	4	8	NA	1500	NA	NA	938		(Opdyke 1979b)
4-Phenylenediamine	24	Petrolatum	0.1	1000	14.5	21	NA	69	NA	NA	16.4	+	(Kligman 1966b)
4-Phenylenediamine	97	Petrolatum	0.01	500,000 ug	5	7.2	NA	NA	NA	10	6.9		(Marzulli and Maibach 1974)
4-Phenylenediamine	24	Petrolatum	10	1000	14.5	100	NA	6897	NA	NA	345		(Kligman 1966a)
4-Phenylenediamine	24	Petrolatum	0.2	1000	14.5	33	NA	138	NA	NA	21		(Kligman 1966a)
Phenylpropionaldehyde	7	Ethanol	2.5	500	6.45	14	NA	NA	NA	1938	692	+	(Akkan et al. 2003)
Potassium dichromate	23	Petrolatum	2	1000	14.5	100	NA	1379	NA	NA	69	+	(Kligman 1966a)
Potassium dichromate	25	Petrolatum	3	1000	14.5	72	NA	2069	NA	NA	144		(Kligman 1966b)
Potassium dichromate	21	Petrolatum	3	1000	14.5	86	NA	2069	NA	NA	120		(Kligman 1966b)
Propylene glycol	24	Petrolatum	25	1000	14.5	0	17241	NA	NA	NA	NA	–	(Kligman 1966a)
Propylene glycol	89	Petrolatum	60	500,000 ug	5	0	NA	NA	60000	NA	NA		(Marzulli and Maibach 1974)
Propylidene phthalate	25	Petrolatum	4	1000	14.5	12	NA	2759	NA	NA	1150	+	(Opdyke 1978)
Propylidene phthalate	NA	EtOH or EtOH/DEP	NA	NA	NA	0	NA	NA	945	NA	NA		(RIFM 2007)
Propylidene phthalate	NA	NA	NA	NA	NA	0	345	NA	NA	NA	NA		(RIFM 2007)
Pyridine	24	Petrolatum	50	1000	14.5	4.2	NA	34483	NA	NA	41051	+	(Kligman 1966a)
Resorcinol	22	Petrolatum	15	1000	14.5	0	10345	NA	NA	NA	NA	–	(Kligman 1966a)
Salicylic acid	25	Petrolatum	20	1000	14.5	0	13793	NA	NA	NA	NA	–	(Kligman 1966a)
Sodium lauryl sulfate	22	Petrolatum	10	1000	14.5	0	6897	NA	NA	NA	NA	–	(Kligman 1966a)

ED_006472_00084346-00300

Test Substance	N	Vehicle	Conc (%)	Test Vol (µL)	Skin Area (cm^2)	Incid (%)	HMT		HRIPT		DSA_{05} (µg/cm^2)	Human Result	Reference
							NOEL (µg/cm^2)	LOEL (µg/cm^2)	NOEL (µg/cm^2)	LOEL (µg/cm^2)			
Spearmint oil	NA	NA	4	NA	NA	0	NA	NA	NA	NA	NA	–	(Opdyke 1978)
Streptomycin	24	Petrolatum	0.1	1000	14.5	4.2	NA	69	NA	NA	82	+	(Kligman 1966b)
Streptomycin	23	Petrolatum	10	1000	14.5	65	NA	6897	NA	NA	769		(Kligman 1966b)
Streptomycin	23	Petrolatum	0.1	1000	14.5	13	NA	69	NA	NA	27		(Kligman 1966b)
Streptomycin	24	Petrolatum	10	1000	14.5	100	NA	6897	NA	NA	345		(Kligman 1966b)
Streptomycin	24	Petrolatum	5	1000	14.5	50	NA	3448	NA	NA	345		(Kligman 1966b)
Streptomycin	25	Petrolatum	25	1000	14.5	80	NA	17241	NA	NA	1078		(Kligman 1966a)
Sulfanilamide	25	Petrolatum	25	1000	14.5	20	NA	17241	NA	NA	4310	+	(Kligman 1966a)
Sulfanilic acid	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	–	(Basketter et al. 1999a)
Tartaric acid	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	–	(Basketter et al. 1999a)
Tea leaf absolute	NA	NA	NA	NA	NA	0	NA	NA	480	NA	NA	–	(RIFM 2007)
Tetrachlorosalicylanilide	25	Petrolatum	0.1	1000	14.5	24	NA	69	NA	NA	14.4	+	(Kligman 1966b)
Tetrachlorosalicylanilide	25	Petrolatum	5	1000	14.5	88	NA	3448	NA	NA	195.9		(Kligman 1966a)
Tetrachlorosalicylanilide	25	Petrolatum	0.5	1000	14.5	26	NA	344.8	NA	NA	14.4		(Kligman 1966b)
Tetrachlorosalicylanilide	23	Petrolatum	0.05	1000	14.5	13	NA	34.5	NA	NA	13		(Kligman 1966b)
Tetramethylthiuram-disulfide	25	Petrolatum	25	1000	14.5	16	NA	17241	NA	NA	5388	+	(Kligman 1966a)
Tetramethylthiuram-disulfide	25	Petrolatum	10	1000	14.5	9	NA	6897	NA	NA	3832		(Kligman 1966b)
Tetramethylthiuram-disulfide	25	Petrolatum	10	1000	14.5	0	6897	NA	NA	NA	NA		(Kligman 1966b)
Thioglycerol	52	Water	1.23	500	4.23	11	NA	NA	NA	1454	661	+	(Voss 1958)
Thioglycerol	24	Petrolatum	50	1000	14.5	100	NA	34483	NA	NA	1724		(Kligman 1966a)
Thioglycerol	24	Petrolatum	20	1000	14.5	42	NA	13793	NA	NA	1642		(Kligman 1966b)
Treemoss	NA	EtOH or EtOH/DEP	NA	NA	NA	0	NA	NA	700	NA	NA	+	(RIFM 2007)
Treemoss	NA	NA	NA	NA	NA	0	6896	NA	NA	NA	NA		(RIFM 2007)
Treemoss	145	EtOH or EtOH/DEP	NA	NA	NA	2.07	NA	NA	NA	1417	3423		(RIFM 2007)
Tween 80	21	Petrolatum	25	1000	14.5	0	17241	NA	NA	NA	NA	–	(Kligman 1966a)

ED_006472_00084346-00301

Test Substance	N	Vehicle	Conc (%)	Test Vol (µL)	Skin Area (cm^2)	Incid (%)	HMT		HRIPT		DSA_{05} (µg/cm^2)	Human Result	Reference
							NOEL (µg/cm^2)	LOEL (µg/cm^2)	NOEL (µg/cm^2)	LOEL (µg/cm^2)			
Xylene	24	Petrolatum	100	1000	14.5	0	68966	NA	NA	NA	NA	–	(Kligman 1966a)
Ylang Ylang	83	NA	NA	NA	NA	2.4	NA	NA	NA	7752	16150	+	(RIFM 2007)
Ylang Ylang	NA	NA	NA	NA	NA	0	6897	NA	1772	NA	NA		(RIFM 2007)
Zinc sulfate	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	–	(Basketter et al. 1999b)

Abbreviations: Conc = concentration; DEP = diethyl phthalate; DSA_{05} = induction dose per skin area, in µg/cm^2, in a human repeat-insult patch test or human maximization test that produces a positive response in 5% of the tested population; EtOH = ethanol; HMT = human maximization test; HRIPT = human repeat-insult patch test; Incid = incidence; LOEL = lowest observed effect level; N = number of subjects tested; NA = not applicable; NOEL = no observed effect level; NR = not reported; PEG = polyethylene glycol; Vol = volume.

+ = An overall positive result was assigned if one or more human skin sensitization tests yielded a positive result.

– = An overall negative result was assigned only if all human skin sensitization tests yielded negative results.

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References

Akkan Z, Kalberlah F, Oltmans J, Schneider K. 2003. Beurteilung der Wirkstärke hautsensibilisierender Chemikalien anhand des Local Lymph Node Assay (LLNA). Dortmund, Germany: Auftrag der Bundesanstalt für Arbeitsschutz und Arbeitsmedizin.

Basketter D, Gerberick GF, Kimber I. 2007. LLNA/EC3 Validation Submission.

Basketter DA, Clapp C, Jefferies D, Safford B, Ryan CA, Gerberick F, et al. 2005. Predictive identification of human skin sensitization thresholds. Contact Dermatitis 53(5): 260-267.

Basketter DA, Gerberick GF, Kimber I. 1998. Strategies for identifying false positive responses in predictive skin sensitization tests. Food Chem Toxicol 36(4): 327-333.

Basketter DA, Lea LJ, Cooper K, Stocks J, Dickens A, Pate I, et al. 1999a. Threshold for classification as a skin sensitizer in the local lymph node assay: a statistical evaluation. Food Chem Toxicol 37(12): 1167-1174.

Basketter DA, Lea LJ, Cooper KJ, Ryan CA, Gerberick GF, Dearman RJ, et al. 1999b. Identification of metal allergens in the local lymph node assay. Am J Contact Dermat 10(4): 207-212.

Basketter DA, Rodford R, Kimber I, Smith I, Wahlberg JE. 1999c. Skin sensitization risk assessment: a comparative evaluation of 3 isothiazolinone biocides. Contact Dermatitis 40(3): 150-154.

Cardin CW, Weaver JE, Bailey PT. 1986. Dose-response assessments of Kathon® biocide. (II) Threshold prophetic patch testing. Contact Dermatitis 15(1): 10-16.

Cocchiara J, Api AM. 2005. A dermal safety evaluation of paratert-butyl-a methyl hydroxy cinnamaldehyde (BMHCA). Toxicologist 72(S1): 301.

Danneman PJ, Booman KA, Dorsky J. 1983. Cinnamic aldehyde: a survey of consumer patch-test sensitization. Food Chem Toxicol 21(6): 721-725.

De Groot AC, Weyland JW, Nater JP. 1994. Unwanted Effects of Cosmetics and Drugs Used in Dermatology. 3rd ed. Amsterdam:Elsevier.

Ford RA, Api AM, Suskind RR. 1988. Allergic contact sensitization potential of hydroxycitronellal in humans. Food Chem Toxicol 26(11-12): 921-926.

Friedmann PS, Moss C, Shuster S, Simpson JM. 1983. Quantitation of sensitization and responsiveness to dinitrochlorobenzene in normal subjects. Br J Dermatol 109(Suppl. 25): 86-88.

Gad SC, Dunn BJ, Dobbs DW. 1986. Development and validation of an alternative dermal sensitization test: the mouse ear swelling test (MEST). Toxicol Appl Pharmacol 84(1): 93-114.

Greif N. 1967. Cutaneous safety of fragrance material as measured by the maximization test. American Perfumer and Cosmetics 82: 54-57.

ED_006472_00084346-00303

Jordan WP, King SE. 1977. Delayed hypersensitivity in females. The development of allergic contact dermatitis in females during the comparison of two predictive patch tests. Contact Dermatitis 3(1): 19-26.

Kligman AM. 1966a. The identification of contact allergens by human assay. 3. The maximization test: a procedure for screening and rating contact sensitizers. J Invest Dermatol 47(5): 393-409.

Kligman AM. 1966b. The identification of contact allergens by human assay. II. Factors influencing the induction and measurement of allergic contact dermatitis. J Invest Dermatol 47(5): 375-392.

Lalko J, Api AM. 2006. Investigation of the dermal sensitization potential of various essential oils in the local lymph node assay. Food Chem Toxicol 44(5): 739-746.

Leyden JJ, Kligman AM. 1977. Contact sensitization to benzoyl peroxide. Contact Dermatitis 3(5): 273-275

Malten KE, Van Ketel WG, Nater JP, Liem DH. 1984. Reactions in selected patients to 22 fragrance materials. Contact Dermatitis 11(1): 1-10.

Marzulli FN, Maibach HI. 1974. The use of graded concentrations in studying skin sensitizers: experimental contact sensitization in man. Food Cosmet Toxicol 12(2): 219-227.

Marzulli FN, Maibach HI. 1976. Contact allergy: predictive testing in man. Contact Dermatitis 2(1): 1-17.

Marzulli FN, Maibach HI. 1980. Contact allergy: predictive testing of fragrance ingredients in humans by Draize and Maximization methods. J Environ Pathol Toxicol 3(5-6): 235-245.

Nethercott JR. 1978. Skin problems associated with multifunctional acrylic monomers in ultraviolet curing inks. Br J Dermatol 98(5): 541-552.

Nguyen LQ, Allen HB. 1979. Reactions to manganese and cadmium in tattoos. Cutis 23(1): 71-72.

Opdyke DL. 1975a. Fragrance raw materials monographs. Clove bud oil. Food Cosmet Toxicol 13(6): 761-763.

Opdyke DL. 1975b. Fragrance raw materials monographs. Clove stem oil. Food Cosmet Toxicol 13(6): 765-767.

Opdyke DL. 1978. Fragrance raw materials monographs. Spearmint oil. Food Cosmet Toxicol 16(Supplement 1): 871-872.

Opdyke DLJ. 1973. Monographs on fragrance raw materials. Benzylidene acetone. Food Cosmet Toxicol 11(6): 1021.

Opdyke DLJ. 1974. Fragrance raw materials monographs. p-Methoxyacetophenone. Food Chem Toxicol 12: 927.

Opdyke DLJ. 1976a. Fragrance raw materials monographs. 6-Methyl coumarin. Food Chem Toxicol 14: 605.

Opdyke DLJ. 1976b. Fragrance raw materials monographs. Isopropyl myristate. Food Cosmet Toxicol 14: 323-325.

Opdyke DLJ. 1979a. Fragrance raw materials monographs. alpha-Methylanisalacetone. Food Chem Toxicol 17: 863.

Opdyke DLJ. 1979b. Fragrance raw materials monographs. Citral. Food Chem Toxicol 17: 259-266.

ED_006472_00084346-00304

Opdyke DLJ. 1982. Fragrance raw materials monographs. Acetyl isovaleryl. Food and Cosmetic Toxicology 20: 637.

Poole RL, Griffith JF, MacMillan FS. 1970. Experimental contact sensitization with benzoyl peroxide. Arch Dermatol 102(4): 400-404.

Rees JL, Friedmann PS, Matthews JNS. 1989. Sex differences in susceptibility to development of contact hypersensitivity to dinitrochlorobenzene (DNCB). Br J Dermatol 120(3): 371-374.

RIFM. 2007. Data submission to NICEATM. Woodcliff Lake, NJ.

Ryan CA, Gerberick GF, Cruse LW, Basketter DA, Lea L, Blaikie L, et al. 2000. Activity of human contact allergens in the murine local lymph node assay. Contact Dermatitis 43(2): 95-102.

Rycroft RJ, Menné T, Frosch PJ, eds. 1993. Textbook of Contact Dermatitis. New York:Springer-Verlag.

SCCNFP. 2003. Opinion concerning methylisothiazolinone. SCCNFP/0625/02. COLIPA.

Steltenkamp RJ, Booman KA, Dorsky J. 1980. Cinnamic alcohol: a survey of consumer patch-test sensitization. Food Cosmet Toxicol 18(4): 419-424.

Voss JG. 1958. Skin sensitization by mercaptans of low molecular weight. J Invest Dermatol 31(5): 273-279.

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Annex II-3

Guinea Pig Data for LLNA Potency Evaluation

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Chemical Name	Guinea Pig Maximization Test				Buehler Test				Most Prevalent GHS Potency Category
	i.d. Induction Conc. (%)	Sensiti-zation Incidence (%)	GHS Potency Category	Reference	Topical Induction Conc. (%)	Sensiti-zation Incidence (%)	GHS Potency Category	Reference	
Abietic acid	0.5	40	Cat 1B	(Basketter and Scholes 1992)	25	0	Unclassified	(Hausen et al. 1989)	Cat 1B
Abietic acid	4	55	Cat 1B	(Karlberg et al. 1980)	50	0	Unclassified	(Hausen et al. 1989)	
Abietic acid	4	40	Cat 1B	(Karlberg et al. 1980)	NA	NA	NA	NA	
Abietic acid	4	5	Unclassified	(Karlberg et al. 1980)	NA	NA	NA	NA	
AE F016382 00 TK71 A101	NA	NA	NA	NA	50	0	Unclassified	(Debruyne 2007)	Unclassified
Aluminum chloride	2	0	Unclassified	(Wahlberg and Boman 1985)	NA	NA	NA	NA	Unclassified
p-Aminobenzoic acid	1	33	Cat 1B	(Gad et al. 1986)	NA	NA	NA	NA	Cat 1B
p-Aminobenzoic acid	1	0	Unclassified	(Wahlberg and Boman 1985)	NA	NA	NA	NA	
3-Aminophenol	1	100	Cat 1A	(Basketter and Scholes 1992)	NA	NA	NA	NA	Cat 1A
Amylcinnamic aldehyde	5	22	Unclassified	(Wahlberg and Boman 1985)	30	100	Cat 1B	(Kimber et al. 2003)	Cat 1B
Aniline	1.5	10	Unclassified	(Wahlberg and Boman 1985)	NA	NA	NA	NA	Cat 1B
Aniline	0.5	90	Cat 1B	(Basketter and Scholes 1992)	NA	NA	NA	NA	
A SC600	NA	NA	NA	NA	100	0	Unclassified	(Debruyne 2007)	Unclassified
Atrazine	30	0	Unclassified	(ECPA 2006)	NA	NA	NA	NA	Unclassified
Benzalkonium chloride	1	0	Unclassified	(Gad et al. 1986)	NA	NA	NA	NA	Unclassified

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Chemical Name	Guinea Pig Maximization Test				Buehler Test				Most Prevalent GHS Potency Category
	i.d. Induction Conc. (%)	Sensiti-zation Incidence (%)	GHS Potency Category	Reference	Topical Induction Conc. (%)	Sensiti-zation Incidence (%)	GHS Potency Category	Reference	
Benzocaine	2	30	Cat 1B	(Wahlberg and Boman 1985)	50	20	Cat 1B	(Basketter et al. 1993)	Cat 1B
Benzocaine	25	60	Cat 1B	(Wahlberg and Boman 1985)	NA	NA	NA	NA	
Benzocaine	1	50	Cat 1B	(Basketter and Scholes 1992)	NA	NA	NA	NA	
Benzocaine	2	28	Unclassified	(Wahlberg and Boman 1985)	NA	NA	NA	NA	
Benzocaine	0.10	5	Unclassified	(Wahlberg and Boman 1985)	NA	NA	NA	NA	
Benzocaine	1	0	Unclassified	(Wahlberg and Boman 1985)	NA	NA	NA	NA	
Benzoic acid	10	0	Unclassified	(Gad et al. 1986)	20	0	Unclassified	(Gad et al. 1986)	Unclassified
Benzoquinone	0.005	100	Cat 1A	(Basketter and Scholes 1992)	NA	NA	NA	NA	Cat 1A
Benzoyl peroxide	NA	NA	NA	NA	10	42	Cat 1B	(Gad et al. 1986)	Cat 1B
Benzyl alcohol	2	< 30	Unclassified	(Wahlberg and Boman 1985)	NA	NA	NA	NA	Unclassified
Benzyl cinnamate	5	> 30	Cat 1B	(Wahlberg and Boman 1985)	NA	NA	NA	NA	Cat 1B
Benzyl salicylate	1	0	Unclassified	(Wahlberg and Boman 1985)	NA	NA	NA	NA	Unclassified
Butyl acrylate	6.4	70	Cat 1B	(Van Der Walle et al. 1982)	NA	NA	NA	NA	Cat 1B
Butyl glycidyl ether	10	50	Cat 1B	(Wahlberg and Boman 1985)	NA	NA	NA	NA	Cat 1B
Chloramine T	0.1	90	Cat 1A	(Basketter and Scholes 1992)	2.5	70	Cat 1A	(Kimber et al. 2003)	Cat 1A
4-Chloroaniline	0.30	50	Cat 1B	(Basketter and Scholes 1992)	NA	NA	NA	NA	Cat 1B

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Chemical Name	Guinea Pig Maximization Test				Buehler Test				Most Prevalent GHS Potency Category
	i.d. Induction Conc. (%)	Sensiti-zation Incidence (%)	GHS Potency Category	Reference	Topical Induction Conc. (%)	Sensiti-zation Incidence (%)	GHS Potency Category	Reference	
(Chloro)methyl-isothiazolinone	0.0001	100	Cat 1A	(Basketter et al. 2005)	0.05	100	Cat 1A	(Chan et al. 1983)	Cat 1A
(Chloro)methyl-isothiazolinone	1 ppm	100	Cat 1A	(Kimber et al. 2003)	0.05	30	Cat 1A	(Chan et al. 1983)	
(Chloro)methyl-isothiazolinone	NA	NA	NA	NA	0.05	0	Unclassified	(Chan et al. 1983)	
(Chloro)methyl-isothiazolinone	NA	NA	NA	NA	0.01	60	Cat 1A	(Chan et al. 1983)	
(Chloro)methyl-isothiazolinone	NA	NA	NA	NA	0.01	7	Unclassified	(Chan et al. 1983)	
(Chloro)methyl-isothiazolinone	NA	NA	NA	NA	0.005	13	Unclassified	(Chan et al. 1983)	
(Chloro)methyl-isothiazolinone	NA	NA	NA	NA	0.005	7	Unclassified	(Chan et al. 1983)	
(Chloro)methyl-isothiazolinone	NA	NA	NA	NA	0.005	0	Unclassified	(Chan et al. 1983)	
(Chloro)methyl-isothiazolinone	NA	NA	NA	NA	0.2	100	Cat 1A	(Chan et al. 1983)	
(Chloro)methyl-isothiazolinone	NA	NA	NA	NA	0.2	20	Cat 1A	(Chan et al. 1983)	
(Chloro)methyl-isothiazolinone	NA	NA	NA	NA	0.2	0	Unclassified	(Chan et al. 1983)	
(Chloro)methyl-isothiazolinone	NA	NA	NA	NA	0.1	80	Cat 1A	(Chan et al. 1983)	
(Chloro)methyl-isothiazolinone	NA	NA	NA	NA	0.1	20	Cat 1A	(Chan et al. 1983)	
(Chloro)methyl-isothiazolinone	NA	NA	NA	NA	0.1	0	Unclassified	(Chan et al. 1983)	

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Chemical Name	Guinea Pig Maximization Test				Buehler Test				Most Prevalent GHS Potency Category
	i.d. Induction Conc. (%)	Sensiti-zation Incidence (%)	GHS Potency Category	Reference	Topical Induction Conc. (%)	Sensiti-zation Incidence (%)	GHS Potency Category	Reference	
Cinnamic aldehyde	5	100	Cat 1B	(Wahlberg and Boman 1985)	10	80	Cat 1A	(Basketter and Gerberick 1996)	Cat 1A
Cinnamic aldehyde	0.20	100	Cat 1A	(Basketter and Scholes 1992)	NA	NA	NA	NA	
Cinnamic aldehyde	2	80	Cat 1B	(Wahlberg and Boman 1985)	NA	NA	NA	NA	
Cinnamyl alcohol	5	0	Unclassified	(Wahlberg and Boman 1985)	30	50	Cat 1B	(Robinson et al. 1990)	Cat 1B
C.I. Reactive Red 231	1	~50	Cat 1B	(Haist et al. 2007)	NA	NA	NA	NA	Cat 1B
C.I. Reactive Red 231	1	~50	Cat 1B	(Haist et al. 2007)	NA	NA	NA	NA	
C.I. Reactive Red 231	1	0	Unclassified	(Haist et al. 2007)	NA	NA	NA	NA	
C.I. Reactive Yellow 174	5	11	Unclassified	(Haist et al. 2007)	NA	NA	NA	NA	Unclassified
Citral	0.2	50	Cat 1B	(Basketter and Scholes 1992)	NA	NA	NA	NA	Cat 1B
Cobalt (II) salts[1]	0.25	100	Cat 1A	(Basketter and Scholes 1992)	NA	NA	NA	NA	Cat 1A
D EC25®	NA	NA	NA	NA	2.5	0	Unclassified	(Debruyne 2007)	Unclassified
D EW 15	NA	NA	NA	NA	100	0	Unclassified	(Debruyne 2007)	Unclassified
Dextran	1.00	0	Unclassified	(Basketter and Scholes 1992)	NA	NA	NA	NA	Unclassified
1,2 Dibromo-2,4-dicyanobutane	0.10	20	Unclassified	(Basketter et al. 2005)	5	5	Unclassified	(Basketter et al. 1999)	Unclassified

[1] Test substance was cobalt chloride.

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Chemical Name	Guinea Pig Maximization Test				Buehler Test				Most Prevalent GHS Potency Category
	i.d. Induction Conc. (%)	Sensiti-zation Incidence (%)	GHS Potency Category	Reference	Topical Induction Conc. (%)	Sensiti-zation Incidence (%)	GHS Potency Category	Reference	
Diethyl phthalate	5	< 30	Unclassified	(Wahlberg and Boman 1985)	NA	NA	NA	NA	Unclassified
Dihydrocoumarin	20	100	Cat 1B	(Wahlberg and Boman 1985)	NA	NA	NA	NA	Cat 1B
1,4-Dihydroquinone	2	100	Cat 1B	(Basketter and Scholes 1992)	NA	NA	NA	NA	Cat 1B
1,4-Dihydroquinone	2	70	Cat 1B	(Goodwin et al. 1981)	NA	NA	NA	NA	
1,4-Dihydroquinone	5.5	50	Cat 1B	(Van Der Walle et al. 1982)	NA	NA	NA	NA	NA
5,5-Dimethyl-3-methylenedihydro-2(3H)-furanone	NR	NR	Unclassified	(Basketter et al. 1999)	NA	NA	NA	NA	Unclassified
Dimethyl sulfoxide	100	0	Unclassified	(Gad et al. 1986)	NA	NA	NA	NA	Unclassified
2,4-Dinitrochlorobenzene	0.10	100	Cat 1A	(Wahlberg and Boman 1985)	0.05	100	Cat 1A	(Buehler 1985)	Cat 1A
2,4-Dinitrochlorobenzene	0.02	100	Cat 1A	(Goodwin et al. 1981)	0.05	30	Cat 1A	(Buehler 1985)	
2,4-Dinitrochlorobenzene	0.05	100	Cat 1A	(Basketter and Scholes 1992)	0.05	0	Unclassified	(Buehler 1985)	
2,4-Dinitrochlorobenzene	0.10	75	Cat 1A	(Wahlberg and Boman 1985)	0.025	60	Cat 1A	(Buehler 1985)	
2,4-Dinitrochlorobenzene	0.5	90	Cat 1A	(Kimber et al. 1991)	0.01	40	Cat 1A	(Buehler 1985)	
2,4-Dinitrochlorobenzene	NA	NA	NA	NA	0.1	100	Cat 1A	(Buehler 1985)	
2,4-Dinitrochlorobenzene	NA	NA	NA	NA	0.3	100	Cat 1A	(Buehler 1985)	
2,4-Dinitrochlorobenzene	NA	NA	NA	NA	0.3	50	Cat 1B	(Buehler 1985)	

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Chemical Name	Guinea Pig Maximization Test				Buehler Test				Most Prevalent GHS Potency Category
	i.d. Induction Conc. (%)	Sensiti-zation Incidence (%)	GHS Potency Category	Reference	Topical Induction Conc. (%)	Sensiti-zation Incidence (%)	GHS Potency Category	Reference	
Dinocap EC	NA	NA	NA	NA	5	55	Cat 1B	(ECPA 2006)	Cat 1B
Ethyl acrylate	5	0	Unclassified	(Wahlberg and Boman 1985)	NA	NA	NA	NA	Unclassified
Ethylenediamine	0.3	90	Cat 1A	(Goodwin et al. 1981)	1	80	Cat 1A	(Basketter and Gerberick 1996)	Cat 1A
Ethylenediamine	0.50	70	Cat 1A	(Gad et al. 1986)	0.5	50	Cat 1B	(Gad et al. 1986)	
Ethylenediamine	0.50	60	Cat 1A	(Wahlberg and Boman 1985)	NA	NA	NA	NA	NA
Ethylene glycol dimethacrylate	5	0	Unclassified	(Wahlberg and Boman 1985)	NA	NA	NA	NA	Unclassified
Eugenol	0.05	60	Cat 1A	(Basketter and Scholes 1992)	100	11	Unclassified	(Basketter and Gerberick 1996)	Cat 1A
Eugenol	0.05	67	Cat 1A	(Kimber et al. 1991)	75	0	Unclassified	(Basketter and Gerberick 1996)	
Eugenol	5.00	50	Cat 1B	(Wahlberg and Boman 1985)	NA	NA	NA	NA	
EXP 10810 A	0.1	100	Cat 1A	(Debruyne 2007)	50	10	Unclassified	(Debruyne 2007)	Cat 1A
EXP 11120 A	NA	NA	NA	NA	100	0	Unclassified	(Debruyne 2007)	Unclassified
FAR01042-00	NA	NA	NA	NA	100	0	Unclassified	(Debruyne 2007)	Unclassified
FAR01060-00	NA	NA	NA	NA	100	0	Unclassified	(Debruyne 2007)	Unclassified
Fatty acid glutamate	NR	NR	Unclassified	(TNO 2006)	NA	NA	NA	NA	Unclassified

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Chemical Name	Guinea Pig Maximization Test				Buehler Test				Most Prevalent GHS Potency Category
	i.d. Induction Conc. (%)	Sensiti-zation Incidence (%)	GHS Potency Category	Reference	Topical Induction Conc. (%)	Sensiti-zation Incidence (%)	GHS Potency Category	Reference	
Fatty alcohol #1	5	0	Unclassified	(TNO 2006)	NA	NA	NA	NA	Unclassified
Fatty alcohol #2	5	0	Unclassified	(TNO 2006)	NA	NA	NA	NA	Unclassified
F & Fo WG 50 + 25	NA	NA	NA	NA	30	0	Unclassified	(Debruyne 2007)	Unclassified
F & Fo WG 50 + 25	NA	NA	NA	NA	15	0	Unclassified	(Debruyne 2007)	
F & Fo WG 50 + 25	NA	NA	NA	NA	5	0	Unclassified	(Debruyne 2007)	
Formaldehyde	0.25	100	Cat 1A	(Kimber et al. 1991)	5	30	Cat 1B	(Gad et al. 1986)	Cat 1B
Formaldehyde	0.1	89.5	Cat 1A	(Wahlberg and Boman 1985)	2.0	30	Cat 1B	(Basketter and Gerberick 1996)	
Formaldehyde	1	0	Unclassified	(Wahlberg and Boman 1985)	7.5	60	Cat 1A	(Buehler 1985)	
Formaldehyde	0.50	90	Cat 1A	(Basketter and Scholes 1992)	7.5	8	Unclassified	(Buehler 1985)	
Formaldehyde	1	10	Unclassified	(Wahlberg and Boman 1985)	5	30	Cat 1B	(Buehler 1985)	
Formaldehyde	1	50	Cat 1B	(Wahlberg and Boman 1985)	1.9	25	Cat 1B	(Buehler 1985)	
Formaldehyde	NA	NA	NA	NA	3.8	45	Cat 1B	(Buehler 1985)	
Formaldehyde	NA	NA	NA	NA	0.9	0	Unclassified	(Buehler 1985)	
Formaldehyde	NA	NA	NA	NA	7.5	42	Cat 1B	(Buehler 1985)	
Formaldehyde	NA	NA	NA	NA	7.5	17	Cat 1B	(Buehler 1985)	
Fumaric acid	5	0	Unclassified	(EFfCI 2006)	NA	NA	NA	NA	Unclassified
Fx + Me EW 69	NA	NA	NA	NA	100	0	Unclassified	(Debruyne 2007)	Unclassified

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Chemical Name	Guinea Pig Maximization Test				Buehler Test				Most Prevalent GHS Potency Category
	i.d. Induction Conc. (%)	Sensiti-zation Incidence (%)	GHS Potency Category	Reference	Topical Induction Conc. (%)	Sensiti-zation Incidence (%)	GHS Potency Category	Reference	
Geraniol	5	> 30	Cat 1B	(Wahlberg and Boman 1985)	NA	NA	NA	NA	Cat 1B
Glutaraldehyde	NA	NA	NA	NA	5	23	Cat 1B	(Gad et al. 1986)	Cat 1B
Glycerol	100	0	Unclassified	(Gad et al. 1986)	100	0	Unclassified	(Gad et al. 1986)	Unclassified
Glyceryl thioglycollate	NR	NR	Unclassified	(TNO 2006)	NA	NA	NA	NA	Unclassified
Hexyl cinnamic aldehyde	0.50	60	Cat 1A	(Basketter et al. 2005)	50	60	Cat 1B	(Basketter and Gerberick 1996)	Cat 1A
Hexyl cinnamic aldehyde	NA	NA	NA	NA	20	70	Cat 1A	(Basketter and Gerberick 1996)	
4-Hydroxybenzoic acid	1	40	Cat 1B	(Goodwin et al. 1981)	NA	NA	NA	NA	Cat 1B
4-Hydroxybenzoic acid	1	20	Unclassified	(Basketter and Scholes 1992)	NA	NA	NA	NA	
Hydroxycitronellal	0.5	60	Cat 1A	(Basketter and Scholes 1992)	30	25	Cat 1B	(Buehler 1985)	Cat 1B
Hydroxycitronellal	5	30	Cat 1B	(Wahlberg and Boman 1985)	30	13	Unclassified	(Buehler 1985)	
Hydroxycitronellal	5	27	Unclassified	(Wahlberg and Boman 1985)	50	20	Cat 1B	(Buehler 1985)	
Hydroxycitronellal	20	27	Unclassified	(Gad et al. 1986)	20	0	Unclassified	(Gad et al. 1986)	
2-Hydroxyethyl acrylate	0.25	70	Cat 1A	(Scholes et al. 1992)	NA	NA	NA	NA	Cat 1A
2-Hydroxypropyl methacrylate	1	0	Unclassified	(Basketter and Scholes 1992)	NA	NA	NA	NA	Unclassified
Imidazolidinyl urea	2.5	80	Cat 1B	(Basketter and Scholes 1992)	NA	NA	NA	NA	Cat 1B

ED_006472_00084346-00316

Chemical Name	Guinea Pig Maximization Test				Buehler Test				Most Prevalent GHS Potency Category
	i.d. Induction Conc. (%)	Sensiti-zation Incidence (%)	GHS Potency Category	Reference	Topical Induction Conc. (%)	Sensiti-zation Incidence (%)	GHS Potency Category	Reference	
Isoeugenol	0.15	100	Cat 1A	(Basketter and Scholes 1992)	NA	NA	NA	NA	Cat 1A
Isoeugenol	1	100	Cat 1A	(Wahlberg and Boman 1985)	NA	NA	NA	NA	
Isopropanol	NR	NR	Unclassified	(Basketter et al. 1999)	NA	NA	NA	NA	Unclassified
d-Limonene	5	> 30	Cat 1B	(Wahlberg and Boman 1985)	NA	NA	NA	NA	Cat 1B
Linoleic acid	5	20	Unclassified	(EFfCI 2006)	NA	NA	NA	NA	Unclassified
Linolenic acid	5	10	Unclassified	(EFfCI 2006)	NA	NA	NA	NA	Unclassified
Maleic acid	0.5	0	Unclassified	(EFfCI 2006)	NA	NA	NA	NA	Unclassified
2-Mercaptobenzothiazole	0.4	80	Cat 1A	(Basketter and Scholes 1992)	75	55	Cat 1B	(Basketter et al. 1994; Basketter and Gerberick 1996)	Cat 1A
2-Mercaptobenzothiazole	0.4	60	Cat 1A	(Goodwin et al. 1981)	NA	NA	NA	NA	
2-Mercaptobenzothiazole	1	40	Cat 1B	(Magnusson and Kligman 1969)	NA	NA	NA	NA	
Mercuric (II) chloride	0.10	32	Cat 1A	(Wahlberg and Boman 1985)	NA	NA	NA	NA	Cat 1A
4-Methylaminophenol sulfate	0.5	90	Cat 1A	(Basketter and Scholes 1992)	NA	NA	NA	NA	Cat 1A
alpha-Methyl cinnamic aldehyde	5	90	Cat 1B	(Wahlberg and Boman 1985)	NA	NA	NA	NA	Cat 1B
Methyl dodecanesulfonate	0.5	100	Cat 1A	(Basketter and Scholes 1992)	NA	NA	NA	NA	Cat 1A

ED_006472_00084346-00317

Chemical Name	Guinea Pig Maximization Test				Buehler Test				Most Prevalent GHS Potency Category
	i.d. Induction Conc. (%)	Sensiti-zation Incidence (%)	GHS Potency Category	Reference	Topical Induction Conc. (%)	Sensiti-zation Incidence (%)	GHS Potency Category	Reference	
Methyl methacrylate	5	30	Cat 1B	(Gad et al. 1986)	5	25	Cat 1B	(Gad et al. 1986)	Cat 1B
Methyl methacrylate	5	15.4	Unclassified	(Wahlberg and Boman 1985)	NA	NA	NA	NA	
Methyl methacrylate	5	0	Unclassified	(Wahlberg and Boman 1985)	NA	NA	NA	NA	
Methyl methacrylate	0.15	0	Unclassified	(Wahlberg and Boman 1985)	NA	NA	NA	NA	
Methyl methacrylate	0.001	0	Unclassified	(Wahlberg and Boman 1985)	NA	NA	NA	NA	
Methyl methacrylate	5	76.9	Cat 1B	(Wahlberg and Boman 1985)	NA	NA	NA	NA	
Methyl methacrylate	5	20	Unclassified	(Wahlberg and Boman 1985)	NA	NA	NA	NA	
Methyl 2-nonynoate	5	>30	Cat 1B	(Wahlberg and Boman 1985)	NA	NA	NA	NA	Cat 1B
Methyl 2-octynoate	5	>30	Cat 1B	(Wahlberg and Boman 1985)	NA	NA	NA	NA	Cat 1B
Methyl salicylate	2.5	0	Unclassified	(Basketter and Scholes 1992)	NA	NA	NA	NA	Unclassified
Methyl salicylate	5	<30	Unclassified	(Wahlberg and Boman 1985)	NA	NA	NA	NA	
NAVY 14 08 723	5	100	Cat 1B	(Haist et al. 2007)	NA	NA	NA	NA	Cat 1B
NAVY 14 08 723	5	95	Cat 1B	(Haist et al. 2007)	NA	NA	NA	NA	
NAVY 14 08 723	5	80	Cat 1B	(Haist et al. 2007)	NA	NA	NA	NA	
Neomycin sulfate	2	30	Cat 1B	(Wahlberg and Boman 1985)	NA	NA	NA	NA	Cat 1B

ED_006472_00084346-00318

Chemical Name	Guinea Pig Maximization Test				Buehler Test				Most Prevalent GHS Potency Category
	i.d. Induction Conc. (%)	Sensiti-zation Incidence (%)	GHS Potency Category	Reference	Topical Induction Conc. (%)	Sensiti-zation Incidence (%)	GHS Potency Category	Reference	
Nickel (II) salts	0.25	30	Cat 1B	(Wahlberg and Boman 1985)	0.1	0	Unclassified	(Gad et al. 1986)	Cat 1B
Nickel (II) salts[2]	1	53.3	Cat 1B	(Wahlberg and Boman 1985)	NA	NA	NA	NA	
Nickel (II) salts[3]	0.25	0	Unclassified	(Goodwin et al. 1981)	NA	NA	NA	NA	
Nickel (II) salts[4]	0.1	35	Cat 1A	(Wahlberg and Boman 1985)	NA	NA	NA	NA	
Nickel (II) salts	0.25	30	Cat 1B	(Basketter and Scholes 1992)	NA	NA	NA	NA	
Nickel (II) salts[5]	0.25	10	Unclassified	(Goodwin et al. 1981)	NA	NA	NA	NA	
Nickel (II) salts[6]	1	55	Cat 1B	(Wahlberg and Boman 1985)	NA	NA	NA	NA	
Nickel (II) salts[7]	2	50	Cat 1B	(Wahlberg and Boman 1985)	NA	NA	NA	NA	
Nickel (II) salts	5	55	Cat 1B	(Gad et al. 1986)	NA	NA	NA	NA	
Octinol	0.25	0	Unclassified	(EFfCI 2006)	NA	NA	NA	NA	Unclassified
Oleic acid	5	20	Unclassified	(EFfCI 2006)	NA	NA	NA	NA	Unclassified
Oxalic Acid	NR	NR	Unclassified	(Montelius et al. 1994)	NA	NA	NA	NA	Unclassified
Oxazolone	NA	NA	NA	NA	1	100	Cat 1A	(Basketter and Gerberick 1996)	Cat 1A

[2] Test substance was nickel chloride.
[3] Test substance was nickel chloride.
[4] Test substance was nickel sulfate.
[5] Test substance was nickel sulfate.
[6] Test substance was nickel sulfate.
[7] Test substance was nickel sulfate.

ED_006472_00084346-00319

Chemical Name	Guinea Pig Maximization Test				Buehler Test				Most Prevalent GHS Potency Category
	i.d. Induction Conc. (%)	Sensiti-zation Incidence (%)	GHS Potency Category	Reference	Topical Induction Conc. (%)	Sensiti-zation Incidence (%)	GHS Potency Category	Reference	
Oxyfluorfen EC	5	26	Unclassified	(ECPA 2006)	NA	NA	NA	NA	Unclassified
Penicillin G	0.3	74	Cat 1A	(Wahlberg and Boman 1985)	NA	NA	NA	NA	Cat 1A
Penicillin G	1	100	Cat 1A	(Basketter and Scholes 1992)	NA	NA	NA	NA	
Penicillin G	3	100	Cat 1B	(Wahlberg and Boman 1985)	NA	NA	NA	NA	
Penicillin G	25	100	Cat 1B	(Wahlberg and Boman 1985)	NA	NA	NA	NA	
Pentaerythritol triacrylate	0.05	87	Cat 1A	(Nethercott 1978)	NA	NA	NA	NA	Cat 1A
Pentaerythritol triacrylate	0.1875	53	Cat 1B	(Wahlberg and Boman 1985)	NA	NA	NA	NA	
Pentaerythritol triacrylate	0.375	100	Cat 1A	(Wahlberg and Boman 1985)	NA	NA	NA	NA	
Pentaerythritol triacrylate	1	67	Cat 1A	(Wahlberg and Boman 1985)	NA	NA	NA	NA	
4-Phenylenediamine	0.25	100	Cat 1A	(Basketter and Scholes 1992)	2	100	Cat 1A	(Buehler 1985)	Cat 1A
4-Phenylenediamine	1	100	Cat 1A	(Kimber et al. 1991)	10	90	Cat 1A	(Basketter and Gerberick 1996)	
4-Phenylenediamine	10	80	Cat 1B	(Wahlberg and Boman 1985)	NA	NA	NA	NA	
Phthalic anhydride	0.10	90	Cat 1A	(Basketter and Scholes 1992)	20	78	Cat 1A	(Gad et al. 1986)	Cat 1A
Phthalic anhydride	NA	NA	NA	NA	25	30	Cat 1B	(Kimber et al. 2003)	

ED_006472_00084346-00320

Chemical Name	Guinea Pig Maximization Test				Buehler Test				Most Prevalent GHS Potency Category
	i.d. Induction Conc. (%)	Sensiti-zation Incidence (%)	GHS Potency Category	Reference	Topical Induction Conc. (%)	Sensiti-zation Incidence (%)	GHS Potency Category	Reference	
Potassium dichromate	0.05	90	Cat 1A	(Basketter and Scholes 1992)	10	20	Cat 1B	(Kimber et al. 2003)	Cat 1A
Potassium dichromate	0.25	100	Cat 1A	(Wahlberg and Boman 1985)	NA	NA	NA	NA	
Potassium dichromate	0.5	90	Cat 1A	(Kimber et al. 1991)	NA	NA	NA	NA	
Potassium dichromate	1	75	Cat 1A	(Gad et al. 1986)	NA	NA	NA	NA	
Produkt P-4G	5	90	Cat 1B	(Haist et al. 2007)	NA	NA	NA	NA	Cat 1B
Propylene glycol	100	0	Unclassified	(Wahlberg and Boman 1985)	NA	NA	NA	NA	Unclassified
Propyl gallate	0.35	100	Cat 1A	(Basketter and Scholes 1992)	NA	NA	NA	NA	Cat 1A
Propylparaben	0.5	0	Unclassified	(Basketter and Scholes 1992)	NA	NA	NA	NA	Unclassified
Quinoxyfen/cyproconazole	NA	NA	NA	NA	10	47	Cat 1B	(ECPA 2007b)	Cat 1B
Quinoxyfen SC	NA	NA	NA	NA	10	0	Unclassified	(ECPA 2006)	Unclassified
Salicylic acid	10	0	Unclassified	(Gad et al. 1986)	NA	NA	NA	NA	Unclassified
Sodium lauryl sulfate	1	0	Unclassified	(Wahlberg and Boman 1985)	0.1	0	Unclassified	(Gad et al. 1986)	Unclassified
Squalene	5	20	Unclassified	(EFfCI 2006)	NA	NA	NA	NA	Unclassified
Streptomycin	10	72	Cat 1B	(Wahlberg and Boman 1985)	NA	NA	NA	NA	Cat 1B
Succinic acid	0.5	0	Unclassified	(EFfCI 2006)	NA	NA	NA	NA	Unclassified
Sulfanilamide	NR	10	Unclassified	(Basketter et al. 1994)	NA	NA	NA	NA	Unclassified
Sulfanilic acid	0.1	90	Cat 1A	(Gad et al. 1986)	NA	NA	NA	NA	Cat 1A

ED_006472_00084346-00321

Chemical Name	Guinea Pig Maximization Test				Buehler Test				Most Prevalent GHS Potency Category
	i.d. Induction Conc. (%)	Sensiti-zation Incidence (%)	GHS Potency Category	Reference	Topical Induction Conc. (%)	Sensiti-zation Incidence (%)	GHS Potency Category	Reference	
Sulfanilic acid	0.5	80	Cat 1A	(Basketter and Scholes 1992)	NA	NA	NA	NA	NA
Tartaric acid	NR	NR	Unclassified	(Basketter et al. 1999)	NA	NA	NA	NA	Unclassified
Tetrachlorosalicylanilide	5	72	Cat 1B	(Wahlberg and Boman 1985)	1	80	Cat 1A	(Buehler 1985)	Cat 1A
Thioglycerol	1	45	Cat 1B	(Gad et al. 1986)	14	60	Cat 1A	(Buehler 1985)	Cat 1A
Toluene 2,4-diisocyanate	1	100	Cat 1A	(Gad et al. 1986)	10	95	Cat 1A	(Basketter and Gerberick 1996)	Cat 1A
Trifluralin EC	NA	NA	NA	NA	5	10	Unclassified	(ECPA 2007a)	Unclassified
Trimellitic anhydride	0.10	50	Cat 1A	(Basketter and Scholes 1992)	25	70	Cat 1B	(Kimber et al. 2003)	Cat 1A
Tween 80[8]	5	0	Unclassified	(Gad et al. 1986)	0.1	0	Unclassified	(Gad et al. 1986)	Unclassified
Undec-10-enal	5	<30	Unclassified	(Wahlberg and Boman 1985)	NA	NA	NA	NA	Unclassified
Undecylenic acid	1	40	Cat 1B	(EFfCI 2006)	NA	NA	NA	NA	Cat 1B
Vanillin	50	60	Cat 1B	(Gad et al. 1986)	2.5	40	Cat 1B	(Kimber et al. 2003)	Cat 1B
Vanillin	5	> 30	Cat 1B	(Basketter and Gerberick 1996)	NA	NA	NA	NA	

[8] Closed patch test results are from Landsteiner Draize test, not Buehler test.

ED_006472_00084346-00322

Chemical Name	Guinea Pig Maximization Test				Buehler Test				Most Prevalent GHS Potency Category
	i.d. Induction Conc. (%)	Sensiti-zation Incidence (%)	GHS Potency Category	Reference	Topical Induction Conc. (%)	Sensiti-zation Incidence (%)	GHS Potency Category	Reference	
YELLOW E-JD 3442	5	10	Unclassified	(Haist et al. 2007)	NA	NA	NA	NA	Unclassified
YELLOW E-JD 3442	5	10	Unclassified	(Haist et al. 2007)	NA	NA	NA	NA	
YELLOW E-JD 3442	5	90	Cat 1B	(Haist et al. 2007)	NA	NA	NA	NA	

Abbreviations: Cat = subcategory of the GHS classification for skin sensitizers; Conc. = concentration; EFfCI = European Federation for Cosmetic Ingredients; ECPA = European Crop Protection Association; GHS = Globally Harmonized System of Classification and Labelling of Chemicals (UN 2009); i.d. = intradermal; NA = not available; NR = not reported.

ED_006472_00084346-00323

References

Basketter DA, Andersen KE, Carola C, Van Loveren H, Boman A, Kimber I, et al. 2005. Evaluation of the skin sensitizing potency of chemicals by using the existing methods and considerations of relevance for elicitation. Contact Dermatitis 52(1): 39-43.

Basketter DA, Gerberick GF. 1996. An interlaboratory evaluation of the Buehler test for the identification and classification of skin sensitizers. Contact Dermatitis 35(3): 146-151.

Basketter DA, Lea LJ, Cooper K, Stocks J, Dickens A, Pate I, et al. 1999. Threshold for classification as a skin sensitizer in the local lymph node assay: a statistical evaluation. Food Chem Toxicol 37(12): 1167-1174.

Basketter DA, Scholes EW. 1992. Comparison of the local lymph node assay with the guinea-pig maximization test for the detection of a range of contact allergens. Food Chem Toxicol 30(1): 65-69.

Basketter DA, Scholes EW, Kimber I. 1994. The performance of the local lymph node assay with chemicals identified as contact allergens in the human maximization test. Food Chem Toxicol 32(6): 543-547.

Basketter DA, Selbie E, Scholes EW, Lees D, Kimber I, Botham PA. 1993. Results with OECD recommended positive control sensitizers in the maximization, Buehler and local lymph node assays. Food Chem Toxicol 31(1): 63-67.

Buehler EV. 1985. A rationale for the selection of occlusion to induce and elicit delayed contact hypersensitivity in the guinea pig. A prospective test. Curr Probl Dermatol 14: 39-58.

Chan PK, Baldwin RC, Parsons RD. 1983. Kathon biocide: manifestation of delayed contact dermatitis in guinea pigs is dependent on the concentration for induction and challenge. J Invest Dermatol 81(5): 409-411.

Debruyne E. 2007. Bayer Crop Science data submission to NICEATM.

ECPA. 2006. Oxyfluorfen EC, Atrazine SC, Quinoxyfen/Cyproconazole SC, Dinocap EC, Quinoxyfen SC and Trifluralin EC: Local Lymph Node Assay in CBA/J Mice. Report by Dow Chemical Company.

ECPA. 2007a. Murine Local Lymph Node Assay: Summary Report. Trifluralin Emulsion Concentrate. Project No. 58H0513/062137. Report by BASF.

ECPA. 2007b. Oxyfluorfen Emulsion Concentrate, Quinoxyfen/Cyproconazole Suspension Concentrate, Dinocap Emulsion Concentrate, and Trifluralin Emulsion Concentrate: Local Lymph Node Assay (LLNA) in Mice. Study No. 20234. Study sponsor: CropLife International/ECPA; report by DuPont.

EFfCI. 2006. Comparative Experimental Study on the Skin Sensitising Potential of Selected Unsaturated Chemicals as Asssessed by the Murine Local Lymph Node Assay (LLNA) and the Guinea Pig Maximisation Test (GPMT). European Federation for Cosmetic Ingredients.

ED_006472_00084346-00324

Gad SC, Dunn BJ, Dobbs DW. 1986. Development and validation of an alternative dermal sensitization test: the mouse ear swelling test (MEST). Toxicol Appl Pharmacol 84(1): 93-114.

Goodwin BFJ, Crevel RWR, Johnson AW. 1981. A comparison of three guinea-pig sensitization procedures for the detection of 19 reported human contact sensitizers. Contact Dermatitis 7(5): 248-258.

Haist I, Brummer D, Albrecht A. 2007. Untersuchung von Textilfarbstoffen im Local Lymph Node Assay (LLNA). Forschung Projekt 1877. Dortmund/Berlin/Dresden.

Hausen BM, Jensen S, Mohnert J. 1989. Contact allergy to colophony. (IV). The sensitizing potency of commercial products: an investigation of French and American modified colophony derivatives. Contact Dermatitis 20(2): 133-143.

Karlberg AT, Boman A, Wahlberg JE. 1980. Allergenic potential of abietic acid, colophony and pine resin-HA. Clinical and experimental studies. Contact Dermatitis 6(7): 481-487.

Kimber I, Basketter DA, Butler M, Gamer A, Garrigue JL, Gerberick GF, et al. 2003. Classification of contact allergens according to potency: proposals. Food Chem Toxicol 41(12): 1799-1809.

Kimber I, Hilton J, Botham PA, Basketter DA, Scholes EW, Miller K, et al. 1991. The murine local lymph node assay: results of an inter-laboratory trial. Toxicol Lett 55(2): 203-213.

Magnusson B, Kligman AM. 1969. The identification of contact allergens by animal assay. The guinea pig maximization test. J Invest Dermatol 52(3): 268-276.

Montelius J, Wahlkvist H, Boman A, Fernström P, Gråbergs L, Wahlberg JE. 1994. Experience with the murine local lymph node assay: inability to discriminate between allergens and irritants. Acta Derm Venereol 74(1): 22-27.

Nethercott JR. 1978. Skin problems associated with multifunctional acrylic monomers in ultraviolet curing inks. Br J Dermatol 98(5): 541-552.

Robinson MK, Nusair TL, Fletcher ER, Ritz HL. 1990. A review of the Buehler guinea pig skin sensitization test and its use in a risk assessment process for human skin sensitization. Toxicology 61(2): 91-107.

Scholes EW, Basketter DA, Sarll AE, Kimber I, Evans CD, Miller K, et al. 1992. The local lymph node assay: results of a final inter-laboratory validation under field conditions. J Appl Toxicol 12(3): 217-222.

TNO. 2006. Limitations of the local lymph node assay (LLNA) as preferred test for skin sensitization: concerns about false positive and false negative test results. V 7217. Zeist, The Netherlands:CESIO (European Committee of Organic Surfactants and their Intermediates).

UN. 2009. Globally Harmonized System for Classification and Labelling of Chemicals. 3rd rev. ed. New York:United Nations. Available: http://www.unece.org/trans/danger/publi/ghs/ghs_rev03/03files_e.html [accessed 21 October 2010].

ED_006472_00084346-00325

Van Der Walle HB, Klecak G, Geleick H, Bensink T. 1982. Sensitizing potential of 14 mono (meth) acrylates in the guinea pig. Contact Dermatitis 8(4): 223-235.

Wahlberg JE, Boman A. 1985. Guinea pig maximization test. Curr Probl Dermatol 14: 59-106.

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Annex II-4

Summary LLNA, Human, and Guinea Pig Data Used in the Regression and Classification Rate Analyses

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Chemical Name	LLNA Results			Human Results		Guinea Pig Results	
	N	GM[1] EC3 (%)	GM[1] EC3 (µg/cm^2)	N	GM[1] DSA_{05} (µg/cm^2)	N	Overall[2] GHS Category
Abietic acid	5	12	2909	NA	NA	6	Cat 1B
Acetyl isovaleryl	1	26	6450	2	3258	NA	NA
AE F016382 00 TK71 A101	1	NC	NC	NA	NA	1	Unclassified
alpha-Amylcinnamyl alcohol	2	NC	NC	1	12308	NA	NA
alpha-Methyl cinnamic aldehyde	1	4.5	1125	2	-	1	Cat 1B
Aluminum chloride	1	NC	NC	NA	-	1	Unclassified
p-Aminobenzoic acid	1	NC	NC	1	-	2	Cat 1B
3-Aminophenol	2	2.4	600	NA	NA	1	Cat 1A
Amylcinnamic aldehyde	5	11	2699	1	-	2	Cat 1B
Aniline	5	33	8129	1	2463	2	Cat 1B
Anisyl alcohol	1	5.9	1475	1	-	NA	NA
A SC600	1	NC	NC	NA	NA	1	Unclassified
Atrazine	2	36	8989	NA	NA	1	Unclassified
Basil oil	1	6.2	1550	1	-	NA	NA
Benzalkonium chloride	1	0.07	17	2	-	1	Unclassified
Benzocaine	7	7.8	1948	4	10140	7	Cat 1B
Benzoic acid	1	NC	NC	1	-	2	Unclassified
Benzoisothiazolione	4	7.79	1948	1	50	NA	NA
Benzoquinone	1	0.01	2.475	NA	NA	1	Cat 1A
Benzoyl peroxide	6	0.07	17	5	1283	1	Cat 1B
Benzyl alcohol	1	NC	NC	1	48670	1	Unclassified
Benzylbenzoate	1	17	4250	2	-	NA	NA
Benzyl cinnamate	1	18	4600	2	-	1	Cat 1B
Benzylidene acetone	1	3.70	925	2	299	NA	NA
Benzyl salicylate	1	2.9	725	2	-	1	Unclassified
Beryllium sulfate	1	0.68	170	1	11	NA	NA
Bourgeonal	1	4.3	1075	1	1541	NA	NA

ED_006472_00084346-00329

Chemical Name	LLNA Results			Human Results		Guinea Pig Results	
	N	GM[1] EC3 (%)	GM[1] EC3 (µg/cm^2)	N	GM[1] DSA_{05} (µg/cm^2)	N	Overall[2] GHS Category
Butanol	1	NC	NC	NA	-	NA	NA
Butyl acrylate	1	11	2750	NA	NA	1	Cat 1B
Butyl glycidyl ether	1	30.9	7725	1	437	1	Cat 1B
C.I. Reactive Red 231	1	0.6	150	NA	NA	3	Cat 1B
C.I. Reactive Yellow 174	1	0.3	75	NA	NA	1	Unclassified
Carvone	4	10	2514	1	19284	NA	NA
Chloramine T	1	0.40	100	NA	NA	2	Cat 1A
4-Chloroaniline	2	NC	NC	NA	NA	1	Cat 1B
(Chloro)methylisothiazolinone	13	0.01	2.8	5	5.0	16	Cat 1A
Chorpromazine	2	5.8	1455	1	1149	NA	NA
Cinnamic aldehyde	27	1.0	254	5	382	4	Cat 1A
Cinnamyl alcohol	2	20	5007	6	3002	2	Cat 1B
Cinnamyl nitrile	1	NC	NC	1	1828	NA	NA
Citral	16	5.0	1246	5	915	1	Cat 1B
Citronella oil	1	NC	NC	1	-	NA	NA
dl-Citronellol	1	44	10875	1	1429	NA	NA
Clove oils	3	7.1	1775	3	-	NA	NA
Cobalt (II) salts	2	0.57	141	2	279	1	Cat 1A
Copper (II) chloride	1	0.40	100	NA	-	NA	NA
Coumarin	2	30	7395	2	14523	NA	NA
Cyclamen aldehyde	1	22	5575	1	-	NA	NA
Damascone	2	1.2	307	1	-	NA	NA
t-alpha Damascone	1	3.3	825	1	-	NA	NA
trans beta Damascone	1	2.4	600	2	-	NA	NA
delta Damascone	3	3.51	877	1	193	NA	NA
D EC25	1	NC	NC	NA	NA	1	Unclassified
D EW 15	1	NC	NC	NA	NA	1	Unclassified

ED_006472_00084346-00330

Chemical Name	LLNA Results			Human Results		Guinea Pig Results	
	N	GM[1] EC3 (%)	GM[1] EC3 (µg/cm^2)	N	GM[1] DSA_{05} (µg/cm^2)	N	Overall[2] GHS Category
d-Limonene	6	32	32	2	-	1	Cat 1B
Dextran	1	NC	NC	NA	NA	1	Unclassified
Dibromodicyanobutane	6	1.9	480	NA	NA	2	Unclassified
Diethyl phthalate	1	NC	NC	1	-	1	Unclassified
Diethylenetriamine	1	3.3	825	1	411	NA	NA
Diethylmaleate	4	3.27	818	2	400	NA	NA
Dihydrocoumarin	2	4.3	1067	2	759	1	Cat 1B
1,4-Dihydroquinone	11	0.12	30	NA	NA	3	Cat 1B
5,5-Dimethyl-3-methylenedihydro-2(3H)-furanone	1	1.8	450	NA	NA	1	Unclassified
Dimethyl sulfoxide	1	72	18000	1	-	1	Unclassified
2,4-Dinitrochlorobenzene	24	0.04	11	2	3.4	13	Cat 1A
Dinocap EC	5	1.2	311	NA	NA	1	Cat 1B
Ethyl acrylate	2	32	8125	3	818	1	Unclassified
Ethyl vanillin	1	NC	NC	NA	-	NA	NA
Ethylene glycol dimethacrylate	3	33	8237	NA	NA	1	Unclassified
Ethylenediamine	2	2.7	684	1	732	5	Cat 1A
Eugenol	23	11	2743	1	5926	5	Cat 1A
EXP 10810 A	1	2.1	527	NA	NA	2	Cat 1A
EXP 11120 A	1	65	16223	NA	NA	1	Unclassified
F & Fo WG 50 + 25	1	0.003	0.78	NA	NA	3	Unclassified
FAR01042-00	1	NC	NC	NA	NA	1	Unclassified
FAR01060-00	1	88	22115	NA	NA	1	Unclassified
Farnesol	2	4.7	1187	1	2593	NA	NA
Fatty acid glutamate	1	75	18750	NA	NA	1	Unclassified
Fatty alcohol #1	1	7.6	1899	NA	NA	1	Unclassified
Fatty alcohol #2	1	8.6	2140	NA	NA	1	Unclassified
Formaldehyde	21	1.4	344	2	191	16	Cat 1B

ED_006472_00084346-00331

Chemical Name	LLNA Results			Human Results		Guinea Pig Results	
	N	GM[1] EC3 (%)	GM[1] EC3 (µg/cm^2)	N	GM[1] DSA_{05} (µg/cm^2)	N	Overall[2] GHS Category
Fumaric acid	1	NC	NC	NA	NA	1	Unclassified
Fx + Me EW 69	1	25	6306	NA	NA	1	Unclassified
Geraniol	7	18	4474	2	1265	1	Cat 1B
Geranium oil	1	NC	NC	1	-	NA	NA
Glutaraldehyde	8	0.16	41	1	1073	1	Cat 1B
Glycerol	1	NC	NC	1	-	2	Unclassified
Glyceryl thioglycolate	1	4.7	1165	NA	NA	1	Unclassified
Glyoxal	3	0.75	187	1	345	NA	NA
Gold chloride	1	0.48	120	1	98.5	NA	NA
Hexane	1	NC	NC	1	-	NA	NA
Hexyl cinnamic aldehyde	27	8.9	2233	1	-	3	Cat 1A
2-Hexylidene cyclopentanone	1	2.4	600	1	255	NA	NA
Hexyl salicylate	1	0.18	45	2	-	NA	NA
Hydrocortisone	1	NC	NC	1	-	NA	NA
4-Hydroxybenzoic acid	4	NC	NC	NA	NA	2	Cat 1B
Hydroxycitronellal	13	23	5764	8	5237	8	Cat 1B
2-Hydroxyethyl acrylate	3	2.4	597	NA	NA	1	Cat 1A
2-Hydroxypropyl methacrylate	4	NC	NC	NA	NA	1	Unclassified
Imidazolidinyl urea	1	24	6000	1	3846	1	Cat 1B
Isocyclemone E	1	25	6285	1	-	NA	NA
Isocyclocitral	1	7.4	1838	2	-	NA	NA
Isocyclogeraniol	1	NC	NC	1	6250	NA	NA
Isoeugenol	66	1.4	342	2.0	1016	2	Cat 1A
Isomethylionone	1	21.8	5450	1	-	NA	NA
Isopropanol	1	NC	NC	1	-	1	Unclassified
Isopropyl myristate	1	44	11000	1	-	NA	NA
Jasmine absolute (grandiflorum)	1	5.9	1475	1	967	NA	NA

ED_006472_00084346-00332

Chemical Name	LLNA Results			Human Results		Guinea Pig Results	
	N	GM[1] EC3 (%)	GM[1] EC3 (µg/cm^2)	N	GM[1] DSA_{05} (µg/cm^2)	N	Overall[2] GHS Category
Jasmine absolute (sambac)	1	36	9100	1	-	NA	NA
Kanamycin	1	NC	NC	1	1874	NA	NA
Lead acetate	1	NC	NC	NA	-	NA	NA
Lemongrass oil	1	6.5	1625	1	-	NA	NA
Lilial	6	8.8	2194	1	335545	NA	NA
Linalool	1	55	13750	3	-	NA	NA
Linoleic acid	1	14	3523	NA	NA	1	Unclassified
Linolenic acid	1	9.9	2463	NA	NA	1	Unclassified
Litsea cubeba oil	1	8.4	2100	1	-	NA	NA
Lyral HMPCC	2	17	4262	2	-	NA	NA
Majantal	1	NC	NC	1	-	NA	NA
Maleic acid	1	7.0	1743	NA	NA	1	Unclassified
Manganese chloride	1	NC	NC	NA	-	NA	NA
Menthadiene-7-methyl formate	2	NC	NC	1	3406	NA	NA
2-Mercaptobenzothiazole	6	2.6	645	2	1930	4	Cat 1A
Mercuric (II) chloride	1	0.39	98	2	225	1	Cat 1A
4-Methoxyacetophenone	1	NC	NC	1	-	NA	NA
Methoxy dicyclopentadiene carboxaldehyde	1	NC	NC	1	-	NA	NA
2-Methoxy-4-methylphenol	1	5.8	1450	1	-	NA	NA
4-Methylaminophenol sulfate	1	0.80	200	NA	NA	1	Cat 1A
Methylanisylidene acetone	1	9.30	2325	1	412	NA	NA
6-Methylcoumarin	2	NC	NC	1	-	NA	NA
Methyl dodecanesulfonate	1	0.39	98	NA	NA	1	Cat 1A
Methylhexanedione	1	26	6500	2	3595	NA	NA
Methylhydrocinnamal	4	18	4417	1	2378	NA	NA
Methylisothiazolinone	2	0.87	218	2	223.5	NA	NA
Methyl methacrylate	2	73	18371	NA	NA	8	Cat 1B

ED_006472_00084346-00333

Chemical Name	LLNA Results			Human Results		Guinea Pig Results	
	N	GM[1] EC3 (%)	GM[1] EC3 (µg/cm^2)	N	GM[1] DSA_{05} (µg/cm^2)	N	Overall[2] GHS Category
Methyl 2-nonynoate	1	2.5	625	1	79	1	Cat 1B
Methyl 2-octynoate	1	0.50	125	1	388	1	Cat 1B
Methyl salicylate	2	17	4239	1	-	2	Unclassified
NAVY 14 08 723	1	0.49	123	NA	NA	3	Cat 1B
Neomycin sulfate	1	NC	NC	5	3466	1	Cat 1B
Nickel (II) salts	8	NC	NC	3	27	10	Cat 1B
Oakmoss	1	3.8	950	1	3374	NA	NA
Octanoic acid	1	NC	NC	1	-	NA	NA
1-Octen-3-yl acetate	1	NC	NC	1	6712	NA	NA
Octinol	1	4.7	1187	NA	NA	1	Unclassified
Oleic acid	1	10	2622	NA	NA	1	Unclassified
Oxalic acid	2	4	958	NA	NA	1	Unclassified
Oxazolone	8	0.002	0.59	NA	NA	1	Cat 1A
Oxyfluorfen EC	3	22	5471	NA	NA	1	Unclassified
Palmarosa oil	1	9.6	2400	1	-	NA	NA
Penicillin G	11	17	4338	4	634	4	Cat 1A
Pentachlorophenol	1	20	5000	1	2155	NA	NA
Pentaerythritol triacrylate	1	NC	NC	1	576	4	Cat 1A
Perillaldehyde	3	7.9	1987	1	1484	NA	NA
Peru balsam absolute	1	2.5	625	1	862	NA	NA
Phenyl benzoate	4	9.5	2366	1	52489	NA	NA
Phenylacetaldehyde	3	4.99	1247	5	329	NA	NA
4-Phenylenediamine	17	0.12	29.7	4	30	5	Cat 1A
Phenylpropionaldehyde	1	6.3	1575	1	692	NA	NA
Phthalic anhydride	1	0.36	90	NA	NA	3	Cat 1A
Potassium dichromate	19	0.12	30	3	106	5	Cat 1A
Produkt P-4G	1	NC	NC	NA	NA	1	Cat 1B

ED_006472_00084346-00334

Chemical Name	LLNA Results			Human Results		Guinea Pig Results	
	N	GM[1] EC3 (%)	GM[1] EC3 (µg/cm^2)	N	GM[1] DSA_{05} (µg/cm^2)	N	Overall[2] GHS Category
Propyl gallate	1	0.32	80	NA	NA	1	Cat 1A
Propylene glycol	1	NC	NC	2	-	1	Unclassified
Propylidene phthalate	1	3.7	925	1	1150	NA	NA
Propylparaben	1	NC	NC	NA	NA	1	Unclassified
Pyridine	1	72	17975	1	41051	NA	NA
Quinoxyfen SC	1	NC	NC	NA	NA	1	Unclassified
Quinoxyfen/cyproconazole	6	21	5223	NA	NA	1	Cat 1B
Resorcinol	2	5.9	1481	1	-	NA	NA
Salicylic acid	1	12	3056	1	-	1	Unclassified
Sodium lauryl sulfate	10	4.0	1001	1	-	2	Unclassified
Spearmint oil	1	8.2	2050	1	-	NA	NA
Squalene	1	7.9	1975	NA	NA	1	Unclassified
Streptomycin	4	NC	NC	6	245	1	Cat 1B
Succinic acid	1	NC	NC	NA	NA	1	Unclassified
Sulfanilamide	1	NC	NC	1	4310	1	Unclassified
Sulfanilic acid	4	NC	NC	NA	-	2	Cat 1A
Tartaric acid	1	NC	NC	NA	-	1	Unclassified
Tea leaf absolute	1	NC	NC	1	-	NA	NA
Tetrachlorosalicylanilide	2	0.04	8.8	4	27	2	Cat 1A
Tetramethylthiruamdisulphide	2	5.6	1396	2	4544	NA	NA
Thioglycerol	1	3.6	895	3	1232	2	Cat 1A
Toluene-2,4-diisocyanate	1	0.11	27.5	NA	NA	2	Cat 1A
trans-2-Hexenal	2	3.78	945	1	49	NA	NA
Treemoss	1	NC	NC	1	3423	NA	NA
Trifluralin EC	5	9.6	2412	NA	NA	1	Unclassified
Trimellitic anhydride	1	0.22	55	NA	NA	2	Cat 1A
Tween 80	1	NC	NC	1	-	2	Unclassified

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Chemical Name	LLNA Results			Human Results		Guinea Pig Results	
	N	GM[1] EC3 (%)	GM[1] EC3 (µg/cm^2)	N	GM[1] DSA_{05} (µg/cm^2)	N	Overall[2] GHS Category
Undec-10-enal	1	6.8	1700	NA	NA	1	Unclassified
Undecylenic acid	1	19	4844	NA	NA	1	Cat 1B
Vanillin	1	NC	NC	NA	NA	3	Cat 1B
Xylene	1	96	23950	1	-	NA	NA
Yellow E-JD 3442	1	NC	NC	NA	NA	3	Unclassified
Ylang ylang	1	6.8	1700	1	16150	NA	NA
Zinc sulfate	1	NC	NC	NA	-	NA	NA

Abbreviations: Cat = subcategory of the GHS classification for skin sensitizers; DSA_{05} = induction dose per skin area, in µg/cm^2, in a human repeat-insult patch test or human maximization test that produces a positive response in 5% of the tested population; EC3 = estimated concentration of a substance expected to produce a stimulation index of 3, which is the threshold value for a substance to be considered a sensitizer in the LLNA; GHS = Globally Harmonized System of Classification and Labelling of Chemicals (UN 2009); GM = geometric mean; LLNA = murine local lymph node assay; N = number of studies; NA = not available; NC = not calculated.

[1] For substances with >1 study.

[2] Based on the most prevalent results for substances with multiple studies.

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Annex III

Physicochemical Properties of Substances Evaluated

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Chemical Name	Synonyms	CASRN	Mol. Weight	Log K_{ow}[1,2]	Physical Form	Peptide Reactivity[3]	Chemical Class[4]	Product Use[5]	Structure
Abietic acid	Sylvic acid	514-10-3	302.46	4.61	Solid	NA	Hydrocarbons, cyclic; Polycyclic compounds	Manufacturing	
Acetyl isovaleryl	5-Methyl-2,3-hexanedione	13706-86-0	128.17	1.42	Liquid	NA	NA	Fragrance agent	
AE F016382 00 TK71 A101	NA	NA	NA	NA	NA	NA	Formulations	Pesticide	NA
Aluminum chloride	Aluminum chloride; anhydrous; Aluminum trichloride	7446-70-0	133.34	NA	Solid	NA	Aluminum compounds; Inorganic chemicals	Intermediate in chemical synthesis; Household products; Manufacturing	
p-Aminobenzoic acid	4-Aminobenzoic acid; PABA	150-13-0	137.14	0.68	Solid	NA	Carboxylic acids	Cosmetics; Food additive; Pharmaceutical	

Chemical Name	Synonyms	CASRN	Mol. Weight	Log K_{ow}[1,2]	Physical Form	Peptide Reactivity[3]	Chemical Class[4]	Product Use[5]	Structure
3-Aminophenol	m-Aminophenol; 3-Hydroxyaniline	591-27-5	109.13	1.17	Solid	Minimal	Amines; Phenols	Cosmetics; Pharmaceuticals	
Amylcinnamic aldehyde	Amyl cinnamic aldehyde; Amylcinnamal; 2 - Benzylideneheptanal; 2-Pentyl cinnamaldehyde	122-40-7	202.30	4.33	Liquid	Minimal	Aldehydes	Food additive	
alpha-Amylcinnamyl alcohol	Amyl cinnamic alcohol; Amylcinnamol; 2-Pentylcinnamic alcohol; 2-Amyl-3-phenyl-2-propen-1-ol; 2-Benzylidene heptanol; Buxinol	101-85-9	204.31	4.35	Liquid	NA	Alcohols	Food additive	
Aniline	Benzenamine	62-53-3	93.13	1.56	Liquid	NA	Amines	Food additive; Manufacturing; Personal care products; Pesticides; Pharmaceuticals	

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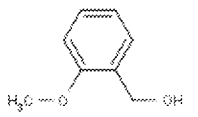
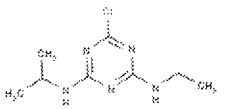
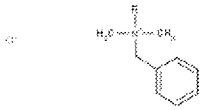
Chemical Name	Synonyms	CASRN	Mol. Weight	Log K_{ow}[1,2]	Physical Form	Peptide Reactivity[3]	Chemical Class[4]	Product Use[5]	Structure
Anisyl alcohol	Benzenemethanol, ar-methoxy-	1331-81-3	138.17	1.16	NA	NA	Ethers; Phenols	Food additive; Fragrance agent; Manufacturing	
A SC600	NA	NA	NA	NA	NA	NA	Formulations	Pesticides	NA
Atrazine	Atrazine SC; 1-Chloro-3-ethylamino-5-isopropylamino-2,4,6-triazine	1912-24-9	215.69	2.82	Solid	NA	Formulations; Heterocyclic compounds	Herbicide	
Basil oil	Ocimum basilicum herb oil	8015-73-4	NA	NA	Liquid	NA	Lipids; Natural complex substances	Food additive; Fragrance agent	NA
Benzalkonium chloride	Alkyl dimethylbenzyl ammonium chloride	8001-54-5	NA	NA	Solid	NA	Amines; Onium compounds	Cosmetics; Manufacturing; Household products; Pharmaceuticals	

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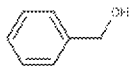
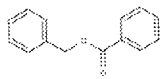
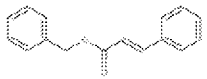
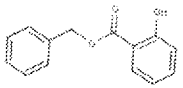
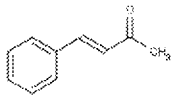
Chemical Name	Synonyms	CASRN	Mol. Weight	Log K_{ow}[1,2]	Physical Form	Peptide Reactivity[3]	Chemical Class[4]	Product Use[5]	Structure
Benzocaine	Benzoic acid, 4-amino-, ethyl ester; Ethyl 4-aminobenzoate; Ethyl p-aminobenzoate	94-09-7	165.19	1.52	Solid	NA	Carboxylic acids	Pharmaceuticals	
Benzoic acid	Benzenecarboxylic acid; Benzeneformic acid; Benzenemethanoic acid; Benzoate	65-85-0	212.20	1.87	Solid	NA	Carboxylic acids	Food additive; Manufacturing; Pharmaceuticals	
Benzoisothia-zolinone	1,2-Benzisothiazol-3(2H)-one; 1,2-Benzisothiazolin-3-one	2634-33-5	151.19	0.64	Solid	High	Sulfur compounds; Heterocyclic compounds	Biocide; Disinfectant; Manufacturing	
Benzoyl peroxide	Dibenzoyl peroxide	94-36-0	242.23	3.46	Solid	High	Carboxylic acids	Manufacturing; Household products	
Benzoquinone	p-Quinone; 1,4-Cyclohexadienedione	106-51-4	108.10	1.17	Solid	High	Quinones	Manufacturing; Pesticides; Pharmaceuticals	

ED_006472_00084346-00342

Chemical Name	Synonyms	CASRN	Mol. Weight	Log K_{ow}[1,2]	Physical Form	Peptide Reactivity[3]	Chemical Class[4]	Product Use[5]	Structure
Benzyl alcohol	Benzenemethanol	100-51-6	108.14	1.1	Liquid	NA	Hydrocarbons, cyclic	Food additive; Fragrance agent; Manufacturing; Pharmaceuticals	
Benzyl benzoate	Benylate; Benzoic acid benzyl ester	120-51-4	212.25	3.14	Liquid	Minimal	Carboxylic acids	Food additive; Fragrance agent; Manufacturing; Pharmaceuticals	
Benzyl cinnamate	2-Propenoic acid, 3-phenyl-phenylmethyl ester; Phenylmethyl 3-phenyl-2-propenoate; Cinnamein	103-41-3	238.29	4.06	Solid	NA	Esters	Food additive; Fragrance agent	
Benzyl salicylate	Phenylmethyl-2-hydroxybenzoate	118-58-1	228.25	4.31	Liquid	NA	Carboxylic acids	Fragrance agent	
Benzylidene acetone	4-Phenyl-3-buten-2-one; Methyl styryl ketone	122-57-6	146.19	2.54	Solid	High	Ketones	Food additive; Manufacturing	

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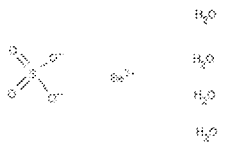
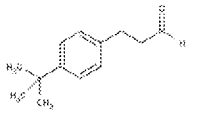
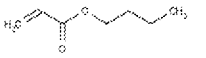
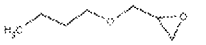
Chemical Name	Synonyms	CASRN	Mol. Weight	Log K_{ow}[1,2]	Physical Form	Peptide Reactivity[3]	Chemical Class[4]	Product Use[5]	Structure
Beryllium sulfate	Beryllium sulfate tetrahydrate	7787-56-6	177.14	NA	Solid	NA	Inorganic chemicals; Metals	Manufacturing	
Bourgenol	p-t-Butyl-dihydro-cinnamaldehyde	18127-01-0	190.28	3.34	Liquid	NA	Aldehydes	Fragrance agent	
1-Butanol	NA	71-36-3	74.12	1.06	Liquid	Minimal	Alcohols; Lipids	Intermediate in chemical synthesis; Household products; Manufacturing; Pharmaceuticals	
Butyl acrylate	n-Butyl acrylate; n-Butyl propenoate; 2-Propenoic acid; Butyl ester	141-32-2	128.17	2.20	Liquid	NA	Carboxylic acids	Intermediate in chemical synthesis; Manufacturing	
Butyl glycidyl ether	NA	2426-08-6	130.19	1.42	Liquid	NA	Ethers	Intermediate in chemical synthesis; Manufacturing	

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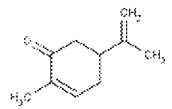
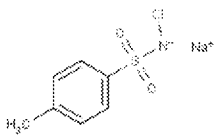
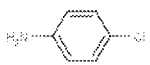
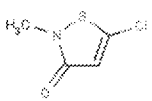
Chemical Name	Synonyms	CASRN	Mol. Weight	Log K_{ow}[1,2]	Physical Form	Peptide Reactivity[3]	Chemical Class[4]	Product Use[5]	Structure
Carvone	2-Cyclohexen-1-one; 2-Methyl-5-(1-methylethenyl)-; p-Mentha-6,8-dien-2-one	99-49-0; 2244-16-8; 6485-40-2	150.22	3.07	Liquid	NA	Hydrocarbons, other	Food additive; Fragrance agent	
Chloramine T	(N-Chloro-p-toluenesulfonamido) sodium	127-65-1	227.65	0.84	Solid	NA	Amides; Hydrocarbons, cyclic; Sulfur compounds	Pesticide; Pharmaceuticals	
4-Chloroaniline	p-Chloroaniline; 1-Amino-4-chlorobenzene; 4-Chloro-1-aminobenzene; 4-Chlorobenzamine; 4-Chlorophenylamine	106-47-8	127.57	1.83	Liquid	NA	Amines	Intermediate in chemical synthesis; Manufacturing; Pesticides; Pharmaceuticals	
(Chloro)methyl-isothiazolinone	CMI; MCI/MI; Kathon CG; 5-Chloro-2-methylisothiazolinone /2-methyl-isothiazolinone; 5 Chloro-2-methyl-3(2H)-isothiazolone and 2-methyl-2H-isothiazolone; Methyl/chloro-methylisothiazolinone	26172-55-4	149.60	-0.34	Liquid	High	Heterocyclic compounds; Sulfur compounds	Biocide; Household products	

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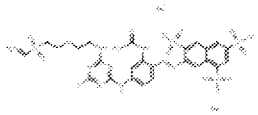
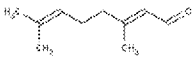
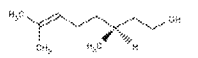
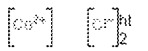
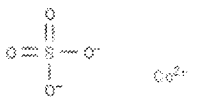
Chemical Name	Synonyms	CASRN	Mol. Weight	Log K_{ow}[1,2]	Physical Form	Peptide Reactivity[3]	Chemical Class[4]	Product Use[5]	Structure
Chlorpromazine	3,4-Dichloroaniline	95-76-1	162.02	2.60	Solid	NA	Amines	Intermediate in chemical synthesis; Manufacturing	
Cinnamic aldehyde	Cinnamaldehyde	104-55-2	132.16	2.29	Liquid	High	Aldehydes	Cosmetics; Food additive; Fragrance agent; Intermediate in chemical synthesis; Personal care products; Pesticides	
Cinnamyl alcohol	3-Phenyl-2-propen-1-ol	104-54-1	134.18	2.29	Solid	NA	Alcohols	Cosmetics; Food additive; Fragrance agent; Intermediate in chemical synthesis; Personal care products	
Cinnamyl nitrile	Cinnamonitrile; 1-Cyano-2-phenylethylene; 2-Propenenitrile, 3-phenyl-; Styryl cyanide; beta-Cyanostyrene; beta-Phenylacrylonitrile	4360-47-8	129.16	1.84	Liquid	NA	Nitriles	Fragrance agent	

ED_006472_00084346-00346

Chemical Name	Synonyms	CASRN	Mol. Weight	Log K_{ow}[1,2]	Physical Form	Peptide Reactivity[3]	Chemical Class[4]	Product Use[5]	Structure
C.I. Reactive Red 231	Potassium/sodium-5-(4-chlor-6(N4-(4-chlor-6-(5-hydroxy-2,7-disulfonato-6-(2-sulfonato-phenylazo)-4 napthylamino)-1,3,5-triazin-2-ylamino)-phenyl-N-methyl)amino)-1,3,5-triazin-2-ylamino)-4-hydroxy-3-(2-sulfonatophenylazo)-naphthalen-2,7-disulfonate	NA	NA	NA	Solid	NA	NA	Dye	NA
C.I. Reactive Yellow 174	1,3,6-Naphthalene-trisulfonic acid, 7-(2-(2-((aminocarbonyl) amino)-4-((4-((2-(2-(ethenyl sulfonyl)ethoxy) ethyl)amino)-6-fluoro-1,3,5-triazin-2-yl) amino) phenyl)diazenyl)-, sodium salt (1:3)	106359-91-5	885.75	NA	Solid	NA	NA	Dye	
Citral	3,7-Dimethyl-2,6-octadienal; Geranial-Neral mixture	5392-40-5	152.23	2.54/ 3.45	Liquid	High	Hydrocarbons, other	Fragrance agent	

ED_006472_00084346-00347

Chemical Name	Synonyms	CASRN	Mol. Weight	Log K_{ow}[1,2]	Physical Form	Peptide Reactivity[3]	Chemical Class[4]	Product Use[5]	Structure
Citronella oil	NA	8000-29-1	NA	NA	Liquid	NA	Lipids; Natural complex substances	NA	NA
dl-Citronellol	3,7-Dimethyl-6-octen-1-ol, (+-)-	26489-01-0	156.27	3.56	Liquid	NA	Hydrocarbons	Fragrance agent	
Clove oil	NA	8000-34-8	NA	NA	Liquid	NA	Natural complex substances	Pharmaceutical	NA
Cobalt chloride	Cobaltous chloride; Cobalt chloride hexahydrate; Cobalt (II) salts	7646-79-9	129.84	0.85	Solid	NA	Elements; Inorganic chemicals; Metals	Manufacturing; Pesticides	
Cobalt sulfate	Cobaltous sulfate; Cobalt (II) salts	10124-43-3	155.00	0.63	Solid	NA	Elements; Inorganic chemicals; Metals	Manufacturing; Pesticides	
Copper (II) chloride	NA	1344-67-8	99.00	NA	Solid	NA	Inorganic chemicals; Metals	Manufacturing	Cu-Cl

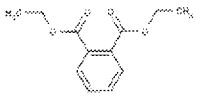
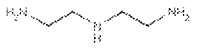
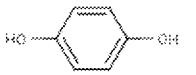
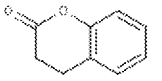
ED_006472_00084346-00348

Chemical Name	Synonyms	CASRN	Mol. Weight	Log K_{ow}[1,2]	Physical Form	Peptide Reactivity[3]	Chemical Class[4]	Product Use[5]	Structure
Coumarin	1,2-Benzopyrone	91-64-5	146.15	1.91	Solid	Minimal	Heterocyclic compounds	Fragrance agent; Manufacturing; Pharmaceuticals	
Cyclamen aldehyde	3-(4-Isopropylphenyl) isobutyraldehyde	103-95-7	190.29	3.28	Liquid	Low	Carboxylic acids	Food additive; Fragrance agent	
Damascenone	1-(2,6,6-Trimethylcyclohexa-1,3-dienyl)-2-buten-1-one; Rose dihydroketone;	23696-85-7	190.28	4.21	Liquid	NA	Hydrocarbons, acyclic	Food additive; Fragrance agent	
trans-alpha-Damascone	(E)-1-(2,6,6-Trimethyl-2-cyclohexen-1-yl)-2-buten-1-one	24720-09-0	192.30	4.29	Liquid	NA	Hydrocarbons, acyclic	Food additive; Fragrance agent	
trans-beta-Damascone	(2E)-1-(2,6,6-Trimethyl-3-cyclohexen-1-yl)-2-buten-1-one	23726-91-2	192.30	4.42	Liquid	NA	Hydrocarbons, acyclic	Food additive; Fragrance agent	

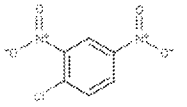
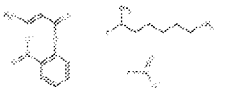
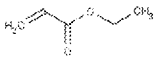
ED_006472_00084346-00349

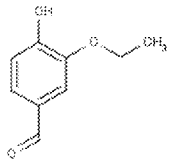
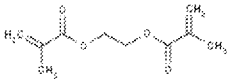
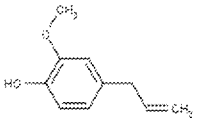
Chemical Name	Synonyms	CASRN	Mol. Weight	Log K_{ow}[1,2]	Physical Form	Peptide Reactivity[3]	Chemical Class[4]	Product Use[5]	Structure
delta-Damascone	delta-1-(2,6,6-Trimethyl-3-cyclohexen-1-yl)-2-buten-1-one	57378-68-4	192.30	4.16	Liquid	NA	NA	Food additive; Fragrance agent	
D EC25®	NA	NA	NA	NA	NA	NA	Formulations	Pesticide	NA
D EW 15	NA	NA	NA	NA	NA	NA	Formulations	Pesticide	NA
Dextran	NA	9004-54-0	Various	Various	Solid	NA	Carbohydrates	Pharmaceuticals	
1,2-Dibromo-2,4-dicyanobutane	2-Bromo-2-(bromomethyl) glutaronitrile; 2-Bromo-2-(bromomethyl) pentanedinitrile; Bromothalonil; Glutaronitrile, Methyldibromo glutaronitrile	35691-65-7	265.93	1.91	Solid	NA	Nitriles	Manufacturing; Personal care products	
Diethyl maleate	Ethyl maleate	141-05-9	172.18	0.89	Liquid	High	Carboxylic acids	Food additive; Intermediate in chemical synthesis	

ED_006472_00084346-00350

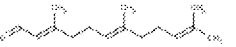
Chemical Name	Synonyms	CASRN	Mol. Weight	Log K_{ow}[1,2]	Physical Form	Peptide Reactivity[3]	Chemical Class[4]	Product Use[5]	Structure
Diethyl phthalate	NA	84-66-2	222.24	1.87	Liquid	Minimal	Carboxylic acids	Household products; Manufacturing	
Diethylenetria-mine	1,2-Ethanediamine, N-(2-aminoethyl)-; 1,4,7-Triazaheptane; 1,5-Diamino-3-azapentane; 2,2'-Diaminodiethylamine; 2,2'-Iminobis(ethanamine); 2-(2-Aminoethylamino)ethylamine; 3-Azapentane-1,5-diamine	111-40-0	103.17	0.29	Liquid	NA	Amines	Intermediate in chemical synthesis	
1,4-Dihydroquinone	1,4-Benzenediol; Hydroquinone p-Hydroxyphenol	123-31-9	110.11	1.17	Solid	NA	Phenols	Manufacturing; Personal care products	
Dihydrocou-marin	1,2-Benzodihydropyrone; 3,4-Dihydrocoumarin; Hydroxydihydro-cinnamic acid lactone; Melilotin	119-84-6	148.16	0.97	Liquid	NA	Heterocyclic compounds	Food additive; Fragrance agent; Pharmaceuticals	

ED_006472_00084346-00351

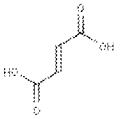
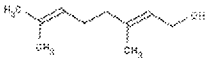
Chemical Name	Synonyms	CASRN	Mol. Weight	Log K_{ow}[1,2]	Physical Form	Peptide Reactivity[3]	Chemical Class[4]	Product Use[5]	Structure
5,5-Dimethyl-3-methylenedihydro-2(3H)-furanone	NA	29043-97-8	126.16	1.42	NA	NA	Heterocyclic compounds; Lactones; Sulfur compounds	NA	NA
Dimethyl sulfoxide	NA	67-68-5	78.13	0.57	Liquid	NA	Sulfur compounds	Manufacturing; Pharmaceuticals	
2,4-Dinitro-chlorobenzene	1-Chloro-2,4-dinitrobenzene; DNCB	97-00-7	202.55	-0.05	Solid	High	Hydrocarbons, halogenated; Nitro compounds; Hydrocarbons, cyclic	Manufacturing; Pesticides	
Dinocap EC	NA	39300-45-3	364.39	5.76	Liquid	NA	Formulations; Hydrocarbons, cyclic; Nitro compounds	Fungicide	
Ethyl acrylate	NA	140-88-5	100.12	0.92/1.22	Liquid	High	Carboxylic acids	Manufacturing	

Chemical Name	Synonyms	CASRN	Mol. Weight	Log K_{ow}[1,2]	Physical Form	Peptide Reactivity[3]	Chemical Class[4]	Product Use[5]	Structure
Ethyl vanillin	3-Ethoxy-4-hydroxybenzalde-hyde; 2-Ethoxy-4-formylphenol	121-32-4	166.18	1.52	Solid	Minimal	Aldehydes	Food additive; Fragrance agent; Manufacturing	
Ethylene glycol dimethacrylate	EGDMA	97-90-5	198.22	1.38	Liquid	High	Carboxylic acids	Manufacturing	
Ethylenediamine	NA	107-15-3	60.10	0.19	Liquid	NA	Amines	Manufacturing	
Eugenol	2-Methoxy-4-(2-propenyl)phenol; 4-Allyl-2-methoxyphenol; 4-Allylguaiacol	97-53-0	164.20	2.15/ 2.73	Liquid	NA	Carboxylic acids	Cosmetics; Food additive; Intermediate in chemical synthesis; Manufacturing; Personal care products; Pharmaceuticals	
EXP 10810 A	NA	NA	NA	NA	NA	NA	Formulations	Pesticides	NA

ED_006472_00084346-00353

Chemical Name	Synonyms	CASRN	Mol. Weight	Log K_{ow}[1,2]	Physical Form	Peptide Reactivity[3]	Chemical Class[4]	Product Use[5]	Structure
EXP 11120 A	NA	NA	NA	NA	NA	NA	Formulations	Pesticides	NA
F & Fo WG 50 + 25	NA	NA	NA	NA	NA	NA	Formulations	Pesticides	NA
FAR01042-00	NA	NA	NA	NA	NA	NA	Formulations	Pesticides	NA
FAR01060-00	NA	NA	NA	NA	NA	NA	Formulations	Pesticides	NA
Fatty acid glutamate	NA	NA	NA	NA	NA	NA	NA	NA	NA
Fatty alcohol #1	NA	NA	NA	NA	NA	NA	NA	NA	NA
Fatty alcohol #2	NA	NA	NA	NA	NA	NA	NA	NA	NA
Farnesal	2,6,10-Dodecatrienal, 3,7,11-trimethyl-, (2E,6E)-	4602-84-0	220.36	3.77	Liquid	Low	Alcohols; Hydrocarbons	Food additive; Fragrance agent; Manufacturing	

ED_006472_00084346-00354

Chemical Name	Synonyms	CASRN	Mol. Weight	Log K_{ow}[1,2]	Physical Form	Peptide Reactivity[3]	Chemical Class[4]	Product Use[5]	Structure
Formaldehyde	NA	50-00-0	30.03	0.33/ 0.35	Liquid	Moderate	Aldehydes	Disinfectant; Manufacturing	
Fumaric acid	Ethylenedicarboxylic acid; 2-Butenedioic acid; Allomaleic acid; Boletic acid; Lichenic acid	110-17-8	116.00	0.03	Solid	NA	Carboxylic acids	Food additive; Household products; Manufacturing	
Geraniol	Rhodinol	106-24-1	154.25	2.54/ 3.47	Liquid	NA	Hydrocarbons, other	Cosmetics; Fragrance agent; Personal care products	
Geranium oil	Pelargonium oil	8000-46-2	NA	NA	Liquid	NA	Natural complex substances	Cosmetics; Fragrance agent; Personal care products; Pesticides	NA
Glutaraldehyde	Glutaral; Pentanedial	111-30-8	100.12	0.92	Liquid	High	Aldehydes	Cosmetics; Disinfectant; Manufactuing; Pesticides	

ED_006472_00084346-00355

Chemical Name	Synonyms	CASRN	Mol. Weight	Log K_{ow}[1,2]	Physical Form	Peptide Reactivity[3]	Chemical Class[4]	Product Use[5]	Structure
Glycerol	1,2,3-Propanetriol	56-81-5	92.09	0.05	Liquid	Minimal	Alcohols; Carbohydrates	Cosmetics; Food additive; Intermediate in chemical synthesis; Manufacturing; Personal care products; Pharmaceuticals; Solvent	
Glyceryl thioglycolate	Acetic acid, mercapto, monoester with 1,2,3-propanetriol; Glycerol monomercaptoacetate; Glyceryl monothioglycolate; Mercaptoacetic acid, monoester with 1,2,3-propanetriol	30618-84-9	166.19	-1.29	NA	NA	Lipids	Personal care products	
Glyoxal	Oxaldehyde; Ethanedial; Biformyl	107-22-2	58.04	0.19	Liquid	High	Aldehydes	Intermediate in chemical synthesis; Manufacturing; Pharmaceuticals	

ED_006472_00084346-00356

Chemical Name	Synonyms	CASRN	Mol. Weight	Log K_{ow}[1,2]	Physical Form	Peptide Reactivity[3]	Chemical Class[4]	Product Use[5]	Structure
Gold chloride	Chloroauric acid; Gold tetrachloride	16903-35-8	339.79	0.16	Solid	NA	Gold compounds; Inorganic chemicals	Manufacturing	
Hexane	NA	110-54-3	86.18	1.94	Liquid	Minimal	Hydrocarbons, acyclic	Manufacturing; Solvent	
trans-2-Hexenal	NA	6728-26-3	98.15	1.56	Liquid	High	Heterocyclic compounds	Fragrance agent	
Hexyl cinnamic aldehyde	HCA; alpha-Hexylcinnamalde-hyde; 2-(Phenylmethylene) octanal	101-86-0	216.32	3.77/ 4.82	Liquid	Minimal	Aldehydes	Food additive; Fragrance agent	
2-Hexylidene cyclopentanone	NA	17373-89-6	166.27	3.71	Liquid	NA	NA	Cosmetics; Fragrance agent	

ED_006472_00084346-00357

Chemical Name	Synonyms	CASRN	Mol. Weight	Log K_{ow}[1,2]	Physical Form	Peptide Reactivity[3]	Chemical Class[4]	Product Use[5]	Structure
Hexyl salicylate	Benzoic acid, 2-hydroxy-, hexyl ester	6259-76-3	222.29	5.06	Liquid	NA	NA	Food additive	
Hydrocortisone	11-beta-Hydrocortisone	50-23-7	362.46	1.16	Solid	NA	Polycyclic compounds	Pharmaceuticals	
4-Hydroxybenzoic acid	4-Carboxyphenol p-Hydroxybenzoic acid p-Salicylic acid	99-96-7	138.12	1.03	Solid	Minimal	Phenols; Carboxylic acids	Intermediate in chemical synthesis; Manufacturing; Pesticides	
Hydroxy-citronellal	NA	107-75-5	172.26	2.15	Liquid	Low	Hydrocarbons, other	Food additive; Fragrance agent; Personal care products	

ED_006472_00084346-00358

Chemical Name	Synonyms	CASRN	Mol. Weight	Log K_{ow}[1,2]	Physical Form	Peptide Reactivity[3]	Chemical Class[4]	Product Use[5]	Structure
2-Hydroxyethyl acrylate	HEA	818-61-1	116.12	0.54	Liquid	High	Carboxylic acids	Intermediate in chemical synthesis; Manufacturing;	
2-Hydroxypropyl methacrylate	2-HPMA	923-26-2	144.17	1.03	Solid	Low	Carboxylic acids	Manufacturing: Plastics; Rubber	
Imidazolidinyl urea	Germall 115; Imidurea	39236-46-9	388.30	-3	Solid	Moderate	Ureas	Cometics; Personal care products; Pesticides	
Isocyclemone E	1-(1,2,3,4,5,6,7,8-Octahydro-2,3,8,8-tetramethyl-2-naphthyl)ethan-1-one; OTNE; Boisvelone	54464-57-2	234.38	5.18	Liquid	NA	Hydrocarbons, cyclic; Polycyclic compounds	Food additive; Fragrance agent; Household products	

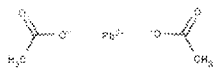
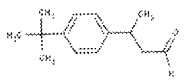
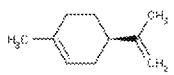
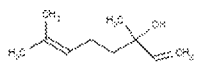
ED_006472_00084346-00359

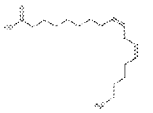
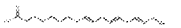
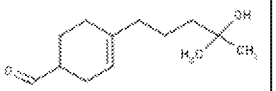
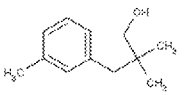
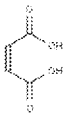
Chemical Name	Synonyms	CASRN	Mol. Weight	Log K_{ow}[1,2]	Physical Form	Peptide Reactivity[3]	Chemical Class[4]	Product Use[5]	Structure
Isocyclocitral	1-Formyl-3,5,6-trimethyl-3-cyclohexene and 1-formyl-2,4,6-trimethyl-3-cyclohexene; 2,4,6-Trimethyl-3-cyclohexen-1-carboxaldehyde	1335-66-6	152.44	3.27	NA	NA	NA	Food additive; Fragrance agent	
Isocyclogeran-iol	3-Cyclohexene-1-methanol, 2,4,6-trimethyl-	68527-77-5	154.25	NA	Liquid	NA	Alcohols	Fragrance agent	
Isoeugenol	2-Methoxy-4-propenylphenol; 4-Propenylguaiacol	97-54-1	164.20	2.15	Liquid	NA	Carboxylic acids	Food additive; Fragrance agent	
Isomethyl ionone	3-Methyl-4-(2,6,6-trimethyl-2-cyclohexen-1-yl)-3-buten-2-one	127-51-5	206.33	NA	NA	NA	Hydrocarbons, acyclic	Food additive; Fragrance agent; Personal care products	

ED_006472_00084346-00360

Chemical Name	Synonyms	CASRN	Mol. Weight	Log K_{ow}[1,2]	Physical Form	Peptide Reactivity[3]	Chemical Class[4]	Product Use[5]	Structure
Isopropanol	Isopropyl alcohol; 2-Propanol	67-63-0	60.10	0.82	Liquid	Minimal	Alcohols	Cosmetics; Disinfectant; Food additive; Intermediate in chemical synthesis; Manufacturing; Personal care products; Pharmaceuticals; Solvent	
Isopropyl myristate	Tetradecanoic acid, 1-methylethyl ester; Myristic acid isopropyl ester	110-27-0	270.46	3.88	Liquid	Minimal	Lipids	Cosmetics; Personal care products; Pharmaceuticals	
Jasmine absolute (grandiflorum)	NA	8022-96-6; 8024-43-9; 90045-94-6; 84776-64-7	NA	NA	NA	NA	Natural complex substances	Food additive; Fragrance agent	NA
Jasmine absolute (sambac)	NA	91770-14-8	NA	NA	NA	NA	Natural complex substances	Food additive; Fragrance agent	NA
Kanamycin	NA	59-01-8; 8063-07-8	484.50	-0.9	Solid	NA	Carbohydrates	Pharmaceuticals	

ED_006472_00084346-00361

Chemical Name	Synonyms	CASRN	Mol. Weight	Log K_{ow}[1,2]	Physical Form	Peptide Reactivity[3]	Chemical Class[4]	Product Use[5]	Structure
Lead acetate	Acetic acid, lead salt	15347-57-6	325.29	-0.08	Solid	NA	Inorganic chemicals; Metals	Cosmetics; Personal care products	
Lemongrass oil	Citral terpenes; Indian melissa oil; Indian oil of verbena; Cymbopogon citratus oil	8007-02-1	NA	NA	Liquid	NA	Lipids; Hydrocarbons, other Natural complex substances	Food additive; Fragrance agent	NA
Lilial	p-tert-Butyl-a-ethyl-hydrocinnamal	80-54-6	204.31	3.52	Liquid	Low	Aldehydes	Fragrance agent	
d-Limonene	1-Methyl-4-(1-methylenethenyl)cyclo hexene; Dipentene; Limonene	5989-27-5	136.24	2.93	Liquid	NA	Hydrocarbons, cyclic	Cosmetics; Food additive; Fragrance agent; Manufacturing	
Linalool	3,7-Dimethyl-2,6-octadienal; Geranial-Neral mixture	78-70-6	154.25	2.54	Liquid	NA	Hydrocarbons	Cosmetics; Food additive; Manufacturing; Personal care products; Pharmaceuticals	

Chemical Name	Synonyms	CASRN	Mol. Weight	Log K_{ow}[1,2]	Physical Form	Peptide Reactivity[3]	Chemical Class[4]	Product Use[5]	Structure
Linoleic acid	9,12-Octadecadienoic acid; Grape seed oil	60-33-3	280.50	7.51	Liquid	NA	Lipids	Food additive; Household products; Manufacturing	
Linolenic acid	(Z,Z,Z)-Octadeca-9,12,15-trienoic acid	463-40-1	278.40	7.30	Liquid	NA	Lipids	Food additive	
Litsea cubeba oil	NA	68855-99-2	NA	NA	Liquid	NA	Natural complex substances	Fragrance agent	NA
Lyral HMPCC	3 and 4-(4-Hydroxy-4-methylpentyl)-3-cyclohexane-1-carboxaldehyde	31906-04-4	210.32	2.89	Liquid	NA	Aldehydes; Hydrocarbons, cyclic	Fragrance agent	
Majantal	Benzenepropanol, beta,beta,3-trimethyl-	103694-68-4	178.27	3.48	NA	NA	NA	Fragrance agent	
Maleic acid	1,2-Ethylene-dicarboxylic acid, (Z); 2-Butenedioic acid, (Z)-; Maleinic acid; Malenic acid; Toxilic acid	110-16-7	116.10	0.05	Solid	NA	Carboxylic acids	Food additive; Intermediate in chemical synthesis; Manufacturing	

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Chemical Name	Synonyms	CASRN	Mol. Weight	Log K_{ow}[1,2]	Physical Form	Peptide Reactivity[3]	Chemical Class[4]	Product Use[5]	Structure
Manganese chloride	Maganese chloride, anhydrous	7773-01-5	125.84	0.85	Solid	NA	Inorganic chemicals; Manganese compounds	Manufacturing	
Menthadiene-7-methyl formate	Menthadienyl formate; Iso-bergamate	68683-20-5	194.28	4.23	NA	NA	NA	Fragrance agent	
2-Mercapto-benzothiazole	Captax	149-30-4	167.25	1.80	Solid	High	Heterocyclic compounds	Manufacturing; Pesticides	
Mercuric (II) chloride	Mercuric chloride	7487-94-7	271.50	0.15	Solid	NA	Inorganic chemicals; Mercury compounds	Fungicide; Insecticide: Intermediate in chemical synthesis; Manufacturing	
4'-Methoxy-acetophenone	4-Acetylanisole; Ethanone, 1-(4-methoxyphenyl)-	100-06-1	150.18	1.91	Solid	Minimal	Ethers; Phenols	Intermediate in chemical synthesis	

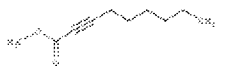
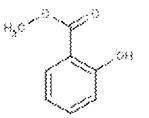
ED_006472_00084346-00364

Chemical Name	Synonyms	CASRN	Mol. Weight	Log K_{ow}[1,2]	Physical Form	Peptide Reactivity[3]	Chemical Class[4]	Product Use[5]	Structure
Methoxy dicyclo-pentadiene carboxaldehyde	Octahydro-5-methoxy-4,7-methano-1H-indene-2-carboxaldehyde; Scentenal	86803-90-9	194.27	2.63	NA	NA	NA	Fragrance agent	
2-Methoxy-4-methylphenol	Creosol	93-51-6	138.17	1.88	Liquid	NA	Phenols	Pharmaceuticals	
4-Methyl-aminophenol sulfate	Metol; Paramethylamino-phenol sulfate	55-55-0	344.39	-0.13	Solid	High	Amines; Phenols	Household products	
Methylanisyl-idene acetone	alpha-Methylanisylidene-acetone; 1-Penten-3-one, 1-(p-methoxyphenyl)-	104-27-8	190.24	2.3	Solid	NA	Ethers	Food additive; Pharmaceuticals	
alpha-Methyl cinnamic aldehyde	2-Propenal, 2-methyl-3-phenyl-	101-39-3	146.19	2.37	NA	NA	DNF	Fragrance agent	
Methyl dodecane-sulfonate	1-Dodecanesulfonic acid, methyl ester	2374-65-4	264.43	2.51	NA	NA	Esters; Sulfur compounds	NA	

ED_006472_00084346-00365

Chemical Name	Synonyms	CASRN	Mol. Weight	Log K_{ow}[1,2]	Physical Form	Peptide Reactivity[3]	Chemical Class[4]	Product Use[5]	Structure
Methylhexane-dione	3-Methyl-2,5-hexanedione	13706-86-0	128.17	-0.39	Liquid	Low	Ketones	NA	
Methylhydro-cinnamal	Benzenepropanal, 4-methyl-	5406-12-2	148.20	2.58	Liquid	NA	NA	NA	
Methylisothia-zolinone	-Methyl-3(2H)-isothiazolone; 2-Methyl-4-isothiazolin-3-one; 3(2H)-Isothiazolone, 2-methyl-; MI	2682-20-4	115.16	-0.83	NA	High	Heterocyclic compounds; Sulfur compounds	Biocide; Household products	
Methyl methacrylate	MMA	80-62-6	100.12	0.70	Liquid	NA	Carboxylic acids	Manufacturing	

ED_006472_00084346-00366

Chemical Name	Synonyms	CASRN	Mol. Weight	Log K_{ow}[1,2]	Physical Form	Peptide Reactivity[3]	Chemical Class[4]	Product Use[5]	Structure
Methyl 2-nonynoate	2-Nonynoic acid methyl ester; Methyl octine carbonate	111-80-8	168.24	2.15	Liquid	High	Lipids	Food additive; Fragrance agent	
Methyl 2-octynoate	Methyl heptene carbonate	111-12-6	154.21	3.30	NA	NA	Carboxylic acids; Lipids	Cosmetics; Food additive; Fragrance agent; Personal care products	
Methyl salicylate	Oil of wintergreen; 2-Hydroxybenzoic acid methyl ester	119-36-8	152.15	1.28/ 2.60	Liquid	Minimal	Carboxylic acids; Phenols	Cosmetics; Disinfectant; Food additive; Fragrance agent; Personal care products; Pharmaceuticals; Solvent	

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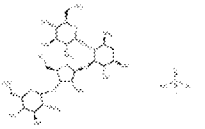
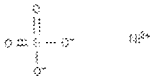
Chemical Name	Synonyms	CASRN	Mol. Weight	Log K_{ow}[1,2]	Physical Form	Peptide Reactivity[3]	Chemical Class[4]	Product Use[5]	Structure
NAVY 14 08 723	Mixture of Sodium/potassium-(3-(4-(5-(5-chlor-2,6-difluor-pyrimidin-4-ylamino)-2-methoxy-3-sulfonatophenylazo)-2-oxido phenylazo)-2,5,7-trisulfonato-4-naphtholato)copper(II) and Sodium/potassium-(3-(4-(5-(5-chlor-4,6-difluor-pyrimidin-2-ylamino)-2-methoxy-3-sulfonatophenylazo) 2,5,7-trisulfonato-4-naphtholato)copper(II)	NA	NA	NA	Solid	NA	NA	Dye	NA
Neomycin sulfate	NA	1405-10-3	712.72	NA	Solid	NA	Carbohydrates	Pharmaceuticals	
Nickel chloride	Nickelous chloride	7718-54-9	129.60	0.05	Solid	NA	Inorganic chemicals; Metals	Manufacturing	
Nickel sulfate	Nickelous sulfate	7786-81-4	154.76	-0.17	Solid	NA	Inorganic chemicals; Metals	Manufacturing	

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Chemical Name	Synonyms	CASRN	Mol. Weight	Log K_{ow}[1,2]	Physical Form	Peptide Reactivity[3]	Chemical Class[4]	Product Use[5]	Structure
Oakmoss	NA	68917-10-2	NA	NA	NA	NA	Natural complex substances	Fragrance agent	NA
Octanoic acid	Caprylic acid; 1-Heptanecarboxylic acid	124-07-2	144.21	1.66	Liquid	Minimal	Carboxylic acids; Lipids	Cosmetics; Disinfectant; Food additive; Pesticides	
1-Octen-3-yl acetate	Amyl crotonyl acetate; Amyl vinyl carbinol acetate; Amyl vinyl carbinyl; Octenyl acetate	2442-10-6	170.25	3.60	Liquid	NA	NA	Food additive; Fragrance agent	
Octinol	1-Octin-3-ol; 1-Octyn-3-ol	818-72-4	128.20	1.96	Liquid	NA	NA	Food additive; Flavor ingredient	
Oleic acid	9-Octadecenoic acid	112-80-1	282.47	7.73	Liquid	NA	Lipids	Food additive; Household products; Manufacturing	
Oxalic Acid	Ethanedioic acid	144-62-7	90.03	-2.22	Solid	Minimal	Carboxylic acids	Household products; Intermediate in chemical synthesis; Manufacturing	

ED_006472_00084346-00369

Chemical Name	Synonyms	CASRN	Mol. Weight	Log K_{ow}[1,2]	Physical Form	Peptide Reactivity[3]	Chemical Class[4]	Product Use[5]	Structure
Oxazolone	4-Ethoxymethylene-2-phenyloxazol-5-one	15646-46-5	217.22	1.87	Solid	High	Heterocyclic compounds	NA	
Oxyfluorfen EC	2-Chloro-1-(3-ethoxy-4-nitrophenoxy)-4-trifluoromethylbenzene; 2-Chloro-alpha,alpha,alpha-trifluoro-p-tolyl-3-ethoxy-4-nitrophenyl ether	42874-03-3	361.70	5.21	Solid	NA	Ethers Formulations	Herbicide	
Palmarosa oil	Cymbopogon martini oil; Geranium oil, East Indian	8014-19-5	NA	NA	Liquid	NA	Natural complex substances	Fragrance agent	NA
Penicillin G	NA	61-33-6	334.39	2.09	Solid	NA	Amides; Sulfur compounds; Heterocyclic compounds	Pharmaceuticals	
Pentachloro-phenol	Penta; PCP	87-86-5	266.34	2.79	Solid	NA	Phenols	Pesticides	

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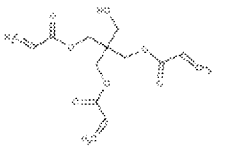
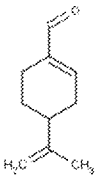
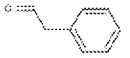
Chemical Name	Synonyms	CASRN	Mol. Weight	Log K_{ow}[1,2]	Physical Form	Peptide Reactivity[3]	Chemical Class[4]	Product Use[5]	Structure
Pentaerythritol triacrylate	2-(Hydroxymethyl)-2-(((1-oxoallyl)oxy)methyl)-1,3-propanediyl diacrylate; 2-Propenoic acid, 1,1'-(2-(hydroxymethyl)-2-(((1-oxo-2-propen-1-yl)oxy)methyl)-1,3-propanediyl) ester	3524-68-3	298.29	0.91	NA	NA	Alcohols; Carboxylic acids	Manufacturing	
Perillaldehyde	p-Mentha-1,8-dien-7-al	2111-75-3	150.22	3.34	Liquid	Moderate	Hydrocarbons	Fragrance agent	
Peru balsam absolute	Myroxylon pereirae oleoresin; Peruvian balsam	8007-00-9	NA	NA	NA	NA	Natural complex substances	Food additive; Fragrance agent; Pharmaceutical	NA
Phenylacet-aldehyde	1-Oxo-2-phenylethane; 2-Phenylethanal; Benzeneacetaldehyde; Benzylcarbox-aldehyde; Hyacinthin; Oxophenylethane; Phenylacetic aldehyde; Phenylethanal; alpha-Tolualdehyde	122-78-1	120.20	2.05	Liquid	Moderate	Aldehydes	Food additive; Fragrance agent	

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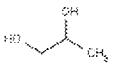
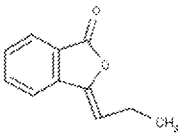
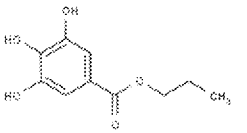
Chemical Name	Synonyms	CASRN	Mol. Weight	Log K_{ow}[1,2]	Physical Form	Peptide Reactivity[3]	Chemical Class[4]	Product Use[5]	Structure
Phenyl benzoate	NA	93-99-2	198.22	2.89	Solid	NA	Carboxylic acids	Manufacturing; Pesticides	
4-Phenylene-diamine	p-PDA; p-Phenylenediamine	106-50-3	108.14	1.17	Solid	NA	Amines	Manufacturing; Personal care products	
Phenylpropion-aldehyde	Hydrocinnamalde-hyde; Propanal, phenyl-; 3-Phenylpropanal	1335-10-0	134.18	2	NA	Moderate	Aldehydes	Food additive; Fragrance agent	
Phthalic anhydride	1,2-Benzene-dicarboxylic acid anhydride; 1,3-Dioxophthalan; 1,3-Benzofurandione; 1,3 Phthalandione	85-44-9	148.12	1.6	Solid	Moderate	Anhydrides	Intermediate in chemical synthesis; Manufacturing	
Potassium dichromate	PDC	7778-50-9	294.18	0.62	Solid	NA	Chromium compounds; Inorganic chemicals; Potassium compounds	Manufacturing	

ED_006472_00084346-00372

Chemical Name	Synonyms	CASRN	Mol. Weight	Log K_{ow}[1,2]	Physical Form	Peptide Reactivity[3]	Chemical Class[4]	Product Use[5]	Structure
Produkt P-4G	Lithium/sodium 4-(5-(4-chloro-6-(3-sulfonatophenylamino)-1,3,5-triazine-2yl-amino)-2-sulfonatophenylazo)-5-oxo-1-(4-sulfonatophenyl)-4,5-dihydro-1H-pyrazole-3-caroxylate	NA	NA	NA	Solid	NA	NA	Dye	NA
Propylene glycol	1,2-Dihydroxypropane; 1,2-Propanediol	57-55-6	76.10	0.43	Liquid	Minimal	Alcohols	Cosmetics; Food additive; Intermediate in chemical synthesis; Personal care products; Pharmaceuticals; Solvent	
Propylidine phthalate	1(3H)-Isobenzofuranone, 3-propylidene-	17369-59-4	174.20	3.10	Liquid	NA	Ketones	NA	
Propyl gallate	3,4,5-Trihydroxybenzoic acid, propyl ester; Benzoic acid, 3,4,5-trihydroxy-, propyl ester	121-79-9	212.20	1.80	Solid	High	Carboxylic acids	Food additive	

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Chemical Name	Synonyms	CASRN	Mol. Weight	Log K_{ow}[1,2]	Physical Form	Peptide Reactivity[3]	Chemical Class[4]	Product Use[5]	Structure
Propylparaben	Propyl 4-hydroxybenzoate	94-13-3	180.20	1.77	Solid	Minimal	Carboxylic acids; Phenols	Cosmetics; Food additive Household products; Pharmaceuticals	
Pyridine	NA	110-86-1	79.10	1.31	Liquid	NA	Heterocyclic compounds	Food additive; Intermediate in chemical synthesis	
Quinoxyfen SC	5,7-dichloro-4-(4-fluorophenoxy) quinoline	124495-18-7	308.10	5.69	Liquid	NA	Formulations; Heterocyclic compounds	Herbicide	
Quinoxyfen/ cyproconazole[6]	5,7-dichloro-4-(4-fluorophenoxy)quino-line/1H-1,2,4-Triazole-1-ethanol, alpha-(4-chlorophenyl)-alpha-(1-cyclopropylethyl)-	124495-18-7/ 113096-99-4	308.10 291.80	5.69/ 3.25	Liquid	NA	Formulations Heterocyclic compounds	Herbicide	
Resorcinol	1,3-Dihydroxybenzene	108-46-3	110.11	1.17	Solid	Minimal	Phenols	Cosmetics; Manufacturing; Personal care products; Pharmaceuticals	

Chemical Name	Synonyms	CASRN	Mol. Weight	Log K_{ow}[1,2]	Physical Form	Peptide Reactivity[3]	Chemical Class[4]	Product Use[5]	Structure
Salicylic acid	2-Hydroxybenzoic acid	69-72-7	138.12	1.03	Solid	NA	Carboxylic acids; Phenols	Food additive; Manufacturing; Pharmaceuticals	
Sodium lauryl sulfate	Sodium dodecyl sulfate; SLS; SDS; Irium	151-21-3	288.38	1.87/ 1.69	Solid	NA	Alcohols; Lipids; Sulfur compounds	Cosmetics; Food additive; Manufacturing; Personal care products; Pesticides; Pharmaceuticals	
Spearmint oil	Mentha spicata oil	8008-79-5	NA	NA	Liquid	NA	Natural complex substances	Food additive; Fragrance agent	NA
Squalene	2,6,10,15,19,23-Hexamethyl-2,6,10,14,18,22-tetracosahexaene	111-02-4	410.73	14.12	Liquid	NA	Hydrocarbons	Food additive	
Streptomycin	D-Streptamine, O-2-deoxy-2-(methylamino)-alpha-L-glucopyranosyl-(1-2)-O-5-deoxy-3-C-formyl-alpha-L-lyxofuranosyl-(1-4)-N,N'-bis(aminoimino-methyl)-, sulfate (2:3) (salt)	3810-74-0	1457.39	-8.50	Solid	NA	Carbohydrates	Pharmaceuticals	

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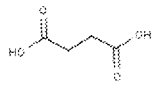
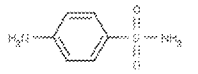
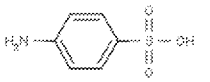
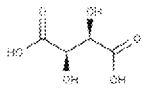
Chemical Name	Synonyms	CASRN	Mol. Weight	Log K_{ow}[1,2]	Physical Form	Peptide Reactivity[3]	Chemical Class[4]	Product Use[5]	Structure
Succinic acid	1,2-Ethanedicarboxylic acid; 1,4-Butanedioic acid	110-15-6	118.09	-0.59	Solid	NA	Carboxylic acids	Food additive; Intermediate in chemical synthesis; Manufacturing	
Sulfanilamide	4-Aminobenzene-sulfonamide, p-Anilinesulfonamide; p-Sulfamidoaniline	63-74-1	172.21	-0.62	Solid	Minimal	Amides; Amines; Sulfur compounds	Pharmaceuticals	
Sulfanilic acid	p-Aminobenzene-sulfonic acid; p-Anilinesulfonic acid	121-57-3	173.19	0.4	Solid	Minimal	Hydrocarbons, cyclic; Sulfur compounds	Pharmaceuticals	
Tartaric acid	[R-(R*,R*)]-2,3-Dihydroxybutane-dioic acid; L-Tartaric acid	87-69-4	150.09	0.87	Solid	NA	Alcohols; Carboxylic acids	Food additive	
Tea leaf absolute	Camelia oleifera extract	84650-60-2	NA	NA	Liquid	NA	Natural complex substances	Cosmetics; Household products	NA

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Chemical Name	Synonyms	CASRN	Mol. Weight	Log K_{ow}[1,2]	Physical Form	Peptide Reactivity[3]	Chemical Class[4]	Product Use[5]	Structure
Tetrachloro-salicylanilide	3,5-Dichloro-N-(3,4-dichlorophenyl)-2-hydroxybenzamide; TCS	1154-59-2	351.02	5.87	NA	Moderate	Amides Amines	Antifungal	
Tetramethyl thiuram disulfide	Thiram; Bis(dimethylthio-carbamoyl) disulfide; TMTD	137-26-8	240.44	1.17	Solid	NA	Carboxylic acids; Sulfur compounds	Fungicide	
Thioglycerol	1-Mercaptoglycerol; 1-Monothioglycerol; 1,2-Propanediol, 3-mercapto-; 1-Mercapto-2,3,-propanediol	96-27-5	108.16	-8.40	Liquid	NA	Alcohols; Carbohydrates ; Sulfur compounds	Manufacturing; Pharmaceuticals	
Toluene 2,4-disocyanate	2,4-Diisocyanato-1-methylbenzene; 2,4-Diiso-cyanatotoluene; 2,4-TDI; 2,4-Toluene diisocyanate	584-84-9	174.16	3.74	Liquid	NA	Hydrocarbons, cyclic; Cyanates	Manufacturing	
Treemoss	Cedar moss extract; Tree moss absolute; Lichen concrete; Treemoss concrete; Treemoss concrete extract; Hyperabsolute	68648-41-9	NA	NA	Liquid	NA	Natural complex substances	Fragrance agent	NA

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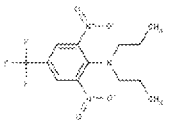
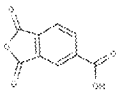
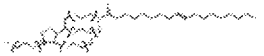
Chemical Name	Synonyms	CASRN	Mol. Weight	Log K_{ow}[1,2]	Physical Form	Peptide Reactivity[3]	Chemical Class[4]	Product Use[5]	Structure
Trifluralin EC	Benzenamine, 2,6-dinitro-N,N-dipropyl-4-(trifluoromethyl)-	1582-09-8	335.28	5.34	Solid	NA	Amines; Formulations; Hydrocarbons	Herbicide	
Trimellitic anhydride	1,2,4-Benzene-tricarboxylic acid, cyclic 1,2-anhydride (8CI); 1,3-Dihydro-1,3-dioxo-5-isobenzofuran-carboxylic acid; 5-Isobenzo-furancarboxylic acid, 1,3-dihydro-1,3-dioxo-; Benzene-1,2,4-tricarboxylic acid 1,2-anhydride	552-30-7	192.10	1.95	Solid	Low	Anhydrides; Carboxylic acids	Intermediate in chemical synthesis; Manufacturing	
Tween 80	Polyethylene glycol sorbitan monooleate; Polyoxyethylene-sorbitan monooleate; Polysorbate 80	9005-65-6	3968.85	NA	Liquid	NA	Alcohols	Food additive; Household products; Pharmaceuticals	

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Chemical Name	Synonyms	CASRN	Mol. Weight	Log K_{ow}[1,2]	Physical Form	Peptide Reactivity[3]	Chemical Class[4]	Product Use[5]	Structure
Undec-10-enal	10-Undecylenaldehyde	112-45-8	168.28	2.79	Liquid	NA	Aldehydes	Food additive	
Undecylenic acid	10-Undecenoic acid	112-38-9	184.38	3.86	Liquid	NA	Lipids	Cosmetics; Fungicide; Pharmaceutical	
Vanillin	NA	121-33-5	152.15	1.28	Solid	Minimal	Aldehydes	Food additive; Fragrance agent	
Xylene	Dimethylbenzene	1330-20-7	107.18	3.16	Liquid	NA	Hydrocarbons, cyclic	Herbicide; Intermediate in chemical synthesis; Manufacturing	
YELLOW E-JD 3442	Benzenesulfonic acid, 3-(2-(2(acetylamino)-4-(2-(4-(2-hydroxybutoxy) phenyl)diazenyl) phenyl)diazenyl)-, sodium salt (1:1)	147703-65-9	NA	NA	Solid	NA	NA	Dye	

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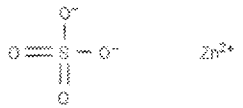
Chemical Name	Synonyms	CASRN	Mol. Weight	Log K_{ow}[1,2]	Physical Form	Peptide Reactivity[3]	Chemical Class[4]	Product Use[5]	Structure
Ylang Ylang	Cananga oil; Canangium odoratum genuina oil	8006-81-3; 68606-83-7; 83863-30-3	NA	NA	Liquid	NA	Natural complex substances	Fragrance agent	NA
Zinc sulfate	NA	7733-02-0	161.46	-0.07	Solid	NA	Inorganic chemicals; Sulfur compounds; Zinc compounds	Intermediate in chemical synthesis; Manufacturing; Pharmaceuticals	

Abbreviation: CASRN = Chemical Abstracts Service Registry Number; Mol. = molecular; NA = not available.

1 K_{ow} represents the estimated octanol-water partition coefficient (expressed on log scale).

2 When two numbers are shown for K_{ow}, the first number is the value calculated by the method of Moriguchi et al. (1994 Chem Pharm Bull 42:976-978) and provided in Gerberick et al. (2005 Dermatitis 16:157-202). The second number was calculated by the method of Meylan and Howard (1995 J Pharm Science 84:83-92). LogP (log K_{ow}) values for GSK chemicals were calculated using the method provided by Daylight Chemical Information Systems (see http://www.daylight.com/dayhtml/doc/clogp/index.html).

3 Peptide reactivity based on Cys (1:10) and Lys (1:50) data as reported in Gerberick et al. 2004 and Gerberick et al. 2007.

4 Chemical classifications based on the Medical Subject Headings classification for chemicals and drugs as developed by the National Library of Medicine at http://www.nlm.nih.gov/mesh/meshhome.html. Chemical classification of pharmaceutical chemicals for the GSK chemicals was suggested by Dr. Michael Olson of GSK, which in spirit captures three types of pharmaceutical active substances (actives, intermediates, and starting materials).

5 Information gathered from the following databases: The Good Scents Company (http://www.thegoodscentscompany.com/); Hazardous Substances Database (http://toxnet.nlm.nih.gov/cgi-bin/sis/htmlgen?HSDB); Haz-Map (http://hazmap.nlm.nih.gov/); Household Products Database (http://hpd.nlm.nih.gov/index.htm); International Programme on Chemical Safety INCHEM database (http://www.inchem.org/); National Library of Medicine Drug Information Portal (http://druginfo.nlm.nih.gov/drugportal/drugportal.jsp?APPLICATION_NAME=drugportal); National Toxicology Program (http://ntp.niehs.nih.gov/).

6 Structure in this row is cyproconazole. For quinoxyfen structure, see the preceding row.

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Annex IV

Analyses to Determine Representative EC3 Values

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1.0 Introduction

The analyses of the 63 murine local lymph node (LLNA) and human sensitizers detailed in **Section 6.0** of this background review document (BRD) include both linear regressions and Spearman correlations of the log-transformed LLNA EC3 values (i.e., estimated concentration of a substance expected to produce a stimulation index of 3, the threshold value for a substance to be considered a sensitizer in the LLNA) and human DSA_{05} values (induction dose per skin area, in $\mu g/cm^2$, in a human repeat-insult patch test or human maximization test that produces a positive response in 5% of the tested population), both in units of $\mu g/cm^2$. This annex describes the analyses performed to evaluate various approaches to calculate the geometric mean EC3 values for substances with multiple LLNA results. The approaches explored (1) the use of negative LLNA results for substances that also produced positive results (i.e., how to account for discordant negative results), (2) the use of vehicle-specific LLNA results for substances that had tests in multiple vehicles, and (3) the use of LLNA results from nonstandard protocols (**Section 5.1** of the BRD). Geometric mean DSA_{05} values were calculated using all available DSA_{05} values for each substance with multiple values.

2.0 Methods and Results

2.1 Combining LLNA Results Tested in Different Vehicles

Although two important factors that contribute to skin sensitization (i.e., the ability of the test substance to traverse the stratum corneum and reach the viable epidermis and the efficiency of Langerhans cell migration) are susceptible to vehicle effects (Basketter et al. 2001; Lea et al. 1999; McGarry 2007; Wright et al. 2001), others have noted that vehicle may have little impact on the accuracy of hazard identification in properly conducted standard test methods (Kimber et al. 2003). With respect to the LLNA potency analyses, while vehicle may be an important determinant of the EC3 value, it may not be important for every substance tested and therefore may have no overall effect on the linear regression analyses that include over 60 substances. To determine if multiple results for individual substances should be evaluated by vehicle (averaging EC3 values for each vehicle and then averaging the vehicle means) or without consideration of vehicle (averaging all EC3 values regardless of vehicle), the National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM) first evaluated whether there was a significant vehicle effect on LLNA EC3 values for the substances in the current database. **Table C-IV-1** shows the 27 vehicles represented in the NICEATM LLNA potency database and the number of LLNA tests for each vehicle. The LLNA vehicle was not specified for 29 tests.

The first analysis was a two-way analysis of variance (ANOVA) (Steel et al. 1997) with substance and vehicle as the factors that influence the EC3 value. EC3 data from four major vehicles represented in the database were used in the analysis: (1) acetone, (2) acetone: olive oil (4:1) (AOO), (3) dimethyl sulfoxide (DMSO), and (4) dimethyl formamide (DMF). All of these vehicles were mentioned as commonly used vehicles in the 1998 Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) evaluation of the LLNA (ICCVAM 1999). AOO, DMSO, and DMF are recommended vehicles in Organisation for Economic Co-operation and Development (OECD) Test Guideline 429 (OECD 2002), the U.S. Environmental Protection Agency (EPA) Health Effects TG 870.2600 (EPA 2003), and the updated ICCVAM-recommended LLNA protocol (ICCVAM 2009). For the two-way ANOVA, all negative LLNA tests that used concentrations that were less than positive tests for the same substance in the same vehicle (n = 10) were deleted. All other negative LLNA tests (i.e., negative at maximum doses that produced positive results in other LLNA tests) were assigned an EC3 = 110% as an arbitrary value for the EC3 (which exceeds the maximum possible value of 100% for a positive response) in order to maximize the available database. Data for substances that were tested in a single vehicle were deleted. The analysis

used data for 28 substances with 261 EC3 values. The two-way ANOVA on the log-transformed data indicated that vehicle was not a significant factor in determining the EC3 value ($F = 1.4758$, $p = 0.2400$) for tests with the four vehicles used in the analysis.

Table C-IV-1 Number of Tests for Each LLNA Vehicle

LLNA Vehicle	Number of Tests	% Total Tests
Acetone: olive oil (4:1)	319	47.9%
Dimethyl formamide	72	10.8%
Pluronic L92	50	7.5%
Ethanol/diethyl phthalate (1:3)	50	7.5%
Dimethyl sulfoxide	45	6.7%
Acetone	30	4.5%
Not specified[1]	29	4.4%
Ethanol/diethyl phthalate (3:1)	9	1.4%
Methyl ethyl ketone	9	1.4%
Propylene glycol	7	1.0%
Diethyl phthalate	6	0.9%
Ethanol	5	0.8%
Ethanol/diethyl phthalate (3:1) + 0.1% Trolox C	4	0.6%
Ethanol/diethyl phthalate (3:1) + acetone: olive oil (3:1) mix[2]	4	0.6%
Water	4	0.6%
Hydroxypropyl cellulose in methanol	4	0.6%
Ethanol/diethyl phthalate (3:1) + 0.1% tocopherol	4	0.6%
Ethanol (10%)	2	0.3%
Ethanol (50%)	2	0.3%
Ethanol/diethyl phthalate (3:1) + 2% tocopherol	2	0.3%
Petrolatum	2	0.3%
Acetone/Water (3:1)	1	0.2%
Dimethyl formamide/water	1	0.2%
Dimethyl sulfoxide/water (9:1)	1	0.2%
Ethanol (25%)	1	0.2%
Ethanol (30%)	1	0.2%
Ethanol (80%)	1	0.2%
Methyl ethyl ketone: olive oil (4:1)	1	0.2%
Grand Total	**666**	

Abbreviations: LLNA = murine local lymph node assay.

[1] Information on the vehicle used was not provided.

[2] Mix = 0.3% butylated hydroxytoluene/tocopherol/eugenol.

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Another two-way ANOVA was then performed using results from all vehicles with five or more tests. Again, substances that were tested in a single vehicle were deleted, as were the negative tests that used concentrations that were less than positive tests for the same substance and vehicle (n = 10). As above, the remaining negative LLNA tests were assigned an EC3 = 110%. The analysis included data for 41 substances, 11 vehicles, and 376 EC3 values. The two-way ANOVA of the log-transformed data indicated that vehicle was a significant factor in determining the EC3 value ($F = 4.0801$, $p = 0.0002$) for vehicles with at least five LLNA tests.

To determine which vehicles were responsible for the significant vehicle effect on the EC3 value, a number of additional two-way ANOVAs were performed for variations of the dataset that excluded one or more vehicles. Excluding propylene glycol and Pluronic L92 removed the significant effect of vehicle ($F = 1.75377$, $p = 0.1000$). The analysis used data for 41 substances, nine vehicles, and 352 EC3 values. Propylene glycol and Pluronic L92 tests accounted for only a small part of the 376-test dataset: 4.5% (17/376) and 1.9% (7/376), respectively.

By excluding only two vehicles, which accounted for a small proportion of the data, there was no significant vehicle effect on EC3 values in the current database. Thus, an EC3 versus DSA_{05} linear regression analysis was performed on the geometric mean EC3 value for each substance regardless of vehicle (see **Section 2.4.1**). A second linear regression was performed by using a geometric mean EC3 value for each substance that was calculated from the geometric mean EC3 values for each vehicle-substance combination (i.e., a geometric mean of the vehicle-substance geometric mean EC3 values). These two linear regressions were compared to provide additional evidence that vehicle has no effect on EC3 values in the current database. Finally, the optimal regression was repeated without propylene glycol and Pluronic L92 to confirm that there is no statistically significant vehicle effect on the EC3 versus DSA_{05} regression (see **Section 2.4.2**).

2.2 Combining LLNA Results for Substances With Both Sensitizer and Nonsensitizer Data

Some substances with multiple LLNA results have both sensitizer (positive) and nonsensitizer (negative) outcomes among the test results. In determining a representative EC3 value for such a substance, how should negative LLNA results be used? Negative LLNA test results could be replaced by an EC3 value that is unattainable in practice and averaged in with the positive tests. Negative LLNA test results could also simply be ignored. Then the EC3 values for only the positive tests for a given substance would be averaged. EC3 versus DSA_{05} linear regressions using geometric means were performed using two approaches for calculating representative EC3 values for each substance: (1) ignoring negative results, and (2) replacing negative results with 110% (see **Section 2.4**).

2.3 The Effect of Nonstandard Protocols on LLNA Results

To address the question of whether LLNA results from nonstandard protocols were different from LLNA results using the standard LLNA protocol (Dean et al. 2001; ICCVAM 1999; OECD 2002), a two-way ANOVA was performed using substance and protocol as the variable factors for the EC3 value. The database for this analysis included 656 LLNA results (10 negative results that used concentrations lower than those required for positive results for the same substance in other tests were excluded). The remaining negative LLNA results were replaced with EC3 values of 110% so that they could be used in the analysis. The analysis included 196 substances and considered three protocol groups: standard (73% [479/656]), nonstandard (18% [120/656]), and not reported (9% [57/656]). The two-way ANOVA of the log-transformed results showed that protocol had no effect on EC3 values ($F = 1.3790$, $p = 0.2600$). To determine whether protocol affects the EC3 versus DSA_{05} linear regression, the optimum regression was repeated using only EC3 results from the standard protocol (see **Section 2.4.2**).

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2.4 Correlation of EC3 with DSA_{05}

The analyses to establish the relationship of EC3 and DSA_{05} values included linear regressions on the log-transformed data expressed in units of $\mu g/cm^2$ and Spearman correlations. Note that the regressions and correlations use only substances that produced positive responses in the LLNA and in human maximization tests (HMT) and/or human repeat-insult patch tests (HRIPT). Although there were 65 substances that produced positive LLNA responses and positive HMT/HRIPT responses, nickel salts and streptomycin were excluded from the regressions because the most prevalent LLNA responses were negative (8/10 tests for nickel salts and 4/5 tests for streptomycin). Thus, the data available for the linear regressions and correlations include the 63 substances that yielded positive results in both the LLNA and human tests.

2.4.1 Approaches for Combining Multiple LLNA Results

The two major approaches for combining multiple results for EC3 and DSA_{05} values for individual substances were to use: (1) the most potent values (i.e., lowest EC3 and DSA_{05}) or (2) the geometric mean values. Other modifications to the geometric mean regression include two approaches to deal with negative LLNA results for substances that also produced positive results (i.e., how to use discordant negative results): (1) ignore the discordant negative results or (2) replace the discordant negative LLNA results with 110% (i.e., 27500 $\mu g/cm^2$). (Note: neither method for discordant negative results is applicable to the regression that uses the most potent values for each substance.) Additional modifications to the geometric mean regression include two approaches to using the vehicle-specific LLNA results for substances that had tests in multiple vehicles: (1) ignore the vehicle when calculating the geometric mean (i.e., pool all of the EC3 values) or (2) calculate a geometric mean of the results for each vehicle (for each substance) and then calculate the geometric mean of the vehicle-specific means for each substance. (Note: this computation is not applicable to the linear regressions using the most potent values for each substance.) For comparison, the linear regression using the optimal approach was repeated to confirm the lack of vehicle effect on the EC3 value by excluding the two vehicles that produced the significant vehicle effect in the two-way ANOVA, propylene glycol and Pluronic L92 (see **Section 2.4.2**). The linear regression using the optimal approach was also repeated to confirm the lack of a protocol effect by using only EC3 results from the standard LLNA protocol (Dean et al. 2001; ICCVAM 1999; OECD 2002) (see **Section 2.4.2**).

The linear regression results are shown in **Table C-IV-2**. Regression 1, which used the most potent results for EC3 and DSA_{05}, produced a slightly lower R^2 (0.382 versus 0.448) than regression 2, which used geometric mean EC3 and DSA_{05} values for substances with multiple results (see **Figure C-IV-1**). For regression 2, the geometric means were calculated across vehicle (i.e., potential vehicle effects were ignored) and discordant negative results were not included in the calculation (i.e., negatives were ignored). The geometric mean regression (3) that combined EC3 values regardless of vehicle and replaced discordant negative LLNA results with values of 27500 $\mu g/cm^2$ (i.e., equivalent to EC3 = 110%) was similar to the geometric mean regression (2) that did not use negative LLNA test results in the geometric mean EC3 values. This similarity is because only 10 negative LLNA results for six substances were replaced with 27500 $\mu g/cm^2$ in regression 3. Regressions 4 and 5 are similar to regressions 2 and 3, respectively, but use a different approach to combining multiple EC3 values. For regressions 4 and 5, a geometric mean was calculated for the EC3 values for each vehicle-substance combination, and then a geometric mean of those combination means was calculated for each substance. Regression 4 had a slightly lower slope than regression 2 (0.718 versus 0.742), possibly because the 10 discordant LLNA negative results exerted more influence on the regression when vehicle-specific results were averaged. In any case, the standard errors for the slopes and y-intercepts easily overlap for all of the geometric mean regressions (i.e., regressions 2, 3, 4, and 5). **Figure C-IV-2** graphically shows the similarity of these regressions. Thus, the inclusion (by

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Table C-IV-2 Linear Regression and Correlation Results[1]

Description of Regression/Correlation	N	Linear Regression				Spearman Correlation	
		Slope (μg/cm²)	Y-intercept	R^2	P-value	r	P-value
Standard, Nonstandard, and Unreported Protocols							
1) Most potent EC3 versus most potent DSA_{05}	63	0.594 ± 0.097	1.164 ± 0.275	0.382	<0.0001	0.594 (0.400-0.737)	<0.0001
2) Geometric mean EC3 versus geometric mean DSA_{05} – (vehicles ignored, negatives ignored)	**63**	**0.747 ± 0.106**	**0.722 ± 0.322**	**0.448**	**<0.0001**	**0.692 (0.530-0.804)**	**<0.0001**
3) Geometric mean EC3 (vehicles ignored, negatives = 27500 μg/cm²) versus geometric mean DSA_{05}	63	0.742 ± 0.105	0.712 ± 0.322	0.451	<0.0001	0.692 (0.531-0.805)	<0.0001
4) Geometric mean EC3 (geometric mean vehicles, negatives = 27500 μg/cm²) versus geometric mean DSA_{05}	63	0.718 ± 0.110	0.765 ± 0.338	0.414	<0.0001	0.646 (0.468-0.773)	<0.0001
5) Geometric mean EC3 (geometric mean vehicles, negatives ignored) versus geometric mean DSA_{05}	63	0.774 ± 0.107	0.639 ± 0.324	0.463	<0.0001	0.678 (0.512-0.796)	<0.0001
6) Optimal regression/correlation (3) repeated without propylene glycol and Pluronic L92 results	63	0.732 ± 0.104	0.773 ± 0.316	0.446	<0.0001	0.692 (0.531-0.805)	<0.0001
Standard Protocols							
7) Optimal regression/correlation (3) repeated with only the standard protocol results	54	0.701 ± 0.121	0.857 ± 0.353	0.393	<0.0001	0.642 (0.4495-0.780)	<0.0001

Boldface text highlights the optimal geometric mean linear regression.

Abbreviations: DSA_{05} = induction dose per skin area, in μg/cm², in a human repeat-insult patch test or human maximization test that produces a positive response in 5% of the tested population; EC3 = estimated concentration of a substance expected to produce a stimulation index of 3, which is the threshold value for a substance to be considered a sensitizer in the LLNA; LLNA = murine local lymph node assay; N = number of substances.

[1] Linear regressions and Spearman correlations used only the substances that were positive in both the LLNA and human tests.

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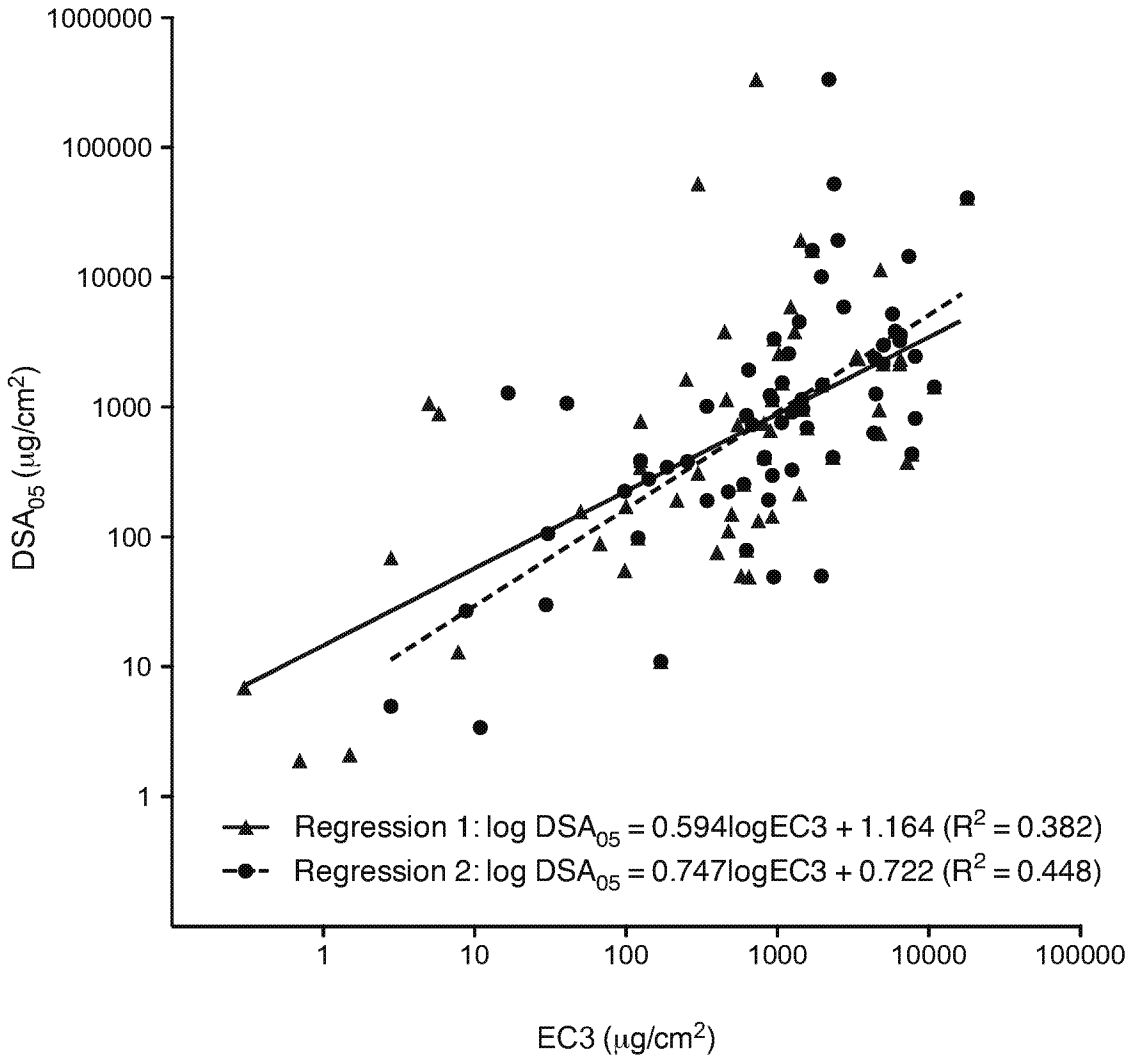
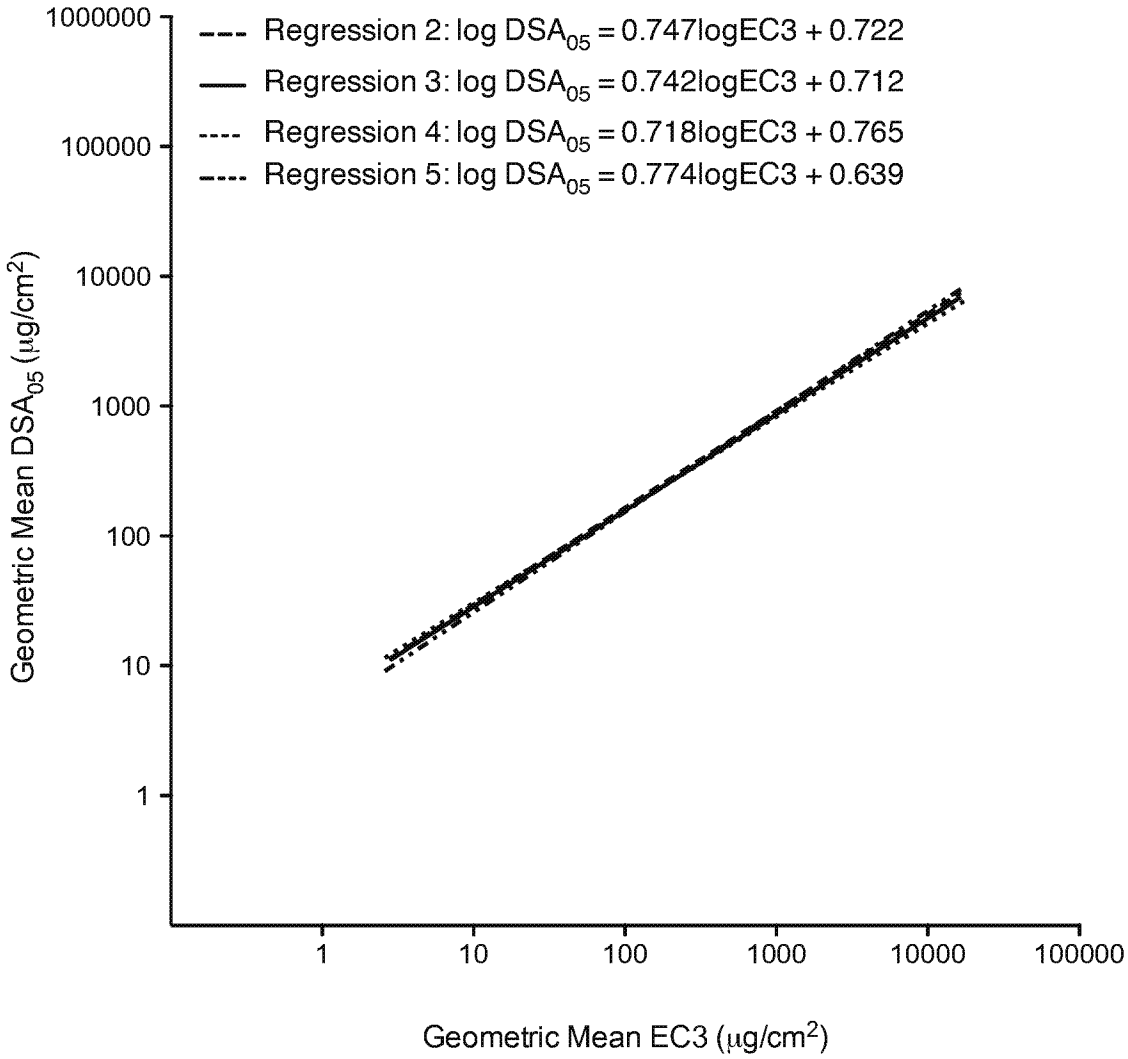
Figure C-IV-1 Most Potent and Geometric Mean Linear Regressions for LLNA EC3 versus Human DSA_{05} for 63 LLNA and Human Skin Sensitizers



Abbreviations: DSA_{05} = induction dose per skin area, in $\mu g/cm^2$, in a human repeat-insult patch test or human maximization test that produces a positive response in 5% of the tested population; EC3 = estimated concentration of a substance expected to produce a stimulation index of 3, which is the threshold value for a substance to be considered a sensitizer in the LLNA; LLNA = murine local lymph node assay.

The regressions correspond to regressions 1 and 2 in **Table C-IV-2**. The triangles and solid line show the data and regression line for the most potent EC3 value versus the corresponding human DSA_{05} (both in $\mu g/cm^2$) for 63 sensitizers. The circles and dashed line show the data and regression line for the geometric mean EC3 versus the corresponding geometric mean human DSA_{05} for the same substances. The geometric mean value was used for substances with more than one value. Geometric mean calculations of the EC3 excluded discordant negative results and ignored vehicle (i.e., results for all vehicles were pooled).

Figure C-IV-2 Geometric Mean Linear Regressions for LLNA EC3 versus Human DSA_{05} for 63 LLNA and Human Skin Sensitizers



Abbreviations: DSA_{05} = induction dose per skin area, in $\mu g/cm^2$, in a human repeat-insult patch test or human maximization test that produces a positive response in 5% of the tested population; EC3 = estimated concentration of a substance expected to produce a stimulation index of 3, which is the threshold value for a substance to be considered a sensitizer in the LLNA; LLNA = murine local lymph node assay.

The regressions of EC3 versus the corresponding human DSA_{05} (both in $\mu g/cm^2$) for 63 sensitizers correspond to regressions 2, 3, 4, and 5 in **Table C-IV-2**. So that the regressions can be viewed with more clarity, the data points, which are very similar for each regression, are not shown.

assigning an EC3 value of 110%) or exclusion of discordant negative tests had no noticeable impact on the regression analyses. All Spearman correlations for regressions 2, 3, 4, and 5 were highly significant ($p < 0.0001$). Correlations 2 and 3 had the highest correlation coefficient, $r = 0.692$.

The multiple linear regression analyses detailed in **Table C-IV-2** were performed to determine the optimum approach to apply in subsequent analyses (i.e., the calculation of correct, under-, and over-classification of human potency category by the EC3 value in **Sections 6.1** and **6.2** of the BRD). All of the geometric mean regressions yielded higher R^2 values than the regression that used the most potent values (**Table C-IV-2**). Because the geometric mean regressions (2, 3, 4, and 5) produced very similar results (**Table C-IV-2**; **Figure C-IV-2**) the optimum approach was considered to be the simplest computational approach, regression 3.

2.4.2 Confirming the ANOVA Results

To confirm the two-way ANOVA results reported in **Sections 2.1** and **2.3**, two additional regressions were performed. These regressions used the same approach as regression 2 for combining LLNA results: discordant negative results and vehicles were ignored in calculating the geometric mean EC3. Regression 6 confirms the two-way ANOVA results in **Section 2.1** that indicated that vehicle was not an important determinant of EC3 value in the current database (**Table C-IV-2**). When LLNA results that used propylene glycol (seven tests for the substances in the regression) and Pluronic L92 (15 tests for the substances used in the regression) were excluded from the analyses, regression 6 was similar to regression 2, which included these tests (**Figure C-IV-3**). For regressions 2 and 6, the standard errors for the slopes and intercepts easily overlapped (**Table C-IV-2**). Thus, the use of multiple LLNA vehicles in deriving the geometric mean EC3 value was confirmed to have no significant effect on the regression results.

Regression 7 was performed to confirm that the use of EC3 values from standard, nonstandard, and unreported protocols would not significantly affect the EC3 versus DSA_{05} regression (**Table C-IV-2**). The two-way ANOVA reported in **Section 2.3** indicated that protocol was not a significant determinant of EC3 value. Regression 7 was performed with only the LLNA results that were generated using standard protocols (Dean et al. 2001; ICCVAM 1999; OECD 2002). Excluding the LLNA tests from nonstandard or unreported protocols reduced the total number of LLNA tests from 375 (for 63 substances) included in regression 2 to 261 (for 54 substances) in regression 7. Nine substances were excluded from regression 7 because they had no positive LLNA tests using a standard protocol. Even with the exclusion of 30% of the LLNA results, regression 7 is similar to regression 2 (**Figure C-IV-4**). The standard errors for the slopes and intercepts easily overlap. Regression 2, however, is the preferred regression because it uses more data and also has a higher R^2.

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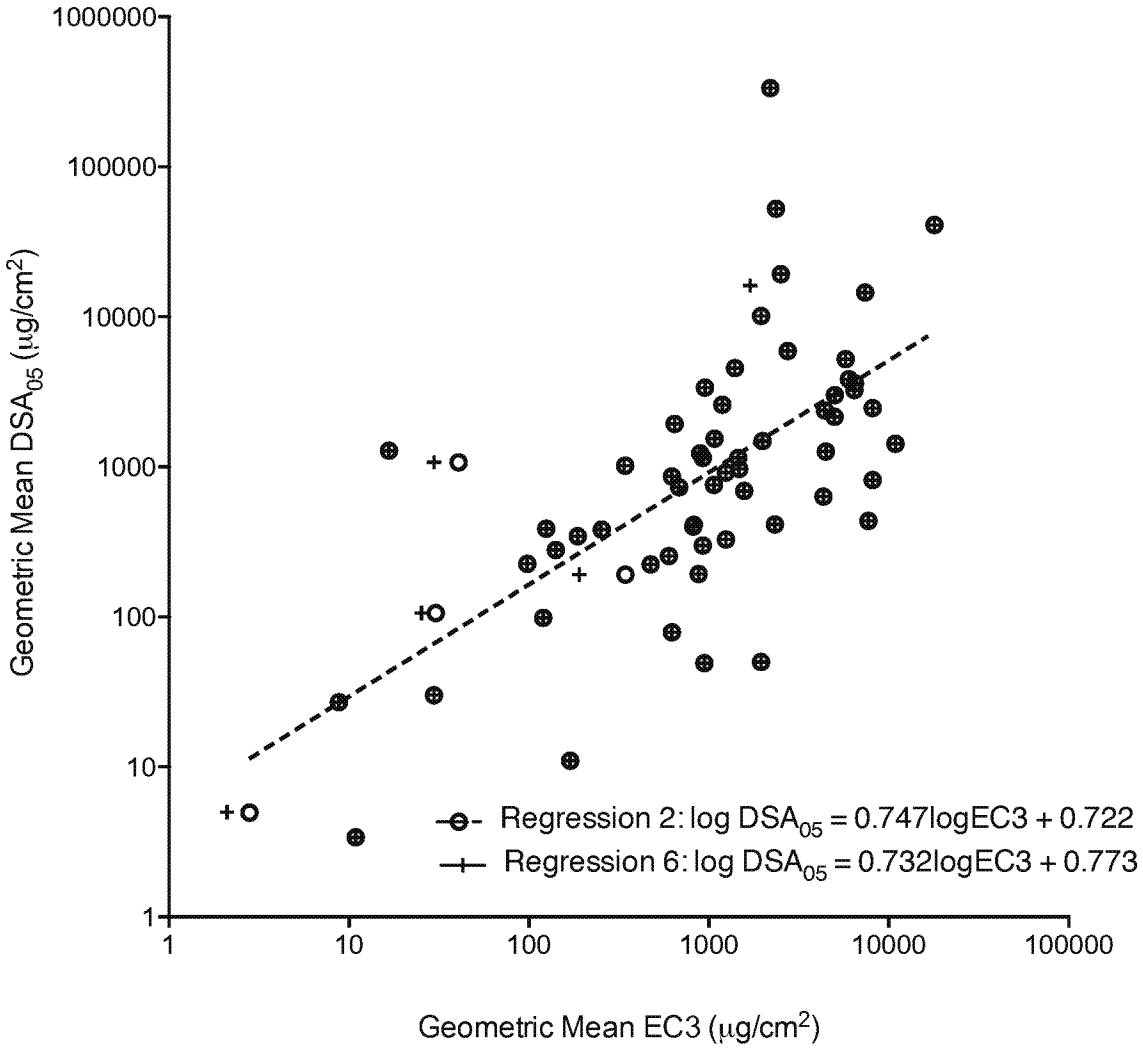
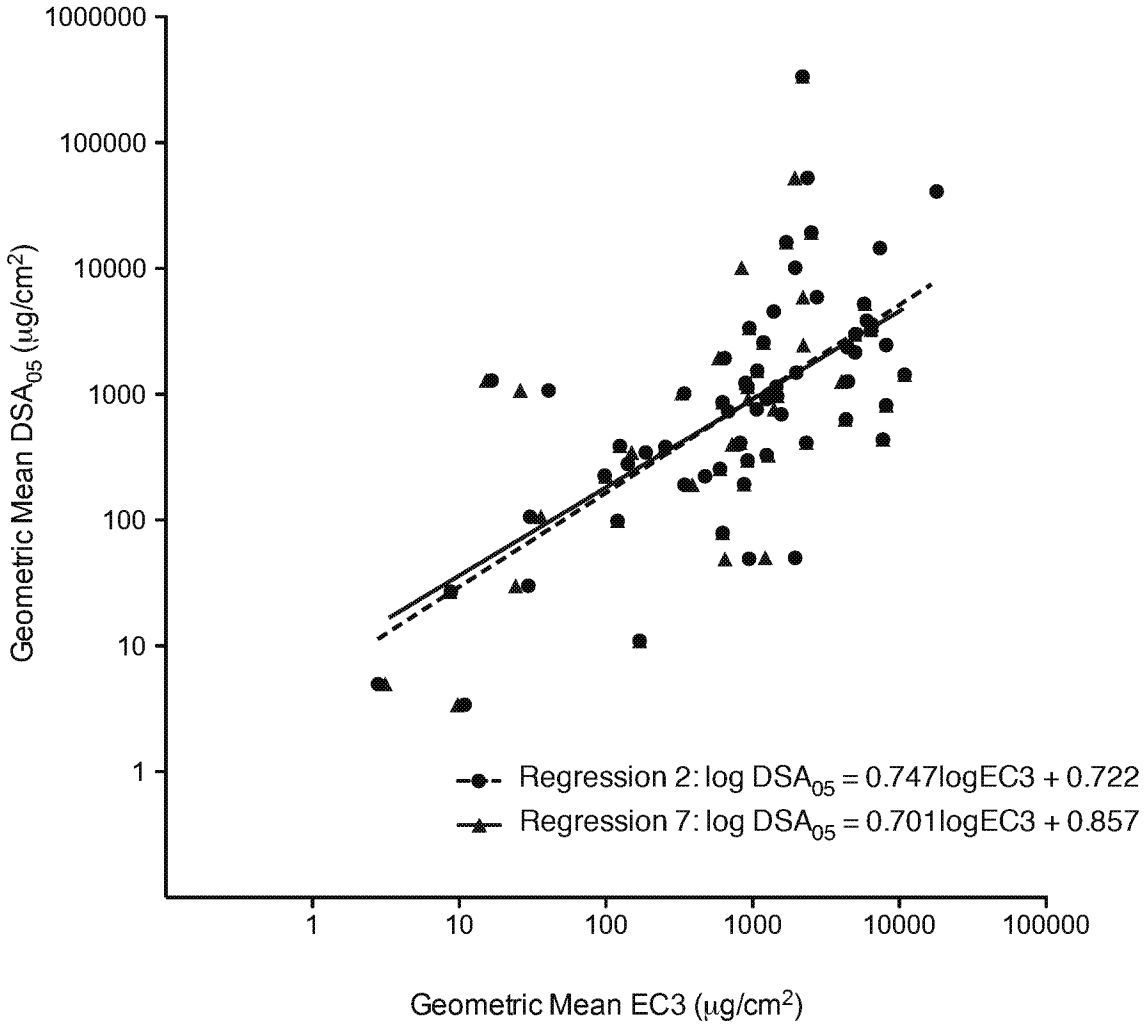
Figure C-IV-3 Geometric Mean Linear Regressions for LLNA EC3 versus Human DSA_{05} for 63 LLNA and Human Skin Sensitizers With or Without Propylene Glycol and Pluronic L92 Tests



Abbreviations: DSA_{05} = induction dose per skin area, in $\mu g/cm^2$, in a human repeat-insult patch test or human maximization test that produces a positive response in 5% of the tested population; EC3 = estimated concentration of a substance expected to produce a stimulation index of 3, which is the threshold value for a substance to be considered a sensitizer in the LLNA; LLNA = murine local lymph node assay.

The regressions correspond to regressions 2 and 6 in **Table C-IV-2**. Both regressions show the geometric mean EC3 value versus the corresponding geometric mean human DSA_{05} (both in $\mu g/cm^2$). The EC3 values for the circles and dashed line regression (2) include LLNA tests that use propylene glycol and Pluronic L92 as vehicles. The EC3 values shown by the diamonds and solid line regression (6) exclude tests that use propylene glycol and Pluronic L92 as vehicles. Many of the data points for the two regressions are coincident.

Figure C-IV-4 Geometric Mean Linear Regressions for LLNA EC3 versus Human DSA_{05} Using Standard (n = 54) or Standard and Nonstandard LLNA Protocols (n = 63)



Abbreviations: DSA_{05} = induction dose per skin area, in $\mu g/cm^2$, in a human repeat-insult patch test or human maximization test that produces a positive response in 5% of the tested population; EC3 = estimated concentration of a substance expected to produce a stimulation index of 3, which is the threshold value for a substance to be considered a sensitizer in the LLNA; LLNA = murine local lymph node assay.

The regressions correspond to regressions 2 and 7 in **Table C-IV-2**. Both regressions show the geometric mean EC3 value versus the corresponding geometric mean human DSA_{05} (both in $\mu g/cm^2$). The EC3 values for the circles and dashed line regression (2) include LLNA tests from both standard and nonstandard protocols. The EC3 values shown by the triangles and solid line regression (7) include only LLNA tests that use the standard LLNA protocol (Dean et al. 2001; ICCVAM 1999; OECD 2002).

3.0 References

Basketter DA, Gerberick GF, Kimber I. 2001. Skin sensitisation, vehicle effects and the local lymph node assay. Food Chem Toxicol 39(6): 621-627.

Dean JH, Twerdok LE, Tice RR, Sailstad DM, Hattan DG, Stokes WS. 2001. ICCVAM evaluation of the murine local lymph node assay: II. Conclusions and recommendations of an independent scientific peer review panel. Regul Toxicol Pharmacol 34(3): 258-273.

EPA. 2003. Health Effects Test Guideline OPPTS 870.2600 - Skin Sensitization. EPA 712-C-03-197. Washington, DC:U.S. Environmental Protection Agency. Available: http://www.regulations.gov/fdmspublic/ContentViewer?objectId=09000064809bc444&disposition=attachment&contentType=pdf [accessed 6 July 2010].

ICCVAM. 1999. The Murine Local Lymph Node Assay: A Test Method for Assessing the Allergic Contact Dermatitis Potential of Chemicals/Compounds. The Results of an Independent Peer Review Evaluation Coordinated by the Interagency Coordinating Committee on the Validation of Alternative Toxicological Methods (ICCVAM) and the National Toxicology Program Center for the Evaluation of Alternative Toxicologial Methods (NICEATM). NIH Publication No. 99-4494. Research Triangle Park, NC:National Institute of Environmental Health Sciences. Available: http://iccvam.niehs.nih.gov/docs/immunotox_docs/llna/llnarep.pdf [accessed 6 July 2010].

ICCVAM. 2009. Recommended Performance Standards: Murine Local Lymph Node Assay. NIH Publication No. 09-7357. Research Triangle Park, NC:National Institute of Environmantal Health Sciences. Available: http://iccvam.niehs.nih.gov/methods/immunotox/llna_PerfStds.htm [accessed 6 July 2010].

Kimber I, Basketter DA, Butler M, Gamer A, Garrigue JL, Gerberick GF, et al. 2003. Classification of contact allergens according to potency: proposals. Food Chem Toxicol 41(12): 1799-1809.

Lea LJ, Warbrick EV, Dearman RJ, Kimber I, Basketter DA. 1999. The impact of vehicle on assessment of relative skin sensitization potency of 1,4-dihydroquinone in the local lymph node assay. Am J Contact Dermat 10(4): 213-218.

McGarry HF. 2007. The murine local lymph node assay: regulatory and potency considerations under REACH. Toxicology 238(2-3): 71-89.

OECD. 2002. Test No. 429. Skin Sensitisation: Local Lymph Node Assay [adopted 24 April 2002]. In: OECD Guidelines for the Testing of Chemicals, Section 4: Health Effects. Paris:OECD Publishing. Available: http://sourceoecd.org/vl=6381888/cl=19/nw=1/rpsv/ij/oecdjournals/1607310x/v1n4/s29/p1 [accessed 30 July 2010].

Steel RGD, Torrie JH, Dickey DA. 1997. Principles and Procedures of Statistics: A Biometrical Approach. 3rd ed. New York:McGraw-Hill.

Wright ZM, Basketter DA, Blaikie L, Cooper KJ, Warbrick EV, Dearman RJ, et al. 2001. Vehicle effects on skin sensitizing potency of four chemicals: assessment using the local lymph node assay. Int J Cosmet Sci 23(2): 75-83.

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Annex V

Performance Characteristics for Use of LLNA EC3 Values to Predict Human Skin Sensitization Potency Categories

Annex V-1
Performance Characteristics for the Use of LLNA EC3 to Classify 63 Sensitizers into Strong Sensitizer ($DSA_{05} \leq 500$ μg/cm^2) and Other Sensitizer ($DSA_{05} > 500$ μg/cm^2) Categories C-323

Annex V-2
Performance Characteristics for the Use of LLNA EC3 to Classify 136 Substances into Strong Sensitizer ($DSA_{05} \leq 500$ μg/cm^2), Other Sensitizer ($DSA_{05} > 500$ μg/cm^2), and Nonsensitizer Categories C-329

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Annex V-1

Performance Characteristics for the Use of LLNA EC3 to Classify 63 Sensitizers into Strong Sensitizer ($DSA_{05} \leq 500$ µg/cm^2) and Other Sensitizer ($DSA_{05} > 500$ µg/cm^2) Categories

The 63 sensitizers in the LLNA and human skin sensitization tests include 25 strong and 38 other human sensitizers based on the criteria of the Globally Harmonized System of Classification and Labelling of Chemicals (UN 2009).

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EC3 Cutoff (%)	Substances Correctly Classified as Strong Sensitizers[1]	Other Sensitizers Overclassified as Strong[1]	Underclassified Strong Sensitizers[1]	Substances Correctly Classified as Other Sensitizers[1]	Correct Classification Rate	Overclassification Rate	Underclassification Rate
0.02	1	0	24	38	62%	0%	96%
0.04	2	0	23	38	63%	0%	92%
0.06	3	0	22	38	65%	0%	88%
0.09	3	1	22	37	63%	3%	88%
0.12	4	1	21	37	65%	3%	84%
0.14	5	1	20	37	67%	3%	80%
0.28	5	2	20	36	65%	5%	80%
0.44	6	2	19	36	67%	5%	76%
0.49	7	2	18	36	68%	5%	72%
0.53	8	2	17	36	70%	5%	68%
0.62	9	2	16	36	71%	5%	64%
0.71	10	2	15	36	73%	5%	60%
0.81	11	2	14	36	75%	5%	56%
0.94	12	2	13	36	76%	5%	52%
1.19	13	2	12	36	78%	5%	48%
1.37	13	3	12	35	76%	8%	48%
1.69	14	3	11	35	78%	8%	44%
2.00	**14**	**3**	**11**	**35**	**78%**	**8%**	**44%**
2.20	14	4	11	34	76%	11%	44%
2.45	15	4	10	34	78%	11%	40%
2.54	16	5	9	33	78%	13%	36%
2.66	16	6	9	32	76%	16%	36%

The 63 sensitizers in the LLNA and human skin sensitization tests include 25 strong and 38 other human sensitizers based on the criteria of the Globally Harmonized System of Classification and Labelling of Chemicals (UN 2009).

Boldface data show the results for the optimal EC3 value of 3.79% and the GHS cutoff EC3 value of 2%.

[1] Values are numbers of substances.

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EC3 Cutoff (%)	Substances Correctly Classified as Strong Sensitizers[1]	Other Sensitizers Overclassified as Strong[1]	Underclassified Strong Sensitizers[1]	Substances Correctly Classified as Other Sensitizers[1]	Correct Classification Rate	Overclassification Rate	Underclassification Rate
3.00	16	7	9	31	75%	18%	36%
3.29	17	7	8	31	76%	18%	32%
3.40	18	7	7	31	78%	18%	28%
3.54	19	7	6	31	79%	18%	24%
3.64	19	8	6	30	78%	21%	24%
3.74	20	9	5	29	78%	24%	20%
3.79	**21**	**9**	**4**	**29**	**79%**	**24%**	**16%**
4.00	21	9	4	29	79%	24%	16%
4.03	21	10	4	28	78%	26%	16%
4.28	21	11	4	27	76%	29%	16%
4.52	21	12	4	26	75%	32%	16%
4.87	21	13	4	25	73%	34%	16%
4.99	21	14	4	24	71%	37%	16%
5.29	22	14	3	24	73%	37%	12%
5.70	22	15	3	23	71%	39%	12%
5.86	22	16	3	22	70%	42%	12%
6.00	22	16	3	22	70%	42%	12%
6.10	22	17	3	21	68%	45%	12%
6.55	22	18	3	20	67%	47%	12%
7.30	22	19	3	19	65%	50%	12%
7.79	23	19	2	19	67%	50%	8%
7.87	23	20	2	18	65%	53%	8%
8.00	23	20	2	18	65%	53%	8%
8.36	23	21	2	17	63%	55%	8%

Boldface data show the results for the optimal EC3 value of 3.79% and the GHS cutoff EC3 value of 2%.

[1] Values are numbers of substances.

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EC3 Cutoff (%)	Substances Correctly Classified as Strong Sensitizers[1]	Other Sensitizers Overclassified as Strong[1]	Underclassified Strong Sensitizers[1]	Substances Correctly Classified as Other Sensitizers[1]	Correct Classification Rate	Overclassification Rate	Underclassification Rate
9.04	23	22	2	16	62%	58%	8%
9.38	24	22	1	16	63%	58%	4%
9.76	24	23	1	15	62%	61%	4%
10.00	24	23	1	15	62%	61%	4%
10.51	24	24	1	14	60%	63%	4%
14.16	24	25	1	13	59%	66%	4%
17.51	24	26	1	12	57%	68%	4%
17.78	24	27	1	11	56%	71%	4%
18.96	24	28	1	10	54%	74%	4%
21.54	24	29	1	9	52%	76%	4%
23.53	24	30	1	8	51%	79%	4%
24.90	24	31	1	7	49%	82%	4%
25.90	24	32	1	6	48%	84%	4%
27.79	24	33	1	5	46%	87%	4%
30.24	24	34	1	4	44%	89%	4%
31.70	25	34	0	4	46%	89%	0%
32.51	25	35	0	3	44%	92%	0%
38.01	25	36	0	2	43%	95%	0%
57.70	25	37	0	1	41%	97%	0%

Boldface data show the results for the optimal EC3 value of 3.79% and the GHS cutoff EC3 value of 2%.

Abbreviations: DSA_{05} = induction dose per skin area, in $\mu g/cm^2$, in a human repeat-insult patch test or human maximization test that produces a positive response in 5% of the tested population; EC3 = estimated concentration of a substance expected to produce a stimulation index of 3, which is the threshold value for a substance to be considered a sensitizer in the LLNA; GHS = Globally Harmonized System of Classification and Labelling of Chemicals (UN 2009); LLNA = murine local lymph node assay.

[1] Values are numbers of substances.

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Annex V-2

Performance Characteristics for the Use of LLNA EC3 to Classify 136 Substances into Strong Sensitizer ($DSA_{05} \leq 500$ µg/cm^2), Other Sensitizer ($DSA_{05} > 500$ µg/cm^2), and Nonsensitizer Categories

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ED_006472_00084346-00404

EC3 Cutoff (%)	Substances Correctly Classified as Strong Sensitizers[1]	Other Sensitizers Overclassified as Strong[1]	Underclassified Strong Sensitizers[1]	Substances Correctly Classified as Other[1]	Other Substances Underclassified as Nonsensitizers[1]	Nonsensitizers Overclassified as Sensitizers[1]	Correctly Classified Nonsensi-tizers[1]	Correct Classifica-tion Rate[2]	Over-classifica-tion Rate[3]	Under-classifica-tion Rate[4]
0.02	1	0	26	38	11	35	25	47%	32%	49%
0.04	2	0	25	38	11	35	25	48%	32%	47%
0.06	3	0	24	38	11	35	25	49%	32%	46%
0.09	3	1	24	37	11	35	25	48%	33%	46%
0.12	4	1	23	37	11	35	25	49%	33%	45%
0.14	5	1	22	37	11	35	25	49%	33%	43%
0.28	5	2	22	36	11	35	25	49%	34%	43%
0.44	6	2	21	36	11	35	25	49%	34%	42%
0.49	7	2	20	36	11	35	25	50%	34%	41%
0.53	8	2	19	36	11	35	25	51%	34%	39%
0.62	9	2	18	36	11	35	25	51%	34%	38%
0.71	10	2	17	36	11	35	25	52%	34%	37%
0.81	11	2	16	36	11	35	25	53%	34%	36%
0.94	12	2	15	36	11	35	25	54%	34%	34%
1.19	13	2	14	36	11	35	25	54%	34%	33%
1.37	13	3	14	35	11	35	25	54%	35%	33%
1.69	14	3	13	35	11	35	25	54%	35%	32%
2.00	**14**	**3**	**13**	**35**	**11**	**35**	**25**	**54%**	**35%**	**32%**
2.20	14	4	13	34	11	35	25	54%	36%	32%
2.45	15	4	12	34	11	35	25	54%	36%	30%
2.54	16	5	11	33	11	35	25	54%	37%	29%
2.66	16	6	11	32	11	35	25	54%	38%	29%

Boldface data show the results for the GHS EC3 cutoff of 2%.
[1] Values are numbers of substances.
[2] Correctly classified human strong sensitizers, human other sensitizers, and human nonsensitizers.
[3] Includes human other sensitizers and nonsensitizers that are overclassified by the LLNA (n = 109).
[4] Includes human strong sensitizers and other sensitizers that are underclassified by the LLNA (n = 76).

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EC3 Cutoff (%)	Substances Correctly Classified as Strong Sensitizers[1]	Other Sensitizers Overclassified as Strong[1]	Underclassified Strong Sensitizers[1]	Substances Correctly Classified as Other[1]	Other Substances Underclassified as Nonsensitizers[1]	Nonsensitizers Overclassified as Sensitizers[1]	Correctly Classified Nonsensi-tizers[1]	Correct Classifica-tion Rate[2]	Over-classifica-tion Rate[3]	Under-classifica-tion Rate[4]
3.00	16	7	11	31	11	35	25	53%	39%	29%
3.29	17	7	10	31	11	35	25	54%	39%	28%
3.40	18	7	9	31	11	35	25	54%	39%	26%
3.54	19	7	8	31	11	35	25	55%	39%	25%
3.64	19	8	8	30	11	35	25	54%	39%	25%
3.74	20	9	7	29	11	35	25	54%	40%	24%
3.79	21	9	6	29	11	35	25	55%	40%	22%
4.00	21	9	6	29	11	35	25	55%	40%	22%
4.03	21	10	6	28	11	35	25	54%	41%	22%
4.28	21	11	6	27	11	35	25	54%	42%	22%
4.52	21	12	6	26	11	35	25	53%	43%	22%
4.87	21	13	6	25	11	35	25	52%	44%	22%
4.99	21	14	6	24	11	35	25	51%	45%	22%
5.29	22	14	5	24	11	35	25	52%	45%	21%
5.70	22	15	5	23	11	35	25	51%	46%	21%
5.86	22	16	5	22	11	35	25	51%	47%	21%
6.00	22	16	5	22	11	35	25	51%	47%	21%
6.10	22	17	5	21	11	35	25	50%	48%	21%
6.55	22	18	5	20	11	35	25	49%	49%	21%
7.30	22	19	5	19	11	35	25	49%	50%	21%
7.79	23	19	4	19	11	35	25	49%	50%	20%
7.87	23	20	4	18	11	35	25	49%	50%	20%

Boldface data show the results for the GHS EC3 cutoff of 2%.

[1] Values are numbers of substances.

[2] Correctly classified human strong sensitizers, human other sensitizers, and human nonsensitizers.

[3] Includes human other sensitizers and nonsensitizers that are overclassified by the LLNA (n = 109).

[4] Includes human strong sensitizers and other sensitizers that are underclassified by the LLNA (n = 76).

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EC3 Cutoff (%)	Substances Correctly Classified as Strong Sensitizers[1]	Other Sensitizers Overclassified as Strong[1]	Underclassified Strong Sensitizers[1]	Substances Correctly Classified as Other[1]	Other Substances Underclassified as Nonsensitizers[1]	Nonsensitizers Overclassified as Sensitizers[1]	Correctly Classified Nonsensi-tizers[1]	Correct Classifica-tion Rate[2]	Over-classifica-tion Rate[3]	Under-classifica-tion Rate[4]
8.00	23	20	4	18	11	35	25	49%	50%	20%
8.36	23	21	4	17	11	35	25	48%	51%	20%
9.04	23	22	4	16	11	35	25	47%	52%	20%
9.38	24	22	3	16	11	35	25	48%	52%	18%
9.76	24	23	3	15	11	35	25	47%	53%	18%
10.00	24	23	3	15	11	35	25	47%	53%	18%
10.51	24	24	3	14	11	35	25	46%	54%	18%
14.16	24	25	3	13	11	35	25	46%	55%	18%
17.51	24	26	3	12	11	35	25	45%	56%	18%
17.78	24	27	3	11	11	35	25	44%	57%	18%
18.96	24	28	3	10	11	35	25	43%	58%	18%
21.54	24	29	3	9	11	35	25	43%	59%	18%
23.53	24	30	3	8	11	35	25	42%	60%	18%
24.90	24	31	3	7	11	35	25	41%	61%	18%
25.90	24	32	3	6	11	35	25	40%	61%	18%
27.79	24	33	3	5	11	35	25	40%	62%	18%
30.24	24	34	3	4	11	35	25	39%	63%	18%
31.70	25	34	2	4	11	35	25	40%	63%	17%
32.51	25	35	2	3	11	35	25	39%	64%	17%
38.01	25	36	2	2	11	35	25	38%	65%	17%
57.70	25	37	2	1	11	35	25	37%	66%	17%

Boldface data show the results for the GHS EC3 cutoff of 2%.

Abbreviations: DSA_{05} = induction dose per skin area, in $\mu g/cm^2$, in a human repeat-insult patch test or human maximization test that produces a positive response in 5% of the tested population; EC3 = estimated concentration of a substance expected to produce a stimulation index of 3, which is the threshold value for a substance to be considered a sensitizer in the LLNA; GHS = Globally Harmonized System of Classification and Labelling of Chemicals (UN 2009); LLNA = murine local lymph node assay.

[1] Values are numbers of substances.

[2] Correctly classified human strong sensitizers, human other sensitizers, and human nonsensitizers.

[3] Includes human other sensitizers and nonsensitizers that are overclassified by the LLNA (n = 109).

[4] Includes human strong sensitizers and other sensitizers that are underclassified by the LLNA (n = 76).

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Appendix D

Independent Scientific Peer Review Panel Assessment

D1 Summary Minutes from the Independent Scientific Peer Review Panel Meeting on March 4-6, 2008 ..... D-3

D2 Peer Review Panel Report: Validation Status of New Versions and Applications of the Murine Local Lymph Node Assay: A Test Method for Assessing the Allergic Contact Dermatitis Potential of Chemicals and Products D-33

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Appendix D1

Summary Minutes from the Independent Scientific Peer Review Panel Meeting on March 4-6, 2008

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Summary Minutes

Independent Scientific Peer Review Panel Meeting

Validation Status of New Versions and Applications of the Murine Local Lymph Node Assay (LLNA): A Test Method for Assessing the Allergic Contact Dermatitis Potential of Chemicals and Products

Consumer Product Safety Commission (CPSC), Headquarters
Bethesda, MD
March 4 – 6, 2008
8:30 a.m. – 5:30 p.m.

Peer Review Panel Members:

Michael Luster, Ph.D. (Peer Review Panel Chair)	Senior Consultant to the NIOSH Health Effects Laboratory, Morgantown, WV, U.S.
Nathalie Alépée, Ph.D.	Associate Research Fellow, Pfizer PDRD MCT Laboratory, France
Anne Marie Api, Ph.D.	Vice President, Human Health Sciences, Research Institute for Fragrance Materials, Woodcliff Lake, NJ, U.S.
Nancy Flournoy, M.S., Ph.D.	Professor and Chair, Dept. of Mathematics and Statistics, University of Missouri-Columbia, Columbia, MO, U.S.
Thomas Gebel, Ph.D.	Regulatory Toxicologist, Federal Institute for Occupational Safety and Health, Dortmund, Germany
Kim Headrick, B. Admin., B.Sc.	International Harmonization Senior Policy Advisor, Health Canada, Ottawa, Ontario, Canada
Dagmar Jírová, M.D., Ph.D.	Toxicologist, Research Manager, Head of Reference Center for Cosmetics, Head of Reference Laboratory for Experimental Immunotoxicology, National Institute of Public Health, Czech Republic
David Lovell, Ph.D.	Reader in Medical Statistics, Postgraduate Medical School, University of Surrey, Guildford, Surrey, U.K.
Howard Maibach, M.D.	Professor, Dept. of Dermatology, University of California-San Francisco, San Francisco, CA, U.S.

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Peer Review Panel Members:

James McDougal, Ph.D.	Professor and Director of Toxicology Research, Dept. of Pharmacology and Toxicology, Boonshoft School of Medicine, Wright State University, Dayton, OH, U.S.
Michael Olson, Ph.D.	Director of Occupational Toxicology, Corporate Environment Health and Safety, GlaxoSmithKline, RTP, NC, U.S.
Raymond Pieters, Ph.D.	Associate Professor, Immunotoxicology Group Leader, Institute for Risk Assessment Sciences, Utrecht University, Utrecht, The Netherlands
Jean Regal, Ph.D.	Professor, Dept. of Pharmacology, University of Minnesota Medical School, Duluth, MN, U.S.
Peter Theran, V.M.D.	Massachusetts Society for the Prevention of Cruelty to Animals, Novato, CA, U.S.
Stephen Ullrich, Ph.D.	Dallas/Ft. Worth Living Legends Professor & Professor of Immunology, Graduate School of Biomedical Science, University of Texas M.D. Anderson Cancer Center, Houston, TX, U.S.
Michael Woolhiser, Ph.D.	Technical Leader - Immunotoxicology, Toxicology and Environmental Research and Consulting Immunology, Dow Chemical, Midland, MI, U.S.
Takahiko Yoshida, M.D., Ph.D.	Professor, Dept. of Health Science, Asahikawa Medical College, Hokkaido, Japan

ICCVAM and ICCVAM IWG Members:

Paul Brown, Ph.D.	FDA, Silver Spring, MD, U.S.
Ruth Barratt, Ph.D., D.V.M.	FDA, Rockville, MD, U.S.
Karen Hamernik, Ph.D.	EPA, Washington, DC, U.S.
Masih Hashim, Ph.D.	EPA, Washington, DC, U.S.
Abigail Jacobs, Ph.D. (IWG Co-Chair)	FDA, Silver Spring, MD, U.S.
Kristina Hatlelid, Ph.D.	CPSC, Bethesda, MD, U.S.
Joanna Matheson, Ph.D. (IWG Co-Chair)	CPSC, Bethesda, MD, U.S.
Tim McMahon, Ph.D.	EPA, Washington, DC, U.S.

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ICCVAM and ICCVAM IWG Members:

Amy Rispin, Ph.D.	EPA, Washington, DC, U.S.
William Stokes, D.V.M., DACLAM	NIEHS, RTP, NC, U.S.
Raymond Tice, Ph.D.	NIEHS, RTP, NC, U.S.
Ron Ward, Ph.D.	EPA, Washington, DC, U.S.
Marilyn Wind, Ph.D. (ICCVAM Chair)	CPSC, Bethesda, MD, U.S.
Jiaqin Yao, Ph.D.	FDA, Silver Spring, MD, U.S.

ECVAM Observer:

David Basketter, Ph.D.	DABMEB Consultancy Ltd., Bedfordshire, U.K.

Invited Experts:

George DeGeorge, Ph.D., DABT	MB Research Laboratories, Spinnerstown, PA, U.S.
Kenji Idehara, Ph.D.	Daicel Chemical Industries, Hyogo, Japan
Masahiro Takeyoshi, Ph.D.	Chemicals Evaluation and Research Institute, Saitama, Japan

Public Attendees:

Odette Alexander	Syngenta Crop Protection, Inc., Greensboro, NC, U.S.
Nancy Beck, Ph.D.	PCRM, Washington, DC, U.S.
Ann Blacker, Ph.D.	Bayer CropScience, RTP, NC, U.S.
Stuart Cagan, Ph.D.	Shell Oil Company, Houston, TX, U.S.
Joan Chapdelaine, Ph.D.	Calvert Laboratories, Inc., Olyphant, PA, U.S.
Adriana Doi, Ph.D.	BASF Corporation, RTP, NC, U.S.
Carol Eisenmann, Ph.D.	Personal Care Products Council, Washington, DC, U.S.
Charles Hastings, Ph.D.	BASF Corporation, RTP, NC, U.S.
Kailash Gupta, D.V.M., Ph.D.	Retired CPSC, Bethesda, MD, U.S.
John Lyssikatos	Hill Top Research, Miamiville, OH, U.S.
Laurence Musset, Ph.D.	OECD, Paris, France
Carol O'Neil	NuPathe, Conshohocken, PA, U.S.

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Public Attendees:

Kui Lea Park, Ph.D.	National Institute of Toxicological Research, KFDA, Seoul, Korea
Rafael Rivas	AFRRI/USUHS, Bethesda, MD, U.S.
Terri Sebree	NuPathe, Conshohocken, PA, U.S.
Libby Sommer	EPA, Washington, DC, U.S.
Merrill Tisdel	Syngenta Crop Protection Inc., Greensboro, NC, U.S.
Jeffrey Toy, Ph.D.	FDA, Rockville, MD, U.S.

NICEATM:

William Stokes, D.V.M., DACLAM	Director
Raymond Tice, Ph.D.	Deputy Director
Debbie McCarley	Special Assistant to the Director

Support Contract Staff— Integrated Laboratory Systems, Inc. (ILS)

David Allen, Ph.D.	Michael Paris
Thomas Burns, M.S.	Eleni Salicru, Ph.D.
Linda Litchfield	Judy Strickland, Ph.D., DABT
Douglas Winters, M.S.	

Abbreviations:

AFRRI = Armed Forces Radiobiology Research Institute

CPSC = U.S. Consumer Product Safety Commission

ECVAM = European Centre for the Validation of Alternative Methods

EPA = U.S. Environmental Protection Agency

FDA = U.S. Food and Drug Administration

ICCVAM = Interagency Coordinating Committee on the Validation of Alternative Methods

ILS = Integrated Laboratory Systems

IWG = Immunotoxicology Working Group

KFDA = Korea Food and Drug Administration

NICEATM = National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods

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NIEHS = National Institute of Environmental Health Sciences

NIOSH = National Institute for Occupational Safety and Health

OECD = Organisation for Economic Co-operation and Development

PCRM = Physicians Committee for Responsible Medicine

USDA = U.S. Department of Agriculture

USUHS = Uniformed Services University of the Health Sciences

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TUESDAY, MARCH 4, 2008

Call to Order and Introductions—

Dr. Michael Luster (Peer Review Panel Chair) called the meeting to order at 8:30 a.m. and introduced himself. He then asked all Peer Review Panel (hereafter Panel) members to introduce themselves and to state their name and affiliation for the record. He then asked all the National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM) staff, the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) members, the ICCVAM Immunotoxicity Working Group (IWG) members, the European Centre for the Validation of Alternative Methods (ECVAM) observer, and members of the public to also introduce themselves. Dr. Luster stated that there would be opportunity for public comments during each of the seven local lymph node assay (LLNA)-related topics. He asked that all those interested in making a comment register at the registration table and provide a written copy of their comments, if available, to NICEATM staff. Dr. Luster emphasized that the comments would be limited to seven minutes per individual and that, while an individual would be welcome to make comments during each commenting period, repeating the same comments at each comment period would be inappropriate. He further stated that the meeting was being recorded and that Panel members should speak directly their microphone. Finally, Dr. Luster noted that if the Panel finished early with the assigned topics on the agenda for that day, they would proceed to the next day's topics if time permitted.

Welcome from the ICCVAM Chair—

Dr. Marilyn Wind, U.S. Consumer Product Safety Commission (CPSC) and Chair of ICCVAM, welcomed everyone to CPSC and to the Panel meeting. Dr. Wind stressed the importance of this Panel's efforts especially considering recent reports that allergies and asthma have increased markedly over the past number of years and that contact dermatitis is the most common occupational illness in the United States. Dr. Wind thanked the Panel members for giving their expertise, time, and effort and acknowledged their important role to the ICCVAM test method evaluation process. Dr. Wind also emphasized the important role of the public and their comments in this process.

Welcome from the Director of NICEATM, and Conflict of Interest Statements—

Dr. William Stokes, Director of NICEATM, stated the Panel meeting was being convened as a National Institutes of Health (NIH) special emphasis panel and was being held in accordance with the Federal Advisory Committee Act regulations. As such, Dr. Stokes indicated that he would serve as the Designated Federal Official for this public meeting. He reminded the Panel that they had signed a conflict-of-interest statement when they were selected for the Panel, in which they identified any potential conflicts of interest. He then read this statement to provide another opportunity for members of the Panel to identify any conflicts not previously declared. Dr. Luster asked the Panel members to declare any direct or indirect conflicts based on Dr. Stokes statements and to recuse themselves from discussion and voting on any aspect of the meeting where there might be a conflict. None of the Panel members declared a conflict of interest.

Overview of the ICCVAM Test Method Evaluation Process

Dr. Stokes provided an overview of the ICCVAM test method evaluation process. He stated that the Panel was made up of 19 different scientists from eight different countries (Canada, Czech Republic, France, Germany, Japan, The Netherlands, United Kingdom, and the United States). Dr. Stokes thanked the Panel members for the significant amount of time and effort that they had devoted to prepare for and attend the meeting. He explained that the purpose of the Panel was to assist ICCVAM by carrying out an independent scientific peer review of the information provided on a series of proposed new versions of the LLNA and some expanded applications of the assay. Dr. Stokes mentioned that the original LLNA peer review panel in 1998 considered the LLNA a valid substitute

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for the guinea pig-based test in most testing situations, but not all. He mentioned that three Panel members from the 1998 review are also on the current Panel (i.e., Drs. Howard Maibach, Jean Regal, and Stephen Ullrich). Dr. Stokes also reviewed the nomination that was received from CPSC in January 2007,[1] which provides the basis for the current evaluation.

Dr. Stokes then identified the 15 Federal agencies that comprise ICCVAM and summarized ICCVAM's mission. He noted that ICCVAM, as an interagency committee, does not carry out research and development or validation studies. Instead, ICCVAM, in conjunction with NICEATM, carries out the critical scientific evaluation of proposed test methods with regard to their usefulness and limitations for regulatory testing and then makes formal recommendations to ICCVAM agencies.

Dr. Stokes provided a brief review of ICCVAM's history and summarized the ICCVAM Authorization Act of 2000,[2] detailing the purpose and duties of ICCVAM. He noted that one of ICCVAM's duties is to review and evaluate new, revised, and alternative test methods applicable to regulatory testing. He stated that all of the reports produced by NICEATM are available on the NICEATM-ICCVAM website or can be obtained upon request from NICEATM. He also mentioned that ICCVAM provides guidance on test method development, validation criteria, and processes, and helps to facilitate not only the acceptance of scientifically valid alternative methods, but also encourages international harmonization.

Dr. Stokes then described the ICCVAM test method evaluation process, which begins with a test method nomination or submission. NICEATM conducts a prescreen evaluation to summarize the extent to which the proposed submission or nomination addresses the ICCVAM prioritization criteria. A report of this evaluation is then provided to ICCVAM, which in turn develops recommendations regarding the priority for evaluation. ICCVAM then seeks input on their recommendations from the Scientific Advisory Committee on Alternative Toxicological Methods (SACATM) and the public. Given sufficient regulatory applicability, sufficient data, resources, and priority, a test method will move forward into a formal evaluation. A draft background review document (BRD), which provides a comprehensive review of all available data and information, is prepared by NICEATM, in conjunction with an ICCVAM working group designated for the relevant toxicity testing area (e.g., the IWG). In addition, ICCVAM considers all of the available information and makes draft test method recommendations on the proposed usefulness and limitations of the test methods, test method protocol, performance standards, and future studies. The BRD and the draft ICCVAM test method recommendations are made available to the Panel and the public for review and comment. The Panel peer reviews the BRD and evaluates the extent to which it supports the draft ICCVAM test method recommendations. A Panel report is published, which is then considered along with public and SACATM comments by ICCVAM in making final recommendations. These final recommendations are forwarded to the ICCVAM member agencies for their consideration and possible incorporation into relevant testing guidelines.

Dr. Stokes reviewed the ICCVAM criteria for adequate validation. He stated that validation is defined by ICCVAM as the process by which the reliability and relevance of a procedure are established for a specific purpose, and that adequate validation is a prerequisite for consideration of a test method by U.S. Federal regulatory agencies. Dr. Stokes listed the ICCVAM acceptance criteria for test method validation and acceptance. He concluded by summarizing the timeline of the review activities beginning with CPSC's nomination in January 2007 and ending with the present Panel meeting.

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[1] http://iccvam.niehs.nih.gov/methods/immunotox/llnadocs/CPSC_LLNA_nom.pdf
[2] http://iccvam.niehs.nih.gov/docs/about_docs/PL106545.pdf

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ICCVAM Charge to the Panel

Dr. Stokes reviewed the charge to the Panel, which was to: (1) review the draft BRDs, the draft Addendum to the traditional[3] LLNA, and the draft performance standards for completeness and identify any errors or omissions; (2) determine the extent to which each of the applicable criteria for validation and regulatory acceptance had been addressed for the proposed revised or modified versions of the LLNA; and (3) consider and provide comment on the extent to which the ICCVAM draft test method recommendations including the proposed use, standardized protocols, performance standards, and additional studies are supported by the information provided in the draft BRDs and draft Addendum.

Dr. Stokes thanked the IWG and ICCVAM for their contributions to this project, and acknowledged the contributions from the participating liaisons from ECVAM and JaCVAM (Japanese Center for the Validation of Alternative Methods). He also acknowledged the NICEATM staff for their support and assistance in organizing the Panel meeting and preparing the materials being reviewed.

Current Regulatory Testing Requirements and Hazard Classification Schemes for Allergic Contact Dermatitis and the Traditional LLNA Procedure

Dr. Joanna Matheson, Chair of the IWG, briefly reviewed the regulatory testing requirements of U.S. Federal agencies for skin-sensitization hazard identification and provided a brief description of the LLNA protocol.

Overview of the Agenda

Dr. Luster provided a brief synopsis of the agenda. He stated that there were six test methods and applications along with the draft LLNA performance standards for review and that the same agenda would be followed for each: (1) introductory summary of the draft ICCVAM recommendations from one of the NICEATM staff members; in addition, test method developers would provide a brief description of the methodology for each of the three nonradioactive tests, (2) presentation of the Evaluation Group draft comments by the Evaluation Group leader, (3) Panel discussion, (4) public comments, (5) recommendations and conclusions by the Panel.

Overview of the Draft LLNA Limit Dose Procedure[4] BRD and Draft ICCVAM Test Method Recommendations

Dr. David Allen, Integrated Laboratory Systems, Inc., the NICEATM support contractor, presented an overview of the draft ICCVAM BRD for the LLNA limit dose procedure. He mentioned that the draft ICCVAM BRD provided a comprehensive review of the available data and information regarding the usefulness and limitations of the LLNA limit dose procedure. The method was reviewed for its accuracy in correctly identifying sensitizers and non-sensitizers, when compared to the traditional LLNA.

NICEATM published a series of *Federal Register* (FR) notices, including an FR notice (72 FR 27815, May 17, 2007) requesting original data from the LLNA. This FR notice was also sent to over 100 potentially interested stakeholders for their input and comment. As a result, data on 255 substances tested in the LLNA were received. The resulting LLNA database consisted of 471 studies of 466 unique substances, 211 of which were included in the original ICCVAM 1999 evaluation. Dr. Allen briefly summarized the performance characteristics of the LLNA limit dose procedure test

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[3] For the purposes of this document, the radioactive LLNA test method, which was first evaluated by ICCVAM in 1999, and subsequently recommended to U.S. Federal agencies as a valid substitute for currently accepted guinea pig test methods to assess the allergic contact dermatitis potential of many, but not all, types of substances, is referred to as the traditional LLNA.

[4] Also known as the reduced LLNA (rLLNA).

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method, which is detailed in the draft ICCVAM BRD,[5] and briefly summarized the draft ICCVAM test method recommendations for the LLNA limit dose procedure.[6]

Panel Evaluation:

Dr. Michael Olson led the Panel discussion on the LLNA limit dose procedure and specifically thanked the members of his Evaluation Group (i.e., Drs. James McDougal, Raymond Pieters, Jonathan Richmond [not present], and Takahiko Yoshida) for their collegial review of the information presented in the draft ICCVAM LLNA Limit Dose Procedure BRD. Dr. Olson also thanked the NICEATM staff for their technical support during the BRD review process. He then presented the draft responses to ICCVAM's questions to the Panel for consideration by the entire Panel. The focus was on review of the BRD for errors and omissions, assessment of the validation status of the test method, and review of draft ICCVAM test method recommendations. The Panel discussion and their recommended revisions to each section of the draft ICCVAM BRD and recommendations are reflected in the *Validation Status of New Versions and Applications of the Murine Local Lymph Node Assay: A Test Method for Assessing the Allergic Contact Dermatitis Potential of Chemicals and Products*, published in May 2008 (hereafter, the Panel report[7]).

During the Panel's evaluation, discussion arose regarding what might have resulted in the inverted-U-shaped dose response that was seen with the false-negative substances in the LLNA limit dose procedure. Dr. Olson responded that although it was difficult to understand what the cause might have been, he speculated that the top dose was either toxic at a systemic-effect level or that those substances were immunosuppressive at the highest dose level. He also stated that there did not seem to be any structural features of the substances that could be attributed for the false negative response in the LLNA limit dose procedure.

The Panel also discussed the use of concurrent versus intermittent positive controls in the LLNA limit dose procedure. Dr. Olson indicated that the Evaluation Group had discussed the possibility to allow intermittent positive controls for laboratories that exhibited repeatable and adequate performance with the LLNA but he indicated that it would be important to describe a set of performance criteria that would determine when this practice would be acceptable. Clearly, if the laboratory was not performing the assay routinely or if there were other reasons to suspect variability in response with any substance, the positive control would be necessary. Dr. Stokes indicated that this discussion was pertinent and indicated that the Panel's suggestions for what the performance criteria might be for intermittent positive control testing would be of interest to the IWG. Dr. Stokes also wanted to clarify that the OECD TG is consistent with the EPA TG and the ICCVAM-recommended test method protocol for the LLNA although the OECD TG allows additional latitude in how tests are run (i.e., four animals per dose group, use of pooled data, and the option to not run a concurrent positive control).

Public Comments:

Dr. Amy Rispin, EPA

Dr. Rispin stated that the ICCVAM LLNA report (1999[8]) and standardized protocol (2001[9]) recommends the use of a concurrent positive control in addition to the concurrent negative control required for each study. Subsequently, the OECD (Organisation for Economic Co-operation and Development) Test Guideline (TG) 429 (Skin Sensitisation: Local Lymph Node Assay) was finalized (2002). She said that originally, OECD TG 429 was drafted without a concurrent positive control but that language was added to include the recommended use of a concurrent positive control until

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[5] http://iccvam.niehs.nih.gov/methods/immunotox/LLNA-LD/LLNAldBRD07Jan08FD.pdf
[6] http://iccvam.niehs.nih.gov/methods/immunotox/LLNA-LD/IWGrecLLNA-LD07Jan08FD.pdf
[7] http://iccvam.niehs.nih.gov/docs/immunotox_docs/LLNAPRPRept2008.pdf
[8] http://iccvam.niehs.nih.gov/docs/immunotox_docs/llna/llnarep.pdf
[9] http://iccvam.niehs.nih.gov/docs/immunotox_docs/llna/LLNAProt.pdf

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laboratories demonstrate competence. Subsequent to that, EPA put forth its LLNA guideline for sensitization,[10] which states that concurrent positive and negative controls are to be included in each study. Dr. Rispin then added that U.S. Federal regulatory agencies, most notably the EPA and FDA, received LLNA data from studies in which the positive control did not achieve the appropriate limits of performance (i.e., the control values were not in the appropriate range) and therefore the studies were deemed unacceptable, underscoring the importance of a concurrent positive control for regulatory acceptance in the United States.

In response to Dr. Rispin's public comment, Drs. Ullrich and Theran asked how competence is determined and if laboratories have difficulties reaching a level of competence, respectively. Dr. Abby Jacobs responded by stating that the FDA has seen large data variations in laboratories that conduct the LLNA. It is often difficult to determine what the variations might be due to (e.g., new technicians, tail vein injection, lymph node removal) and these variations have been seen both in laboratories that are established and those that are not.

Dr. David Basketter, ECVAM Observer

Dr. Basketter said that the main point he wanted to address is that efforts should be made to harmonize the LLNA protocol with that described in OECD TG 429. He stated that although there is referral to the "ICCVAM protocol" throughout the BRDs under consideration, OECD TG 429 is more globally recognized for regulatory use of the LLNA and therefore should be the referenced protocol. Dr. Basketter further stated that if the LLNA limit dose procedure followed the ICCVAM protocol using five animals per group instead of following OECD TG 429, which allows using four animals per group, there would only be a savings of one animal for substances that were negative. He stated that the goal of ECVAM was actually to halve the number of animals by omitting the mid- and low-dose groups and that this would achieve significant animal savings since the likely prevalence of non-sensitizers is approximately two-thirds of chemicals tested and non-sensitizers would not require further testing even if dose response information for sensitizers was needed.

Dr. Basketter also mentioned that the retrospective evaluation of the LLNA being presented to the Panel analyzed whether the top dose could identify a substance as a sensitizer and how that compares to the traditional LLNA's performance. Since the traditional LLNA assay was determined to be positive or negative based on a stimulation index (SI) of 3, it is problematic if the focus is on statistics when using the five-animal model as this would require also going back and re-evaluating all the preceding data using the statistical approach.

Dr. McDougal responded to Dr. Basketter's comment by stating that one wouldn't have to go back and retrospectively re-evaluate previous data but that new data generated could be analyzed statistically. This approach would include determining if the treatment group was statistically different from the vehicle control group and then determining the biological relevance. This might help to eliminate irritants.

Panel Conclusions and Recommendations:

Dr. Luster asked the Panel to review the conclusions and recommendations for the LLNA limit dose procedure they had discussed earlier and to make any revisions, if necessary. One particular question that was asked during the Panel's conclusions and recommendations was whether an OECD TG existed for the LLNA limit dose procedure. Dr. Stokes indicated that the OECD TG would need to be updated to allow for the provision of a limit dose procedure and that's why the Panel's conclusions and recommendations are even more relevant. Dr. Stokes indicated that ICCVAM has already submitted a proposal to update the OECD TG based on the outcome of these deliberations and recommendations from the IWG.

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[10] http://www.epa.gov/opptsfrs/publications/OPPTS_Harmonized/870_Health_Effects_Test_Guidelines/Revised/870r-2600.pdf

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The Panel agreed to use the term *weight-of-evidence* to refer to existing information that would aid the LLNA limit dose procedure in identifying a substance as a sensitizer or a non-sensitizer. The Panel also discussed the use of concurrent positive controls and recommended that a laboratory that is proficient at conducting the limit dose procedure can test a positive control at routine intervals rather than concurrently (although the Panel did not identify what constituted routine intervals). The Panel also discussed the use of individual versus pooled data and agreed with the ICCVAM-recommended protocol that individual animal data should always be collected. The Panel concluded that individual animal response data are necessary in order to allow for statistical analyses of any differences between treated and control data. In addition, having data from individual animals also allows for identification of technical problems and outlier animals within a dose group. Dr. Luster asked the Panel if they agreed with the changes and revisions made at this point and with the Panel conclusions and recommendations as presented and revised. The Panel unanimously agreed. The Panel's detailed recommendations and conclusions on the LLNA limit dose procedure are included in their final Panel report.[11]

Overview of the Draft Addendum for the Applicability Domain of the LLNA and Draft ICCVAM Test Method Recommendations

Dr. Eleni Salicru, Integrated Laboratory Systems, Inc. (the NICEATM support contractor), summarized the information provided in the draft ICCVAM Addendum to the ICCVAM LLNA report (1999). This Addendum provided an updated assessment of the validity of the LLNA for testing the sensitizing potential of mixtures, metals, and aqueous solutions. The database used for this evaluation contained traditional LLNA data submitted as part of the original LLNA evaluation (ICCVAM 1999), data extracted from peer-reviewed articles published after the original evaluation, and data submitted to NICEATM in response to the FR notice (72 FR 27815, May 17, 2007) requesting such data. Dr. Salicru then summarized the performance characteristics of the LLNA when used to test mixtures, metals, and aqueous solutions,[12] as well as the draft ICCVAM test method recommendations for each of the three categories of test substances.[13]

Panel Evaluation:

Dr. McDougal, on behalf of his Evaluation Group, presented for consideration by the entire Panel the draft responses to the questions asked of the Panel by ICCVAM. The Panel then discussed the completeness of the draft ICCVAM Addendum, identified any errors and omissions, and reviewed the draft ICCVAM test method recommendations with regard to the ability of the LLNA to be used to test the sensitizing potential of mixtures, metals, and aqueous solutions. The Panel discussion and their recommended revisions to each section of the draft ICCVAM Addendum are reflected in the Panel report, published in May 2008.[14] During the Panel's evaluation of the LLNA's applicability domain, the difficulty of testing metals in the LLNA was discussed and Dr. Woolhiser asked if testing metals was also problematic in the guinea pig. Dr. Api indicated that with the metals, most of the data has come from the clinical experience because animal studies are not predicting accurately what is happening in the clinic. Dr. Maibach indicated that metals have been tested in the guinea pig and that they are sensitized easily. Dr. Maibach further commented that metals in man need to be patch-tested for clinical relevance at a level close to the irritant dose and that a thoughtful series of algorithms is necessary to determine this. He also pointed out that patch test results to some metals (e.g., nickel, palladium) may indicate that a cell mediated reaction is occurring (i.e., contact allergy) but it needs to be sorted out if this cell mediated reaction actually results in a disease (i.e., allergic contact dermatitis) and this is where the LLNA could prove useful.

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[11] http://iccvam.niehs.nih.gov/docs/immunotox_docs/LLNAPRPRept2008.pdf
[12] http://iccvam.niehs.nih.gov/methods/immunotox/LLNA-app/LLNAappADD19Jan08FD.pdf
[13] http://iccvam.niehs.nih.gov/methods/immunotox/LLNA-app/LLNAappRecs19Jan08FD.pdf
[14] http://iccvam.niehs.nih.gov/docs/immunotox_docs/LLNAPRPRept2008.pdf

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With regard to mixtures, Dr Api commented that based on her experience, when the mixture tested in the LLNA contains a predominant material (loosely defined that as greater than 70 percent) then the LLNA for the mixture mirrors what occurs for that one material. When evidence indicates that the substance is a true mixture, some times the LLNA does what is expected and other times the results are unexpected. In those cases, a weight-of-evidence approach (e.g., structure-activity relationships, clinical evidence) is employed.

Public Comments:

Dr. Charles Hastings, BASF Corporation

Dr. Hastings, representing CropLife America (an industry association of companies in the crop protection business), provided an overview of current activities in industry related to the use of the LLNA to detect dermal sensitizers and the global issues that are of importance. Dr. Hastings mentioned that CropLife America's primary concern is the testing of pesticide mixtures and formulations. He stated that they support the use of the LLNA for testing the dermal sensitization of mixtures and formulations as well as single ingredients.

Dr. Hastings mentioned that in the United States, EPA OPPTS (Office of Prevention, Pesticides and Toxic Substances) Guideline 870.2600[15] allows for the use of the LLNA as the preferred alternative to the standard guinea pig test. Based on this recommendation, member companies of CropLife America conducted a large number of LLNA studies for both active ingredients and formulations in the European Union (E.U.) and were at the point of submitting data in the United States, as well. Then, in early 2007, they were informed that EPA had concerns about the validity of using the LLNA to test mixtures and formulations, and were advised to discontinue using this test method for that purpose until it had been adequately validated. Dr. Hastings stated that, in contrast to the EPA, E.U. regulators consider the LLNA acceptable for testing pesticide formulations and actually prefer it to a guinea pig test.

Dr. Pieters asked if the E.U. has conducted any evaluations of the validity of the LLNA for testing mixtures and formulations. Dr. Hastings replied that he was not certain if they had performed an extensive evaluation or not but that the E.U. considered the LLNA a validated method and therefore likely considered it appropriate to test not only the active ingredient but also the formulation or mixture.

Dr. Hastings mentioned that one concern in terms of using the LLNA for testing mixtures or formulations, particularly in the E.U., is the testing of aqueous substances. Many of the industry formulations are aqueous-based and may be incompatible with traditional LLNA vehicles. The European Crop Protection Association sponsored a study that evaluated the use of an aqueous vehicle known as Pluronic L92, which helps adhere the test material to the mouse ear. In the study, they tested three aqueous pesticide formulations that contained known sensitizers, using Pluronic L92 as the vehicle. As expected, the test results demonstrated sensitizing activity. Regarding global considerations, Dr. Hastings mentioned that if the LLNA is not accepted for mixture/formulation testing in the United States, industry will have no choice but to conduct both the LLNA, with 18 to 24 animals, and a guinea pig test, with 20 to 30 animals, for each formulation they may develop for global distribution. This scenario counters the ICCVAM goal of "reducing, refining, and replacing" animal use in regulatory safety testing.

Dr. Hastings ended with the following conclusions:

- CropLife America believes the LLNA test can be used for pesticide formulations.

[15] http://www.epa.gov/opptsfrs/publications/OPPTS_Harmonized/870_Health_Effects_Test_Guidelines/Revised/870r-2600.pdf

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- CropLife America supports the efforts of EPA and ICCVAM to confirm the validity of the LLNA for testing mixtures/formulations and encourages a quick evaluation.
- CropLife America is willing to help, as needed.
- If and, when, it is determined that the LLNA is acceptable, CropLife America requests that EPA notify them so they can then begin conducting the LLNA again for the United States.

Dr. Api asked if CropLife America has data comparing pesticides that have been evaluated in the LLNA and in guinea pigs and/or humans. Dr. Hastings replied that they do and that generally there is not much discrepancy with guinea pig test results. Occasionally they might see a false positive compared to a guinea pig test, but he did not recall ever seeing a false negative. In most cases, they would feel comfortable accepting an occasional false positive because human health is still protected.

Dr. David Basketter, ECVAM Observer

Dr. Basketter stated that he had personal reservations about testing complex mixtures and formulations in assays that were designed for testing substances (e.g., the LLNA) since no single test has ever been validated for testing mixtures. On another point, he stated that most of the metals of importance have been tested in both the guinea pig and the LLNA and the "right" answers have been generated. Thus, it does not seem worthwhile to produce new tests with revised protocols for hazard and potency categorization for testing metals.

Panel Conclusions and Recommendations:

Dr. Luster asked the Panel if they agreed with the comments and recommendations that were made earlier during the Panel discussion. The Panel agreed with the draft ICCVAM recommendation for continued collection of information from traditional LLNA evaluations of mixtures, metals, and aqueous solutions with comparative data for guinea pig (i.e., guinea pig maximization test [GPMT] or Buehler test [BT]) and human (i.e., human maximization test [HMT] or human repeat insult patch test [HRIPT]) tests. However, the Panel suggested that, given resource limitations, it would be important to organize the recommendations based on relative priority. Dr. Luster asked the Panel if they agreed with this suggestion about prioritization of activities; all members of the Panel agreed with one abstention. Dr. Howard Maibach abstained from voting stating that he hoped this public meeting and the subsequent Panel report would emphasize to industry the need for them to submit more data on mixtures, metals, and aqueous substances in order to provide a clearer evidence of the validity of the LLNA in testing these types of substances. The Panel's detailed recommendations and conclusions on the applicability domain of the LLNA are included in their final Panel report.[16]

Method Description and Overview of the LLNA: Daicel Adenosine Triphosphate (LLNA: DA) Test Method

Dr. Kenji Idehara, Daicel Chemical Industries, Ltd. (private limited company), summarized the technical aspects of the LLNA: DA test method. He described the LLNA: DA as a non-radioisotopic version of the LLNA method in which lymph node adenosine triphosphate (ATP) content is used as a measure of cell proliferation instead of radiolabeled thymidine incorporation. Dr. Idehara indicated that the LLNA: DA was developed six years ago at Daicel Chemical Industries, Ltd., and that they use the test method regularly for in-house assessments of the skin-sensitization potential of chemical materials, intermediates, or products. He summarized the protocol differences between the LLNA: DA and the traditional LLNA. In the LLNA: DA, the application site is treated with 1% sodium lauryl sulfate (SLS) one hour before each test substance (or vehicle control) application, and the test substance is applied to the test site on day 7 as well as on days 1, 2, and 3. The auricular lymph nodes are excised from individual animals on day 8 rather than on day 6 and the amount of ATP in the

[16] http://iccvam.niehs.nih.gov/docs/immunotox_docs/LLNAPRPRept2008.pdf

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lymph nodes is measured with a luciferin-luciferase assay. Dr. Idehara mentioned that these modifications (i.e., 1% SLS pretreatment and additional application on day 7) enhance lymph node cell proliferation in order to achieve an SI = 3 in the LLNA: DA, which allows for a more direct comparison to the traditional LLNA.

Dr. Idehara mentioned that after excision, ATP content gradually decreased with time. Therefore, the overall assay time for measuring ATP content needs to be similar (i.e., within approximately 30 minutes) among all test animals. He noted that this was an important point for this method and recommended that the LLNA: DA be conducted by at least two persons. Dr. Idehara mentioned that ATP content assays are conducted using commercially available kits, and his laboratory has experience with two different commercial sources in Japan, Kikkoman and Lonzar.

Overview of the Draft LLNA: DA BRD and Draft ICCVAM Test Method Recommendations

Dr. Allen then presented an overview of the draft ICCVAM BRD for the LLNA: DA test method. He mentioned that the draft ICCVAM BRD provided a comprehensive review of the available data and information regarding the usefulness and limitations of the LLNA: DA to distinguish between sensitizers and non-sensitizers, compared to the traditional LLNA. The objective of the BRD was to describe the current validation status of the LLNA: DA test method, including its relevance and reliability, scope of substances tested, and the availability of a standardized protocol.

Dr. Allen mentioned that the data analyzed in the BRD included data provided by Daicel Chemical Industries, Ltd., on 31 substances tested at their laboratories. In addition, data for 14 different coded substances were generated from a two-phased interlaboratory validation study that included 17 total labs. Taken together, the total database represented in the LLNA: DA BRD included 33 different substances. Dr. Allen briefly summarized the performance characteristics of the LLNA: DA test method, which is detailed in the draft ICCVAM BRD.[17] Dr. Allen concluded by briefly summarizing the draft ICCVAM test method recommendations for the LLNA: DA test method.[18]

Panel Evaluation:

Dr. Michael Woolhiser thanked the Panel members of his Evaluation Group (i.e., Drs. Nathalie Alépeé, Thomas Gebel, Sidney Green [not present], and Jean Regal) for their tireless efforts in reviewing their Evaluation Group's assigned documents. He also thanked the NICEATM staff for their technical support during the review process. Dr. Woolhiser then presented the draft responses to ICCVAM's questions about this test method for consideration by the entire Panel. This included their review of the draft BRD for errors and omissions, their overall assessment of the validation status of the test method, and their comments on the draft ICCVAM test method recommendations. The Panel discussion and their recommended revisions to each section of the draft ICCVAM BRD are reflected in the Panel report, published in May 2008.[19]

Adjournment—

The meeting was adjourned for the day at 5:03 p.m., to reconvene at 8:30 a.m., Wednesday, March 5, 2008.

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[17] http://iccvam.niehs.nih.gov/methods/immunotox/llna-DA/LLNA-DAbrd07Jan08FD.pdf
[18] http://iccvam.niehs.nih.gov/methods/immunotox/llna-DA/LLNA-DARecs07Jan08FD.pdf
[19] http://iccvam.niehs.nih.gov/docs/immunotox_docs/LLNAPRPRept2008.pdf

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WEDNESDAY, MARCH 5, 2008

Reconvening of the Panel Meeting

Dr. Luster reconvened the Panel Meeting at 8:30 a.m. He introduced himself and then asked that all Panel members, followed by all others in attendance, introduce themselves as well.

Overview of the Draft LLNA: DA BRD and Draft ICCVAM Test Method Recommendations

Panel Evaluation:

Dr. Woolhiser continued his presentation from the previous day of the draft responses to ICCVAM's questions to the Panel, for consideration by the entire Panel. The Panel discussion and their recommended revisions to each section of the draft ICCVAM BRD are reflected in the Panel report, published in May 2008.[20] Dr. Woolhiser indicated that the Evaluation Group had two main concerns with the LLNA: DA test method. The first concern related to pretreatment with 1% SLS and understanding how this impacted the biology of the response. Second, the time course of the study was different than the traditional LLNA because it extended the study by one day and included an additional challenge. This brought forth a question about the immunology of the response as it relates to the potential for elicitation and whether or not that is a significant change from the traditional LLNA, which is purely an induction model.

Public Comments:

Dr. George DeGeorge, MB Research Laboratories
In response to a question raised during the Panel discussion, Dr. DeGeorge commented that using lymph node weight as the readout to differentiate between sensitizers and non-sensitizers in the LLNA is problematic because although there are more lymph node cells packed into a node, each cell has less cytoplasm. The lymph nodes swell to a point, and then excrete water and become smaller lymphocytes that are countable. He cited examples from his laboratory with several different sensitizers, which demonstrate that lymphocytes in the node are smaller when a large SI (e.g., SI = 25) is obtained relative to when a smaller SI (e.g., SI = 3) is obtained.

Dr. DeGeorge also commented that he agreed with a point made during the Panel discussion that the LLNA: DA method and the LLNA: Bromodeoxyuridine Detected by ELISA (LLNA: BrdU-ELISA) method should be considered separately, because they are so dissimilar.

In his final comment, Dr. DeGeorge stated that in the traditional LLNA, in the LLNA: Bromodeoxyuridine Detected by Flow Cytometry (LLNA: BrdU-FC), and probably also in the LLNA: DA, strong sensitizing substances do not need to be administered three times. For instance, if one administers a single, moderately high dose of dinitrochlorobenzene (DNCB) (i.e., one that would induce an SI of 20 to 40) and then measures lymph node cell proliferation on day 1, 2, 3, or 4, an increase in the number of cells in the node and the number of cells that are positive for BrdU would likely be observed. Thus, administrations of additional applications have the potential to cause cumulative irritation. Dr. DeGeorge stated that the LLNA: DA method, which extends the assay to eight days instead of six days, should evaluate what happens to lymph node cell number at earlier sample times. In addition, if the animals receive just one application using a high dose, with or without the SLS, is there an increase in the SI? If so, that would lead to the possibility that the extra applications are not necessary and might lead to cumulative irritation.

Dr. David Basketter, ECVAM Observer
Dr. Basketter made a statement that from a clinical perspective, substances are typically described as significant sensitizers or not significant sensitizers, and within that latter group some of the substances

[20] http://iccvam.niehs.nih.gov/docs/immunotox_docs/LLNAPRPRept2008.pdf

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may indeed be non-sensitizing. Thus, just because a substance has been shown in an isolated case report to be a human sensitizer does not mean that there is sufficient evidence to consider it as positive for comparison with outcomes of predictive assays. It has to be of sufficient importance (i.e., potency) to trigger a positive classification. Dr. Basketter mentioned SLS, methyl salicylate, and isopropanol, as substances which will always be positive in some human cases although they shouldn't be positive in a predictive assay.

Dr. Basketter also commented that caution should be given to making sensitization assumptions based on chemical class references. As an example, eugenol and isoeugenol are structurally similar and have similar physical properties, but they act by different chemical reaction mechanisms and could fit into distinctly different chemical classes.

Dr. Basketter's last comment acknowledged that much work has been done in terms of validating the traditional LLNA. If one makes minor changes to the LLNA in terms of a different readout for proliferation, then they benefit from all the experience generated in validating the traditional LLNA and less effort is needed to prove that the minor modification is valid. In contrast, if more significant modifications are made, one cannot rely on that same experience. Dr. Basketter cautioned that more importance should be placed on distinguishing whether something has changed substantially enough such that you can no longer rely on the traditional LLNA as a reference.

Dr. Masahiro Takeyoshi, Chemicals Evaluation and Research Institute
Dr. Takeyoshi made a short presentation about differences in LLNA sensitization responsiveness among different strains of mice. He mentioned that this was an important issue when evaluating the modified LLNA methods being developed in Japan. He showed differences in responsiveness among three different mouse strains commonly used in Japan (i.e., BALB/cAnN, CBA/JN, and CD-1) tested with parabenzoquinone in his group's non-radioactive LLNA (i.e., LLNA: BrdU-ELISA). The data indicated that the CBA/JN mouse strain exhibited a higher responsiveness, as indicated by an increased SI, to parabenzoquinone than the other two mouse strains tested. Based on these results, CBA/JN mice were chosen for testing substances in the LLNA: BrdU-ELISA test method.
Dr. Takeyoshi also indicated that based on evaluating different SI cutoffs in the LLNA: BrdU-ELISA, 2-mercaptobenzothiazole, 3-(4-isopropylphenyl)isobutyraldehyde, and hydroxycitronellal had low responsiveness (i.e., SI values). He noted that 2-mercaptobenzothiazole is an OECD TG 429 recommended positive control for the LLNA; however, repeat tests could not detect this substance as positive when using an SI value of 1.7 or more. Dr. Takeyoshi suggested that a substance-specific lower response might exist in the test system. Dr. Takeyoshi also summarized LLNA data by Dr. Ullmann and coworkers with the contract lab RCC, Ltd. in which they investigated the responsiveness of six different mouse strains (CBA/CaOlaHsd, CBA/Ca (CruBR), CBA/Jlbm (SPF), CBA/JNCrj, BALB/c and NMRI) to 25% 2-mercaptobenzothiazole. The data indicated that CBA/JNCrj mice showed markedly lower responsiveness compared to the other strains tested. These studies indicate that strain related differences would not be negligible with regard to measuring different endpoints of cellular proliferation in the LLNA because depending on the chemicals tested, responsiveness might be potentially impacted. For instance, some of the discordance seen in the LLNA: DA test method (e.g., 2-mercaptobenzothiazole) could be a strain specific effect.

Panel Conclusions and Recommendations:

Dr. Luster asked the Panel to review their conclusions and recommendations and discuss any revisions, if necessary. The Panel viewed the difference in treatment schedule between the LLNA: DA and the traditional LLNA to potentially be significant if the treatment schedule for the LLNA: DA corresponds to entering the elicitation phase of skin sensitization. The Panel was concerned that the 1% SLS pretreatment step in the LLNA: DA might modify the inherent sensitivity of the LLNA. They recommended that the test method developer (Daicel Chemical Industries, Ltd.) justify the use of 1% SLS or consider an alternative decision criterion (i.e., an SI threshold other than three) such that the 1% SLS pretreatment is no longer necessary. Dr. Luster asked the Panel if they

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agreed with the recommendations and conclusions that the Panel made along with the revisions; unanimously, the Panel agreed. The Panel's detailed recommendations and conclusions on the LLNA: DA test method are included in their final Panel report.[21]

Method Description and Overview of the LLNA: BrdU-FC Test Method

Dr. George DeGeorge, MB Research Laboratories, presented an overview of the LLNA: BrdU-FC test method. He stated that mice are dosed topically on the ears once daily for three consecutive days (i.e., days 1, 2, and 3), just like the traditional LLNA protocol. On day 6, the mice receive an intraperitoneal injection with bromodeoxyuridine (BrdU), and five hours later, the auricular lymph nodes are removed. The lymph nodes from individual animals are processed and, using flow cytometry, the number of BrdU-positive cells are counted from treated animals and compared to control animals as a measure of lymph node cell proliferation.

Dr. DeGeorge described in detail how the cells are processed and gated for flow cytometric analysis. He mentioned that the cells are also permeabilized and treated with propidium iodide which allows gates to be drawn around the G_0, G_1, S, and G_2M phases of the cell cycle. Dr. DeGeorge projected specific examples of flow cytometry plots and histograms for DNCB, hexyl cinnamic aldehyde (HCA), and positive and negative control data.

Dr. DeGeorge also described the tiered protocol for the assessment of sensitization potential using the LLNA: BrdU-FC and how ear swelling measurements and additional immunophenotypic endpoints (i.e., the enhanced LLNA: BrdU-FC) aid in distinguishing skin irritants from an irritating sensitizer.

Overview of the Draft LLNA: BrdU-FC BRD and Draft ICCVAM Test Method Recommendations

Dr. Judy Strickland, Integrated Laboratory Systems, Inc. (the NICEATM support contractor), presented an overview of the draft ICCVAM BRD for the LLNA: BrdU-FC test method. She stated that the draft ICCVAM BRD provided a comprehensive review of the available data and information regarding the usefulness and limitations of the LLNA: BrdU-FC test method. Specifically, the test method was reviewed for its ability to distinguish between sensitizers and non-sensitizers compared with the traditional LLNA. The objective of the BRD was to describe the current validation status of the LLNA: BrdU-FC test method, including its relevance and reliability, scope of substances tested, and the availability of a standardized protocol.

Dr. Strickland indicated that MB Research Laboratories submitted data to NICEATM for the 48 substances analyzed in the BRD in response to an FR notice (72 FR 27815, May 17, 2007) that requested such data. Dr. Strickland briefly summarized the performance characteristics of the LLNA: BrdU-FC test method, which is detailed in the draft ICCVAM BRD,[22] and the draft ICCVAM test method recommendations for the LLNA: BrdU-FC test method.[23]

<u>Panel Evaluation:</u>

Dr. Raymond Pieters, on behalf of his Evaluation Group, presented the Evaluation Group's review of the draft BRD and the draft test method recommendations for the LLNA: BrdU-FC test method. Specifically, he presented the draft responses to ICCVAM's questions to the Panel for consideration by the entire Panel. This included their review of the draft BRD for errors and omissions, their overall assessment of the validation status of this test method, and their comments on the draft ICCVAM test method recommendations. The Panel discussion and their recommended revisions to each section of

[21] http://iccvam.niehs.nih.gov/docs/immunotox_docs/LLNAPRPRept2008.pdf
[22] http://iccvam.niehs.nih.gov/methods/immunotox/fcLLNA/FC-LLNAbrd07Jan08FD.pdf
[23] http://iccvam.niehs.nih.gov/methods/immunotox/fcLLNA/FCLLNARecs07Jan08FD.pdf

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the draft ICCVAM BRD are reflected in the Panel report, published in May 2008.[24] The applicability of the draft ICCVAM-recommended LLNA performance standards to the LLNA: BrdU-FC test method was discussed, particularly with regard to the number of substances tested in the LLNA: BrdU-FC method and whether more data would be necessary for review before the validation status of the assay could be determined. Dr. Stokes reminded the Panel that the proposed LLNA performance standards didn't exist when the studies for the LLNA: BrdU-FC test method were performed. The questions should be whether the adequacy of the substances that have been tested is sufficient or if more studies need to be done to cover any gaps that might exist (e.g., range of potencies or activity, chemical classes).

Public Comments

Dr. David Basketter, ECVAM Observer

Dr. Basketter commented on the statement that Dr. DeGeorge made during his overview of the LLNA: BrdU-FC test method that HCA is irritating. He said that he is not convinced it is a significant irritant. Based on previous data, they had to use 50% HCA in a 48 hour occlusive application in the guinea pig in order to produce a mildly irritating response. Dr. Api added to Dr. Basketter's comment by stating that RIFM has also not found HCA to be an irritant when tested up to 20% in humans.

Dr. Basketter also commented that in the draft BRD for the LLNA: BrdU-FC, resorcinol was noted to be negative in the traditional LLNA and this is not correct. Dr. Basketter's group published results in 2007 in the journal Contact Dermatitis that resorcinol is clearly positive in the traditional LLNA when tested at higher concentrations and therefore this should be corrected for the record.

Dr. George DeGeorge, MB Research Laboratories

Dr. DeGeorge wanted to clarify that the LLNA: BrdU-FC test method was compared to the traditional LLNA to determine if the LLNA: BrdU-FC was more predictive of skin-sensitization potential. He stated that in some cases it was better while in others it wasn't, but overall, using human data as the gold standard reference, the LLNA: BrdU-FC exceeded the traditional LLNA predictivity values and accuracy. He also noted that the additional endpoints included in the LLNA: BrdU-FC allow for them to distinguish irritating substances that typically are considered false positives in the LLNA.

Dr. DeGeorge also noted that since the LLNA: BrdU-FC is so similar to the traditional LLNA the issue of refinement and reduction in animal use is not immediately apparent but if the assay is done in as few as four mice per group with a periodic positive control (e.g., every six months) this represents a significant decrease in animal numbers compared to guinea pig tests. Furthermore, there is a refinement since mice are phylogenetically lower than guinea pigs, and undergo less pain and distress during the assay than guinea pigs undergo.

With regard to the discussion of coefficients of variation (CVs) and the 0.5x to 2.0x EC3 (i.e., the estimated concentration expected to produce a stimulation index of 3) range, Dr. DeGeorge suggested that a larger range might be more reasonable because the current range is likely too restrictive.

Dr. George also noted that ICCVAM requires interlaboratory validation if a test method is to be transferred to other laboratories. With regard to the LLNA: BrdU-FC, it is a "me-too" assay and only has "minor" changes from the traditional LLNA and is currently only used in one laboratory. Therefore, the current dataset should suffice for determining the validity of the LLNA: BrdU-FC. In response to Dr. DeGeorge's comment, Dr. Stokes stated that if a method is only proposed to be used by one laboratory, having only intralaboratory data certainly would suffice but if it was proposed for broader use (e.g., adopted or endorsed by regulatory authorities), then other laboratories would have to demonstrate interlaboratory reproducibility. Dr. Luster asked if there was any mechanism available so that a company or small laboratory could apply for funding to help support an interlaboratory validation. Dr. Stokes

[24] http://iccvam.niehs.nih.gov/docs/immunotox_docs/LLNAPRPRept2008.pdf

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indicated that they could nominate the test method for additional validation studies to ICCVAM. It would go through a nomination review process and a prioritization would be given to that. The nomination would then be considered by the member agencies as to whether funding would be provided.

Panel Conclusions and Recommendations:

Dr. Luster asked the Panel to review their conclusions and recommendations and discuss any revisions, if necessary. The Panel suggested that the utility of ear swelling or other methods to detect inflammation appeared warranted for inclusion in every variation of the LLNA (including the traditional LLNA), but should be further investigated before routine inclusion in the protocol is recommended. The Panel further agreed that the draft ICCVAM test method recommendations for future studies highlighted the unanswered questions raised by the available data set. Specifically, conducting interlaboratory studies as a part of the validation process is important.

The Panel considered the immunological markers suggested for the LLNA: BrdU-FC to be appropriate, but noted that other immunological markers for discrimination of irritant versus sensitization phenomena were also available. In general, for any future work, efforts should be made to decrease the variability and to thereby increase the power of the test in order to ensure that more animals were not needed relative to the traditional LLNA or other modified LLNA protocols.

Dr. Luster asked the Panel to indicate if they agreed with the recommendations and conclusions that the Panel made along with the revisions; the Panel unanimously agreed. The Panel's detailed recommendations and conclusions on the LLNA: BrdU-FC test method are included in their final Panel report.[25]

Method Description and Overview of the LLNA: BrdU-ELISA Test Method

Dr. Masahiro Takeyoshi, Chemicals Evaluation and Research Institute, presented an overview of the LLNA: BrdU-ELISA test method. He stated that the LLNA: BrdU-ELISA test method is very similar to the traditional LLNA test method. Unique to the LLNA: BrdU-ELISA test method, after test substance applications on days 1, 2, and 3, BrdU is injected interperitoneally on day 5. Approximately 24 hours after the BrdU injection, lymph nodes are collected, and detection of the amount of BrdU incorporated into the DNA of lymph node cells is conducted with an ELISA.

In the development process of this method, experiments were conducted to detect the most efficient injection schedule of BrdU. Based on the various injection schedules tested, a single injection protocol on day four was identified as the optimal injection schedule for BrdU administration.

Dr. Takeyoshi then showed a video of laboratory personnel preparing the lymph node cells for BrdU detection by ELISA. He went on to describe data for the LLNA: BrdU-ELISA compared to the traditional LLNA and how performance could be improved using alternative decision criteria (i.e., an SI other than 3 as the threshold for a positive response).

Overview of the Draft LLNA: BrdU-ELISA BRD and Draft ICCVAM Test Method Recommendations

Dr. Salicru presented an overview of the draft ICCVAM BRD for the LLNA: BrdU-ELISA test method. She noted that the draft ICCVAM BRD provided a comprehensive review of the available data and information regarding the usefulness and limitations of the LLNA: BrdU-ELISA test method. Specifically, the test method was reviewed for its ability to distinguish between sensitizers and non-sensitizers compared with the traditional LLNA and guinea pig test methods. The objective of the BRD was to describe the current validation status of the LLNA: BrdU-ELISA test method, including its relevance and reliability, scope of substances tested, and the availability of a standardized protocol.

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[25] http://iccvam.niehs.nih.gov/docs/immunotox_docs/LLNAPRPRept2008.pdf

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Dr. Salicru stated that data from a total of 29 substances were considered in the accuracy analysis for the LLNA: BrdU-ELISA, and they were all tested in one laboratory. Dr. Salicru briefly summarized the performance characteristics of the LLNA: BrdU-ELISA test method, which are detailed in the draft ICCVAM BRD,[26] and the draft ICCVAM test method recommendations for the LLNA: BrdU-ELISA test method.[27]

Panel Evaluation:

Ms. Kim Headrick presented her Evaluation Group's (Drs. Anne Marie Api, Howard Maibach, Peter Theran, and Stephen Ullrich) review of the draft BRD and draft ICCVAM test method recommendations for the LLNA: BrdU-ELISA test method. Specifically, she presented the draft responses to ICCVAM's questions to the Panel for consideration by the entire Panel. This included their review of the draft BRD for errors and omissions, their overall assessment of the validation status of the test method, and their comments on the draft ICCVAM test method recommendations. The Panel discussion and their recommended revisions to each section of the draft ICCVAM BRD are reflected in the Panel report, published in May 2008.[28]

Public Comments:

Dr. David Basketter, ECVAM Observer

Dr. Basketter noted that when the traditional LLNA was first suggested as an alternative to the guinea pig tests, it went through a comprehensive validation process, and one of the concerns was that it should perform reliably and distinctly better than the guinea pig assays. He emphasized that this point should be kept in mind when thinking about the modified LLNA protocols with alternative endpoints that are currently being reviewed. He stated that the current rigor of examination for the modified LLNA protocols being reviewed for validation is higher than that for the traditional LLNA. He speculated that in the not-too-distant future, *in vitro* alternatives are likely to be going through a similar review process and it is going to become ever more difficult to put these alternatives in place, not because there is ill-will against the selections but because of the high standard of being good scientists. Thus, it is important that pragmatic decisions are made using the tools that are available.

Dr. George DeGeorge, MB Research Laboratories

Dr. DeGeorge commented that he agreed with Dr. Basketter's statements. He said that based on his experience in this peer review process, it is unlikely that he would bring any of the three *in vitro* test methods that MB Research Laboratories is developing for consideration by ICCVAM, given the many high hurdles that have to be negotiated.

In response to the comments by Drs. Basketter and DeGeorge, Dr. McDougal commented that it does not seem unreasonable to raise the bar for what is expected of new or modified tests. Dr. Luster added that understandably, the focus on animal refinement and reduction is paramount, but that as scientists we have to ensure that the bar is maintained sufficiently high so that as the years go by scientific quality is not compromised.

Panel Conclusions and Recommendations:

Dr. Luster asked the Panel to review their conclusions and recommendations and discuss any revisions, if necessary. The Panel concluded that the available data and test method performance for the LLNA: BrdU-ELISA support the draft ICCVAM test method recommendations that it may be useful for identifying substances as potential skin sensitizers and non-sensitizers, but that more information and existing data must be made available before the LLNA: BrdU-ELISA can be recommended for use. The Panel also stated that a detailed protocol was needed, in addition to sufficient quantitative data for broader analysis on a larger set of balanced reference substances that

[26] http://iccvam.niehs.nih.gov/methods/immunotox/llna-ELISA/BrdUELISAbrd07Jan08.pdf
[27] http://iccvam.niehs.nih.gov/methods/immunotox/llna-ELISA/BrdUELISARecs07Jan08FD.pdf
[28] http://iccvam.niehs.nih.gov/docs/immunotox_docs/LLNAPRPRept2008.pdf

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take into account physicochemical properties and sensitization potency, as well as an appropriate evaluation of interlaboratory reproducibility.

The Panel's main concern with this test method was that the accuracy of the LLNA: BrdU-ELISA at SI $\geq$ 3 was inadequate and not equivalent to the traditional LLNA. Furthermore, although using a decision criterion of SI $\geq$ 1.3 improved the test's performance in identifying sensitizers from non-sensitizers, it did not resolve concerns about the test method, particularly considering that power calculations suggest a much larger number of animals per group would be required to identify a positive response. Thus, the Panel also concluded that it might be more appropriate to use a statistically based decision criterion rather than a stimulation index to classify substances as sensitizers, and that this should be further investigated. Dr. Luster asked the Panel to indicate if they agreed with the recommendations and conclusions that the Panel made along with the revisions; unanimously, the Panel agreed. The Panel's detailed recommendations and conclusions on the LLNA: BrdU-ELISA test method are included in their final Panel report.[29]

Overview of the Draft ICCVAM Performance Standards for the LLNA

Dr. Allen presented an overview of the draft ICCVAM Performance Standards for the LLNA. He briefly summarized the overall purpose of performance standards (i.e., to provide a basis for evaluating the performance of a proposed test method that is mechanistically and functionally similar to the validated test method) and the three elements encompassed within such performance standards (i.e., essential test method components, a minimum list of reference substances, and accuracy/reliability values). He noted that the proposed applicability of these draft ICCVAM LLNA performance standards is for the evaluation of LLNA protocols that deviate from the ICCVAM-recommended LLNA protocol only with respect to the method for assessing lymphocyte proliferation (e.g., using non-radioactive instead of radioactive reagents). Dr. Allen then provided an overview of the essential test method components, the minimum list of reference substances, and the accuracy/reliability values as detailed in the draft ICCVAM LLNA Performance Standards.[30]

Panel Evaluation:

Dr. Woolhiser, on behalf of his Evaluation Group, presented the Evaluation Group's responses to the ICCVAM questions asked about the draft ICCVAM LLNA Performance Standards for the entire Panel to consider. The overall question for the Panel was whether these performance standards were considered adequate for assessing the accuracy and reliability of test method protocols that were based on similar scientific principles and that measured the same biological effect as the traditional LLNA. The Panel discussion and their recommended revisions to the draft ICCVAM LLNA Performance Standards are reflected in the Panel report published in May 2008.[31]

Adjournment—

The meeting was adjourned at 5:42 p.m., to reconvene at 8:30 a.m., Thursday, March 6, 2008.

THURSDAY, MARCH 6, 2008

Reconvening of the Panel Meeting

Dr. Luster reconvened the Panel Meeting at 8:30 a.m. He introduced himself and then asked that all Panel members and all others in attendance introduce themselves as well.

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[29] http://iccvam.niehs.nih.gov/docs/immunotox_docs/LLNAPRPRept2008.pdf
[30] http://iccvam.niehs.nih.gov/methods/immunotox/PerfStds/LLNAPerfStd07Jan08FD.pdf
[31] http://iccvam.niehs.nih.gov/docs/immunotox_docs/LLNAPRPRept2008.pdf

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Overview of the Draft ICCVAM LLNA Performance Standards

Panel Evaluation:

Dr. Woolhiser reviewed some of the important points highlighted during the previous day's discussion on this topic, and then continued to summarize the remaining comments of his Evaluation Group on the questions asked by ICCVAM on the draft ICCVAM LLNA Performance Standards for consideration by the entire Panel. As mentioned above, the Panel discussion and their recommended revisions to the draft ICCVAM LLNA Performance Standards are reflected in the Panel report published in May 2008.[32]

Dr. Woolhiser noted that there were general comments on the topic order for the Panel's review. He asked if Dr. Stokes would comment on the rationale for the topic order. Dr. Stokes indicated that as the IWG deliberated the order of topics for this review, consideration was given to the fact that the three non-radioactive methods had undergone validation studies prior to the creation of LLNA performance standards. Thus, the non-radioactive test methods were reviewed before the performance standards, so as to not bias the Panel's assessment of each test method's performance. The performance standards could then be considered for their application to future test methods.

Public Comments:

Dr. Amy Rispin, EPA

Dr. Rispin stated that her intent was to provide some additional regulatory perspective on some of the points that have been discussed. When Federal agencies evaluate the validation status of a test method under ICCVAM, they conduct a comprehensive analysis of overall performance (i.e., accuracy and reliability) in the context of making regulatory decisions with data from the test method. Thus, in a regulatory situation, equal or greater accuracy compared to the reference test method is the expectation. If the number of animals can be decreased only at the expense of accuracy, the acceptability of such a test method for the particular regulatory purpose would need to be carefully considered. Certain methods, instead of being complete replacements, might have to be relegated to the role of screens, where positives would be accepted, but negatives would require further testing - a less than ideal situation.

Dr. Rispin commented that performance standards are the regulating agencies' basis for the acceptability of variations of accepted test methods. If an agency receives data from a modified LLNA method that has not been reviewed and validated in the ICCVAM process, there is unlikely to be a comprehensive peer review of it within the agency, given resource limitations. Therefore, the question of major versus minor departures from the functional criteria is important to ICCVAM and its member agencies. One cannot anticipate that there will be anything other than these performance standards to adequately evaluate the usefulness and limitations of a new method.

Dr. David Basketter, ECVAM Observer

Dr. Basketter first commented on a point that Dr. Thomas Gebel alluded to during the Panel's discussion of the draft ICCVAM LLNA Performance Standards, which was that if a new laboratory performed the traditional LLNA to assess 18 or 22 chemicals, they probably wouldn't get a complete match. Dr. Basketter disagreed with Dr. Gebel's statement and viewed that a competent laboratory performing the LLNA would get it 100% correct.

Dr. Basketter then provided some comments that he stated were "from the ECVAM perspective." He stated that the ECVAM performance standards tried to address adhering to a standard protocol and that any change to the protocol other than the method for evaluating lymph node proliferation (e.g., strain, species, number of applications, time) was considered not to be minor, and therefore such a protocol would not be applied to these performance standards. By restricting the performance standards to minor

[32] http://iccvam.niehs.nih.gov/docs/immunotox_docs/LLNAPRPRept2008.pdf

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changes, ECVAM was trying to minimize the number of chemicals required to evaluate sensitivity. Furthermore, the EC3 value could be used to see if the test method could classify substances in the appropriate range of sensitization potency.

ECVAM initially chose their reference substances in order to determine whether a modified method (differing only in the method for measuring cell proliferation) would give the same answer as the traditional LLNA. Thus, there was no intent to compare to the guinea pig or human data.

Dr. Basketter speculated that it is doubtful that data from multiple LLNA studies on the same substance are available and therefore it is unlikely that much larger sample sizes from which to calculate mean EC3 values and associated ranges will be obtained.

Dr. Basketter concluded by stating that ECVAM will not include more false positives and false negatives in its list. It has included one false positive and false negative in order to harmonize with ICCVAM but they don't see an added statistical value of just having one more false positive and false negative.

Dr. Karen Hamernik, EPA

Dr. Hamernik concurred with the comments that Dr. Rispin made previously, that performance standards, if developed such that they are too generalized with respect to minor versus major changes, would be problematic for regulatory agencies when they are reviewing submissions that include data from a modified LLNA protocol. Dr. Hamernik also asked for clarification from the Panel on a statement made during their discussions that a test for concordance for measuring the accuracy of classification (i.e., yes/no answer) should be done and that a chemical-for-chemical match is not necessary. Dr. Flournoy responded that concordance is not absolute but a continuum. Dr. Luster further clarified that the Panel discussion was based on the fact that the traditional LLNA is not a perfect match when compared to the guinea pig tests. Because there are false negatives and false positives compared to the guinea pig, there should be some flexibility so that an absolute chemical-by-chemical match is not required. In addition, a scientifically valid explanation can be provided for any discordance. Dr. Stokes emphasized that this was an important point and that additional clarity on the differences between a chemical-by-chemical match and overall accuracy need to be carefully considered before the final test method accuracy requirements are defined.

Panel Conclusions and Recommendations:

Dr. Luster asked the Panel to review the conclusions and recommendations for the ICCVAM LLNA performance standards they had discussed earlier and to make any revisions, if necessary. The Panel indicated that modified LLNA protocols that are undergoing validation should contain essential test method components that follow the ICCVAM-recommended protocol,[33] unless adequate scientific rationale for deviating from this protocol was provided. The Panel also identified aspects of the LLNA that should be required as part of the test method validation process, if more extensive changes to the protocol are being considered: (1) application of the test substance to the skin with sampling of the lymph nodes draining that site, (2) measurement of cell proliferation in the draining lymph node, (3) absence of a skin reaction that could be indicative of the onset of the elicitation phase of skin sensitization, (4) data collected at the level of the individual animal to allow for an estimate of the variance within control and treatment groups,[34] and (5) if dose response information is needed, there are an adequate number of dose groups ($n \geq 3$) with which to accurately characterize the dose response for a given test substance.

The Panel also recommended that statistical tests to analyze the data might allow for a more accurate interpretation. They recommended that a suitable variance-stabilizing transformation (e.g., log

[33] http://iccvam.niehs.nih.gov/docs/immunotox_docs/llna/LLNAProt.pdf

[34] Individual animal data will allow the application of a formal statistical test, if deemed necessary, and will also allow power calculations associated with the modified LLNA test.

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transformation, square root transformation) be applied in all statistical analyses and in reporting summary standard deviations. The Panel also recommended that a more rigorous evaluation be conducted of what would be considered an appropriate range of ECt values (i.e., estimated concentration expected to produce a stimulation index that is indicative of a positive response) to include as a requirement. This would be a statistical evaluation that considers the variability of ECt values generated among the sensitizers included on the performance standards reference substances list and the statistical multiple comparisons problem.
Dr. Luster asked the Panel if they agreed with the changes and revisions made at this point and with the Panel conclusions and recommendations as presented and revised. The members of the Panel agreed with one abstention; Dr. McDougal abstained from voting stating that he still had a concern about what constitutes a "major/minor" change. The Panel's detailed recommendations and conclusions on the ICCVAM LLNA performance standards are included in their final Panel report.[35]

Overview of the Draft LLNA Potency Determinations BRD and Draft ICCVAM Test Method Recommendations

Dr. Strickland presented an overview of the draft ICCVAM BRD for the use of the LLNA to determine skin-sensitization potency. She mentioned that the draft ICCVAM BRD provided a comprehensive review of the available data and information regarding the usefulness and limitations of the LLNA as a stand-alone assay for hazard categorization of skin-sensitization potency. In the BRD, the LLNA was evaluated for its ability to categorize substances for skin-sensitization potency using EC3 values.

Dr. Strickland noted that the analyses conducted in the BRD were based on LLNA studies obtained from ICCVAM (1999), the published literature, and data received in response to an FR notice (72 FR 27815, May 17, 2007) requesting original data from the LLNA. As a result, the analyzed data included 170 substances with LLNA, human, and/or guinea pig data. Dr. Strickland noted that three sets of data were analyzed and briefly summarized the results which are detailed in the draft ICCVAM BRD.[36] Dr. Strickland also briefly summarized the draft ICCVAM test method recommendations for potency determinations.[37]

Panel Evaluation:

Ms. Headrick presented her Evaluation Group's draft responses to ICCVAM's questions to the Panel for consideration by the entire Panel. These included their review of the draft BRD for errors and omissions, their overall assessment of the validation status of the test method, and their comments on the draft ICCVAM test method recommendations. The Panel discussion and their recommended revisions to each section of the draft ICCVAM BRD and recommendations are reflected in the Panel report published in May 2008.[38]

During the course of the discussion on the potency applicability of the LLNA, Dr. Woolhiser asked what the basis for the human threshold concentration cutoff values of 250 and 500 $\mu g/cm^2$ were. Dr. Wind replied that a number of experts and clinicians from throughout the world went back and looked at what, in their countries, they demarcated as strong sensitizers. The proposed Globally Harmonized System of Classification and Labelling of Chemicals (GHS) subcategory guidance values for the LLNA, guinea pig tests (GPMT, BT) and human data (HMT and HRIPT) were made on the basis of an impact analysis of 175 chemicals. In addition, the two proposed cut-offs were evaluated by the GHS Expert Group on Sensitization based upon chemicals already regulated as strong sensitizers to ensure their inclusion within the GHS categorization scheme. Clinical members of the Expert Group

[35] http://iccvam.niehs.nih.gov/docs/immunotox_docs/LLNAPRPRept2008.pdf
[36] http://iccvam.niehs.nih.gov/methods/immunotox/LLNA-pot/LLNApotency18Jan08FD.pdf
[37] http://iccvam.niehs.nih.gov/methods/immunotox/LLNA-pot/LLNAPotencyRecs18Jan08FD.pdf
[38] http://iccvam.niehs.nih.gov/docs/immunotox_docs/LLNAPRPRept2008.pdf

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also confirmed relevance of the cut-off values such that clinically important skin sensitizers fell into the appropriate subcategory. The proposed guidance values were also in line with the European Commission's Expert Working Group recommendations.

Public Comments:

Dr. David Basketter, ECVAM Observer

Dr. Basketter commented that reviewing the potency data by splitting it into pooled and unpooled groups could be interesting but might be difficult since the majority of available data likely comes from pooled groups. Furthermore, much of the deliberation concluding that individual animal data must be used was derived from analyses based only or largely on pooled data from four animals.

Dr. Basketter further stated that he viewed the analyses, which make the assumption that the human threshold data is the gold standard, as fundamentally flawed. Human data comes from studies conducted at different times, with different protocols, according to varying quality standards, and by different people. Therefore, there is no definitive knowledge of the reproducibility of the data. However, he considers the analyses adequate for recommending the LLNA as a part of a weight-of-evidence decision on human sensitization potency categorizations.

Dr. Amy Rispin, EPA

Dr. Rispin noted that there has been much discussion about various ways of handling the potency data. The OECD expert task force on skin sensitization needs to see an analytical comparison of what is considered to be the most appropriate approach for evaluating the data. The question for categorization purposes is, *What is the ideal testing modality for separating strong versus weak sensitizers for potency categorization*? A regulator who must assign a categorization is going to be confronted with all available test data and must know which data should be given the greatest weight in their evaluation.

Dr. Rispin noted that the OECD task force also reviewed the draft BRD on potency determinations and sent a list of several questions to the Panel, some of which have been answered, many of which have not been. One of the questions is, can the LLNA protocols be refined (e.g., by selection of solvents or choice of other test parameters) to improve correlation? She concluded by noting that she hopes that the additional analyses that the Panel has suggested will bring some clarity to the matter.

Panel Conclusions and Recommendations:

Dr. Luster asked the Panel to review the conclusions and recommendations for the LLNA potency determinations they had discussed earlier and to make any revisions, if necessary. The Panel agreed with the draft ICCVAM recommendation that the LLNA should not be used as a stand-alone assay for categorizing skin sensitizers as strong versus weak, but that it could be used as part of a weight-of-evidence evaluation (e.g., along with quantitative structure-activity relationships, peptide reactivity, human evidence, historical data from other experimental animal studies) for this purpose. The Panel also agreed with ICCVAM's recommendation that any LLNA studies conducted for the purpose of evaluating skin-sensitization potency should use the ICCVAM-recommended LLNA protocol. In addition, the Panel stated that the relevant testing guidelines for the traditional LLNA should be revised to include the procedure for calculating an EC3 value. Dr. Luster asked the Panel if they agreed with the changes and revisions made at this point and with the Panel conclusions and recommendations as presented and revised; the Panel unanimously agreed. The Panel's detailed recommendations and conclusions on the LLNA potency determinations are included in their final Panel report.[39]

[39] http://iccvam.niehs.nih.gov/docs/immunotox_docs/LLNAPRPRept2008.pdf

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Concluding Remarks—

Dr. Luster, on behalf of the Panel, thanked the NICEATM-ICCVAM staff for their continued assistance during the review process and the Panel meeting. He also thanked Drs. Joanna Matheson and Abby Jacobs, the IWG co-chairs, and Dr. Marilyn Wind, ICCVAM Chair and IWG member, for the hard work they put into the project. Dr. Luster also thanked the Panel and the Panel Chairs for their involvement in the huge task of reviewing seven topics. He commented that, for future reference for ICCVAM, the Panel in their individual groups were able to do a good job in reviewing the materials, but because they were so focused on their particular topics due to serious time constraints, there may not have been the full benefit of their expertise for other topics in all cases.
Drs. Wind and Stokes thanked the Panel again for their hard work, thoughtful and objective deliberations, and advice. Dr. Stokes further thanked the invited test method developers for their excellent summaries of their method for the benefit of the Panel, and CPSC for hosting the Panel meeting. He mentioned that there has been discussion about obtaining additional existing data (i.e., on mixtures, on one or more of the non-radiolabeled test methods), and that should these data become available in a timely manner and if NICEATM is able to assimilate and analyze the data, the Panel might be reconvened by teleconference to review the data. Dr. Stokes concluded by saying he looked forward to further working with the Panel members to complete their Panel report.

Adjournment—

The meeting was adjourned and concluded at 3:20 p.m.

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William S. Stokes, D.V.M.
NIEHS
P.O. Box 12233
MD-EC17
Research Triangle Park, NC 27709

Dear Dr. Stokes,

The Meeting Summary Minutes, Independent Scientific Peer Review Panel Meeting, Validation Status of New Versions and Applications of the Murine Local Lymph Node Assay (LLNA): A Test Method for Assessing the Allergic Contact Dermatitis Potential of Chemicals and Products, accurately summarizes the Peer Review Panel meeting of March 4-6, 2008, in Bethesda, MD.

Sincerely,

Signature	Printed Name	Date
	MICHAEL I LUSTER	11-18-08

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Appendix D2

Peer Review Panel Report: Validation Status of New Versions and Applications of the Murine Local Lymph Node Assay: A Test Method for Assessing the Allergic Contact Dermatitis Potential of Chemicals and Products

The full document is available electronically at:
http://iccvam.niehs.nih.gov/docs/immunotox_docs/LLNAPRPRept2008.pdf

The document is also available on request from NICEATM:

NICEATM
National Institute of Environmental Health Sciences
P.O. Box 1233, MD K2-16
Research Triangle Park, NC 27709 USA
Telephone: 919-541-2384, Fax: 919-541-0947
E-mail: niceatm@niehs.nih.gov

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Appendix E

GHS Materials on Skin Sensitization Potency

E1 Meetings of the Sub-Committee of Experts on the Globally Harmonized System of Classification and Labelling of Chemicals Relevant to Subcategorization of Skin Sensitizers E-3

E2 ST/SG/AC.10/C.4.2006/16, Strong versus Weak Sensitizers E-9

E3 UN/SCEGHS/12/INF.16 (OECD), Progress Report on OECD Work Since the Last Sub-Committee Meeting E-23

E4 UN/SCEGHS/15/INF.14 (OECD), Proposal for Revising Chapter 3.4 with Respect to Strong versus Weak Sensitizers: Explanatory Notes and Revised Chapter with Visible Changes E-27

E5 ST/SG/AC.10/C.4/2008/18 (Secretariat), Revision of Chapter 3.4 with Respect to Strong versus Weak Sensitizers E-43

E6 ST/SG/AC.10/C.4/2008/18/Add.1 (Secretariat), Revision of Chapter 3.4 with Respect to Strong versus Weak Sensitizers, Addendum E-55

E7 UN/SCEGHS/16/INF.3 (Secretariat), Section 3.4.2 of Chapter 3.4 ..... E-63

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Appendix E1

Meetings of the Sub-Committee of Experts on the Globally Harmonized System of Classification and Labelling of Chemicals Relevant to Subcategorization of Skin Sensitizers

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Meetings[1] of the Sub-Committee of Experts on the Globally Harmonized System of Classification and Labelling of Chemicals Relevant to Subcategorization of Skin Sensitizers

Meeting Date	Excerpt of Meeting Minutes Relevant to Skin Sensitization
11th Meeting **July 12-14, 2006**	***Strong versus weak sensitizers*** Document: ST/SG/AC.10/C.4.2006/16, *Strong vs. weak sensitizers*[2] (See **Appendix E2**) 26. The Sub-Committee took note of the progress of work on strong versus weak sensitizers and agreed to defer the decision to the next session, when the results of the WHO/IPCS international workshop on skin sensitization risk assessment to be held in October 2006 are available.
12th Meeting **December 12-14, 2006**	***Strong versus weak sensitizers*** Informal document: UN/SCEGHS/12/INF.16 (OECD), *Progress report on OECD work since the last Sub-Committee meeting*[3] (See **Appendix E3**) 11. The Sub-Committee took note of the decision of the OECD expert group to restart the work on the development of a proposal for classification of strong versus weak sensitizers and decided to keep this item on its programme of work for the biennium 2007-2008 (see annex 2).

[1] Meeting reports can be obtained at http://www.unece.org/trans/main/dgdb/dgsubc4/c4rep.html
[2] http://www.unece.org/trans/main/dgdb/dgsubc4/c42006.html
[3] http://www.unece.org/trans/main/dgdb/dgsubc4/c4inf12.html

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Meeting Date	Excerpt of Meeting Minutes Relevant to Skin Sensitization
13th Meeting July 9-10, 2007	***Strong versus weak sensitizers*** 27. The representative of the OECD updated the Sub-Committee about progress on the development of classification criteria for strong and weak sensitizers. She said that the expert group had reached a general agreement on having one general category for "skin sensitizers", with the same criteria as the existing classification criteria in the GHS, and a new sub-category (to be used by some competent authorities only) for "strong sensitizers". However, she said that the expert group had not yet decided on how both categories would be accommodated into the hazard class "skin sensitizers" in the GHS. 28. She announced that the final proposal would be submitted as an information document to the Sub-Committee at its fifteenth session (July 2008) provided that the expert group was able to reach consensus on the draft proposal by March 2008. Otherwise, the agreed proposal would be submitted for consideration by the Sub-Committee at its December 2008 session.
14th Meeting December 12-14, 2007	**4. Classification of strong versus weak sensitizers** 23. The representative of the OECD said that the expert group had developed a proposal for revising the classification criteria in chapter 3.4 of the GHS which will be further discussed at the beginning of March and if agreed, will be submitted to the OECD Task Force on classification and labelling in April 2008 for approval.

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Meeting Date	Excerpt of Meeting Minutes Relevant to Skin Sensitization
15th Meeting **July 9-11, 2008**	**7. Strong versus weak sensitizers** Informal documents: UN/SCEGHS/15/INF.13 (OECD), *Proposal for revising Chapter 3.4 with respect to strong versus weak sensitizers*[4] UN/SCEGHS/15/INF.14 (OECD), *Proposal for revising Chapter 3.4 with respect to strong versus weak sensitizers: Explanatory notes and revised chapter with visible changes*[5] (See **Appendix E4**) 31. The Sub-Committee adopted in principle the proposal intended to revise the classification criteria in chapter 3.4 to allow the sub-categorization of skin and respiratory sensitizers where data are sufficient and where required by a competent authority. 32. One expert noted that some editorial corrections were needed in paragraphs 3.4.2.2.1.4 and 3.4.2.2.1.5. The Sub-Committee agreed on the proposed corrections to those paragraphs. 33. The secretariat was invited to issue the OECD proposal (including the agreed corrections) as an official document to be considered for final adoption at the next session of the Sub-Committee.

[4] http://www.unece.org/trans/main/dgdb/dgsubc4/c4inf15.html
[5] http://www.unece.org/trans/main/dgdb/dgsubc4/c4inf15.html

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Meeting Date	Excerpt of Meeting Minutes Relevant to Skin Sensitization
16th Meeting December 10-12, 2008	**1. Strong versus weak sensitizers: amendments to Chapter 3.4** Documents: ST/SG/AC.10/C.4/2008/18 (Secretariat), *Revision of Chapter 3.4 with respect to strong versus weak sensitizers*[6] (See **Appendix E5**) ST/SG/AC.10/C.4/2008/18/Add.1 (Secretariat), *Revision of Chapter 3.4 with respect to strong versus weak sensitizers, Addendum*[7] (See **Appendix E6**) Informal documents: UN/SCEGHS/16/INF.3 (Secretariat), *Section 3.4.2 of Chapter 3.4*[8] (See **Appendix E7**) INF.23 (Australia), *Proposal for amendment of Table 3.4.3 in document ST/SG/AC.10/C.4/2008/18: Cut-off values concentration limits of ingredients of a mixture classified as either skin sensitizers or respiratory sensitizers that would trigger classification of the mixture*[9] 23. The proposals for amendment to Chapter 3.4 contained in the documents from the secretariat were adopted with a minor editorial change to paragraph 3.4.2.1.1.3 (see annex I). 24. Regarding the inconsistencies in the hazard statements for respiratory and skin sensitization currently used in the GHS, as noted by the secretariat in annex III to document ST/SG/AC.10/C.4/2008/18/Add.1, the Sub-Committee agreed that only the statements used in Chapter 3.4 were deemed to be correct (see annex I). 25. The proposal in document INF.23 for the amendment of Table 3.4.3, to make reference to sub-categories 1A and 1B in the cells containing the cut-off values was not adopted. The expert from Germany said that sub-categorization was based on potency and explained that the OECD expert group who developed the criteria had decided to refer only to Category 1 because data for the sub-categorization of mixtures in sub-categories 1A or 1B was not generally available.

[6] http://www.unece.org/trans/main/dgdb/dgsubc4/c42008.html
[7] http://www.unece.org/trans/main/dgdb/dgsubc4/c42008.html
[8] http://www.unece.org/trans/main/dgdb/dgsubc4/c4inf16.html
[9] http://www.unece.org/trans/main/dgdb/dgsubc4/c4inf16.html

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Appendix E2

ST/SG/AC.10/C.4.2006/16, Strong versus Weak Sensitizers

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COMMITTEE OF EXPERTS ON THE TRANSPORT OF DANGEROUS GOODS AND ON THE GLOBALLY HARMONIZED SYSTEM OF CLASSIFICATION AND LABELLING OF CHEMICALS

Sub-Committee of Experts on the Globally Harmonized System of Classification and Labelling of Chemicals

Eleventh session, 12 (p.m) - 14 July 2006
Item 2(b) of the provisional agenda

UPDATING OF THE GLOBALLY HARMONIZED SYSTEM OF CLASSIFICATION AND LABELLING OF CHEMICALS (GHS)

Health hazards

Strong vs. weak sensitizers

Transmitted by the Organization for Economic Co-operation and Development (OECD)

This paper briefly refers to respiratory sensitization, but the bulk of the paper addresses skin sensitization.

Introduction

1. Currently, most countries/sectors regulate sensitization hazards, without additional differentiation into strong or weak sensitizers. However, in the U.S., consumer products falling under the Federal Hazardous Substances Act (FHS) are regulated only if they are deemed strong sensitizers

2. The GHS calls for classification of substances as respiratory or dermal sensitizers without differentiation regarding sensitization strength. Dermal sensitizers may be classified using animal or human data. However, there is no animal model for regulatory use to identify respiratory sensitizers. Classification for that endpoint is based primarily on human data. Positive results in animal studies are considered to provide additional indications of respiratory sensitization potential. In many cases, weight of evidence reasoning using expert judgment must

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be used to classify dermal or respiratory sensitizers (GHS Par. 1.3.2.4.8). "For classification purposes, reliable epidemiological data and experience on the effects of chemicals on humans (e.g. occupational data, data from accident data bases) should be taken into account in the evaluation of human health hazards of a chemical. Testing on humans solely for hazard identification purposes is generally not acceptable." (GHS Par. 1.3.2.4.7).

3. When harmonizing existing systems for hazard classification, the OECD Task Force for Classification and Labeling treated sensitization without additional differentiation, noting that there was no internationally accepted animal test at the time which could be used to determine sensitization strength (GHS Chapter 3.4). However, as documented in the OECD *Harmonized Integrated Classification System for Human Health and Environmental Hazards of Chemical Substances and Mixtures* in the appendix to chapter 2.4, the terms strong/weak include consideration of severity of allergic manifestations in humans or animals, as well as frequency in exposed populations. The following text was provided as "Background Information":

> "118. Categorization of sensitizers accounting for differences in sensitizing capacity among substances would be a useful concept to develop. It may be appropriate to allocate both respiratory and dermal sensitizers to, for example, one of the following categories:
>
> Category 1, Strong Sensitizer:
>
> A strong sensitizer would be indicated by:
> - a high frequency of occurrence and/or severity of occurrence within an exposed population; or
> - a probability of occurrence of a high sensitization rate in humans based on animal or other tests.
>
> Category 2, Sensitizer:
>
> A low to moderate sensitizer would be indicated by:
> - a low or moderate frequency or severity of occurrence within an exposed population; or
> - a probability of occurrence of a low to moderate sensitization rate in humans based on animal or other tests.
>
> 119. Some authorities currently categorize strong sensitizers. However, at present, animal or other test systems to subcategorize sensitizers as indicated above, have not been validated and accepted. Work is going on to develop such models for the potency evaluation of contact allergens."

4. In 2002, the IOMC Coordinating Group for the Harmonization of Classification and Labelling noted that the sensitization criteria for substances should be re-opened to consider the inclusion of new information and evolving testing approaches that address the question of distinguishing strong sensitizers from those that are weaker. Appropriate hazard communication should be considered along with the discussions on the criteria and the availability of an appropriate test method. The UN mandate (ST/SG/AC.10/C.4/2002/19, December 2002, UN Sub-Committee HCL) directed OECD to consider use of "strong vs. weak" sensitizers in the

GHS. This mandate was extended for the biennium 2005 - 2006 (ST.SG/AC.10/C.4/16). The mandate is stated as follows:

> "Sensitization - Strong versus weak
> Objective: To examine the available information concerning strong vs. weak sensitizers and, if appropriate, propose revisions to the classification criteria for respiratory and/or dermal sensitization."

Background Information on Approaches for Determining Sensitization Strength

German Panel of Experts

5. The approach articulated in the paper by Schlede et al summarized work performed in the course of 15 years by a panel of German experts. In this publication, more than 200 contact sensitizers were ranked. (Schlede et al. Chemical substances and contact allergy - 244 substances ranked according allergenic potency. (2003), Toxicology 193, 219-259). Schlede et al include prevalence, strength of sensitization in animals and humans and severity of response and cross-reactivity to rank sensitizers. Weight-of-evidence determinations used human clinical data and patch test results as well as animal data when available.

1. Significant allergen: (1) proven strong allergenic effect in humans after short and/or almost negligible exposure taking into account existing animal data; (2) frequently proven contact allergenic effect in humans. Remarks: data on humans demonstrate that in larger collectives 1% or more of the patients react positive and that several independent case studies and experimental data on humans are available.

2. Solid-based indication for contact allergenic effects: (1) less frequently proven contact allergenic effect in humans taking into account existing positive animal data; (2) the capacity of substances to induce cross-reactions in humans without being a significant allergen itself. Remarks: data on humans demonstrate that in collectives less than 1% of the patients react positively and that independent case studies and/or experimental data on humans are available.

3. Insignificant contact allergen or questionable contact allergenic effect because of: (1) rarely proven contact allergenic effect in humans; (2) doubtful effect in humans; no or non-appropriate animal data; (3) no data on humans but positive animal data. Remarks: data on humans include isolated positive test results and isolated case studies and experimental data.

EU Expert Group on Sensitization

6. For induction of skin sensitization, the EU Expert Group proposed a 3-level potency scheme based primarily on potency scores from any of the Local Lymph Node Assay (LLNA), Guinea Pig Maximization Test (GPMT), and Buehler tests. According to the report of the EU Expert Group (4-6 November 2002):

ST/SG/AC.10/C.4/2006/16
page 4

> "The design of the LLNA makes it better suited than the guideline guinea pig assays to the assignment of skin sensitizers into specific potency categories. This is because the LLNA focuses on induction of sensitization only, incorporates a dose response assessment, and has an objective and quantitative endpoint."
>
> "The majority of skin sensitizing chemicals would then fall into the category corresponding to the current default value of 1% for labelling of preparation with R43. An additional 2 categories should be defined for substances with higher potency; these identify strong (>0.1%) and extreme (>0.001%) sensitizers, respectively. With regard to preparations, moderate and strong skin sensitizers would be listed on the label when present in a concentration of 10 ppm or greater, and extreme skin sensitizers when in a concentration of 1 ppm or greater."
>
> "Elicitation thresholds correlate only poorly with induction potency. Variation in elicitation thresholds between individuals is very large and depends on numerous factors of which the sensitizing potency of the substance is only one. Other factors affecting elicitation include the duration, extent and site of exposure, status of the skin and degree of specific sensitization. For this reason, the Expert Group considered that it would be inappropriate to define elicitation thresholds as a function of skin sensitizing potency."
>
> "Human data should normally only be used to re-categorize a substance into a higher potency category. The EU Expert Group proposal does not consider questions of severity of response or cross-reactivity." (Report from the Expert Group on Sensitization, 18-19 April 2002 and 4-6 November 2002)."

European Center for Ecotoxicology and Toxicology of Chemicals (ECETOC)

7. Report # 87 *Contact Sensitization: Classification according to Potency* includes the following definition of potency:

> "Potency in the context of allergic contact dermatitis is best defined as the amount of chemical required for the acquisition of skin sensitization in a previously naive individual (induction phase), or the amount of chemical necessary to elicit a clinically discernable cutaneous reaction in previously sensitized subjects."

U.S.: Consumer Products

8. In regulating consumer products that are strong sensitizers according to FHSA, the US uses severity of response, frequency of responses in exposed populations, and dose at which allergic reactions occur. In addition, Canada has expressed interest in such considerations.

9. The statutory definition in use by the Consumer Products Safety Commission (CPSC) for consumer products in the US combines these elements to determine strength of sensitization. The CPSC definition is as follows:

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> "A strong sensitizer means a substance which will cause on normal living tissue through an allergic or photodynamic process a hypersensitivity which becomes evident on reapplication of the same substance and which is designated as such by the Commission. Before designating any substance as a strong sensitizer, the Commission, upon consideration of the frequency of occurrence and severity of the reaction, shall find that the substance has significant potential for causing hypersensitivity."

10. Supplementary guidance issued by a Technical Advisory Panel on Allergic Sensitization follows:

> "a sensitizer is a substance that will induce an immunologically-mediated (allergic) response, including allergic photosensitivity. This allergic reaction will become evident upon re-exposure to the same substance. Occasionally, a sensitizer will induce and elicit an allergic response on first exposure by virtue of active sensitization."

11. In addition, the panel recommended that when determining that a substance is a "strong" sensitizer and a substance's sensitizing potential, available data is to be considered (i.e.; frequency of occurrence, severity of reactions in healthy or susceptible populations, human and animal experimental data with human taking precedence, bioavailability of sensitizers, data on cross-reacting substances, human threshold sensitivity). The severity of reaction was qualified as a "clinically important reaction", one producing substantial illness (i.e.; physical discomfort, distress, hardship, functional or structural impairment).

12. CPSC has assembled an expert panel, reflecting academia, industry and government regulators from Europe and North America to address the issue of need and criteria for identifying strong sensitizers for their statutorily mandated regulation. This panel is taking the GHS definition of sensitizers into account as part of its deliberations.

Testing for Sensitization

13. Human and animal testing and evaluation methods are described in the Annex to this document.

Animal data:

14. Traditional test methods used for regulation of sensitizers have focused on determining whether or not a substance is a sensitizer. In the Guinea Pig test methods, the determination is based on results in excess of a pre-determined percent of animals eliciting a response after repeated applications of the substance. In the LLNA test in mice, determination that a substance is a sensitizer is based on results exceeding a pre-determined ratio of effect in test animals versus controls.

15. Overall, the accuracy of the guinea pig tests as methods of predicting human sensitization is considered 88%. According to the ICCVAM LLNA peer review report [ref.: NIH(1999), NIH Publication No. 99-4494], the LLNA performed at least as well as currently accepted guinea pig methods (GPMT/BA) for the hazard identification of strong to moderate chemical sensitizing agents. The performance of the LLNA and the GPMT/BA was similar when each was compared

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to human data (HMT/HPTA). The accuracy of the LLNA vs. human data was 72% (N=74), GPMT/BA was 72% (N=57), and all guinea pig tests (GPT) vs. human was 73% (N=62).

16. In traditional guinea pig tests submitted for regulatory review, the dose at which responses occur is not generally recorded, although in some cases, such information is available. The guinea pig test are not designed for looking at potency; however, by a modified protocol using multiple induction doses, described by Andersen, potency can be assessed.

17. With the recent development of the mouse Local Lymph Node Assay, the dose at which the EC3 (discriminating level) is exceeded is normally available.

18. Many animal tests and human data/information actually observe elicitation, which is an indicator of both the induction and elicitation phases of sensitization. Some experts consider however, that animal tests are performed primarily to identify induction of sensitivity.

Human data:

19. Epidemiological evidence judges prevalence of effects based on frequency of response in humans, consideration of severity of response and its significance for regulatory purposes. (Prevalence in the general population is a reflection of intrinsic potency and the degree of exposure.) Human testing for epidemiological or diagnostic purposes normally measure elicitation responses in subjects who have been previously exposed (annex).

Issues to be addressed by the OECD

20. GHS paragraph 1.3.2.4.9.3 says that "generally data of good quality and reliability in humans takes precedence over other data." And the GHS paragraph 3.4.2.2.2.2 states "Positive effects seen in either humans or animals will normally justify classification. Evidence from animal studies is usually much more reliable than evidence from human exposure. However, in cases where evidence is available from both sources, and there is conflict between the results, the quality and reliability of the evidence from both sources must be assessed in order to resolve the question of classification on a case-by-case basis." Respiratory sensitization is classified primarily based on human data because there is no standardized animal model for this end point. Therefore, the scheme proposed by the EU Expert Group for induction of skin sensitization may not be applicable for respiratory sensitization.

21. Potentiation of skin sensitizers in mixtures can occur due to other ingredients which might enhance absorption or "toxicity" of the sensitizer. Solvents can have up to 20 fold influence on the measured potency of an allergen. Product matrices can also affect responses due to availability of the sensitizing ingredient. What are vehicle effects on dose-response? How would classification of untested mixtures with 3 or 4 potency levels address this? Are these factors playing roles different from their roles in the determination if the substance/mixture is a sensitizer or not a sensitizer?

22. Many animal tests and human data/information actually observe elicitation, which is an indicator of both the induction and elicitation phases of sensitization. Elicitation generally occurs at lower doses than induction and can occur at lower doses as exposure is repeated. The variable

nature of such data would lead to special challenges for comparison among chemicals of sensitization potency if it were decided to link potency to induction and elicitation rather than to induction only. The GHS includes both induction and elicitation in defining a sensitizer: should potency be connected to induction only?

23. Given that prevalence is not necessarily an end by itself, but is a measurement used when evaluating human data, how can intrinsic potency be teased out from exposure when both factors contribute to prevalence? What considerations are needed regarding use of prevalence data in humans as part of the weight of evidence to discriminate between strong and weak sensitizers? How significant is consideration of the type of exposed population, i.e. general population, sensitive population, occurrence of atopic individuals? Can intrinsic strength of sensitizers be adequately distinguished when there is likely to be a range of exposures?

24. The severity of allergic reactions varies. Allergic contact dermatitis can range from mild local reactions to erythroderma, which affects most of the body surface. Immediate hypersensitivity reactions can range from mild rhinitis to local hives (contact urticaria) to severe asthma and anaphylactic shock. Can such responses be related to strong vs. weak sensitizers? Can such manifestation be predicted from responses in animals? How can manifested responses in humans be distinguished from dose to which they are exposed? When can a severe response be determined to be caused by intrinsic properties of a chemical or high exposure or individual susceptibility? Can interspecies extrapolation be used to relate animal responses to severe responses in humans?

25. For some animal data, the sensitization response rate can be measured. How can such measurements be related to response rate in humans?

26. Can animal tests be correlated with probable human sensitization responses in order to distinguish strong from weak sensitizers?

Discussion

27. The OECD Expert Group is exploring development of a scientifically defensible way to define strong versus weak sensitizers with sufficient clarity for classification purposes.

28. Harmonization must take into consideration generally hazard based existing systems. In the U.S., consumer products falling under the FHSA are regulated only if they are deemed strong sensitizers (taking into consideration frequency of sensitization in an exposed population, severity of response and dose at which the sensitization occurs).

29. The GHS defines sensitization hazards as including both induction and elicitation and advocates the use of animal and human data as and when available. "Generally, data of good quality in humans will have precedence over other data. However, even well designed and conducted epidemiological studies may lack sufficient numbers of subjects to detect relatively rare but still significant effects, or to assess potentially confounding effects. Positive results from well-conducted animal studies are not necessarily negated by the lack of positive human experience but require an assessment of the robustness and quality of both the human and animal data relative to the expected frequency of occurrence of effects and the impact of potentially

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confounding factors." (GHS Par. 1.3.2.4.9.3).

30. The approach under consideration in the EU is to define strong versus weak sensitizers based on the intrinsic capacity of the chemical to induce sensitization in animals. The current regulatory approach in the US is to consider, in defining strong versus weak sensitizers, data in animals and humans, with the latter based on the intrinsic capacity of the chemical to induce sensitivity, to elicit responses in sensitized individuals, and on the actual exposure encountered in the population.

31. An approach like that of the German panel (Schlede et al) merges human and animal data and takes into consideration severity, dose, and frequency of response.

32. At the second OECD Expert Group meeting (5-6 May 2004), "The Expert Group agreed that the LLNA is very important for ranking (contact) sensitizers but that additional factors (human data and animal data) also have to be taken into account".

33. The approach under consideration in Europe leads to categories based on potency for induction of sensitization. If dose leading to sensitization is to be used as a single parameter, in order to reflect the US system in use currently, it must be shown to correlate with human response in a way which takes into account severity and frequency of response as well. For such a correlation to be validated, it is essential to have a reference list of chemicals characterized for sensitization in humans with consensus values for potency and other relevant parameters including frequency and severity of response. In addition, since many test or observational methods actually measure elicitation responses, agreement is needed on a consistent way to assess elicitation thresholds (noting that elicitation responses often are expressed at lower concentrations with repeated exposures).

34. The EU Expert Group advocates ranking chemicals primarily by means of LLNA. This is because the LLNA focuses on induction of sensitization only, incorporates a dose response assessment, and has an objective and quantitative endpoint. However, GPMT and Buehler evaluate both phases of sensitization – induction and elicitation, as included in the GHS, while LLNA evaluates primarily induction. The response rates of GPMT and Buehler have been used by some authorities for potency determination.

35. Classification should be able to be used for both existing and new chemicals. The approach based on animal test model (in particular the LLNA) is predictive (proactive) attempting to identify and rank the effect before human use and does not rely on human exposure information. When sensitizers are ranked based on human data, the approach is retroactive. This approach relies on human exposure information considering magnitude and frequency of exposure and severity of response among exposed individuals during the actual use of the marketed (commercially) available product(s). This is not an approach that is applicable for new substances before they are available on the market. It is necessary to decide on the advantages /disadvantages of these two approaches in relation to the requirements of the GHS which is based on hazard classification. Animal test data may be used to identify a sensitizer before human use. When human data becomes available it should be considered in evaluating the hazard.

36. Harmonized Categories proposed for classification must be able to be strongly differentiated such that substances can be classified consistently in the various UN nations.

37. The comprehensive examination of the current science by an international panel being undertaken for consumer products in the U.S. is expected to provide new insights into the question of strong vs. weak sensitizers.

Annex

Sensitization Evaluation Methods

Guinea Pig Maximization Test: Typically the highest concentration of a chemical causing mild to moderate irritation is multiply injected intradermally (with and with out adjuvant) on a shaven shoulder, 7 days later a patch containing the same highest to moderate irritating concentration of chemical is applied for 48hrs as a booster. At 14 days post induction, challenge (with a maximal non-irritating dose of the chemical) is carried out on the flank with occlusion for 24hr. The area of erythema and edema is evaluated at 24 and 48 hrs post challenge. A chemical is classified as a sensitizer if at least 30% of the animals have a positive response (grade 1 or higher). Past concerns with the GPMT regarded occurrences of false positive responses and interpretation of weak responses. Dose-responses are typically utilized with the challenge dose. The GPMT has not been formally validated for potency determination.

Buehler Test: The chemical is applied to a shaven flank at a minimal irritating dose and occluded for 6hrs. The procedure is repeated on day 7 and day 14. Chemical challenge is carried out 2 weeks later on the opposite shaven flank and occluded for 24hrs (though some may occlude for 6hrs) taking the highest non-irritating. Upon removal of the patch, the area is evaluated at 24hr and 48hrs for edema and erythema. A chemical is classified as a sensitizer if 15% of the animals demonstrate a positive response (grade 1 or higher). This protocol is considered less sensitive than the GPMT but is less prone to false positive results. The major differences from the GPMT lie in the induction phase, with the lack of utilization of both adjuvant and intradermal application. The Buehler test has not been formally validated for potency determination.

Local Lymph Node Assay: A 3 day repeated application of the chemical is applied to the ear dorsum. On day 5, tritiated-thymidium is injected (i.v.) and 5hrs later lymph nodes are excised and counted. A chemical is classified as a skin sensitizer if it induces at least a 3-fold increase in proliferative counts compared to vehicle-treated controls (the stimulation index – SI). The concentration of chemical which produces at least a SI of 3 is the EC3 value. A concern regarding the LLNA is that it is more appropriate for the class of chemicals considered Type IV sensitizers, which act through T-cell mediated mechanisms, and less so for Type I sensitizers whose mechanistic effects are antibody mediated. The LLNA has been validated for dermal sensitization hazard identification; the LLNA has not been formally validated for potency determination.

Epidemiological Data: Much of the population data is derived from diagnostic patch testing in dermatitis patients. However, this provides common exposure patterns such that a typical list of 25-35 compounds is maintained for universal patch test studies (standard series). Data from samples of the general population and exposed groups are also available, however more limited. Thus, the epidemiological data generated may not be representative of the general population. Nevertheless, the data permit recognition of allergens with high sensitizing potential.

Human Testing: Diagnostic patch testing is the procedure used for detection of contact allergy (skin sensitization) to substances in humans. Patch testing is performed in individuals

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with dermatitis, and in experimental and epidemiological studies. The test procedure is standardized, while different patch test systems are in use. The patches are applied on the back for 2 days and grading of reactions is recommended to be done 2, 3/4 and 5/7 days after application. A typical patch test system is aluminium chambers (Finn chamber®) on adhesive tape with test substances at set concentrations (the standard series, other series etc.), generally in petrolatum. TRUE® test is comprised of chemical gel matrix patches put on with adhesive tape. Patch test dose-response studies have been carried out, which thereby demonstrate elicitation thresholds under certain circumstances (e.g.; the fragrance allergen isoeugenol).

Additional testing procedures may also be used, but they should not be used to replace patch testing. These include the open test, the semi-open test and use tests. Use tests with the products (e.g. the provocative use test, PUT) were originally intended to mimic the actual use situation without the goal to differentiate between allergic and irritant skin reactions. Nowadays they are most commonly used to evaluate the clinical relevance of a patch test reaction. The repeated open application test (ROAT) is a standardized method of use testing. The test substance, either a commercial product, as is, or a special test substance is applied twice daily for one week or longer. The value of ROAT has been verified in cases with positive, negative or questionable reactions at initial patch testing and in animal studies.

In addition to patch tests for contact allergy, diagnostic analysis for respiratory sensitization includes tests such as the Skin Prick test, intradermal tests, and serological immunological tests for the presence of specific antibodies (e.g; RAST test). Less commonly, challenge testing via oral, inhaled or other routes.

While human diagnostic tests only test for whether an individual has been pre-sensitized by prior exposure or to determine an elicitation threshold in a sensitized individual, the Human Repeat Insult Patch Test (HRIPT) is a test of allergenic potential and more comparable in function to the guinea pig test or the LLNA. It involves a chemical patch applied (to the same site) three times a week, occluded for 24 hours, for a three week period. Two weeks later, the same site is challenged with the chemical and responses noted. The amount of consecutive induction patches can vary. The HRIPT provides an exaggeration of product use and testing higher than use concentrations (typically a mild irritating dose).

Human predictive sensitization tests in volunteers is, in Europe, not considered ethical to perform due to the risk that patch test sensitization may elicit clinical disease in the subject. In addition, GHS Paragraph 1.3.2.4.7 says: "Testing on humans solely for hazard identification purposes is generally not acceptable."

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Appendix E3

UN/SCEGHS/12/INF.16 (OECD), Progress Report on OECD Work Since the Last Sub-Committee Meeting

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COMMITTEE OF EXPERTS ON THE TRANSPORT OF DANGEROUS GOODS AND ON THE GLOBALLY HARMONIZED SYSTEM OF CLASSIFICATION AND LABELLING OF CHEMICALS

Sub-Committee of Experts on the Globally Harmonized System of Classification and Labelling of Chemicals

Twelfth session, 12 (p.m.)-14 December 2006
Agenda item 2 of the provisional agenda

UPDATING OF THE GLOBALLY HARMONIZED SYSTEM OF CLASSIFICATION AND LABELLING OF CHEMICALS

Progress report on OECD work since the last Sub-Committee meeting

Transmitted by the Organization for Economic Co-operation and Development (OECD)

Issues under discussion

1. The remaining work for the OECD Task Force on Harmonization of Classification and Labelling (HCL) is the submission of (i) a proposal for classification and labelling for chronic aquatic hazards, (ii) a proposal for ozone depleting chemicals, (iii) a (summary) validation report of the T/D Protocol and (iv) a proposal related to strong versus weak sensitizers.

Chronic aquatic hazards

2. The Sub-Committee requested the OECD to further develop the classification scheme to accommodate chronic toxicity to aquatic organisms for assigning a chronic hazard category, starting with the submission of a scientific issue paper. The OECD expert group on Aquatic Hazards made considerable efforts to develop the scientific issue paper that was submitted to the Sub-Committee in July 2006, and the related proposal. The expert group had three meetings (Spain, 2004; OECD, 2005 and United States, 2006), and a number of conference calls. The scheme for classification of substances was agreed at the 2006 Task Force meeting. However, the expert group had to further work on the classification scheme for mixtures. A proposal for replacing GHS Chapter 4.1 and Sections A9.1-A9.3 was recently agreed by the expert group on Aquatic Hazards. It was sent to the Task Force on HCL and to the Sub-Committees on Transport on Dangerous goods and on GHS for any comments by 26 December 2006. An OECD proposal should be submitted for approval at the July Sub-Committee meeting.

Ozone depleting chemicals

3. The OECD submitted a detailed comparison of classification and labelling systems for ozone depleting chemicals at the July Sub-Committee meeting. The OECD Secretariat informed the Ozone Secretariat of the conclusions of the Sub-Committee, i.e. that the work should continue and that it would consider any proposal or option in this respect for final decision. The OECD expert group on ozone depleting chemicals had two conference calls on 13 October and 30 November 2006. A proposal for classification criteria and labelling of ozone depleting chemicals was agreed by the experts on 30 November. This proposal has been circulated to the expert group for any

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comments from the experts who were unable to attend the last conference call. By the end of December, the Task Force on HCL and the two sub-Committees should receive a draft proposal for comments. The Ozone Secretariat will be informed as soon as that the proposal is available for the Parties to the Montreal Protocol. It is expected that a proposal will be submitted for approval at the July Sub-Committee meeting.

Validation of the Transformation/Dissolution Protocol

4. A fourth Validation Management Group (VMG) of the Transformation/Dissolution Protocol was held in Brussels on 28-29 September 2006. The draft report of the validation (assessing reproducibility and repeatability) includes a description of the experimental work and a statistical analysis of the results. This draft report is being finalized and the VMG agreed that it should be sent to the OECD Task Force on HCL and to the two Sub-Committees before the end of the year for any comments. A summary report should be submitted for endorsement at the July Sub-Committee meeting. Further work is ongoing to study the relevance of the protocol (phase 2). The VMG already discussed the content of the phase 2 report, which should also include a statistical analysis. It is expected that the phase 2 report will be ready for endorsement by the Sub-Committee at the July or December 2007 meeting.

Strong versus weak sensitizers

5. The OECD submitted an issue paper on strong versus weak sensitizers at the last Sub-Committee meeting. This paper suggested waiting the IPCS/WHO workshop before developing a proposal for strong versus weak sensitizers. The OECD expert group agreed to restart its work considering the following new elements: the IPCS/WHO workshop held on 17-18 October 2006, the US CPSC Staff Report on the draft Proposed Revision of the FHSA "Strong Sensitizer" Supplemental Definition, ICCVAM possible validation of the LLNA for potency categorization, and the EU forthcoming development of a guidance document including sensitizers classification. An expert group meeting is planned in February in the United States.

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Appendix E4

UN/SCEGHS/15/INF.14 (OECD), Proposal for Revising Chapter 3.4 with Respect to Strong versus Weak Sensitizers: Explanatory Notes and Revised Chapter with Visible Changes

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UN/SCEGHS/15/INF.14

COMMITTEE OF EXPERTS ON THE TRANSPORT OF DANGEROUS GOODS AND ON THE GLOBALLY HARMONIZED SYSTEM OF CLASSIFICATION AND LABELLING OF CHEMICALS

Sub-Committee of Experts on the Globally Harmonized System of Classification and Labelling of Chemicals

Fifteenth session,
Geneva, 9-11 July 2008
Item 2 (b) of the provisional agenda

UPDATING OF THE SECOND REVISED EDITION OF THE GLOBALLY HARMONIZED SYSTEM OF CLASSIFICATION AND LABELLING OF CHEMICALS (GHS)

Health hazards

Proposal for revising Chapter 3.4 with respect to strong versus weak sensitizers: Explanatory notes and revised chapter with visible changes

Transmitted by the Organisation for Economic Co-operation and Development (OECD)

This document includes explanatory notes to the proposal for revising Chapter 3.4 of the GHS and the proposed revised Chapter 3.4 with visible changes (Annex).

UN/SCEGHS/15/INF.14
page 2

Explanatory notes

1. In December 2002, the UN Sub-Committee of Experts on the GHS (UN SCEGHS) requested that the OECD examine the available information concerning strong versus weak sensitizers and, if appropriate, propose revisions to the classification criteria for respiratory and/or dermal sensitization.

2. The proposal includes two new subcategories 1A and 1B for respiratory sensitization and two subcategories 1A and 1B for skin sensitization. New sections 3.4.2.1.1 (for respiratory sensitization) and 3.4.2.2.1 (for skin sensitization) describe the subcategories.

3. Sentences are added at the beginning of the new paragraph 3.4.2.1.1.3 for respiratory sensitization, and at the beginning of the new paragraph 3.4.2.2.1.3 for skin sensitization to strengthen the weight of evidence approach.

4. On the basis of an impact analysis, the proposed guidance value for skin sensitization Subcategory 1A is: $\leq 2\%$ for the LLNA (Table 3.4.1) and: $\leq 500\ \mu g/cm^2$ for human data (3.4.2.2.1.4).

5. The subcategories 1A and 1B may be used for substances and for mixtures that are not classified on the basis of data available for all or some ingredients, when data are sufficient and when required by a competent authority (Sections 3.4.2.1.1 for respiratory sensitization and 3.4.2.2.1 for skin sensitization). For mixtures that are classified on the basis of data available for all or some ingredients, specific cut-off values are provided for the subcategories 1A and 1B (Table 3.4.3); subcategories 1A and 1B cannot be used for such mixtures that can be classified as Category 1 only.

6. Whatever the category or subcategory, the label remains the same as it is in the current GHS for respiratory and skin sensitization.

7. The six notes to the table of Section 3.4.3.3 are replaced with a single Note 1; the six notes were confusing (repeating what is the normal consequence of classification: an SDS and a label would normally be expected), and the same text was repeated in several notes.

8. Given the inclusion of two subcategories, two paragraphs that apply for more than one category only are inserted in Section 3.4.3.2, under the bridging principles (new Paragraphs 3.4.3.2.3 and 3.4.3.2.4), and the existing Paragraph 3.4.3.2.2 on dilution is slightly revised.

9. The proposal includes a few additional changes: in 3.4.2.2.2.2, the first sentence is deleted as it is now in the new paragraph 3.4.2.2.1.3, and a sentence is added to note the possible impact of vehicles; the footnote 2 is revised to delete the example that is not considered relevant; in 3.4.2.2.4.1, the criteria for a positive response with the LLNA is inserted and the last sentence is deleted as it is considered to include too specific information. Paragraphs 3.4.2.2.4.2 and 3.4.2.2.4.3 are deleted as they are no longer relevant; the subcategories 1A and 1B are mentioned in the table of Section 3.4.4; Paragraph 3.4.4.2 is slightly revised to extend its application to classified mixtures; slight change to the Decision Logic (new footnote 6).

Annex

PROPOSED REVISED CHAPTER 3.4
(WITH VISIBLE CHANGES)

"RESPIRATORY OR SKIN SENSITIZATION

3.4.1 Definitions and general considerations

3.4.1.1 A *respiratory sensitizer* is a substance that will lead to hypersensitivity of the airways following inhalation of the substance[1].

A *skin sensitizer* is a substance that will lead to an allergic response following skin contact[1].

3.4.1.2 For the purpose of this chapter, sensitization includes two phases: the first phase is induction of specialized immunological memory in an individual by exposure to an allergen. The second phase is elicitation, i.e. production of a cell-mediated or antibody-mediated allergic response by exposure of a sensitized individual to an allergen.

3.4.1.3 For respiratory sensitization, the pattern of induction followed by elicitation phases is shared in common with skin sensitization. For skin sensitization, an induction phase is required in which the immune system learns to react; clinical symptoms can then arise when subsequent exposure is sufficient to elicit a visible skin reaction (elicitation phase). As a consequence, predictive tests usually follow this pattern in which there is an induction phase, the response to which is measured by a standardized elicitation phase, typically involving a patch test. The local lymph node assay is the exception, directly measuring the induction response. Evidence of skin sensitization in humans normally is assessed by a diagnostic patch test.

3.4.1.4 Usually, for both skin and respiratory sensitization, lower levels are necessary for elicitation than are required for induction. Provisions for alerting sensitized individuals to the presence of a particular sensitizer in a mixture can be found at section 3.4.4.

3.4.2 Classification criteria for substances

3.4.2.1 *Respiratory sensitizers*

3.4.2.1.1 *Hazard ~~category~~ categories*

~~Substances shall be classified as respiratory sensitizers (Category 1) in accordance with the criteria given below:~~

~~(a) If there is evidence in humans that the substance can lead to specific respiratory hypersensitivity and/or~~
~~(b) If there are positive results from an appropriate animal test.~~

[1] *This is a working definition for the purpose of this document*

3.4.2.1.1.1 Respiratory sensitizers shall be classified in Category 1 where subcategorization is not required by a competent authority or where data are not sufficient for subcategorization.

3.4.2.1.1.2 Where data are sufficient and where required by a competent authority, a refined evaluation allows the allocation of respiratory sensitizers into subcategory 1A, strong sensitizers, or subcategory 1B for other respiratory sensitizers.

3.4.2.1.1.3 Effects seen in either humans or animals will normally justify classification in a weight of evidence approach for respiratory sensitizers. Substances are allocated to one of the two subcategories 1A or 1B using a weight of evidence approach in accordance with the criteria given in figure 3.4.1 and on the basis of reliable and good quality evidence from human cases or epidemiological studies and/or observations from appropriate studies in experimental animals.

Figure 3.4.1: Hazard category and subcategories for respiratory sensitizers

CATEGORY 1:	**Respiratory sensitizer**
	A substance is classified as a respiratory sensitizer -if there is evidence in humans that the substance can lead to specific respiratory hypersensitivity and/or -if there are positive results from an appropriate animal test[2].
Subcategory 1A:	Substances showing a high frequency of occurrence in humans; or a probability of occurrence of a high sensitization rate in humans based upon animal or other tests[2]. Severity of reaction may also be considered.
Subcategory 1B:	Substances showing a low to moderate frequency of occurrence in humans; or a probability of occurrence of a low to moderate sensitization rate in humans based upon animal or other tests[2]. Severity of reaction may also be considered.

3.4.2.1.2 *Human evidence*

3.4.2.1.2.1 Evidence that a substance can ~~induce~~ lead to induce specific respiratory hypersensitivity will normally be based on human experience. In this context, hypersensitivity is normally seen as asthma, but other hypersensitivity reactions such as rhinitis/conjunctivitis and alveolitis are also considered. The condition will have the clinical character of an allergic reaction. However, immunological mechanisms do not have to be demonstrated.

3.4.2.1.2.2 When considering the human evidence, it is necessary for a decision on classification to take into account, in addition to the evidence from the cases:

(a) the size of the population exposed;

(b) the extent of exposure.

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[2] *At present recognized and validated animal models for the testing of respiratory hypersensitivity are not available. Under certain circumstances, ~~animal testing may be used, e.g. a modification of the guinea pig maximization test for determination of relative allergenicity of proteins. However, these tests still need further validation.~~ data from animal studies may provide valuable information in a weight of evidence assessment.*

3.4.2.1.2.3 The evidence referred to above could be:

(a) clinical history and data from appropriate lung function tests related to exposure to the substance, confirmed by other supportive evidence which may include:

(i) *in vivo* immunological test (e.g. skin prick test);

(ii) *in vitro* immunological test (e.g. serological analysis);

(iii) studies that may indicate other specific hypersensitivity reactions where immunological mechanisms of action have not been proven, e.g. repeated low-level irritation, pharmacologically mediated effects;

(iv) a chemical structure related to substances known to cause respiratory hypersensitivity;

(b) data from positive bronchial challenge tests with the substance conducted according to accepted guidelines for the determination of a specific hypersensitivity reaction.

3.4.2.1.2.4 Clinical history should include both medical and occupational history to determine a relationship between exposure to a specific substance and development of respiratory hypersensitivity. Relevant information includes aggravating factors both in the home and workplace, the onset and progress of the disease, family history and medical history of the patient in question. The medical history should also include a note of other allergic or airway disorders from childhood, and smoking history.

3.4.2.1.2.5 The results of positive bronchial challenge tests are considered to provide sufficient evidence for classification on their own. It is however recognized that in practice many of the examinations listed above will already have been carried out.

3.4.2.1.3 *Animal studies*

Data from appropriate animal studies[2] which may be indicative of the potential of a substance to cause sensitization by inhalation in humans[3] may include:

(a) measurements of Immunoglobulin E (IgE) and other specific immunological parameters, for example in mice;

(b) specific pulmonary responses in guinea pigs.

---

[2] *At present recognized and validated animal models for the testing of respiratory hypersensitivity are not available. Under certain circumstances, animal testing may be used, e.g. a modification of the guinea pig maximization test for determination of relative allergenicity of proteins. However, these tests still need further validation. data from animal studies may provide valuable information in a weight of evidence assessment..*

[3] *The mechanisms by which substances induce symptoms of asthma are not yet fully known. For preventative measures, these substances are considered respiratory sensitizers. However, if on the basis of the evidence, it can be demonstrated that these substances induce symptoms of asthma by irritation only in people with bronchial hyperreactivity, they should not be considered as respiratory sensitizers.*

UN/SCEGHS/15/INF.14
page 6
Annex

3.4.2.2 ***Skin sensitizers***

3.4.2.2.1 *Hazard ~~category~~ categories*

~~Substances shall be classified as contact sensitizers (Category 1) in accordance with the criteria below:~~

~~(a) If there is evidence in humans that the substance can lead to sensitization by skin contact in a substantial number of persons, or~~

~~(b) If there are positive results from an appropriate animal test.~~

3.4.2.2.1.1 Skin sensitizers shall be classified in Category 1 where subcategorization is not required by a competent authority or where data are not sufficient for subcategorization.

3.4.2.2.1.2 Where data are sufficient and where required by a competent authority, a refined evaluation according to 3.4.2.2.1.3 allows the allocation of skin sensitizers into subcategory 1A, strong sensitizers, or subcategory 1B for other skin sensitizers.

3.4.2.2.1.3 Effects seen in either humans or animals will normally justify classification in a weight of evidence approach for skin sensitizers as described in 3.4.2.2.2. Substances may be allocated to one of the two subcategories 1A or 1B using a weight of evidence approach in accordance with the criteria given in figure 3.4.2 and on the basis of reliable and good quality evidence from human cases or epidemiological studies and/or observations from appropriate studies in experimental animals according to the guidance values provided in 3.4.2.2.1.4 for Subcategory 1A and in 3.4.2.2.1.5 for Subcategory 1B.

Figure 3.4.2: Hazard category and subcategories for skin sensitizers

CATEGORY 1:	**Skin sensitizer**
	A substance is classified as a skin sensitizer -if there is evidence in humans that the substance can lead to sensitization by skin contact in a substantial number of persons, or -if there are positive results from an appropriate animal test.
Subcategory 1A:	Substances showing a high frequency of occurrence in humans and/or a high potency in animals can be presumed to have the potential to produce significant sensitization in humans. Severity of reaction may also be considered.
Subcategory 1B:	Substances showing a low to moderate frequency of occurrence in humans and/or a low to moderate potency in animals can be presumed to have the potential to produce sensitization in humans. Severity of reaction may also be considered.

3.4.2.2.1.4 Human evidence for Subcategory 1A can include positive responses at ≤ 500 μg/cm^2 (HRIPT, HMT – induction threshold); diagnostic patch test data where there is a relatively high and substantial incidence of reactions in a defined population in relation to relatively low exposure then that is indicative of a strong sensitizer; other epidemiology evidence where there is a relatively high and substantial incidence of allergic contact dermatitis in relation to relatively low exposure then that is indicative of a strong sensitizer.

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Animal test results for Subcategory 1A can include data with values indicated in Table 3.4.1 below:

Table 3.4.1: Animal test results for Subcategory 1A

Assay	Criteria
Local Lymph Node Assay	EC3 value ≤ 2%
Guinea Pig Maximisation Test	≥ 30% responding at ≤ 0.1% intradermal induction dose or ≥ 60% responding at > 0.1% to ≤ 1% intradermal induction dose
Buehler Assay	≥15% responding at ≤ 0.2% topical induction dose or ≥ 60% responding at > 0.2% to ≤ 20% topical induction dose

3.4.2.2.1.5 Human evidence for subcategory 1B can include any positive response at > 500 µg/cm2 (HRIPT, HMT – induction threshold); diagnostic patch test data where there is a relatively low but substantial incidence of reactions in a defined population in relation to relatively high exposure then that is indicative of a skin sensitizer of subcategory 1B; other epidemiology evidence where there is a relatively low but substantial incidence of allergic contact dermatitis in relation to relatively high exposure then that is indicative of a skin sensitizer of subcategory 1B.

Animal test results for Subcategory 1B can include data with values indicated in Table 3.4.2 below:

Table 3.4.2: Animal test results for Subcategory 1B

Assay	Criteria
Local Lymph Node Assay	EC3 value > 2%
Guinea Pig Maximisation Test	≥ 30% to < 60% responding at > 0.1% to ≤ 1% intradermal induction dose or ≥ 30% responding at > 1% intradermal induction dose
Buehler Assay	≥15% to < 60% responding at > 0.2% to ≤ 20% topical induction dose or ≥ 15% responding at > 20% topical induction dose

3.4.2.2.2 *Specific considerations*

3.4.2.2.2.1 For classification of a substance, evidence should include any or all of the following using a weight of evidence approach:

(a) Positive data from patch testing, normally obtained in more than one dermatology clinic;

(b) Epidemiological studies showing allergic contact dermatitis caused by the substance; Situations in which a high proportion of those exposed exhibit characteristic symptoms are to be looked at with special concern, even if the number of cases is small;

(c) Positive data from appropriate animal studies;

(d) Positive data from experimental studies in man (see Chapter 1.3, para. 1.3.2.4.7);

(e) Well documented episodes of allergic contact dermatitis, normally obtained in more than one dermatology clinic.

(f) Severity of reaction may also be considered.

3.4.2.2.2.2 ~~Positive effects seen in either humans or animals will normally justify classification.~~ Evidence from animal studies is usually much more reliable than evidence from human exposure. However, in cases where evidence is available from both sources, and there is conflict between the results, the quality and reliability of the evidence from both sources must be assessed in order to resolve the question of classification on a case-by-case basis. Normally, human data are not generated in controlled experiments with volunteers for the purpose of hazard classification but rather as part of risk assessment to confirm lack of effects seen in animal tests. Consequently, positive human data on contact sensitization are usually derived from case-control or other, less defined studies. Evaluation of human data must therefore be carried out with caution as the frequency of cases reflect, in addition to the inherent properties of the substances, factors such as the exposure situation, bioavailability, individual predisposition and preventive measures taken. Negative human data should not normally be used to negate positive results from animal studies. For both animal and human data, consideration should also be given to the impact of vehicle.

3.4.2.2.2.3 If none of the above mentioned conditions are met, the substance need not be classified as a skin ~~contact~~ sensitizer. However, a combination of two or more indicators of skin ~~contact~~ sensitization as listed below may alter the decision. This shall be considered on a case-by-case basis.

(a) Isolated episodes of allergic contact dermatitis;

(b) Epidemiological studies of limited power, e.g. where chance, bias or confounders have not been ruled out fully with reasonable confidence;

(c) Data from animal tests, performed according to existing guidelines, which do not meet the criteria for a positive result described in 3.4.2.2.4.1, but which are sufficiently close to the limit to be considered significant;

(d) Positive data from non-standard methods;

(e) Positive results from close structural analogues.

3.4.2.2.3 *Immunological contact urticaria*

Substances meeting the criteria for classification as respiratory sensitizers may in addition cause immunological contact urticaria. Consideration should be given to classifying these substances also as skin ~~contact~~ sensitizers. Substances which cause immunological contact urticaria without meeting the criteria for respiratory sensitizers should also be considered for classification as skin ~~contact~~ sensitizers.

There is no recognized animal model available to identify substances which cause immunological contact urticaria. Therefore, classification will normally be based on human evidence which will be similar to that for skin sensitization.

3.4.2.2.4 *Animal studies*

3.4.2.2.4.1 For Category 1, w~~W~~hen an adjuvant type test method for skin sensitization is used, a response of at least 30% of the animals is considered as positive. For a non-adjuvant Guinea pig test

method a response of at least 15% of the animals is considered positive. For Category 1, a stimulation index of 3 or more is considered a positive response in the Local Lymph Node Assay. Test methods for skin sensitization are described in the OECD Guideline 406 (the Guinea Pig Maximisation test and the Buehler guinea pig test) and Guideline 429 (Local Lymph Node Assay). Other methods may be used provided that they are well-validated and scientific justification is given. ~~The Mouse Ear Swelling Test (MEST), appears to be a reliable screening test to detect moderate to strong sensitizers, and can be used as a first stage in the assessment of skin sensitization potential. In case of a positive result in this latter test it may not be necessary to conduct a further guinea pig test.~~

~~3.4.2.2.4.2 When evaluating animal data, produced by testing according to the OECD or equivalent Guidelines for skin sensitization, the rate of sensitized animals may be considered. This rate reflects the sensitizing capacity of a substance in relation to its mildly irritating dose. This dose may vary between substances. A more appropriate evaluation of the sensitizing capacity of a substance could be carried out if the dose response relationship was known for the substance. This is an area that needs further development.~~

~~3.4.2.2.4.3 There are substances that are extremely sensitizing at low doses where others require high doses and long time of exposure for sensitization. For the purpose of hazard classification it may be preferable to distinguish between strong and moderate sensitizers. However, at present animal or other test systems to subcategorize sensitizers have not been validated and accepted. Therefore, sub-categorization should not yet be considered as part of the harmonized classification system.~~

3.4.3 Classification criteria for mixtures

3.4.3.1 ***Classification of mixtures when data are available for the complete mixture***

When reliable and good quality evidence from human experience or appropriate studies in experimental animals, as described in the criteria for substances, is available for the mixture, then the mixture can be classified by weight of evidence evaluation of these data. Care should be exercised in evaluating data on mixtures that the dose used does not render the results inconclusive. (For special labelling required by some competent authorities, see Notes 1~~, 3 and 5~~ to Table 3.4.3~~.4.1~~ of this chapter~~.)~~and 3.4.4.2).

3.4.3.2 ***Classification of mixtures when data are not available for the complete mixture: bridging principles***

3.4.3.2.1 Where the mixture itself has not been tested to determine its sensitizing properties, but there are sufficient data on the individual ingredients and similar tested mixtures to adequately characterize the hazards of the mixture, these data will be used in accordance with the following agreed bridging principles. This ensures that the classification process uses the available data to the greatest extent possible in characterizing the hazards of the mixture without the necessity for additional testing in animals.

3.4.3.2.2 *Dilution*

If a mixture is diluted with a diluent which is not a sensitizer and which is not expected to affect the sensitization of other ingredients, then the new mixture may be classified as equivalent to the original mixture.

3.4.3.2.3 *Concentration of mixtures of the highest sensitizing Category/subcategory*

If a mixture is classified in Category 1 or subcategory 1A, and the concentration of ingredients of the mixture that are in Category 1 and subcategory 1A is increased, the new mixture should be classified in Category 1 or subcategory 1A without additional testing.

3.4.3.2.4 *Interpolation within one category/subcategory*

For three mixtures with identical ingredients, where A and B are in the same category/subcategory and mixture C has the same sensitizing ingredients with concentrations intermediate to the concentrations of those ingredients in mixtures A and B, then mixture C is assumed to be in the same category/subcategory as A and B.

3.4.3.2.~~3~~5 *Batching*

The sensitizing properties of one production batch of a complex mixture can be assumed to be substantially equivalent to that of another production batch of the same commercial product and produced by or under the control of the same manufacturer, unless there is reason to believe there is significant variation such that the ~~sensitization~~ sensitizing properties of the batch has changed. If the latter occurs, a new classification is necessary.

3.4.3.2.~~4~~6 *Substantially similar mixtures*

Given the following:

(a) Two mixtures: (i) A + B;
(ii) C + B;

(b) The concentration of ingredient B is essentially the same in both mixtures;

(c) The concentration of ingredient A in mixture (i) equals that of ingredient C in mixture (ii);

(d) Ingredient B is a sensitizer and ingredients A and C are not sensitizers;

(e) A and C are not expected to affect the sensitizing properties of B.

If mixture (i) is already classified by testing, then mixture (ii) can be assigned the same hazard category.

3.4.3.2.~~5~~7 *Aerosols*

An aerosol form of the mixture may be classified in the same hazard category as the tested non-aerosolized form of the mixture provided that the added propellant does not affect the sensitizing properties of the mixture upon spraying.

3.4.3.3 ***Classification of mixtures when data are available for all ingredients or only for some ingredients of the mixture***

The mixture should be classified as a respiratory or skin sensitizer when at least one ingredient has been classified as a respiratory or skin sensitizer and is present at or above the appropriate cut-off value/concentration limit for the specific endpoint as shown in Table 3.4.~~1~~3 for solid/liquid and gas respectively.

Table 3.4.~~1~~3: Cut-off values/concentration limits of ingredients of a mixture classified as either skin sensitizers or respiratory sensitizers that would trigger classification of the mixture

INGREDIENT CLASSIFIED AS:	**Cut-off values/concentration limits triggering classification of a mixture as:**		
	Skin sensitizer Category 1	**Respiratory sensitizer Category 1**	
	All physical states	Solid/Liquid	Gas
Skin sensitizer Category 1	≥ 0.1% (Note 1)		
	≥ 1.0% ~~(Note 2)~~		
Skin sensitizer Subcategory 1A	≥ 0.1%		
Skin sensitizer Subcategory 1B	≥ 1.0%		
Respiratory sensitizer Category 1		≥ 0.1% (Note ~~3~~1)	≥ 0.1% (Note ~~5~~1)
		≥ 1.0 % ~~(Note 4)~~	≥ 0.2% ~~(Note 6)~~
Respiratory sensitizer Subcategory 1A		≥ 0.1%	≥ 0.1%
Respiratory sensitizer Subcategory 1B		≥ 1.0 %	≥ 0.2%

NOTE 1*: ~~If a skin sensitizer is present in the mixture as an ingredient at a concentration between 0.1% and 1.0%, both an SDS and a label would generally be expected. In addition, some~~ Some competent authorities may require SDS and/or supplemental labelling only, as described in 3.4.4.2 for mixtures containing a sensitizing ingredient at concentrations ~~above 0.1%. The label warning for skin sensitizers~~ between 0.1% and 1.0% (or between 0.1% ~~may differ from the label warning for skin sensitizers ≥ 1.0%,~~ and 0.2% for a gaseous respiratory sensitizer ~~depending on competent authority requirements.~~ While the current cut-off values reflect existing systems, all recognize that special cases may require information to be conveyed below that level.*

~~***NOTE 2****: If a skin sensitizer is present in the mixture as an ingredient at a concentration of ≥ 1.0%, both an SDS and a label would generally be expected.***~~

~~***NOTE 3****: If a solid or liquid respiratory sensitizer is present in the mixture as an ingredient at a concentration between 0.1% and 1.0%, both an SDS and a label would generally be expected. In addition, some competent authorities may require supplemental labelling for mixtures containing a sensitizing ingredient at concentrations above 0.1%. The label warning for solid or liquid respiratory***

~~*sensitizers between 0.1% and 1.0% may differ from the label warning for solid or liquid respiratory sensitizers ≥ 1.0%, depending on competent authority requirements. While the current cut-off values reflect existing systems, all recognize that special cases may require information to be conveyed below that level.*~~

~~***NOTE 4**: If a solid or liquid respiratory sensitizer is present in the mixture as an ingredient at a concentration of ≥ 1.0%, both an SDS and a label would generally be expected.*~~

~~***NOTE 5**: If and 0.2% for a gaseous respiratory sensitizeris present in the mixture as an ingredient at a concentration between 0.1% and 0.2%, both an SDS and a label would generally be expected. In addition, some competent authorities may require supplemental labelling for mixtures containing a sensitizing ingredient at concentrations above 0.1%. The label warning for gaseous respiratory sensitizers between 0.1% and 0.2% may differ from the label warning for gaseous respiratory sensitizers ≥ 0.2%, depending on competent authority requirements. While the current cut-off values reflect existing systems, all recognize that special cases may require information to be conveyed below that level.*~~

~~***NOTE 6**: If a gaseous respiratory sensitizer is present in the mixture as an ingredient at a concentration of ≥ 0.2%, both an SDS and a label would generally be expected.*~~

3.4.4 Hazard communication

3.4.4.1 General and specific considerations concerning labelling requirements are provided in *Hazard communication: Labelling* (Chapter 1.4). Annex 2 contains summary tables about classification and labelling. Annex 3 contains examples of precautionary statements and pictograms which can be used where allowed by the competent authority. Table 3.4.~~2~~4 below presents specific label elements for substances and mixtures that are classified as respiratory and skin sensitizers based on the criteria in this chapter.

Table 3.4.~~2~~4: Label elements for respiratory or skin sensitization

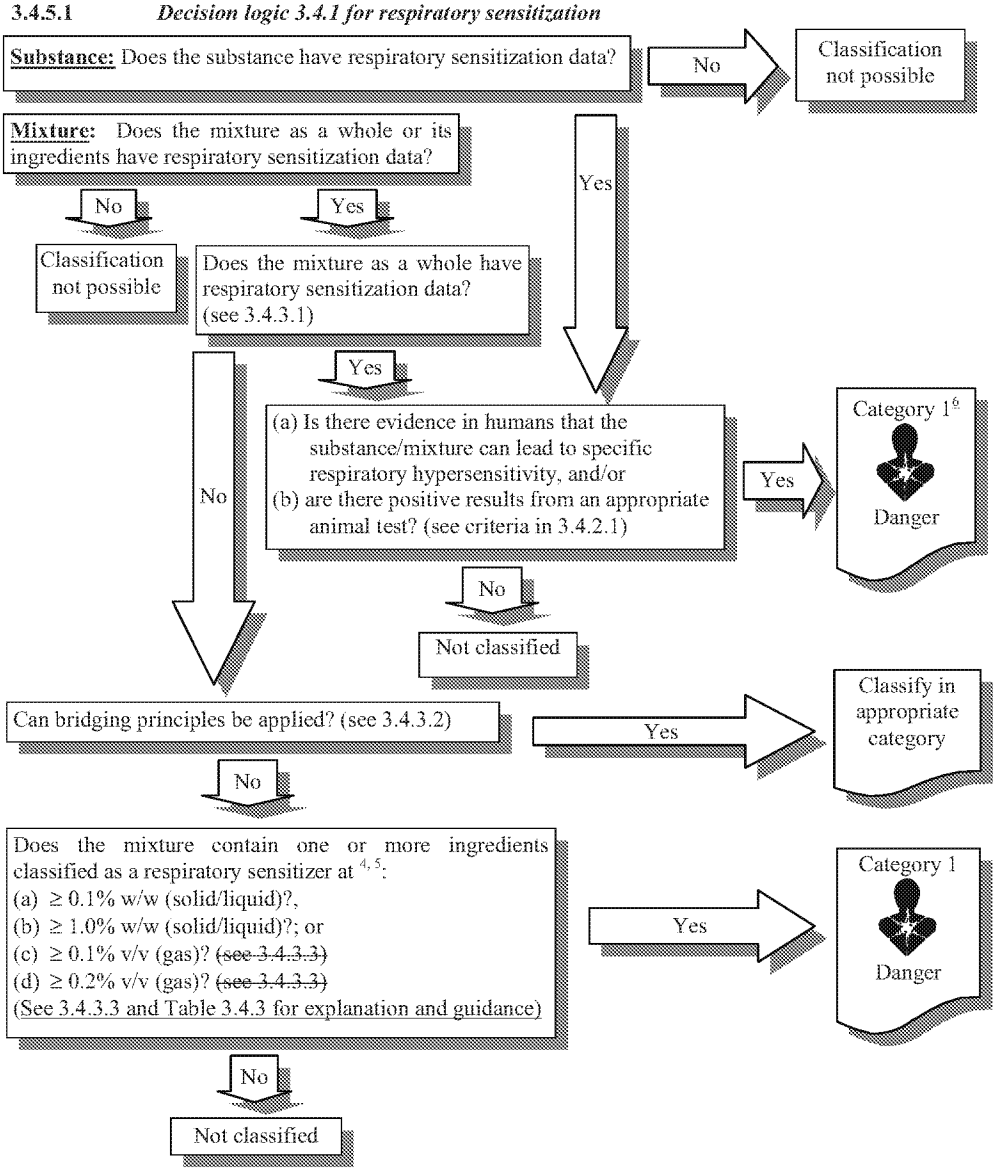
	Respiratory sensitization Category 1 and subcategories 1A and 1B	**Skin sensitization Category 1 and Subcategories 1A and 1B**
Symbol	Health hazard	Exclamation mark
Signal word	Danger	Warning
Hazard statement	May cause allergy or asthma symptoms or breathing difficulties if inhaled	May cause an allergic skin reaction

3.4.4.2 Some chemicals that are classified as sensitizers may elicit a response, when present in a mixture in quantities below the cut-offs established in Table 3.4.~~1~~3, in individuals who are already sensitized to the chemicals. To protect these individuals, certain authorities may choose to require the name of the ingredient as a supplemental label element ~~even though~~ whether or not the mixture as a whole is ~~not~~ classified as sensitizer. ~~Others may choose to classify and label the mixture as a sensitizer in accordance with notes 1, 3 and 5 to Table 3.4.1.~~

3.4.5 Decision logic

The decision logics which follow are not part of the harmonized classification system but are provided here as additional guidance. It is strongly recommended that the person responsible for classification study the criteria before and during use of the decision logics.

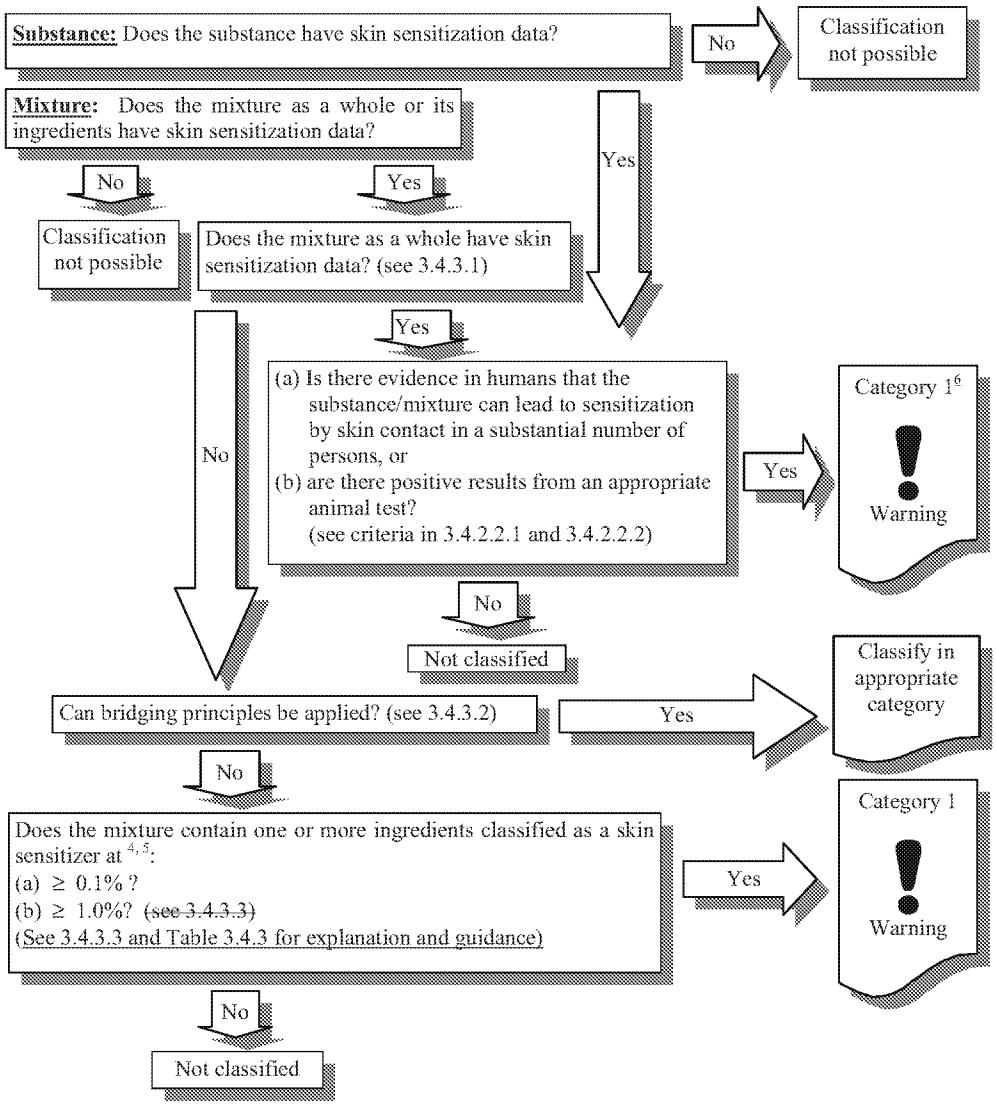
3.4.5.1 ***Decision logic 3.4.1 for respiratory sensitization***



[4] *For specific concentration limits, see "The use of cut-off values/concentration limits" in Chapter 1.3, para. 1.3.3.2.*
[5] *See 3.4.4.2.*
[6] *See 3.4.2.1.1 for details on use of Category 2 subcategories.*

UN/SCEGHS/15/INF.14
page 14
Annex

3.4.5.2 ***Decision logic 3.4.2 for skin sensitization***



[4] *For specific concentration limits, see "The use of cut-off values/concentration limits" in Chapter 1.3, para. 1.3.3.2.*
[5] *See 3.4.4.2.*
[6] *See 3.4.2.1.1 for details on use of Category 2 subcategories.*

Appendix E5

ST/SG/AC.10/C.4/2008/18 (Secretariat), Revision of Chapter 3.4 with Respect to Strong versus Weak Sensitizers

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COMMITTEE OF EXPERTS ON THE TRANSPORT OF DANGEROUS GOODS AND ON THE GLOBALLY HARMONIZED SYSTEM OF CLASSIFICATION AND LABELLING OF CHEMICALS

Sub-Committee of Experts on the Globally
Harmonized System of Classification
and Labelling of Chemicals

Sixteenth session
Geneva, 10 -12 (a.m) December 2008
Item 2 (b) of the provisional agenda

UPDATING OF THE SECOND REVISED EDITION OF THE GLOBALLY HARMONIZED SYSTEM OF CLASSIFICATION AND LABELLING OF CHEMICALS (GHS)

Health hazards

Revision of Chapter 3.4 with respect to strong versus weak sensitizers

Note by the secretariat

At the request of the Sub-Committee (ST/SG/AC.10/C.4/30, para. 33) the secretariat reproduces hereafter the proposal for amendment to Chapter 3.4 of the GHS, initially submitted by the Organization for the Economic Co-operation and Development (OECD) as information document UN/SECGHS/15/INF.13, and provisionally adopted by the Sub-Committee (with some editorial corrections) at its fifteenth session.

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ST/SG/AC.10/C.4/2008/18
page 2

Proposal

Chapter 3.4

3.4.2.1.1 Amend to read as follows:

"3.4.2.1.1 *Hazard categories*

3.4.2.1.1.1 Respiratory sensitizers shall be classified in Category 1 where sub-categorization is not required by a competent authority or where data are not sufficient for sub-categorization.

3.4.2.1.1.2 Where data are sufficient and where required by a competent authority, a refined evaluation allows the allocation of respiratory sensitizers into sub-category 1A, strong sensitizers, or sub-category 1B for other respiratory sensitizers.

3.4.2.1.1.3 Effects seen in either humans or animals will normally justify classification in a weight of evidence approach for respiratory sensitizers. Substances are allocated to one of the two sub-categories 1A or 1B using a weight of evidence approach in accordance with the criteria given in figure 3.4.1 and on the basis of reliable and good quality evidence from human cases or epidemiological studies and/or observations from appropriate studies in experimental animals.

Figure 3.4.1: Hazard category and sub-categories for respiratory sensitizers

CATEGORY 1:	**Respiratory sensitizer**
	A substance is classified as a respiratory sensitizer - if there is evidence in humans that the substance can lead to specific respiratory hypersensitivity and/or - if there are positive results from an appropriate animal test[2].
Sub-category 1A:	Substances showing a high frequency of occurrence in humans; or a probability of occurrence of a high sensitization rate in humans based on animal or other tests[2]. Severity of reaction may also be considered.
Sub-category 1B:	Substances showing a low to moderate frequency of occurrence in humans; or a probability of occurrence of a low to moderate sensitization rate in humans based on animal or other tests[2]. Severity of reaction may also be considered."

Add the following footnote 2:

"[2] At present recognized and validated animal models for the testing of respiratory hypersensitivity are not available. Under certain circumstances data from animal studies may provide valuable information in a weight of evidence assessment."

3.4.2.1.2.1 In the first sentence, replace "induce" with "lead to".

3.4.2.1.3 Amend the text of related footnote 2 to read as follows:

"At present recognized and validated animal models for the testing of respiratory hypersensitivity are not available. Under certain circumstances data from animal studies may provide valuable information in a weight of evidence assessment."

3.4.2.2.1 Amend to read as follows:

"3.4.2.2.1 *Hazard categories*

3.4.2.2.1.1 Skin sensitizers shall be classified in Category 1 where sub-categorization is not required by a competent authority or where data are not sufficient for sub-categorization.

3.4.2.2.1.2 Where data are sufficient and where required by a competent authority, a refined evaluation according to 3.4.2.2.1.3 allows the allocation of skin sensitizers into sub-category 1A, strong sensitizers, or sub-category 1B for other skin sensitizers.

3.4.2.2.1.3 Effects seen in either humans or animals will normally justify classification in a weight of evidence approach for skin sensitizers as described in 3.4.2.2.2. Substances may be allocated to one of the two sub-categories 1A or 1B using a weight of evidence approach in accordance with the criteria given in figure 3.4.2 and on the basis of reliable and good quality evidence from human cases or epidemiological studies and/or observations from appropriate studies in experimental animals according to the guidance values provided in 3.4.2.2.1.4 for sub-category 1A and in 3.4.2.2.1.5 for sub-category 1B.

Figure 3.4.2: Hazard category and sub-categories for skin sensitizers

CATEGORY 1:	**Skin sensitizer**
	A substance is classified as a skin sensitizer - if there is evidence in humans that the substance can lead to sensitization by skin contact in a substantial number of persons, or - if there are positive results from an appropriate animal test.
Sub-category 1A:	Substances showing a high frequency of occurrence in humans and/or a high potency in animals can be presumed to have the potential to produce significant sensitization in humans. Severity of reaction may also be considered.
Sub-category 1B:	Substances showing a low to moderate frequency of occurrence in humans and/or a low to moderate potency in animals can be presumed to have the potential to produce sensitization in humans. Severity of reaction may also be considered.

ST/SG/AC.10/C.4/2008/18
page 4

3.4.2.2.1.4 Human evidence for sub-category 1A can include:

(a) positive responses at ≤ 500 μg/cm^2 (HRIPT, HMT – induction threshold);

(b) diagnostic patch test data where there is a relatively high and substantial incidence of reactions in a defined population in relation to relatively low exposure;

(c) other epidemiology evidence where there is a relatively high and substantial incidence of allergic contact dermatitis in relation to relatively low exposure.

Animal test results for sub-category 1A can include data with values indicated in Table 3.4.1 below:

Table 3.4.1: Animal test results for sub-category 1A

Assay	Criteria
Local lymph node assay	EC3 value ≤ 2%
Guinea pig maximisation test	≥ 30% responding at ≤ 0.1% intradermal induction dose or ≥ 60% responding at > 0.1% to ≤ 1% intradermal induction dose
Buehler assay	≥15% responding at ≤ 0.2% topical induction dose or ≥ 60% responding at > 0.2% to ≤ 20% topical induction dose

3.4.2.2.1.5 Human evidence for sub-category 1B can include:

(a) positive responses at > 500 μg/cm^2 (HRIPT, HMT – induction threshold);

(b) diagnostic patch test data where there is a relatively low but substantial incidence of reactions in a defined population in relation to relatively high exposure;

(c) other epidemiology evidence where there is a relatively low but substantial incidence of allergic contact dermatitis in relation to relatively high exposure.

Animal test results for sub-category 1B can include data with values indicated in Table 3.4.2 below:

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Table 3.4.2: Animal test results for sub-category 1B

Assay	Criteria
Local lymph node assay	EC3 value > 2%
Guinea pig maximisation test	≥ 30% to < 60% responding at > 0.1% to ≤ 1% intradermal induction dose or ≥ 30% responding at > 1% intradermal induction dose
Buehler assay	≥15% to < 60% responding at > 0.2% to ≤ 20% topical induction dose or ≥ 15% responding at > 20% topical induction dose

3.4.2.2.2.1 Insert "using a weight of evidence approach" after "any or all of the following";
Add: "(f) Severity of reaction may also be considered" at the end of the paragraph.

3.4.2.2.2.2 Delete the first sentence.

Add the following sentence at the end of the paragraph:
"For both animal and human data, consideration should be given to the impact of vehicle."

3.4.2.2.2.3 Add a comma after "met" and replace "contact sensitizer" with "skin sensitizer" twice, in the second and third line.

3.4.2.2.3 Replace "contact sensitizer" with "skin sensitizer" twice, in the third and last line.

3.4.2.2.4.1 In the first line, replace "When an adjuvant type test method" with "For Category 1, when an adjuvant type test method";

After the second sentence, insert the following sentence:
"For Category 1, a stimulation index of 3 or more is considered a positive response in the local lymph node assay.".

Delete the last sentence.

3.4.2.2.4.2 Delete.

3.4.2.2.4.3 Delete.

3.4.3.1 Replace the last sentence with the following:
"(For special labelling required by some competent authorities, see Note 1 to Table 3.4.3 of this chapter and 3.4.4.2).

3.4.3.2.3 and 3.4.3.2.4 Insert the following paragraphs as new 3.4.3.2.3 and 3.4.3.2.4:

"3.4.3.2.3 Concentration of mixtures of the highest sensitizing category/sub-category

If a mixture is classified in Category 1 or sub-category 1A, and the concentration of ingredients of the mixture that are in Category 1 and sub-category 1A is increased, the new mixture should be classified in Category 1 or sub-category 1A without additional testing.

3.4.3.2.4 Interpolation within one category/sub-category

For three mixtures with identical ingredients, where A and B are in the same category/sub-category and mixture C has the same sensitizing ingredients with concentrations intermediate to the concentrations of those ingredients in mixtures A and B, then mixture C is assumed to be in the same category/sub-category as A and B."

Current paragraphs 3.4.3.2.3, 3.4.3.2.4 and 3.4.2.3.5 become new paragraphs 3.4.3.2.5, 3.4.3.2.6 and 3.4.3.2.7 respectively.

3.4.3.2.5 In the first sentence, replace "sensitization of the batch" with "sensitizing properties of the batch". In the last sentence, add "a" before "new classification".

3.4.3.3 In the paragraph, replace "Table 3.4.1" with "Table 3.4.3";

Replace the entire table and its six notes with a new table and a single note 1, as follows:

"Table 3.4.3: Cut-off values/concentration limits of ingredients of a mixture classified as either skin sensitizers or respiratory sensitizers that would trigger classification of the mixture

Ingredient classified as:	**Cut-off values/concentration limits triggering classification of a mixture as:**		
	Skin sensitizer Category 1	**Respiratory sensitizer Category 1**	
	All physical states	Solid/Liquid	Gas
Skin sensitizer Category 1	≥ 0.1% (Note 1)		
	≥ 1.0%		
Skin sensitizer Sub-category 1A	≥ 0.1%		
Skin sensitizer Sub-category 1B	≥ 1.0%		
Respiratory sensitizer Category 1		≥ 0.1% (Note 1)	≥ 0.1% (Note 1)
		≥ 1.0 %	≥ 0.2%
Respiratory sensitizer Sub-category 1A		≥ 0.1%	≥ 0.1%
Respiratory sensitizer Sub-category 1B		≥ 1.0 %	≥ 0.2%

***NOTE 1**: Some competent authorities may require SDS and/or supplemental labelling only, as described in 3.4.4.2 for mixtures containing a sensitizing ingredient at concentrations between 0.1 and 1% (or between 0.1 and 0.2% for a gaseous respiratory sensitizer). While the current cut-off values reflect existing systems, all recognize that special cases may require information to be conveyed below that level."*

3.4.4.1 In the last sentence, replace "Table 3.4.2" with "Table "3.4.4".

In the new Table 3.4.4, add "and Sub-categories 1A and 1B" after "Category 1" in the first row of the two last columns.

3.4.4.2 In the first sentence, replace "Table 3.4.1" with "Table 3.4.3".

Amend the second sentence to read as follows:
"To protect these individuals, certain authorities may choose to require the name of the ingredients as a supplemental label element whether or not the mixture as a whole is classified as sensitizer."

Delete the last sentence.

3.4.5.1 Add a footnote 6 to "Category 1" above the first exclamation mark, as follows:

"[6] *See 3.4.2.1.1 for details on use of Category 1 subcategories*"

In the last but one box on the left, delete the two references into brackets and insert at the bottom of the box: "(See 3.4.3.3 and Table 3.4.3 for explanation and guidance)".

3.4.5.2 Add a footnote 6 to "Category 1" above the first exclamation mark, as follows:

"[6] *See 3.4.2.2.1 for details on use of Category 1 subcategories*"

In the last but one box on the left, delete the reference into brackets and insert at the bottom of the box: "(See 3.4.3.3 and Table 3.4.3 for explanation and guidance)".

Consequential amendments to Annexes 1 and 2

Annex 1

Amend the tables for respiratory and skin sensitization (page 254 of the English version to read as follows:

For respiratory sensitization, add two columns similar to the first one, but replace "Category 1" with "Category 1A" in the second column and with "Category 1B" in the third column.

ST/SG/AC.10/C.4/2008/18
page 8

For skin sensitization, add two columns similar to the first one, but replace "Category 1" with "Category 1A" in the second column and with "Category 1B" in the third column.

Annex 2

A2.20 Replace the text under "1. *For substances and tested mixtures*" with:

"(a) if there is evidence in humans that the substance can lead to specific respiratory hypersensitivity and /or

(b) if there are positive results from an appropriate animal test."

Add the following columns and rows:

Hazard sub-category	Criteria	Hazard communication elements	
1A (where data are sufficient and where required by a competent authority)	1. *For substances and tested mixtures* showing a high frequency of occurrence in humans; or a probability of occurrence of a high sensitization rate in humans based on animal or other tests. Severity of reaction may also be considered. 2. *If data for the complete mixture are not available*, apply bridging principles (see 3.4.3.2). 3. *If bridging principles do not apply*, classify the mixture as respiratory sensitizer if it contains at least one ingredient classified as sub-category 1A at the following concentrations: (a) Solids or liquids: ≥ 0.1% w/w (b) Gases: ≥ 0.1% v/v	Symbol	
		Signal word	Danger
		Hazard statement	May cause allergic or asthma symptoms or breathing difficulties if inhaled
1B (where data are sufficient and where required by a competent authority)	1. *For substances and tested mixtures* showing a low to moderate frequency of occurrence in humans; or a probability of occurrence of a low to moderate sensitization rate in humans based on animal or other tests. Severity of reaction may also be considered. 2. *If data for the complete mixture are not available*, apply bridging principles (see 3.4.3.2). 3. *If bridging principles do not apply*, classify the mixture as respiratory sensitizer if it contains at least one ingredient classified as sub-category 1B at the following concentrations: (a) Solids or liquids: ≥ 1% w/w (b) Gases: ≥ 0.2% v/v	Symbol	
		Signal word	Danger
		Hazard statement	May cause allergic or asthma symptoms or breathing difficulties if inhaled

A2.21 Replace the text under "1. *For substances and tested mixtures*" with:

(a) if there is evidence in humans that the substance can lead to sensitization by skin contact in a substantial number of persons, or

(b) if there are positive results from an appropriate animal test.

Add the following columns and rows:

Hazard category	**Criteria**	**Hazard communication elements**	
1A (where data are sufficient and where required by a competent authority)	1. *For substances and tested mixtures* showing a high frequency of occurrence in humans and/or a high potency in animals, which can be presumed to have the potential to produce significant sensitization in humans. Severity of reaction may also be considered. 2. *If data for the complete mixture are not available*, apply bridging principles (see 3.4.3.2) 3. *If bridging principles do not apply*, classify the mixture as skin sensitizer if it contains at least one ingredient classified as sub-category 1A at a concentration ≥ 0.1%.	Symbol	!
		Signal word	Warning
		Hazard statement	May cause allergic skin reaction
1B (where data are sufficient and where required by a competent authority)	1. *For substances and tested mixtures* showing a low to moderate frequency of occurrence in humans and/or a low to moderate potency in animals, which can be presumed to have the potential to produce sensitization in humans. 2. *If data for the complete mixture are not available*, apply bridging principles (see 3.4.3.2) 3. *If bridging principles do not apply*, classify the mixture as skin sensitizer if it contains at least one ingredient classified as sub-category 1B at a concentration ≥ 1.0%.	Symbol	!
		Signal word	Warning
		Hazard statement	May cause allergic skin reaction

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Appendix E6

ST/SG/AC.10/C.4/2008/18/Add.1 (Secretariat), Revision of Chapter 3.4 with Respect to Strong versus Weak Sensitizers, Addendum

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Sub-Committee of Experts on the Globally Harmonized System of Classification and Labelling of Chemicals

Sixteenth session,
Geneva, 10 -12 (a.m) December 2008
Item 2 (b) of the provisional agenda

UPDATING OF THE SECOND REVISED EDITION OF THE GLOBALLY HARMONIZED SYSTEM OF CLASSIFICATION AND LABELLING OF CHEMICALS (GHS)

Health hazards

Revision of Chapter 3.4 with respect to strong versus weak sensitizers

Addendum

Note by the secretariat

1. At the request of the Sub-Committee (ST/SG/AC.10/C.4/30, para. 33) the secretariat reproduced in document ST/SG/AC.10/C.4/2008/18 a proposal for amendment of Chapter 3.4 of the GHS provisionally adopted at the 15th session. The secretariat has noted some inconsistencies in these proposed amendments and therefore proposes corrections and additional amendments in annexes I, II and III to this document, as follows:

- Annex I: Corrections to the list of amendments in document ST/SG/AC.10/C.4/2008/18;
- Annex II: Proposed additional amendments to the text of Chapter 3.4 and consequential amendments to Annex 3; and
- Annex III: Draft corrections to the text of current Chapter 3.4 and Annexes 1, 2 and 3 to the GHS (to be included in a corrigendum to the second revised edition of the GHS)

2. The Sub-Committee may wish to consider the proposed corrections and consequential amendments listed hereafter.

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ST/SG/AC.10/C.4/2008/18/Add.1
Annex I
page 2

Annex I

Corrections to the list of amendments in ST/SG/AC.10/C.4/2008/18

3.4.2.2.1.4 (c) and 3.4.2.2.1.5 (c) Replace "epidemiology evidence" with "epidemiological evidence"

3.4.2.2.2.2 Add the following amendment:

In the fourth sentence, replace "contact sensitization" with "skin sensitization".

3.4.2.2.2.3 The amendment should read:

In the first sentence, insert a comma after "met" and replace "contact sensitizer" with "skin sensitizer". In the second sentence, replace "contact sensitization" with "skin sensitization".

3.4.3.3 In the table, replace "Note 1" with "see note" and in the note, replace "NOTE 1" with "NOTE".

Note by the secretariat: In the table, the rows and columns related to respiratory sensitization should appear before those related to skin sensitization, to be consistent with the order followed through the whole chapter, where the criteria and decision logics for respiratory sensitization are always listed before that related to skin sensitization.

3.4.5.1 The first amendment should read:

"Add a reference to a new footnote 6 in the first box from the top for "Category 1", as follows: "Category 1[6]"

Add a new footnote "6" to read as follows: "[6] *See 3.4.2.1.1 for details on use of Category 1 sub-categories*"

(Justification: The reference to the exclamation mark symbol for respiratory sensitizers is wrong)

3.4.5.2 The amendment should read:

"Add a reference to a new footnote 7 in the first box from the top for "Category 1", as follows: "Category 1[7]"

Add a new footnote "7" to read as follows: "[7] *See 3.4.2.2.1 for details on use of Category 1 sub-categories*"

(Justification: It cannot be footnote 6 as initially proposed since the text of the footnote is not the same).

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Annex 2

A2.20 The amendment starting with "Replace the text" should read:

For Category 1:

- In paragraph 1 (a), replace "human evidence that the individual substance leads" with "evidence in humans that the substance can lead";
- In paragraph 1 (b), replace "Where" with "If";
- In paragraphs 3 (a) (i) and (ii) and 3 (b) (i) and (ii), delete figures "3" ,"4", "5" and "6" after "note" in the text between brackets.

(Justification: notes 3, 4 ,5 and 6 have been deleted)

A2.21 The amendment starting with "Replace the text" should read:

For Category 1:

- In paragraph 1 (b), replace "Where" with "If"
- In paragraphs 3 (a) and (b) delete figures "1" and "2" after "note" in the text between brackets.

(Justification: notes 1 and 2 have been deleted)

Note by the secretariat: For comments regarding the hazard statements see Annex III to this document.

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ST/SG/AC.10/C.4/2008/18/Add.1
Annex II
page 4

Annex II

Proposed additional amendments to the text of Chapter 3.4 and consequential amendments to Annex 3

3.4.2.1.1.2 Insert "according to 3.4.2.1.1.3" after "a refined evaluation"

(Justification: Consistency with the equivalent paragraph for skin sensitizers, where a reference to the paragraph where the evaluation criteria can be found has been included)

Figures 3.4.1 and 3.4.2

Rename as "Table 3.4.1" and "Table 3.4.2" and renumber subsequent tables accordingly.

Annex 3

Section 1: In table A3.1.2, for codes H317 and H334, replace "1" with "1, 1A, 1B" in column (4).

Section 2: In table A3.2.2, replace "1" with "1, 1A, 1B" in column (4) against each reference to chapter 3.4 in column (3). (Apply to codes P261, P272, P280 and P285)

In table A3.2.3, replace "1" with "1, 1A, 1B" in column (4) against each reference to chapter 3.4 in column (3). (Apply to codes P302, P304, P311, P313, P321, P333, P341, P342, P352, P363, P302+P352, P304+P341, P333+P313 and P342+P311).

In table A3.2.5, replace "1" with "1, 1A, 1B" in column (4) against each reference to chapter 3.4 in column (3).

Section 3: In the matrix for respiratory and skin sensitization (pages 379 and 380 of the English version of the GHS), under "hazard category" replace "1" with "1, 1A, 1B".

Structure of new section 3.4.2.2 for skin sensitizers

The secretariat would like to bring the attention of the Sub-Committee to the fact that the structure of sections 3.4.2.1 and 3.4.2.2, as amended in accordance with document ST/SG/AC.10/C.4/2008/18, is not consistent (see list hereafter):

Section 3.4.2.1 for respiratory sensitizers

3.4.2.1 Respiratory sensitizers
- 3.4.2.1.1 Hazard categories
- 3.4.2.1.2 Human evidence
- 3.4.2.1.3 Animal studies

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Section 3.4.2.2 for skin sensitizers (as amended by ST/SG/AC.10/C.4/2008/18)

3.4.2.2 Skin sensitizers
- 3.4.2.2.1 Hazard categories
  - 3.4.2.2.1.4 Human evidence for 1A (includes also animal evidence under the same heading)
  - 3.4.2.2.1.5 Human evidence for 1B (includes also animal evidence under the same heading)
- 3.4.2.2.2 Specific considerations
- 3.4.2.2.3 Immunological contact urticaria
- 3.4.2.2.4 Animal studies (applicable only to animal evidence for Category 1 skin sensitizers)

In order to rationalize the structure of section 3.4.2.2, the secretariat suggests reorganizing the paragraphs regarding the classification criteria for skin sensitizers, as follows:

3.4.2.2 Skin sensitizers

3.4.2.2.1 Hazard categories (includes paragraphs 3.4.2.2.1.1 to 3.4.2.2.1.3)

3.4.2.2.2 (new) Human evidence
(new heading)

3.4.2.2.2.1 (new) (former 3.4.2.2.1.4) Human evidence for sub-category 1A (including subparagraphs (a) to (c) but excluding the last sentence applicable to animal evidence and table 3.4.1)

3.4.2.2.2.2 (new) (former 3.4.2.2.1.5) Human evidence for sub-category 1B (including subparagraphs (a) to (c) but excluding the last sentence applicable to animal evidence and table 3.4.2)

3.4.2.2.3 (new) Animal studies
(heading from paragraph 3.4.2.2.4 of the GHS, renumbered)

3.4.2.2.3.1 (new) Evidence from animal studies for Category 1
(Text from paragraph 3.4.2.2.4.1 of the GHS, renumbered)

3.4.2.2.3.2 (new) Evidence from animal studies for 1A
(includes last sentence from former 3.4.2.2.1.4 and table 3.4.1)

3.4.2.2.3.3 (new) Evidence from animal studies for 1B
(includes last sentence from former 3.4.2.2.1.5 and table 3.4.2)

3.4.2.2.4 (new) Specific considerations
(includes heading and text from sub-sections 3.4.2.2.2 and 3.4.2.2.3 (Immunological urticaria) of the GHS, renumbered)

The text of section 3.4.2.2, as amended in accordance with document ST/SG/AC.10/C4/2008/18, and reorganized as explained above, may be found in information document UN/SCEGHS/16/INF.3.

Annex III

Draft corrections to the current text of Chapter 3.4 and Annexes 1, 2 and 3 (to be included in a corrigendum to the second revised edition of the GHS)

3.4.1.4 Replace "at section 3.4.4" with "in 3.4.4.2".

3.4.5.2 In decision logic 3.4.2 for skin sensitization, in the sentence "Does the mixture as a whole have respiratory sensitization data? (see 3.4.3.1)" replace "respiratory" with "skin".

Annex 2

A2.20 and A2.21 The secretariat notes that there are currently two different wordings for the same hazard statements relating to respiratory and skin sensitization.

It is also noted that the proposal in document ST/SG/AC.10/C.4/2008/18 introduces a third wording for respiratory sensitization (see table below).

	Respiratory sensitization	Skin sensitization
Chapter 3.4, Annex 1 and Annex 3 (codes H334 and H317)	May cause allergy or asthma symptoms or breathing difficulties if inhaled	May cause an allergic skin reaction
Annex 2 (A2.20 and A2.21)	May cause allergic or asthmatic symptoms or breathing difficulties if inhaled	May cause allergic skin reaction
As proposed in – C4/2008/18	May cause allergic or asthma symptoms or breathing difficulties if inhaled	May cause allergic skin reaction

The Sub-Committee is invited to specify the appropriate wording to be used for respiratory and skin sensitization hazard statements.

Appendix E7

UN/SCEGHS/16/INF.3 (Secretariat), Section 3.4.2 of Chapter 3.4

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UN/SCEGHS/16/INF.3

COMMITTEE OF EXPERTS ON THE TRANSPORT OF DANGEROUS GOODS AND ON THE GLOBALLY HARMONIZED SYSTEM OF CLASSIFICATION AND LABELLING OF CHEMICALS

Sub-Committee of Experts on the Globally Harmonized System of Classification and Labelling of Chemicals

Sixteenth session
Geneva, 10 -12 (a.m) December 2008
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UPDATING OF THE SECOND REVISED EDITION OF THE GLOBALLY HARMONIZED SYSTEM OF CLASSIFICATION AND LABELLING OF CHEMICALS (GHS)

Health hazards

Section 3.4.2 of Chapter 3.4
(refer to documents ST/SG/AC.10/C.4/2008/18 and -2008/18/Add.1)

Note by the secretariat

This document contains the text of section 3.4.2 of Chapter 3.4 as amended by ST/SG/AC.10/C.4/2008/18 and rearranged as proposed in ST/SG/AC.10/C.4/2008/18/Add.1, Annex II.

The corrections and additional amendments to section 3.4.2, proposed in ST/SG/AC.10/C.4/2008/18/Add.1 are shown in visible mode, between square brackets.

ED_006472_00084346-00507

UN/SCEGHS/16/INF.3
page 2

"3.4.2 Classification criteria for substances

3.4.2.1 *Respiratory sensitizers*

3.4.2.1.1 *Hazard categories*

3.4.2.1.1.1 Respiratory sensitizers shall be classified in Category 1 where sub-categorization is not required by a competent authority or where data are not sufficient for sub-categorization.

3.4.2.1.1.2 Where data are sufficient and where required by a competent authority, a refined evaluation [according to 3.4.2.1.1.3] allows the allocation of respiratory sensitizers into sub-category 1A, strong sensitizers, or sub-category 1B for other respiratory sensitizers.

3.4.2.1.1.3 Effects seen in either humans or animals will normally justify classification in a weight of evidence approach for respiratory sensitizers. Substances are allocated to one of the two sub-categories 1A or 1B using a weight of evidence approach in accordance with the criteria given in [~~figure~~ Table 3.4.1] and on the basis of reliable and good quality evidence from human cases or epidemiological studies and/or observations from appropriate studies in experimental animals.

[Table~~Figure~~ 3.4.1]: Hazard category and sub-categories for respiratory sensitizers

CATEGORY 1:	Respiratory sensitizer
	A substance is classified as a respiratory sensitizer: - if there is evidence in humans that the substance can lead to specific respiratory hypersensitivity and/or - if there are positive results from an appropriate animal test[2].
Sub-category 1A:	Substances showing a high frequency of occurrence in humans; or a probability of occurrence of a high sensitization rate in humans based on animal or other tests[2]. Severity of reaction may also be considered.
Sub-category 1B:	Substances showing a low to moderate frequency of occurrence in humans; or a probability of occurrence of a low to moderate sensitization rate in humans based on animal or other tests[2]. Severity of reaction may also be considered.

3.4.2.1.2 *Human evidence*

3.4.2.1.2.1 Evidence that a substance can lead to specific respiratory hypersensitivity will normally be based on human experience. In this context, hypersensitivity is normally seen as asthma, but other hypersensitivity reactions such as rhinitis/conjunctivitis and alveolitis are also considered. The condition will have the clinical character of an allergic reaction. However, immunological mechanisms do not have to be demonstrated.

[2] *At present recognized and validated animal models for the testing of respiratory hypersensitivity are not available. Under certain circumstances, data from animal studies may provide valuable information in a weight of evidence assessment.*

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3.4.2.1.2.2 When considering the human evidence, it is necessary for a decision on classification to take into account, in addition to the evidence from the cases:

(a) the size of the population exposed;

(b) the extent of exposure.

3.4.2.1.2.3 The evidence referred to above could be:

(a) clinical history and data from appropriate lung function tests related to exposure to the substance, confirmed by other supportive evidence which may include:

(i) *in vivo* immunological test (e.g. skin prick test);

(ii) *in vitro* immunological test (e.g. serological analysis);

(iii) studies that may indicate other specific hypersensitivity reactions where immunological mechanisms of action have not been proven, e.g. repeated low-level irritation, pharmacologically mediated effects;

(iv) a chemical structure related to substances known to cause respiratory hypersensitivity;

(b) data from positive bronchial challenge tests with the substance conducted according to accepted guidelines for the determination of a specific hypersensitivity reaction.

3.4.2.1.2.4 Clinical history should include both medical and occupational history to determine a relationship between exposure to a specific substance and development of respiratory hypersensitivity. Relevant information includes aggravating factors both in the home and workplace, the onset and progress of the disease, family history and medical history of the patient in question. The medical history should also include a note of other allergic or airway disorders from childhood, and smoking history.

3.4.2.1.2.5 The results of positive bronchial challenge tests are considered to provide sufficient evidence for classification on their own. It is however recognized that in practice many of the examinations listed above will already have been carried out.

3.4.2.1.3 *Animal studies*

Data from appropriate animal studies[2] which may be indicative of the potential of a substance to cause sensitization by inhalation in humans[3] may include:

(a) measurements of Immunoglobulin E (IgE) and other specific immunological parameters, for example in mice;

(b) specific pulmonary responses in guinea pigs.

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[2] *At present recognized and validated animal models for the testing of respiratory hypersensitivity are not available. Under certain circumstances, data from animal studies may provide valuable information in a weight of evidence assessment.*

[3] *The mechanisms by which substances induce symptoms of asthma are not yet fully known. For preventative measures, these substances are considered respiratory sensitizers. However, if on the basis of the evidence, it can be demonstrated that these substances induce symptoms of asthma by irritation only in people with bronchial hyperreactivity, they should not be considered as respiratory sensitizers.*

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3.4.2.2 ***Skin sensitizers***

3.4.2.2.1 *Hazard categories*

3.4.2.2.1.1 Skin sensitizers shall be classified in Category 1 where sub-categorization is not required by a competent authority or where data are not sufficient for sub-categorization.

3.4.2.2.1.2 Where data are sufficient and where required by a competent authority, a refined evaluation according to 3.4.2.2.1.3 allows the allocation of skin sensitizers into sub-category 1A, strong sensitizers, or sub-category 1B for other skin sensitizers.

3.4.2.2.1.3 Effects seen in either humans or animals will normally justify classification in a weight of evidence approach for skin sensitizers as described in [3.4.2.2.~~2~~4]. Substances may be allocated to one of the two sub-categories 1A or 1B using a weight of evidence approach in accordance with the criteria given in ~~figure~~Table 3.4.2 and on the basis of reliable and good quality evidence from human cases or epidemiological studies and/or observations from appropriate studies in experimental animals according to the guidance values provided in [3.4.2.2.~~1.4~~2.1 and 3.4.2.2.3.2] for sub-category 1A and in [3.4.2.2.~~1.~~2.2~~5~~ and 3.4.2.2.3.3] for sub-category 1B.

[Table~~Figure~~ 3.4.2]: Hazard category and sub-categories for skin sensitizers

CATEGORY 1:	**Skin sensitizer**
	A substance is classified as a skin sensitizer - if there is evidence in humans that the substance can lead to sensitization by skin contact in a substantial number of persons, or - if there are positive results from an appropriate animal test.
Sub-category 1A:	Substances showing a high frequency of occurrence in humans and/or a high potency in animals can be presumed to have the potential to produce significant sensitization in humans. Severity of reaction may also be considered.
Sub-category 1B:	Substances showing a low to moderate frequency of occurrence in humans and/or a low to moderate potency in animals can be presumed to have the potential to produce sensitization in humans. Severity of reaction may also be considered.

[3.4.2.2.~~1.~~42 *Human evidence*]

[3.4.2.2.2.1] Human evidence for sub-category 1A can include:

(a) positive responses at ≤ 500 µg/cm^2 (HRIPT, HMT – induction threshold);

(b) diagnostic patch test data where there is a relatively high and substantial incidence of reactions in a defined population in relation to relatively low exposure;

(c) other ~~epidemiology~~ epidemiological evidence where there is a relatively high and substantial incidence of allergic contact dermatitis in relation to relatively low exposure.

[3.4.2.2.4~~.5~~2.2] Human evidence for sub-category 1B can include:

(a) positive responses at > 500 µg/cm^2 (HRIPT, HMT – induction threshold);

(b) diagnostic patch test data where there is a relatively low but substantial incidence of reactions in a defined population in relation to relatively high exposure;

(c) other ~~epidemiology~~ epidemiological evidence where there is a relatively low but substantial incidence of allergic contact dermatitis in relation to relatively high exposure.

[3.4.2.2.43] *Animal studies*

[3.4.2.2.43.1] For Category 1, when an adjuvant type test method for skin sensitization is used, a response of at least 30% of the animals is considered as positive. For a non-adjuvant Guinea pig test method a response of at least 15% of the animals is considered positive. For Category 1, a stimulation index of 3 or more is considered a positive response in the Local Nymph Node Assay. Test methods for skin sensitization are described in the OECD Guideline 406 (the Guinea Pig Maximisation test and the Buehler guinea pig test) and Guideline 429 (Local Lymph Node Assay). Other methods may be used provided that they are well-validated and scientific justification is given. The Mouse Ear Swelling Test (MEST), appears to be a reliable screening test to detect moderate to strong sensitizers, and can be used as a first stage in the assessment of skin sensitization potential.

[3.4.2.2.3.2] Animal test results for sub-category 1A can include data with values indicated in [Table 3.4.~~1~~3] below:

[Table 3.4.~~1~~3]: Animal test results for sub-category 1A

Assay	Criteria
Local lymph node assay	EC3 value ≤ 2%
Guinea pig maximisation test	≥ 30% responding at ≤ 0.1% intradermal induction dose or ≥ 60% responding at > 0.1% to ≤ 1% intradermal induction dose
Buehler assay	≥15% responding at ≤ 0.2% topical induction dose or ≥ 60% responding at > 0.2% to ≤ 20% topical induction dose

[3.4.2.2.3.3] Animal test results for sub-category 1B can include data with values indicated in [Table 3.4.~~2~~4] below:

Table [3.4.~~2~~4]: Animal test results for sub-category 1B

Assay	Criteria
Local lymph node assay	EC3 value > 2%
Guinea pig maximisation test	≥ 30% to < 60% responding at > 0.1% to ≤ 1% intradermal induction dose or ≥ 30% responding at > 1% intradermal induction dose
Buehler assay	≥15% to < 60% responding at > 0.2% to ≤ 20% topical induction dose or ≥ 15% responding at > 20% topical induction dose"

UN/SCEGHS/16/INF.3
page 6

[3.4.2.2.~~2~~4] *Specific considerations*

[3.4.2.2.~~2~~4.1] For classification of a substance, evidence should include any or all of the following, using a weight of evidence approach:

(a) Positive data from patch testing, normally obtained in more than one dermatology clinic;

(b) Epidemiological studies showing allergic contact dermatitis caused by the substance; Situations in which a high proportion of those exposed exhibit characteristic symptoms are to be looked at with special concern, even if the number of cases is small;

(c) Positive data from appropriate animal studies;

(d) Positive data from experimental studies in man (see Chapter 1.3, para. 1.3.2.4.7);

(e) Well documented episodes of allergic contact dermatitis, normally obtained in more than one dermatology clinic;

(f) Severity of reaction may also be considered

[3.4.2.2.~~2~~4.2] Evidence from animal studies is usually much more reliable than evidence from human exposure. However, in cases where evidence is available from both sources, and there is conflict between the results, the quality and reliability of the evidence from both sources must be assessed in order to resolve the question of classification on a case-by-case basis. Normally, human data are not generated in controlled experiments with volunteers for the purpose of hazard classification but rather as part of risk assessment to confirm lack of effects seen in animal tests. Consequently, positive human data on ~~contact~~ [skin] sensitization are usually derived from case-control or other, less defined studies. Evaluation of human data must therefore be carried out with caution as the frequency of cases reflect, in addition to the inherent properties of the substances, factors such as the exposure situation, bioavailability, individual predisposition and preventive measures taken. Negative human data should not normally be used to negate positive results from animal studies. For both animal and human data, consideration should be given to the impact of vehicle.

[3.4.2.2.~~2~~4.3] If none of the above mentioned conditions are met, the substance need not be classified as a skin sensitizer. However, a combination of two or more indicators of skin sensitization as listed below may alter the decision. This shall be considered on a case-by-case basis.

(a) Isolated episodes of allergic contact dermatitis;

(b) Epidemiological studies of limited power, e.g. where chance, bias or confounders have not been ruled out fully with reasonable confidence;

(c) Data from animal tests, performed according to existing guidelines, which do not meet the criteria for a positive result described in [3.4.2.2.4~~3~~.1], but which are sufficiently close to the limit to be considered significant;

(d) Positive data from non-standard methods;

(e) Positive results from close structural analogues.

[3.4.2.2.~~3~~4.4] *Immunological contact urticaria*

Substances meeting the criteria for classification as respiratory sensitizers may in addition cause immunological contact urticaria. Consideration should be given to classifying these substances also as skin sensitizers. Substances which cause immunological contact urticaria without meeting the criteria for respiratory sensitizers should also be considered for classification as skin sensitizers.

There is no recognized animal model available to identify substances which cause immunological contact urticaria. Therefore, classification will normally be based on human evidence which will be similar to that for skin sensitization."

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Appendix F

Federal Register Notices and Public Comments

F1 *Federal Register* Notices F-3
F2 Public Comments Received in Response to *Federal Register* Notices F-17
F3 SACATM Comments: SACATM Meeting on June 18-19, 2008 ..... F-93

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Appendix F1

Federal Register Notices

72 FR 27815 (May 17, 2007)
The Murine Local Lymph Node Assay: Request for Comments, Nominations of Scientific Experts, and Submission of Data F-5

72 FR 52130 (September 12, 2007)
Draft Performance Standards for the Murine Local Lymph Node Assay: Request for Comments F-8

73 FR 1360 (January 8, 2008)
Announcement of an Independent Scientific Peer Review Panel Meeting on the Murine Local Lymph Node Assay; Availability of Draft Background Review Documents; Request for Comments F-10

73 FR 25754 (May 7, 2008)
Meeting of the Scientific Advisory Committee on Alternative Toxicological Methods (SACATM) F-13

73 FR 29136 (May 20, 2008)
Peer Review Panel Report on the Validation Status of New Versions and Applications of the Murine Local Lymph Node Assay (LLNA): A Test Method for Assessing the Allergic Contact Dermatitis Potential of Chemicals and Products: Notice of Availability and Request for Public Comments F-15

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Federal Register / Vol. 72, No. 95 / Thursday, May 17, 2007 / Notices 27815

DEPARTMENT OF HEALTH AND HUMAN SERVICES

National Toxicology Program (NTP) Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM); the Murine Local Lymph Node Assay: Request for Comments, Nominations of Scientific Experts, and Submission of Data

AGENCY: National Institute of Environmental Health Sciences (NIEHS), National Institutes of Health (NIH).

ACTION: Request for comments, submission of relevant data, and nominations of scientific experts.

SUMMARY: The Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) received a nomination from the U.S. Consumer Product Safety Commission (CPSC) to evaluate the validation status of: (1) The murine local lymph node assay (LLNA) as a stand-alone assay for determining potency (including severity) for the purpose of hazard classification; (2) the "cut-down" or "limit dose" LLNA approach; (3) non-radiolabeled LLNA methods; (4) the use of the LLNA for testing mixtures, aqueous solutions, and metals; and (5) the current applicability domain (i.e., the types of chemicals and substances for which the LLNA has been validated). ICCVAM reviewed the nomination, assigned it a high priority, and proposed that NICEATM and ICCVAM carry out the following activities in its evaluation: (1) Initiate a review of the current literature and available data, including the preparation of a comprehensive background review document, and (2) convene a peer review panel to review the various proposed LLNA uses and procedures for which sufficient data and information are available to adequately assess their validation status. ICCVAM also recommends development of performance standards for the LLNA. At this time, NICEATM requests: (1) Public comments on the appropriateness and relative priority of these activities, (2) nominations of expert scientists to consider as members of a possible peer review panel, and (3) submission of data for the LLNA and/or modified versions of the LLNA.

DATES: Submit comments, data, and nominations by June 15, 2007. Relevant data will also be accepted after this date and considered when feasible.

ADDRESSES: Dr. William S. Stokes, NICEATM Director, NIEHS, P.O. Box 12233, MD EC–17, Research Triangle Park, NC 27709, (fax) 919–541–0947, (e-mail) *niceatm@niehs.nih.gov.* Courier address: NICEATM, 79 T.W. Alexander Drive, Building 4401, Room 3128, Research Triangle Park, NC 27709. Responses can be submitted electronically at the ICCVAM–NICEATM Web site: *http://iccvam.niehs.nih.gov/contact/FR_pubcomment.htm* or by e-mail, mail, or fax.

FOR FURTHER INFORMATION CONTACT: Other correspondence should be

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directed to Dr. William S. Stokes (919–541–2384 or *niceatm@niehs.nih.gov*).

SUPPLEMENTARY INFORMATION:

Background

ICCVAM previously evaluated the validation status of the LLNA as a stand-alone alternative method to the Guinea Pig Maximization Test (GPMT) and the Buehler Assay (NIH publication No. 99–4494; available at *http://iccvam.niehs.nih.gov/methods/immunotox/llna.htm*). Based on this evaluation, ICCVAM recommended the LLNA as a valid substitute for the guinea pig methods for most testing situations. The Environmental Protection Agency, Food and Drug Administration, and the CPSC subsequently accepted the method as a valid substitute. The OECD also adopted the LLNA as OECD Test Guideline 429.

In January 2007, the CPSC submitted a nomination to NICEATM (*http://iccvam.niehs.nih.gov/SuppDocs/submission.htm*) requesting that ICCVAM assess the validation status of:

- The LLNA as a stand-alone test for potency determinations (including severity) for the purpose of hazard classification.
- LLNA protocols that do not require the use of radioactive materials.
- The LLNA "cut-down" or "limit dose" procedure.
- The ability of the LLNA to test mixtures, aqueous solutions, and metals.
- The current applicability domain (i.e., the types of chemicals and substances for which the LLNA has been determined to be useful).

Since 2003, ICCVAM has routinely developed performance standards for test methods; however, they were not developed for the LLNA, which was reviewed in 1999. Accordingly, ICCVAM proposes to now develop performance standards for the LLNA. Performance standards communicate the basis by which new proprietary and nonproprietary test methods have been determined to have sufficient relevance and reliability for specific testing purposes. Performance standards based on test methods accepted by regulatory agencies can be used to evaluate the reliability and relevance of other test methods that are based on similar scientific principles and measure or predict the same biological or toxic effect. On January 24, 2007, ICCVAM unanimously endorsed with a high priority: (1) Developing performance standards for the LLNA and (2) initiating a review of the available data and information associated with the CPSC nominated activities. A determination of which (if any) of the nominated activities will move forward will be made subsequent to this review and after consideration of comments by the public and the Scientific Advisory Committee on Alternative Toxicological Methods (SACATM). If a decision is made to proceed with evaluation of these test methods, ICCVAM and NICEATM propose convening a peer review panel to review the usefulness and limitations of each of the LLNA methods listed above. The panel would also formulate conclusions on the adequacy of draft ICCVAM performance standards, any proposed future validation studies, and draft ICCVAM-proposed standardized test method protocols.

Request for Public Comments and Nominations of Scientific Experts

NICEATM requests public comments on the appropriateness and relative priority of the nominated activities. NICEATM also requests the nominations of scientists with relevant knowledge and experience to serve on the panel if a panel meeting occurs. Areas of relevant expertise include, but are not limited to: physiology, pharmacology, immunology, skin sensitization testing in animals, development and use of in vitro methodologies, biostatistics, knowledge about the use of chemical datasets for validation of toxicity studies, and hazard classification of chemicals and products. Each nomination should include the person's name, affiliation, contact information (i.e., mailing address, e-mail address, telephone and fax numbers), curriculum vitae, and a brief summary of relevant experience and qualifications.

Request for Data

NICEATM invites the submission of data from standard LLNA testing (i.e., OECD TG 429) with mixtures, aqueous solutions, and/or metals, as well as corresponding data from human and other animal studies. In addition, NICEATM invites the submission of data supporting the use of (1) the LLNA as a stand-alone test for determining potency (including severity) for the purpose of hazard classification, (2) the LLNA "cut-down" or "limit dose" procedure, and (3) LLNA protocols that do not require the use of radioactivity. Although data can be accepted at any time, data submitted by June 15, 2007, will be considered during the ICCVAM evaluation process. Submitted data will be used to further evaluate the usefulness and limitations of the LLNA and may be incorporated into future NICEATM and ICCVAM reports and publications as appropriate. The data will also be included in a database to support the investigation of other test methods for assessing skin sensitization.

When submitting chemical and protocol information/test data, please reference this **Federal Register** notice and provide appropriate contact information (name, affiliation, mailing address, phone, fax, e-mail, and sponsoring organization, as applicable).

NICEATM prefers data to be submitted as copies of pages from study notebooks and/or study reports, if available. Raw data and analyses available in electronic format may also be submitted. Each submission for a chemical should preferably include the following information, as appropriate:

- Common and trade name.
- Chemical Abstracts Service Registry Number (CASRN).
- Chemical class.
- Product class.
- Commercial source.
- LLNA protocol used.
- Individual animal responses.
- The extent to which the study complied with national or international Good Laboratory Practice (GLP) guidelines.
- Date and testing organization.
- Sensitization data from other test methods.

Consideration by SACATM

On June 12, 2007, SACATM will meet at the Marriott Bethesda North Hotel and Conference Center in Bethesda, Maryland. The agenda includes consideration of the nominated LLNA activities, priorities, and proposed activities *http://ntp.niehs.nih.gov/go/7441*) and an opportunity for oral public comments. The SACATM meeting was announced in a separate **Federal Register** notice (**Federal Register** Vol. 72, No. 83, pp. 23831–32, May 1, 2007).

Background Information on ICCVAM and NICEATM

ICCVAM is an interagency committee composed of representatives from 15 federal regulatory and research agencies that use or generate toxicological information. ICCVAM conducts technical evaluations of new, revised, and alternative methods with regulatory applicability and promotes the scientific validation and regulatory acceptance of toxicological test methods that more accurately assess the safety and hazards of chemicals and products and that refine, reduce, or replace animal use. The ICCVAM Authorization Act of 2000 (42 U.S.C. 285l–3, available at *http://iccvam.niehs.nih.gov/about/PL106545.htm*) establishes ICCVAM as a permanent interagency committee of the

NIEHS under NICEATM. NICEATM administers ICCVAM and provides scientific and operational support for ICCVAM-related activities. NICEATM and ICCVAM work collaboratively to evaluate new and improved test methods applicable to the needs of federal agencies. Additional information about ICCVAM and NICEATM is available on the following Web site: *http://iccvam.niehs.nih.gov.*

Dated: May 8, 2007.

David A. Schwartz,

Director, National Institute of Environmental Health Sciences and National Toxicology Program.

[FR Doc. E7–9544 Filed 5–16–07; 8:45 am]

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52130 Federal Register / Vol. 72, No. 176 / Wednesday, September 12, 2007 / Notices

DEPARTMENT OF HEALTH AND HUMAN SERVICES

National Toxicology Program (NTP) Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM); Draft Performance Standards for the Murine Local Lymph Node Assay: Request for Comments

AGENCY: National Institute of Environmental Health Sciences (NIEHS), National Institutes of Health (NIH).

ACTION: Request for comments.

SUMMARY: The Murine Local Lymph Node Assay (LLNA) is the first alternative test method evaluated and recommended by the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM). It was subsequently accepted by regulatory authorities to determine the allergic contact dermatitis potential of chemicals and products. In January 2007, the U.S. Consumer Product Safety Commission (CSPC) submitted a nomination requesting that NICEATM and ICCVAM assess the validation status of (1) The LLNA as a stand-alone assay for potency determination for hazard classification purposes; (2) modified LLNA protocols; (3) the LLNA limit test; (4) the use of LLNA to test mixtures, aqueous solutions, and metals; and (5) the applicability domain for LLNA. In order to facilitate the review of the modified LLNA protocols, ICCVAM proposed developing performance standards for the LLNA. In May 2007, a **Federal Register** notice was published (Vol. 72, No. 95, pages 27815–27817, May 17, 2007) requesting comments and data relevant to these nominated activities. In June 2007, the Scientific Advisory Committee on Alternative Toxicological Methods (SACATM) endorsed the nominated activities as high priorities for ICCVAM. In response to SACATM comments, along with those provided by the public in response to the previous **Federal Register** notice, ICCVAM also endorsed these activities as high priorities. ICCVAM subsequently prepared draft performance standards for the LLNA and now requests public comments on this draft document, which is available on the NICEATM/ICCVAM Web site at: (*http://iccvam.niehs.nih.gov/methods/immunotox/immunotox.htm*) or by contacting NICEATM (see **FOR FURTHER INFORMATION CONTACT** below).

DATES: Submit comments on or before October 29, 2007.

ADDRESSES: Dr. William S. Stokes, NICEATM Director, NIEHS, P.O. Box 12233, MD EC–17, Research Triangle Park, NC 27709, (fax) 919–541–0947, (e-mail) *niceatm@niehs.nih.gov*. Courier address: NICEATM, 79 T.W. Alexander Drive, Building 4401, Room 3128, Research Triangle Park, NC 27709. Responses can be submitted electronically at the ICCVAM–NICEATM Web site: *http://iccvam.niehs.nih.gov/contact/FR_pubcomment.htm* or by e-mail, mail, or fax.

FOR FURTHER INFORMATION CONTACT: Other correspondence should be directed to Dr. William S. Stokes (919–541–2384 or *niceatm@niehs.nih.gov*).

SUPPLEMENTARY INFORMATION:

Background

The LLNA is an alternative test method used for skin sensitization testing that reduces the number of animals needed, reduces the time required for testing, and can substantially reduce or avoid pain and distress associated with traditional guinea pig testing methods. The LLNA was the first alternative test method evaluated and recommended by ICCVAM and based on the recommendations of ICCVAM and an independent scientific peer review panel, the LLNA has been accepted by U.S. and international regulatory authorities as an alternative to the guinea pig maximization test and Buehler test for assessing allergic contact dermatitis (EPA 2003; ISO 2002; OECD 2002). Since 2003, ICCVAM has routinely developed performance standards for test methods; however, because the concept of performance standards was not developed by ICCVAM until 2003, they were not developed during the ICCVAM evaluation of the LLNA in 1998 (NIH Publication No. 99–4494, available: (*http://iccvam.niehs.nih.gov/docs/immunotox_docs/llna/llnarep.pdf*).

In January 2007, CSPC submitted a nomination requesting that NICEATM and ICCVAM assess the validation status of (1) The LLNA as a stand-alone assay for potency determination for classification purposes; (2) modified LLNA protocols; (3) the LLNA limit test; (4) the use of LLNA to test mixtures, aqueous solutions, and metals; and (5) the applicability domain for LLNA. ICCVAM endorsed the nomination and also decided to develop performance standards to facilitate evaluation of modified LLNA protocols to the traditional LLNA. In May 2007, a **Federal Register** notice was published requesting comments and data relevant to these activities (Vol. 72, No. 95, pages 27815–27817, May 17, 2007; available,

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http://iccvam.niehs.nih.gov/SuppDocs/FedDocs/FR/FR_E7_9544.pdf). In June 2007, SACATM endorsed these activities as high priorities for ICCVAM. In response to SACATM comments, along with those provided by the public in response to the previous **Federal Register** notice, ICCVAM endorsed these activities, including the development of performance standards, as high priorities. ICCVAM subsequently prepared draft performance standards for the LLNA, which are available on the NICEATM/ICCVAM Web site at: (*http://iccvam.niehs.nih.gov/methods/immunotox/immunotox.htm*).

These draft test method performance standards are proposed to evaluate the performance of LLNA test methods that incorporate specific modifications to the measurement of lymphocyte proliferation in the traditional LLNA. These modifications focus specifically on incorporating non-radioactive procedures to evaluate lymphocyte proliferation in the draining auricular lymph nodes rather than incorporation of radioactivity (i.e., ^{3}H-thymidine), which is used in the traditional LLNA.

Public comments received in response to the draft LLNA performance standards will be considered by ICCVAM during development of a revised draft version of this document. A public meeting is planned for early 2008 where an international, independent, peer review panel will evaluate the revised draft LLNA performance standards and review the other nominated LLNA related activities. Following this meeting, the recommendations of the peer review panel will be made available for public and SACATM comment. ICCVAM will consider the panel report and public and SACATM comments in preparing final LLNA performance standards.

Request for Public Comments

NICEATM invites the submission of written comments on the draft LLNA performance standards. When submitting written comments, please refer to this **Federal Register** notice and include appropriate contact information (name, affiliation, mailing address, phone, fax, e-mail, and sponsoring organization, if applicable). All comments received by the deadline listed above will be placed on the NICEATM/ICCVAM Web site (*http://ntp-apps.niehs.nih.gov/iccvampb/searchPubCom.cfm*) and made available to the peer review panel and ICCVAM.

Background Information on ICCVAM and NICEATM

ICCVAM is an interagency committee composed of representatives from 15 federal regulatory and research agencies that use or generate toxicological information. ICCVAM conducts technical evaluations of new, revised, and alternative methods with regulatory applicability and promotes the scientific validation and regulatory acceptance of toxicological test methods that more accurately assess the safety and hazards of chemicals and products and that refine, reduce, or replace animal use. The ICCVAM Authorization Act of 2000 (42 U.S.C. 285*l*–3, available at *http://iccvam.niehs.nih.gov/about/PL106545.htm*) establishes ICCVAM as a permanent interagency committee of the NIEHS under NICEATM. NICEATM administers ICCVAM and provides scientific and operational support for ICCVAM-related activities. NICEATM and ICCVAM work collaboratively to evaluate new and improved test methods applicable to the needs of federal agencies. Additional information about ICCVAM and NICEATM is available on the following Web site: *http://iccvam.niehs.nih.gov.*

Dated: September 5, 2007.

Samuel H. Wilson,

Acting Director, National Institute of Environmental Health Sciences and National Toxicology Program.

[FR Doc. E7–18011 Filed 9–11–07; 8:45 am]

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1360 Federal Register / Vol. 73, No. 5 / Tuesday, January 8, 2008 / Notices

DEPARTMENT OF HEALTH AND HUMAN SERVICES

National Institutes of Health

National Toxicology Program (NTP); NTP Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM); Announcement of an Independent Scientific Peer Review Panel Meeting on the Murine Local Lymph Node Assay; Availability of Draft Background Review Documents; Request for Comments

AGENCY: National Institute of Environmental Health Sciences (NIEHS), National Institutes of Health (NIH).

ACTION: Meeting announcement and request for comments.

SUMMARY: NICEATM in collaboration with the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) announces an independent scientific peer review panel meeting to evaluate modifications and new applications for the Murine Local Lymph Node Assay (LLNA). The LLNA is an alternative test method that can be used to determine the allergic contact dermatitis potential of chemicals and products. The panel will review the following:

- The validation status of three modified LLNA test method protocols that use non-radioactive probe chemicals.
- The validation status of a LLNA limit dose procedure.
- The use of the LLNA to test mixtures, aqueous solutions, and metals (applicability domain for the LLNA).
- The use of the LLNA to determine potency (potential for causing allergic contact dermatitis).
- Revised draft recommended performance standards for the LLNA.

At this meeting, the panel will peer review the draft background review documents and revised draft LLNA performance standards for each topic and evaluate the extent that established validation and acceptance criteria have been appropriately addressed. The panel will also comment on the extent

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that the review documents support draft ICCVAM recommendations on proposed test method protocols, proposed uses of the LLNA, and the revised draft LLNA performance standards.

NICEATM invites public comments on the draft background review documents, draft ICCVAM test recommendations, draft test method protocols, and revised draft LLNA performance standards. All documents will be available on the NICEATM–ICCVAM Web site at *http://iccvam.niehs.nih.gov/methods/immunotox/immunotox.htm* by January 8, 2008.

DATES: The meeting is scheduled for March 4–6, 2008, from 8:30 a.m. to 5 p.m. each day. The meeting is open to the public free of charge, with attendance limited only by the space available. In order to facilitate planning for this meeting, persons wishing to attend are asked to register by February 20, 2008, via the NICEATM–ICCVAM Web site (*http://iccvam.niehs.nih.gov/contact/reg_LLNAPanel.htm*). The deadline for written comments is February 22, 2008.

ADDRESSES: The meeting will be held at the U.S. Consumer Product Safety Commission (CPSC) Headquarters, Bethesda Towers Bldg., 4330 East West Highway, Bethesda, MD.

FOR FURTHER INFORMATION CONTACT: Comments may also be submitted via the NICEATM–ICCVAM Web site at *http://iccvam.niehs.nih.gov/contact/FR_pubcomment.htm*. Comments or other correspondence can be sent to Dr. William S. Stokes, NICEATM Director, NIEHS, P.O. Box 12233, MD EC–17, Research Triangle Park, NC, 27709, (phone) 919–541–2384, (fax) 919–541–0947, (e-mail) *niceatm@niehs.nih.gov*. Courier address: NICEATM, 79 T.W. Alexander Drive, Building 4401, Room 3128, Research Triangle Park, NC 27709.

SUPPLEMENTARY INFORMATION:

Background

The LLNA is a reduction and refinement alternative test method for skin sensitization testing because it reduces the number of animals needed and can substantially reduce or avoid pain and distress compared to traditional guinea pig testing methods for sensitization. The LLNA was the first alternative test method evaluated and recommended by ICCVAM (NIH Publication No. 99–4494, available at: *http://iccvam.niehs.nih.gov/docs/immunotox_docs/llna/llnarep.pdf*). Based on the recommendations of ICCVAM and an independent scientific peer review panel, U.S. and international regulatory authorities have accepted the LLNA as an alternative to the guinea pig maximization test and Buehler test for assessing allergic contact dermatitis (ISO 2002; OECD 2002; EPA 2003). This review will evaluate the potential for broader use of the LLNA for regulatory testing of chemicals and products for allergic contact dermatitis potential, enabling further reduction and refinement (less pain and suffering) of animal use for this purpose. In January 2007, the CPSC submitted a nomination requesting that NICEATM and ICCVAM assess the validation status of (1) the LLNA as a stand-alone assay for potency determination for hazard classification purposes; (2) modified LLNA protocols; (3) the LLNA limit test; (4) the use of the LLNA to test mixtures, aqueous solutions, and metals; and (5) the applicability domain for the LLNA. In June 2007, the Scientific Advisory Committee on Alternative Toxicological Methods (SACATM) endorsed these activities as high priorities for ICCVAM. NICEATM on behalf of ICCVAM also sought input from the public on these activities (**Federal Register**: Vol. 72, No. 95, pages 27815–27817, May 17, 2007). After considering these inputs, ICCVAM endorsed these activities as high priorities. ICCVAM is also developing performance standards to facilitate evaluation of modified LLNA protocols compared to the traditional LLNA. Although ICCVAM has routinely developed performance standards for test methods since 2003, they were not developed as part of the ICCVAM evaluation of the LLNA in 1998. These draft performance standards for the LLNA were made public and comments were requested via the **Federal Register** (Vol. 72, No. 176, pages 52130–52131, Sept. 12, 2007). The May 2007 **Federal Register** notice requested data from studies using the LLNA or modified versions of the LLNA.

Drawing on the submitted data and literature sources, ICCVAM and NICEATM drafted background review documents for each of the modifications and new applications of the LLNA. ICCVAM has also developed draft test method recommendations regarding the proposed usefulness, limitations, and validation status of these test methods. ICCVAM will convene an independent scientific panel to peer review the draft background review documents for the test methods and determine whether the data and analyses in the draft documents support the draft ICCVAM test method recommendations. The panel will also be asked to comment on the adequacy of the revised draft performance standards, proposed future studies, draft standardized test method protocols, and recommended reference substances. NICEATM will ask the panel to consider all available information, including the scientific studies cited in the draft review documents, public comments, and any new information identified during the peer review, for developing their conclusions and recommendations.

Peer Review Panel Meeting

The purpose of this meeting is to conduct a scientific peer review of the revised draft performance standards and an evaluation of modifications and new applications for the LLNA. The LLNA is an alternative test method that can be used to determine the allergic contact dermatitis potential of chemicals and products. The panel will review the following:

- The LLNA as a stand-alone assay for potency determination for hazard classification purposes
- Modified LLNA protocols
- The LLNA limit test
- The use of the LLNA to test mixtures, aqueous solutions, and metals (applicability domain for the LLNA)
- The use of the LLNA to determine potency (potential for causing allergic contact dermatitis).

The panel will consider the draft background review documents for each of these methods and evaluate the extent that established validation and acceptance criteria are appropriately addressed for each test method (as described in the ICCVAM document, *Validation and Regulatory Acceptance of Toxicological Test Methods: A Report of the ad hoc Interagency Coordinating Committee on the Validation of Alternative Methods,* NIH Publication No. 97–981, available at *http://iccvam.niehs.nih.gov/docs/about_docs/validate.pdf*). The panel will then comment on the extent to which the draft ICCVAM recommendations are supported by the information provided in the background review document for each topic. It is anticipated that the panel will address the topics in the following order:

1. The LLNA limit test.
2. The applicability domain of the LLNA including its suitability for mixtures, aqueous solutions, and metals.
3. The LLNA as a stand-alone assay for potency determination for hazard classification.
4. The revised draft performance standards for the LLNA.
5. The modified LLNA test method protocols using non-radioactive materials.

Additional information about the meeting, including a roster of the panel members and the draft agenda, will be made available two weeks prior to the meeting on the NICEATM-ICCVAM Web site (*http://iccvam.niehs.nih.gov*). This information will also be available after that date by contacting NICEATM (see **FOR FURTHER INFORMATION CONTACT** above).

Attendance and Registration

This public meeting will take place March 4–6, 2008, at the CPSC Headquarters, Bethesda Towers Bldg., 4330 East West Highway, Bethesda, MD (an area map, driving directions, and CPSC contact information are available at *http://www.cpsc.gov/about/contact.html*). The meeting will begin at 8:30 a.m. and is scheduled to conclude at approximately 5 p.m. each day, although adjournment on March 6 may occur earlier or later depending upon the time needed for the expert panel to complete its work. It is also possible that the panel may conclude its deliberations on March 5 and not need to meet on March 6. Persons needing special assistance in order to attend, such as sign language interpretation or other reasonable accommodation, should contact 919–541–2475 (voice), 919–541–4644 TTY (text telephone, through the Federal TTY Relay System at 800–877–8339), or e-mail *niehsoeeo@niehs.nih.gov*. Requests should be made at least seven days in advance of the event.

Availability of the Draft Background Review Documents and Draft ICCVAM Recommendations

NICEATM prepared draft background review documents on each of these modifications or applications of the LLNA that describe the current validation status of the modified test methods and applications and contain all of the data and analyses supporting this proposed validation status. The draft background review documents, draft ICCVAM test method recommendations, draft test method protocols, and revised draft test method performance standards are available from the NICEATM–ICCVAM Web site (*http://iccvam.niehs.nih.gov/methods/immunotox/immunotox.htm*) or by contacting NICEATM (see **FOR FURTHER INFORMATION CONTACT** above).

Request for Public Comments

NICEATM invites the submission of written comments on the draft background review documents, draft ICCVAM test method recommendations, draft test method protocols, and revised draft test method performance standards. Written comments should be submitted preferably electronically via the NICEATM–ICCVAM Web site or by e-mail (*niceatm@niehs.nih.gov*); the deadline for submission of written comments is February 22, 2008. When submitting written comments, please refer to this **Federal Register** notice and include appropriate contact information (name, affiliation, mailing address, phone, fax, e-mail, and sponsoring organization, if applicable). Written comments may also be sent by mail, fax, or e-mail to Dr. William Stokes (see **FOR FURTHER INFORMATION CONTACT** above). All comments received will be placed on the NICEATM–ICCVAM Web site (*http://iccvam.niehs.nih.gov*) and identified by the individual's name and affiliation or sponsoring organization (if applicable). Comments will also be sent to the panel and ICCVAM agency representatives and made available at the meeting.

This meeting is open to the public, and time will be provided for the presentation of oral comments by the public at designated times during the peer review. Members of the public who wish to present oral statements at the meeting should contact NICEATM (see **FOR FURTHER INFORMATION CONTACT** above) no later than February 20, 2008, and provide contact information (name, affiliation, mailing address, phone, fax, e-mail, and sponsoring organization, if applicable). Up to seven minutes will be allotted per speaker, one speaker per organization. Persons registering to make comments are asked to provide NICEATM a written copy of their statement by February 27, 2008, so that copies can be distributed to the panel prior to the meeting. If this is not possible, please bring 40 copies of your comments to the meeting for distribution and to supplement the record. Written statements can supplement and expand the oral presentation.

Summary minutes and the panel's final report will be available following the meeting on the NICEATM–ICCVAM Web site (*http://iccvam.niehs.nih.gov*). ICCVAM will consider the panel's conclusions and recommendations and any public comments received when finalizing their test method recommendations and performance standards for these methods.

Background Information on ICCVAM and NICEATM

ICCVAM is an interagency committee composed of representatives from 15 Federal regulatory and research agencies that use or generate toxicological information. ICCVAM conducts technical evaluations of new, revised, and alternative methods with regulatory applicability, and promotes the scientific validation and regulatory acceptance of toxicological test methods that more accurately assess the safety and hazards of chemicals and products and that refine, reduce, or replace animal use. The ICCVAM Authorization Act of 2000 (42 U.S.C. 285l–3, available at *http://iccvam.niehs.nih.gov/docs/about_docs/PL106545.pdf*) establishes ICCVAM as a permanent interagency committee of the NIEHS under NICEATM. NICEATM administers ICCVAM and provides scientific and operational support for ICCVAM-related activities. NICEATM and ICCVAM work collaboratively to evaluate new and improved test methods applicable to the needs of Federal agencies. Additional information about ICCVAM and NICEATM is available on the NICEATM–ICCVAM Web site at *http://iccvam.niehs.nih.gov*.

References

EPA. 2003. EPA OPPTS 870.2600 Test Guideline—Skin Sensitization. Available: *http://www.epa.gov/opptsfrs/publications/OPPTS_Harmonized/870_Health_Effects_Test_Guidelines/Drafts/870–2600.pdf*.

ISO. 2002. ISO 10993–10 Biological evaluation of medical devices—Part 10: Tests for irritation and delayed-type hypersensitivity. Geneva: International Organization for Standardization.

OECD. 2002. OECD Guideline for the Testing of Chemicals—Test Guideline 429: Skin Sensitization: Local Lymph Node Assay (adopted 24 April 2002). Paris: Organisation for Economic Co-operation and Development.

Dated: December 19, 2007.

Samuel H. Wilson,

Acting Director, National Institute of Environmental Health Sciences and National Toxicology Program.

[FR Doc. E7–25553 Filed 1–7–08; 2:42 pm]

BILLING CODE 4140–01–P

25754 **Federal Register** / Vol. 73, No. 89 / Wednesday, May 7, 2008 / Notices

DEPARTMENT OF HEALTH AND HUMAN SERVICES

National Toxicology Program (NTP); Office of Liaison, Policy and Review; Meeting of the Scientific Advisory Committee on Alternative Toxicological Methods (SACATM)

AGENCY: National Institute of Environmental Health Sciences (NIEHS), National Institutes of Health (NIH).

ACTION: *COM057*Meeting announcement and request for comment.

SUMMARY: Pursuant to section 10(a) of the Federal Advisory Committee Act, as amended (5 U.S.C. Appendix 2), notice is hereby given of a meeting of SACATM on June 18–19, 2008, at the

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Radisson Hotel Research Triangle Park, 150 Park Drive, Research Triangle Park, NC 27709. The meeting is scheduled from 8:30 a.m. to 5:30 p.m. on June 18 and 8:30 a.m. until adjournment on June 19. The meeting is open to the public with attendance limited only by the space available. SACATM advises the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM), the NTP Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM), and the Director of the NIEHS and NTP regarding statutorily mandated duties of ICCVAM and activities of NICEATM.

DATES: The SACATM meeting will be held on June 18 and 19, 2008. All individuals who plan to attend are encouraged to register online at the NTP Web site (*http://ntp.niehs.nih.gov/go/7441*) by June 10, 2008. In order to facilitate planning, persons wishing to make an oral presentation are asked to notify Dr. Lori White, NTP Executive Secretary, via online registration, phone, or email by June 10, 2008 (see **ADDRESSES** below). Written comments should also be received by June 10 to enable review by SACATM and NIEHS/NTP staff before the meeting.

ADDRESSES: The SACATM meeting will be held at the Radisson Hotel Research Triangle Park, 150 Park Drive, Research Triangle Park, NC 27709 [hotel: (919) 549–8631]. Public comments and other correspondence should be directed to Dr. Lori White (NTP Office of Liaison, Policy and Review, NIEHS, P.O. Box 12233, MD A3–01, Research Triangle Park, NC 27709; telephone: 919–541–9834 or e-mail: *whiteld@niehs.nih.gov*). Courier address: NIEHS, 111 T.W. Alexander Drive, Room A326, Research Triangle Park, NC 27709. Persons needing interpreting services in order to attend should contact 301–402–8180 (voice) or 301–435–1908 (TTY). Requests should be made at least 7 days in advance of the meeting.

SUPPLEMENTARY INFORMATION:

Preliminary Agenda Topics and Availability of Meeting Materials

Preliminary agenda topics include:

- NICEATM–ICCVAM Update;
- Overview of NICEATM–ICCVAM 5–Year Plan;
- NRC Report: Toxicity Testing in the 21st Century;
- Presentations from Federal Agencies on Research, Development, Translation, and Validation Activities Relevant to the NICEATM–ICCVAM Five-Year Plan;
- Report on the ICCVAM–NICEATM Independent Scientific Peer Review Meeting: Validation Status of New Versions and Applications of the Murine Local Lymph Node Assay (LLNA), a Test Method for Assessing the Contact Dermatitis Potential of Chemicals and Products;
- Report on the ICCVAM–NICEATM–ECVAM–JACVAM Scientific Workshop on Acute Chemical Safety Testing: Advancing In Vitro Approaches and Humane Endpoints for Systemic Toxicity Evaluations;
- Nominations to ICCVAM: NTP Rodent Bioassay for Carcinogenicity;
- Proposal for International Cooperation on Alternative Test Methods;
- Update from the Japanese Center for the Validation of Alternative Methods;
- Update from the European Center for the Evaluation of Alternative Methods,

A copy of the preliminary agenda, committee roster, and additional information, when available will be posted on the NTP Web site (*http://ntp.niehs.nih.gov/go/7441*) or available upon request (see **ADDRESSES** above). Following the SACATM meeting, summary minutes will be prepared and available on the NTP website or upon request.

Request for Comments

Both written and oral public input on the agenda topics is invited. Written comments received in response to this notice will be posted on the NTP Web site. Persons submitting written comments should include their name, affiliation (if applicable), and sponsoring organization (if any) with the document. Time is allotted during the meeting for presentation of oral comments and each organization is allowed one time slot per public comment period. At least 7 minutes will be allotted for each speaker, and if time permits, may be extended up to 10 minutes at the discretion of the chair. Registration for oral comments will also be available on-site, although time allowed for presentation by on-site registrants may be less than for pre-registered speakers and will be determined by the number of persons who register at the meeting.

Persons registering to make oral comments are asked to do so through the online registration form (*http://ntp.niehs.nih.gov/go/7441*) and to send a copy of their statement to Dr. White (see **ADDRESSES** above) by June 10 to enable review by SACATM, NICEATM–ICCVAM, and NIEHS/NTP staff prior to the meeting. Written statements can supplement and may expand the oral presentation. If registering on-site and reading from written text, please bring 40 copies of the statement for distribution and to supplement the record.

Background Information on ICCVAM, NICEATM, and SACATM

ICCVAM is an interagency committee composed of representatives from 15 Federal regulatory and research agencies that use, generate, or disseminate toxicological information. ICCVAM conducts technical evaluations of new, revised, and alternative methods with regulatory applicability and promotes the development, scientific validation, regulatory acceptance, implementation, and national and international harmonization of new, revised, and alternative toxicological test methods that more accurately assess the safety and hazards of chemicals and products and that refine, reduce, and replace animal use. The ICCVAM Authorization Act of 2000 [42 U.S.C. 285l–3] established ICCVAM as a permanent interagency committee of the NIEHS under NICEATM. NICEATM administers ICCVAM and provides scientific and operational support for ICCVAM-related activities. NICEATM and ICCVAM work collaboratively to evaluate new and improved test methods applicable to the needs of U.S. Federal agencies. Additional information about ICCVAM and NICEATM can be found on their Web site (*http://iccvam.niehs.nih.gov*).

SACATM was established in response to the ICCVAM Authorization Act [Section 285l–3(d)] and is composed of scientists from the public and private sectors. SACATM advises ICCVAM, NICEATM, and the Director of the NIEHS and NTP regarding statutorily mandated duties of ICCVAM and activities of NICEATM. SACATM provides advice on priorities and activities related to the development, validation, scientific review, regulatory acceptance, implementation, and national and international harmonization of new, revised, and alternative toxicological test methods. Additional information about SACATM, including the charter, roster, and records of past meetings, can be found at *http://ntp.niehs.nih.gov/go/167*.

Dated: April 28, 2008.

Samuel H. Wilson,

Acting Director, National Institute of Environmental Health Sciences and National Toxicology Program.

[FR Doc. E8–10010 Filed 5–6–08; 8:45 am]

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29136 Federal Register / Vol. 73, No. 98 / Tuesday, May 20, 2008 / Notices

DEPARTMENT OF HEALTH AND HUMAN SERVICES

National Toxicology Program (NTP); NTP Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM); Peer Review Panel Report on the Validation Status of New Versions and Applications of the Murine Local Lymph Node Assay (LLNA): A Test Method for Assessing the Allergic Contact Dermatitis Potential of Chemicals and Products: Notice of Availability and Request for Public Comments

AGENCY: National Institute of Environmental Health Sciences (NIEHS), National Institutes of Health (NIH).

ACTION: Request for comments.

SUMMARY: NICEATM, in collaboration with the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM), convened an independent international scientific peer review panel on March 4–6, 2008 to evaluate new versions and applications of the LLNA for assessing the allergic contact dermatitis potential of chemicals and products. The peer review panel ("the Panel") report from this meeting is now available. The report contains (1) the Panel's evaluation of the validation status of the methods and (2) the Panel's comments and conclusions on draft ICCVAM test method recommendations. NICEATM invites public comment on the Panel's report. The report is available on the NICEATM–ICCVAM Web site at *http://iccvam.niehs.nih.gov/methods/immunotox/llna_PeerPanel.htm* or by contacting NICEATM at the address given below.

DATES: Written comments on the Panel report should be received by July 7, 2008.

ADDRESSES: Comments should be submitted preferably electronically via the NICEATM–ICCVAM Web site at *http://iccvam.niehs.nih.gov/contact/FR_pubcomment.htm*. Comments can also be submitted by e-mail to *niceatm@niehs.nih.gov*. Written comments can be sent by mail or fax to Dr. William S. Stokes, Director, NICEATM, NIH/NIEHS, P.O. Box 12233, MD EC–17, Research Triangle Park, NC 27709, (phone) 919–541–2384, (fax) 919–541–0947. Courier address: NICEATM, 79 T.W. Alexander Drive, Building 4401, Room 3128, Research Triangle Park, NC 27709.

FOR FURTHER INFORMATION CONTACT: Dr. William S. Stokes, Director, NICEATM (919–541–2384 or *niceatm@niehs.nih.gov*).

SUPPLEMENTARY INFORMATION:

Background

In January 2007, the Consumer Product Safety Commission submitted a nomination to NICEATM and ICCVAM to assess the validation status of (1) The use of the LLNA to determine potency for hazard classification purposes; (2) LLNA protocols using non-radioactive procedures; (3) the LLNA limit dose procedure; and (4) the use of the LLNA to test mixtures, aqueous solutions, and metals (*i.e.*, an updated assessment of the applicability domain of the LLNA). In June 2007, the Scientific Advisory Committee on Alternative Toxicological Methods (SACATM) endorsed these activities as high priorities for ICCVAM. NICEATM, on behalf of ICCVAM, also sought input from the public on these activities and requested data from studies using the LLNA or modified versions of the LLNA (**Federal Register** Vol. 72, No. 95, pages 27815–27817, May 17, 2007). After considering all comments received, ICCVAM endorsed carrying out these activities as high priorities. ICCVAM also developed draft LLNA performance standards to facilitate evaluation of modified LLNA protocols that are functionally and mechanistically similar to the traditional LLNA. These draft LLNA performance standards were made public and comments were requested via the **Federal Register** (Vol. 72, No. 176, pages 52130–52131, Sept. 12, 2007).

ICCVAM and NICEATM prepared draft background review documents (BRDs) that provided comprehensive reviews of available data and relevant information for each of the modifications and new applications of the LLNA. ICCVAM also developed draft test method recommendations regarding the proposed usefulness and limitations, standardized protocols, and future studies. Both the draft BRDs and draft recommendations were made available for public comment, and a public peer review meeting was announced in the **Federal Register** (Vol. 73, No. 5, pages 1360–1362, Jan. 8, 2008).

The Panel met in public session on March 4–6, 2008. The Panel reviewed the draft ICCVAM BRDs for completeness, errors, and omissions of any existing relevant data or information. The Panel evaluated the information in the BRDs to determine the extent to which each of the applicable criteria for validation and acceptance of toxicological test methods (ICCVAM, 2003) had been appropriately addressed. The Panel then considered the ICCVAM draft test method

recommendations (*i.e.*, proposed test method uses, proposed recommended standardized protocol, proposed test method performance standards, and proposed additional studies) and commented on whether the recommendations were supported by the information provided in the draft BRDs.

The Panel's conclusions and recommendations are detailed in the *Peer Review Panel Final Report: Validation Status of New Versions and Applications of the Murine Local Lymph Node Assay (LLNA): A Test Method for Assessing the Allergic Contact Dermatitis Potential of Chemicals and Products* (available at *http://iccvam.niehs.nih.gov/methods/immunotox/llna_PeerPanel.htm*). The draft BRDs, draft ICCVAM test method recommendations, and the draft LLNA Performance Standards are available at *http://iccvam.niehs.nih.gov/methods/immunotox/immunotox.htm*.

Request for Comments

NICEATM invites the submission of written comments on the Panel's report. When submitting written comments, please refer to this **Federal Register** notice and include appropriate contact information (name, affiliation, mailing address, phone, fax, e-mail, and sponsoring organization, if applicable). All comments received will be made publicly available on the NICEATM–ICCVAM Web site at *http://ntp-apps.niehs.nih.gov/iccvampb/searchPubCom.cfm*. In addition, there will be an opportunity for oral public comments on the Panel's report during an upcoming meeting of SACATM scheduled for June 18–19, 2008. Information concerning the SACATM meeting will be published in a separate **Federal Register** notice and available on the SACATM Web site at *http://ntp.niehs.nih.gov/go/7441*.

ICCVAM will consider the Panel report along with SACATM and public comments when finalizing test method recommendations. An ICCVAM test method evaluation report, which will include the final ICCVAM recommendations, will be forwarded to relevant Federal agencies for their consideration. The evaluation report will also be available to the public on the NICEATM–ICCVAM Web site and by request from NICEATM (see ADDRESSES above).

Background Information on ICCVAM, NICEATM, and SACATM

ICCVAM is an interagency committee composed of representatives from 15 Federal regulatory and research agencies that use, generate, or disseminate toxicological information. ICCVAM conducts technical evaluations of new, revised, and alternative methods with regulatory applicability and promotes scientific validation, regulatory acceptance, and national and international harmonization of toxicological test methods that more accurately assess safety and hazards of chemicals and products and that refine, reduce, and replace animal use. The ICCVAM Authorization Act of 2000 (42 U.S.C. 285*l*-3, available at *http://iccvam.niehs.nih.gov/docs/about_docs/PL106545.pdf*) established ICCVAM as a permanent interagency committee of the NIEHS under NICEATM. NICEATM administers ICCVAM and provides scientific and operational support for ICCVAM-related activities. NICEATM and ICCVAM work collaboratively to evaluate new and improved test methods applicable to the needs of Federal agencies. Additional information about ICCVAM and NICEATM can be found at the NICEATM–ICCVAM Web site (*http://iccvam.niehs.nih.gov*).

Additional information about SACATM, including the charter, roster, and records of past meetings, can be found at *http://ntp.niehs.nih.gov/go/167*.

References

ICCVAM, 2003, ICCVAM Guidelines for the Nomination and Submission of New, Revised, and Alternative Test Methods. NIH Publication No. 03–4508. Research Triangle Park, NC: NIEHS. Available at: *http://iccvam.niehs.nih.gov*.

Dated: May 8, 2008.

Samuel H. Wilson,

Acting Director, National Institute of Environmental Health Sciences and National Toxicology Program.

[FR Doc. E8–11195 Filed 5–19–08; 8:45 am]

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Appendix F2

Public Comments Received in Response to *Federal Register* Notices

The public comments listed below are available in printed copies of this document and on request from NICEATM.

72 FR 27815 (May 17, 2007)
The Murine Local Lymph Node Assay: Request for Comments, Nominations of Scientific Experts, and Submission of Data

- Eric Debruyne, Ph.D. (BAYER CropScience) F-19
- H.-W. Vohr, Ph.D. (Bayer HealthCare AG) F-21
- H.-W. Vohr, Ph.D. (Bayer HealthCare AG) F-26
- H.-W. Vohr, Ph.D. (Bayer HealthCare AG) F-31
- Kirill Skirda, Ph.D. (CESIO) F-36
- Mark S. Maier, Ph.D., DABT (CropLife America) F-37
- Phil Botham, Ph.D. (European Crop Protection Association) F-38
- Peter Ungeheuer, Ph.D. (European Federation for Cosmetic Ingredients) F-41
- Dori Germolec, Ph.D. (NIEHS) F-43
- Dori Germolec, Ph.D. (NIEHS) F-44
- Robert L. Guest, B.Sc., CBiol, MlBiol (Safepharm Laboratories Ltd.) F-45
- Daniel R. Cerven, M.S. and Melissa K. Kirk, Ph.D. (MB Research Laboratories) F-47
- Daniel Marsman, D.V.M., Ph.D. (The Procter & Gamble Company) F-51
- Michael J. Olson, Ph.D. (GlaxoSmithKline) F-52
- Anne Marie Api, Ph.D. (Research Institute for Fragrance Materials) F-54
- Peter S. Thorne, Ph.D. (The University of Iowa) F-56
- Catherine Willett, Ph.D. (People for the Ethical Treatment of Animals), Sara Amundson (Humane Society Legislative Fund), Martin Stephens, Ph.D. (Humane Society of the United States), Kristie Stoick, M.P.H. (Physicians Committee for

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Responsible Medicine), Sue A. Leary (Alternatives Research & Development Foundation), and Tracie Letterman, Esq. (American Anti-Vivisection Society) F-57

72 FR 52130 (September 12, 2007)
Draft Performance Standards for the Murine Local Lymph Node Assay: Request for Comments

- Ann-Therese Karlberg, Ph.D. (Goteborg University) F-62
- Jon Richmond, MB ChB, FRCSEd (U.K. Home Office) F-63
- Henk van Loveren, Ph.D. (National Institute of Public Health and the Environment, the Netherlands) F-65
- Catherine Willett, Ph.D. (People for the Ethical Treatment of Animals), Sara Amundson (Humane Society Legislative Fund), Martin Stephens, Ph.D. (Humane Society of the United States), Kristie Stoick, M.P.H. (Physicians Committee for Responsible Medicine), Sue A. Leary (Alternatives Research & Development Foundation), and Tracie Letterman, Esq. (American Anti-Vivisection Society) F-67

73 FR 1360 (January 8, 2008)
Announcement of an Independent Scientific Peer Review Panel Meeting on the Murine Local Lymph Node Assay; Availability of Draft Background Review Documents; Request for Comments

- David Basketter, Ph.D. (DABMEB Consultancy Ltd.) F-70
- David Basketter, Ph.D. (DABMEB Consultancy Ltd.) F-71
- Kenneth T. Bogen, Dr.P.H., DABT (Exponent) F-72
- G. Frank Gerberick, Ph.D. (The Procter & Gamble Company) F-73
- Laurence Musset, Ph.D. (OECD) F-82
- B. Schau F-85
- Catherine Willett, Ph.D. (People for the Ethical Treatment of Animals) and Kristie Stoick, M.P.H. (Physicians Committee for Responsible Medicine) F-86

73 FR 25754 (May 7, 2008)
Meeting of the Scientific Advisory Committee on Alternative Toxicological Methods (SACATM)

- B. Sachau F-91

73 FR 29136 (May 20, 2008)
Peer Review Panel Report on the Validation Status of New Versions and Applications of the Murine Local Lymph Node Assay (LLNA): A Test Method for Assessing the Allergic Contact Dermatitis Potential of Chemicals and Products: Notice of Availability and Request for Public Comments

- No responses received

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Appendix F3

SACATM Comments:

SACATM Meeting on June 18-19, 2008

The original report contained excerpts from the final minutes and speaker presentations of the SACATM meeting convened on June 18-19, 2008. The full meeting minutes are available online at: https://ntp.niehs.nih.gov/ntp/about_ntp/sacatm/archives/2008/minutes20080619_508.pdf

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Appendix G

Relevant Skin Sensitization Regulations and Testing Guidelines

G1 Table of Relevant Skin Sensitization Test Regulations G-3

G2 EPA Health Effects Test Guidelines OPPTS 870.2600: Skin Sensitization (March 2003) G-7

G3 International Organization for Standardization – ISO 10993-10: Biological Evaluation of Medical Devices Part 10: Tests for Irritation and Skin Sensitization (July 2010) G-25

G4 OECD Test Guideline 429: Skin Sensitization – Local Lymph Node Assay (Adopted July 2010) G-27

G5 OECD Test Guideline 406: Skin Sensitisation (Adopted July 1992) G-49

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Appendix G1

Table of Relevant Skin Sensitization Test Regulations

Note to the Reader:
Regulations may be updated in the future. It is recommended that users review the most current version of all regulations identified.

Electronic versions of United States Code (U.S.C.) can be obtained at:
http://www.gpoaccess.gov/uscode/index.html

Electronic versions of the Code of Federal Regulations (CFR) can be obtained at:
http://www.gpoaccess.gov/cfr/index.html

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Skin Sensitization Testing: Relevant US Federal Laws, Regulations, Guidelines, and Recommendations				
Agency, Center, or Office	**Regulated Products**	**Statutory Requirements**	**Regulations**	**Guidelines and Recommendations**
FDA/CDER	Pharmaceuticals	Federal Food, Drug, and Cosmetic Act (U.S.C. Title 21, Chapter 9) Public Health Service Act (U.S.C. Title 42, Chapter 6A)	21 CFR 312 21 CFR 314	Guidance for Industry Immunotoxicology Evaluation of Investigational New Drugs (2002)
EPA/OPPTS	Chemicals as defined by Section 5 of the Act Pesticides	Toxic Substances Control Act (U.S.C. Title 15, Chapter 53) Federal Insecticide, Fungicide, and Rodenticide Act (U.S.C. Title 7, Chapter 6)	40 CFR 158.50 40 CFR 158.100 40 CFR 158.340 40 CFR 700-799	OPPTS 870.2600 (2003) (see **Appendix G2**)
CPSC	Consumer Products	Federal Hazardous Substances Act (U.S.C. Title 15, Chapters 1261-1278)	16 CFR 1500.3	No Specific Guidelines, Guidances, or Recommendations
OSHA	Chemicals	Occupational Safety and Health Act of 1970 (U.S.C. Title 29, Chapter 15)	29 CFR 1910.1200	No Specific Guidelines, Guidances, or Recommendations

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Relevant Skin Sensitization Regulations and Guidelines Europe		
Agency, Center, or Office	**Regulated Products**	**Regulations and Directives**
EU	Dangerous Preparations (Chemicals and Chemical Mixtures)	Directive 1999/45/EC of the European Parliament and of the Council of 31 May 1999 Annex V to Directive 67/548/EEC of 27 June 1967
	Pesticides	Directive 91/414/EEC of the European Parliament and of the Council of 15 July 1991

Relevant Skin Sensitization Regulations and Guidelines International			
Organizations	**Regulated Products**	**Legal Instruments and Recommendations**	**Guidelines, Guidance, and Recommendations**
GHS	Chemicals	GHS Part 3, Chapter 3.4	No Specific Guidelines, Guidances, or Recommendations
ISO	Medical Devices	NA	ISO 10993-10 (2010) (see **Appendix G3**)
OECD	Chemicals	NA	OECD Test Guideline 429 (2010) (see **Appendix G4**) OECD Test Guideline 406 (1992) (see **Appendix G5**)
ICH	NA	NA	No Specific Guidelines, Guidances, or Recommendations

Abbreviations: CDER = Center for Drug Evaluation and Research; CFR = Code of Federal Regulations; CPSC = U.S. Consumer Product Safety Commission; EC = European Community; EEC = European Economic Community; EPA = U.S. Environmental Protection Agency; EU = European Union; FDA = U.S. Food and Drug Administration; GHS = Globally Harmonized System of Classification and Labelling of Chemicals; ICH = International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use; ISO = International Organization for Standardization; NA = not available; OECD = Organisation for Economic Co-operation and Development; OPPTS = Office of Prevention, Pesticides and Toxic Substances; OSHA = U.S. Occupational Safety and Health Administration; US = United States; U.S.C. = United States Code.

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Appendix G2

EPA Health Effects Test Guidelines OPPTS 870.2600: Skin Sensitization (March 2003)

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United States
Environmental Protection
Agency

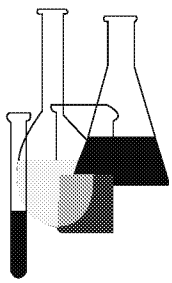
Prevention, Pesticides
and Toxic Substances
(7101)

EPA 712–C–03–197
March 2003

EPA

Health Effects Test Guidelines

OPPTS 870.2600
Skin Sensitization



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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.*).

Final Guideline Release: This guideline is available from the U.S. Government Printing Office, Washington, DC 20402 on disks or paper copies: call (202) 512–0132. This guideline is also available electronically in PDF (portable document format) from EPA's Internet Web site at http://www.epa.gov/opptsfrs/home/guidelin.htm.

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 ^{3}H-methyl thymidine or ^{125}I-iododeoxyuridine (^{125}IU) 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 ≥ 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 ≥ 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 μL/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 µL of sterile phosphate buffered saline (PBS) containing 20 µCi of ^{3}H-methyl thymidine or 250 µL PBS containing 2 µCi ^{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 ^{o}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 ± 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 ^{3}H-methyl thymidine or ^{125}IU 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 alter 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 l58 (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 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)

(3) 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).

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

(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).

(6) Basketter, D.A. et al. Identification of metal allergens in the local lymph node assay. *American Journal of Contact Dermatitis* 10 (4):207–212 (1999).

(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).

(9) Buehler, L.V. Occlusive patch method for skin sensitization in guinea pigs: the Buehler method. *Food and Chemical Toxicology* 32:97–101 (1994).

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

(11) Magnusson, B. and Kligman, A.M. The identification of contact allergens by animal assay. The guinea pig maximization test. *Journal of Investigative Dermatology* 52: 268–276 (1969).

(12) Magnusson, B. and Kligman, A.M. *Allergic contact dermatitis in the guinea pig.* Charles C. Thomas, Springfield, IL (1970).

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

(14) Magnusson, B. et al. Determination of skin sensitization potential of chemicals. Predictive testing in guinea pigs. *Arbete och Halsa:* 26(E) (1979).

(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 (l986).

(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).

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Appendix G3

International Organization for Standardization - ISO 10993-10: Biological Evaluation of Medical Devices Part 10: Tests for Irritation and Skin Sensitization (July 2010)

Document available from the ISO website:

http://www.iso.org/iso/iso_catalogue/catalogue_ics/catalogue_detail_ics.htm?csnumber=40884

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Appendix G4

OECD Test Guideline 429: Skin Sensitization – Local Lymph Node Assay (Adopted July 2010)

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Appendix G5

OECD Test Guideline 406: Skin Sensitisation
(Adopted July 1992)

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406
Adopted:
17.07.92

OECD GUIDELINE FOR TESTING OF CHEMICALS

Adopted by the Council on 17th July 1992

Skin Sensitisation

INTRODUCTION

1. OECD Guidelines for Testing of Chemicals are periodically reviewed in light of scientific progress. In such reviews, special attention is given to possible improvements in relation to animal welfare. This updated version of the original guideline 406, adopted in 1981, is the outcome of a meeting of OECD experts held in Paris in May 1991.

2. Currently, quantitative structure-activity relationships and *in vitro* models are not yet sufficiently developed to play a significant role in the assessment of the skin-sensitisation potential of substances which therefore must continue to be based on *in vivo* models.

3. The guinea pig has been the animal of choice for predictive sensitisation tests for several decades. Two types of tests have been developed: adjuvant tests in which sensitisation is potentiated by the injection of Freunds Complete Adjuvant (FCA), and non-adjuvant tests. In the original guideline 406, four adjuvant tests and three non-adjuvant tests were considered to be acceptable. In this updated version, the Guinea Pig Maximisation Test (GPMT) of Magnusson and Kligman which uses adjuvant (1)(2)(3)(4) and the non-adjuvant Buehler Test (5)(6) are given preference over other methods and the procedures are presented in detail. It is recognised, however, that there may be circumstances where other methods may be used to provide the necessary information on sensitisation potential.

4. The immune system of the mouse has been investigated more extensively than that of the guinea pig. Recently, mouse models for assessing sensitisation potential have been developed that offer the advantages of an endpoint which is measured objectively, short duration and minimal animal treatment. The mouse ear swelling test (MEST) and the local lymph node assay (LLNA) appear to be promising. Both assays have undergone validation in several laboratories (7)(8)(9)(10)(11) and it has been shown that they are able to detect reliably moderate to strong sensitisers. The LLNA or the MEST can be used as a first stage in the assessment of skin sensitisation potential. If a positive result is seen in either assay, a test substance may be designated as a potential sensitiser, and it may not be necessary to conduct a further guinea pig test. However, if a negative result is seen in the LLNA or MEST, a guinea pig test (preferably a GPMT or Buehler Test) must be conducted using the procedure described in this guideline.

5. Definitions used are set out in the Annex.

GENERAL PRINCIPLE OF SENSITISATION TESTS IN GUINEA PIGS

6. The test animals are initially exposed to the test substance by intradermal injection and/or epidermal application (induction exposure). Following a rest period of 10 to 14 days (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 in the test animals is compared with that demonstrated by control animals which undergo sham treatment during induction and receive the challenge exposure.

ELEMENTS COMMON TO SENSITISATION TESTS IN GUINEA PIGS

Sex of animals

7. Male and/or female healthy young adult animals can be used. If females are used they should be nulliparous and non-pregnant.

Housing and feeding conditions

8. The temperature of the experimental animal room should be 20°C (± 3°C) and the relative humidity 30-70 per cent. Where the lighting is artificial, the sequence should be 12 hours light, 12 hours dark. For feeding, 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.

Preparation of the animals

9. Animals are acclimatised to the laboratory conditions for at least 5 days prior to the test. Before the test, animals are randomised and assigned to the treatment groups. Removal of hair is by clipping, shaving or possibly by chemical depilation, depending on the test method used. Care should be taken to avoid abrading the skin. The animals are weighed before the test commences and at the end of the test.

Reliability check

10. The sensitivity and reliability of the experimental technique used should be assessed every six months by use of substances which are known to have mild-to-moderate skin sensitisation properties.

11. In a properly conducted test, a response of at least 30% in an adjuvant test and at least 15% in a non-adjuvant test should be expected for mild/moderate sensitisers. Preferred substances are hexyl cinnamic aldehyde (CAS No. 101-86-0), mercaptobenzothiazole (CAS No. 149-30-4) and benzocaine (CAS No. 94-09-7). There may be circumstances where, given adequate justification, other control substances meeting the above criteria may be used.

Removal of the test substance

12. If removal of the test substance is considered necessary, this should be achieved using water or an appropriate solvent without altering the existing response or the integrity of the epidermis.

DESCRIPTION OF THE GUINEA-PIG MAXIMISATION TEST METHOD

Number of animals

13. A minimum of 10 animals is used in the treatment group and at least 5 animals in the control group. When fewer than 20 test and 10 control guinea pigs have been used, and it is not possible to conclude that the test substance is a sensitiser, testing in additional animals to give a total of at least 20 test and 10 control animals is strongly recommended.

Dose levels

14. The concentration of test substance used for each induction exposure should be well-tolerated systemically and should be the highest to cause mild-to-moderate skin irritation. The concentration used for the challenge exposure should be the highest nonirritant dose. The appropriate concentrations can be determined from a pilot study using two or three animals. Consideration should be given to the use of FCA-treated animals for this purpose.

Induction: Intradermal Injections

Day 0 - treated group

15. Three pairs of intradermal injections of 0.1 ml volume are given in the shoulder region which is cleared of hair so that one of each pair lies on each side of the midline.

Injection 1: a 1:1 mixture (v/v) FCA/water or physiological saline

Injection 2: the test substance in an appropriate vehicle at the selected concentration

Injection 3: the test substance at the selected concentration formulated in a 1:1 mixture (v/v) FCA/water or physiological saline.

16. In injection 3, water soluble substances are dissolved in the aqueous phase prior to mixing with FCA. Liposoluble or insoluble substances are suspended in FCA prior to combining with the aqueous phase. The concentration of test substance shall be equal to that used in injection 2.

17. Injections 1 and 2 are given close to each other and nearest the head, while 3 is given towards the caudal part of the test area.

Day 0 - control group

18. Three pairs of intradermal injections of 0.1 ml volume are given in the same sites as in the treated animals.

Injection 1: a 1:1 mixture (v/v) FCA/water or physiological saline

Injection 2: the undiluted vehicle

Injection 3: a 50% w/v formulation of the vehicle in a 1:1 mixture (v/v) FCA/water or physiological saline.

Induction: Topical Application

Day 5-7 - treated and control groups

19. Approximately twenty-four hours before the topical induction application, if the substance is not a skin irritant, the test area, after close-clipping and/or shaving is painted with 0.5 ml of 10% sodium lauryl sulphate in vaseline, in order to create a local irritation.

Day 6-8 - treated group

20. The test area is again cleared of hair. A filter paper (2 x 4 cm) is fully-loaded with test substance in a suitable vehicle and applied to the test area and held in contact by an occlusive dressing for 48 hours. The choice of the vehicle should be justified. Solids are finely pulverised and

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incorporated in a suitable vehicle. Liquids can be applied undiluted, if appropriate.

Day 6-8 - control group

21. The test area is again cleared of hair. The vehicle only is applied in a similar manner to the test area and held in contact by an occlusive dressing for 48 hours.

Challenge: Topical Application

Day 20-22 - treated and control groups

22. The flanks of treated and control animals are cleared of hair. A patch or chamber loaded with the test substance is applied to one flank of the animals and, when relevant, a patch or chamber loaded with the vehicle only may also be applied to the other flank. The patches are held in contact by an occlusive dressing for 24 hours.

Observations - treated and control groups

23.
- approximately 21 hours after removing the patch the challenge area is cleaned and closely-clipped and/or shaved or depilated if necessary;
- approximately 3 hours later (approximately 48 hours from the start of the challenge application) the skin reaction is observed and recorded according to the grades shown below;
- approximately 24 hours after this observation a second observation (72 hours) is made and once again recorded.

Blind reading of test and control animals is encouraged.

TABLE: MAGNUSSON AND KLIGMAN GRADING SCALE FOR THE EVALUATION OF CHALLENGE PATCH TEST REACTIONS

0 = no visible change

1 = discrete or patchy erythema

2 = moderate and confluent erythema

3 = intense erythema and swelling

Rechallenge

24. If it is necessary to clarify the results obtained in the first challenge, a second challenge (i.e. a rechallenge), where appropriate with a new control group, should be considered approximately one week after the first one. A rechallenge may also be performed on the original control group.

Clinical observations

25. All skin reactions and any unusual findings, including systemic reactions, resulting from induction and challenge procedures should be observed and recorded. Other procedures, e.g.

histopathological examination, the measurement of skin fold thickness, may be carried out to clarify doubtful reactions.

DESCRIPTION OF THE BUEHLER TEST METHOD

Number of animals

26. A minimum of 20 animals is used in the treatment group and at least 10 animals in the control group.

Dose levels

27. The concentration of test substance used for each induction exposure should be the highest to cause mild irritation. The concentration used for the challenge exposure should be the highest non-irritating dose. The appropriate concentration can be determined from a pilot study using two or three animals.

28. For water soluble test materials, it is appropriate to use water or a dilute non-irritating solution of surfactant as the vehicle. For other test materials 80% ethanol/water is preferred for induction and acetone for challenge.

Induction: Topical application

Day 0 - treated group

29. One flank is cleared of hair (closely-clipped). The test patch system should be fully loaded with test substance in a suitable vehicle (the choice of the vehicle should be justified; liquid test substances can be applied undiluted, if appropriate). The test patch system is applied to the test area and held in contact with the skin by an occlusive patch or chamber and a suitable dressing for 6 hours.

30. The test patch system must be occlusive. A cotton pad is appropriate and can be circular or square, but should approximate 4-6 cm^2. Restraint using an appropriate restrainer is preferred to assure occlusion. If wrapping is used, additional exposures may be required.

Day 0 - control group

31. One flank is cleared of hair (closely-clipped). The vehicle only is applied in a similar manner to that used for the treated group. The test patch system is held in contact with the skin by an occlusive patch or chamber and a suitable dressing for 6 hours. If it can be demonstrated that a sham control group is not necessary, a naive control group may be used.

Days 6-8 and 13-15 - treated and control groups

32. The same application as on day 0 is carried out on the same test area (cleared of hair if necessary) of the same flank on day 6-8, and again on day 13-15.

Challenge

Day 27-29 - treated and control groups

33. The untreated flank of treated and control animals is cleared of hair (closely-clipped). An occlusive patch or chamber containing the appropriate amount of test substance is applied, at the maximum non-irritant concentration, to the posterior untreated flank of treated and control animals.

When relevant, an occlusive patch or chamber with vehicle only is also applied to the anterior untreated flank of both treated and control animals. The patches or chambers are held in contact by a suitable dressing for 6 hours.

Observations - treated and control groups

34. - approximately 21 hours after removing the patch the challenge area is cleared of hair;

- approximately three hours later (approximately 30 hours after application of the challenge patch) the skin reactions are observed and recorded according to the grades shown in the Guinea-Pig Maximisation Test (see paragraph 23);

- approximately 24 hours after the 30 hour observation (approximately 54 hours after application of the challenge patch) skin reactions are again observed and recorded.

Blind reading of test and control animals is encouraged.

Rechallenge

35. If it is necessary to clarify the results obtained in the first challenge, a second challenge (i.e. a rechallenge), where appropriate with a new control group, should be considered approximately one week after the first one. The rechallenge may also be performed on the original control group.

Clinical observations

36. All skin reactions and any unusual findings, including systemic reactions, resulting from induction and challenge procedures should be observed and recorded. Other procedures, e.g. histopathological examination, measurement of skin fold thickness, may be carried out to clarify doubtful reactions.

DATA AND REPORTING (GPMT and Buehler Test)

Data

37. Data should be summarised in tabular form, showing for each animal the skin reactions at each observation.

Test report

38. The test report must include the following information:

Test substance:

- physical nature and, where relevant, physicochemical properties;
- identification data.

Vehicle:

- justification of choice of vehicle.

Test animals:

- strain of guinea-pig used;

- number, age and sex of animals;
- source, housing conditions, diet, etc.;
- individual weights of animals at the start and at the conclusion of the test.

Test conditions:

- technique of patch site preparation;
- details of patch materials used and patching technique;
- result of pilot study with conclusion on induction and challenge concentrations to be used in the test;
- details of test substance preparation, application and removal;
- vehicle and test substance concentrations used for induction and challenge exposures and the total amount of substance applied for induction and challenge.

Reliability check:

- a summary of the results of the latest reliability check including information on substance, concentration and vehicle used.

Results:

- on each animal including grading system;
- narrative description of the nature and degree of effects observed;
- any histopathological findings.

Discussion of the results.

If a screening assay is performed before the guinea pig test the description or reference of the test, including details of the procedure, must be given together with results obtained with the test and reference substances.

LITERATURE

(1) Magnusson B. and Kligman A.M. (1969). The identification of contact allergens by animal assay. The guinea pig maximisation test. J. Invest. Dermatol., 52, 268.

(2) Magnusson B. and Kligman A.M. (1970). Allergic Contact Dermatitis in the Guinea Pig. Charles G. Thomas; Springfield, Illinois.

(3) Magnusson B. (1980). Identification of contact sensitizers by animal assay. Cont. Derm., 6, 46.

(4) Magnusson B., Fregert S. and Wahlberg J. (1979). Determination of skin sensitization potential of chemicals. Predictive testing in guinea pigs. Arbete och Hälsa, 26(E).

(5) Buehler E.V. (1965). Delayed contact hypersensitivity in the guinea pig. Arch. Dermatol., 91, 171.

(6) Ritz H.L. and Buehler E.V. (1980). Procedure for conducting the guinea pig assay. Current Concepts in Dermatology, Drill V.A. and Lazar P. (eds), Academic Press, New York, N.Y., 25-40.

(7) Kimber I., Hilton J. and Weisenberger C. (1989). The murine local lymph mode assay for identification of contact allergens: a preliminary evaluation of in situ measurement of lymphocyte proliferation Contact Dermatitis, 21, 215-220.

(8) Kimber I., Hilton J. and Botham P.A. (1990). 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(3), 173-180.

(9) Kimber I., Hilton J., Botham P.A., Basketter D.A., Scholes E.W., Miller K., Robbins M.C., Harrison P.T.C., Gray T.J.B. and Waite S.J. (1991). The murine local lymph node assay: results of an inter-laboratory trial. Toxicology Letters, 55, 203-213.

(10) Basketter D.A., Scholes E.W., Kimber I., Botham P.A., Hilton J., Miller K., Robbins M.C., Harrison P.T.C. and Waite S.J. (1991). Interlaboratory evaluation of the local lymph node assay with 25 chemicals and comparison with guinea pig test data. Toxicology Methods, 1, 30-43.

(11) Gad S.C., Dunn B.J., Dobbs D.W., Reilly C. and Walsh R.D. (1986). Development and validation of an alternative dermal sensitisation test: the mouse ear swelling test (MEST). Toxicol. Appl. Pharmacol., 84, 93-114.

ANNEX

DEFINITIONS

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

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

Induction period: a period of at least one week following an induction exposure during which a hypersensitive state may develop.

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

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